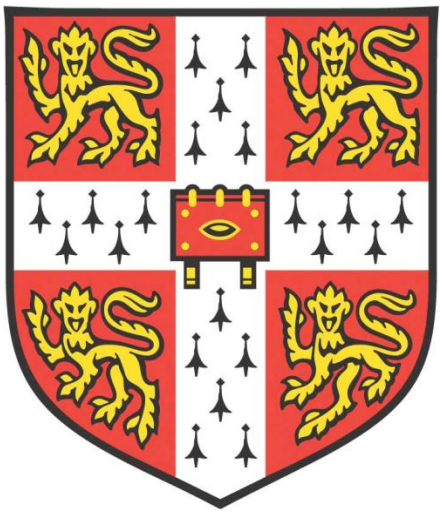
Neurochemical and Neuroanatomical Basis of Reversal Learning in the Rat



Leanne Young

Newnham College, Cambridge

Department of Psychology

University of Cambridge

This thesis is submitted for the degree of Doctor of Philosophy

November 2017

DECLARATION

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NEUROCHEMICAL AND NEUROANATOMICAL BASIS OF REVERSAL LEARNING IN THE RAT – LEANNE YOUNG

ABSTRACT

The aim of this thesis was to investigate the neural and neurochemical substrates of cognitive flexibility using a novel touchscreen task involving serial reversal of visual discrimination in rats. Much evidence has implicated frontostriatal circuitry in the mediation of reversal learning and this thesis sought to further delineate the role of these structures. Although dopamine has been implicated in cognitive flexibility in psychopharmacological studies in primates, there are relatively few studies in the rat. Consequently, the behavioural effects of a dopamine D_2/D_3 receptor antagonist (raclopride) were assessed, both systemically and via intracerebral infusions into different regions of the striatum. Systemic raclopride had no specific effects initially on serial reversal learning, but continued treatment with a low dose did impair retention of a novel visual discrimination, and its subsequent reversal. Intracerebral infusions of raclopride into the dorsomedial and dorsolateral striatum produced a dissociation during separate phases of reversal learning, dorsomedial infusions affecting new learning, and dorsolateral infusions producing perseveration in the early phase. By contrast, raclopride infusions into the anterior dorsomedial striatum produced a general slowing of responding, whereas infusions into the nucleus accumbens core region had no significant effects.

The second part of the thesis investigated the role of prefrontal cortical projections to the striatum in serial reversal learning, focusing on the lateral and medial orbitofrontal cortex, as well as regions of the medial prefrontal cortex. Local temporary inactivation of these structures via infusion of a muscimol/baclofen mixture produced dissociable effects. Inactivation of the infralimbic cortex led to a significant general improvement in reversal learning regardless of phase, while inactivation of the medial orbitofrontal cortex led specifically to a significant reduction in the number of errors during perseveration. By contrast, inactivation of the lateral orbitofrontal cortex caused a significant increase in the number of errors

and omissions during perseveration, whereas prelimbic cortex inactivation had no major effects.

The findings of the thesis are discussed in terms of the separate roles for the different phases of reversal learning of different sectors of the frontal lobe and striatum in the rat, and the modulatory role of the striatal D_2/D_3 receptors. Possible clinical, as well as functional, implications of the results are also considered.

To Guy

& my parents, Roy and Lorna

"Flexibility is a requirement for survival."

Roger Von Oech

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ACKNOWLEDGEMENTS

This thesis would not have been possible without the help and guidance of my supervisor, Professor Trevor Robbins. Trevor has been a constant source of knowledge and support, whose door and inbox were always open. His guidance and mentorship has always pushed me in the right direction, and he has always seen the bigger picture and impact of this research.

I would also like to thank my advisor, Dr Johan Alsiö. I am very grateful for the training Johan provided, and answering all of my questions surrounding both behavioural and surgical techniques. I am thankful for our conversations over lunch that triggered ideas for further work, and his patience and help when I was unable to grasp ideas.

I thank Colin McKenzie, our tireless lab manager. Colin provided his generous and valuable assistance in performing histology, providing his training and experience to get the best results in the wetlab.

Julie Gautrey, Chris Cardinal, Ali, and Hayley, and all of the rest of the animal care staff who provided excellent care for all of my rats, and were always available to answer any questions regarding the overall health and welfare of my animals.

I would like to thank all members of the lab, with special thanks to Ben Phillips, Beth Fisher and Craig Mantanona for providing moral support and being heads to bounce ideas and theories off of. Thanks to: Laith Alexander, Amal Zbib, Júlia Sala Bayo, Emil Gidlund, and Tadej Božič, your assistance in the lab has been invaluable and I wish all of you luck in your careers.

I am not sure I would be here without the support of dear friends, with special mentions to Lucy Pickworth, James Quarrington, and James King. Your moral support has been invaluable, along with the constant supply of tea and baked goods to get me through the long days and make my time at Cambridge more memorable.

Finally, thanks to the BBSRC and Wellcome Trust for funding this research and making this degree possible. And of course I would like to thank the people this thesis is dedicated to, Guy and my parents, for always supporting me and your unconditional love and encouragement.

LIST OF PUBLICATIONS

Please note publications are in the name L Fiddian.

Chapter 4 contributes towards:

Dorsal and ventral striatal dopamine D1 and D2 receptors differentially modulate distinct phases of reversal learning, *Neuropsychopharmacology (submitted)*. J Sala-Bayo; L Fiddian; SRO Nilsson; ME Hervig; C McKenzie; A Mareschi; M Boulos; P Zhukovsky; J Nicholson; JW Dalley; J Alsiö; TW Robbins.

Chapter 6 contributes towards:

Dissociable and Paradoxical Roles of Rat Medial and Lateral Orbitofrontal Cortex in Visual Serial Reversal Learning, *Cerebral Cortex (2019)*. ME Hervig, L Fiddian, L Piilgaard, T Božič, M Blanco-Pozo, C Knudsen, SF Olesen, J Alsiö, TW Robbins.

LIST OF ABBREVIATIONS AND ACRONYMS

The following abbreviations are used in this thesis

5,7-DHT	**5,7-dihydroxytryptamine**
5-HT	**5-hydroxytryptamine**
6-OHDA	**6-hydroxydopamine**
ANOVA	**Analysis of Variance**
CS	**Conditioned stimulus**
DLS	**Dorsolateral striatum**
DMS	**Dorsomedial striatum**
DREADDs	**Designer Receptor Exclusively Activated by Designer Drugs**
GABA	**γ-Aminobutyric acid**
IL	**Infralimbic cortex**
ITI	**Inter-trial interval**
lOFC	**Lateral orbitofrontal cortex**
mOFC	**Medial orbitofrontal cortex**
MSNs	**Medium Spiny Neurons**
NAc	**Nucleus accumbens**
OCD	**Obsessive-compulsive disorder**
OFC	**Orbitofrontal cortex**
PFC	**Prefrontal cortex**
PrL	**Prelimbic cortex**
SEM	**Standard Error of the Mean**

Chapter 1: General Introduction

1.1 Executive function and reversal learning

1.1.1 Measures of executive function

Executive function allows calculated, goal-directed interactions with the world, relying on several cognitive operations that are mediated by sub-regions of the prefrontal cortex. In humans, executive functions, such as decision making, behavioural adaptation, and planning and organising, depend on an intact prefrontal cortex (PFC) (Fuster, 1988; Robbins *et al.*, 1996), with damage to different sectors of the PFC leading to dissociable deficits in decision making tasks (Manes *et al.*, 2002). Anatomical homology between the rodent, non-human primate, and humans has been difficult to establish (Preuss, 1995; Uylings, Groenewegen and Kolb, 2003; Wise, 2008); however, a number of studies reveal similar functional compartmentalisations (for review, see Chudasama and Robbins 2006). Analogous tests of executive function can be tailored to the abilities of the animals, allowing for evaluation of cognitive elements, such as planning, attention, working memory, conditional learning, and response sequencing (Robbins, 1998; Hvoslef-Eide *et al.*, 2015). In general, rodent tests of executive function can be split into three classes, models of working memory, models of attention, and models of decision making (for review, see Chudasama 2011).

1.1.1.1 Models of working memory

Damage to the PFC is characterised by an inability to keep track of information (Fuster, 1985, 1997), manifested by impairments in planning and preparing, recalling a specific order of events, or remembering which stimuli was presented most recently (Chudasama, 2011). Non-human primate and rat models to assess working memory include spatial delay discounting, spatial search, and Trial-Unique Nonmatching-to-Location (TUNL)(Bussey *et al.*, 2012).

A spatial delay discounting task requires the monkey to remember the location of food; the animal observes the experimenter place some food into a well, the wells are then covered and obscured from view, after a delay the animal is required to retrieve the food from the covered well. Jacobsen demonstrated that monkeys with lesions to the anterior PFC are unable to retain the spatial location of the food, even with a minimal delay (Jacobsen, 1936); more recent studies have shown that the dorsolateral region of the primate PFC as the critical region for spatial working memory (Bauer and Fuster, 1976; Levy and Goldman-Rakic, 2000). Similarly, Owen et al demonstrated significant impairments in a paradigm designed to assess spatial working memory in patients with unilateral or bilateral frontal lobe ablation (Owen *et al.*, 1990). A rodent model of spatial delay discounting utilises a T-Maze; rats must alternate their responding to alternating arms of the T-Maze on successive trials, with trials separated by a delay (Dudchenko, 2004).

A spatial search task requires an increased level of complexity compared to a spatial delay model. Frontal lobe dysfunction has been shown to affect both rhesus monkeys in a spatial search task (Passingham, 1985) and humans in a subject-ordered task (Petrides and Milner, 1982). A task has been developed that can be used in both monkeys and humans alike (Owen *et al.*, 1990); using touchscreen technology the subject can "search" boxes to find a token, this task has been shown to be sensitive to frontal lobe damage in both humans and non-human primates (Collins *et al.*, 1998). Using a radial maze; on any individual trial, only a limited number of the arms contain reward, then after a delay rats must enter the previously unrewarded arms, requiring them to remember which arms had been previously visited (Olton and Samuelson, 1976).

The TUNL task was developed by Talpos et al. (2010) as a computer-automated touchscreen paradigm. Using a touchscreen method for spatial separation, stimulus options are no longer limited to the location of two levers (as with the previous Delayed Non-Matching in Position task). Briefly, one stimulus is displayed, and after a variable period of delay the previous stimulus and a novel location are presented; this testing paradigm allows for variations in both delayed presentation and spatial pattern separation.

Measures of working memory have been shown to be dependent on the dorsolateral PFC and dopamine signalling in the PFC (for review, see Chudasama 2011).

1.1.1.2 Models of attention

The most widely used method of assessing attention is the five-choice reaction time task, developed by Carli et al (1983). Briefly, food restricted rats were trained to respond to a brief (0.5 second) flash of light, presented randomly in one of five pre-determined locations with a fixed inter-trial interval. Rats were rewarded for correct responses and mildly punished for incorrect responses with a time-out. As well as being able to measure response accuracy, this task also measures premature responding and perseverative responding, two aspects of behavioural inhibition. The 5-choice task has been shown to be sensitive to lesions of the prelimbic (PrL) cortex, infralimbic (IL) cortex, and areas of the orbital PFC (Chudasama, Bussey and Muir, 2001; Passetti, Chudasama and Robbins, 2002; Chudasama *et al.*, 2003); the 5-choice task has also been shown to be sensitive to both monoaminergic and cholinergic neurotransmitter modulation (Chudasama and Robbins, 2004). The 5-choice task is also part of the touchscreen testing battery of tests (Bussey *et al.*, 2012; Mar *et al.*, 2013; Hvoslef-Eide *et al.*, 2015).

A set-shifting task has been designed for rats (Birrell and Brown, 2000), in which rats are required dig in a selected bowl to retrieve a reward. Rats were presented with two bowls, only one of which contained reward, and the rat had to select a bowl based on discrimination of odour (cinnamon vs cumin), the filling in the bowl (leaf tea vs ground tea), or the texture covering the bowl (fine vs course sandpaper). As with the monkey version of the task, rats were able to perform numerous discriminations, including an intra-dimensional shift, an extradimensional shift, and reversal learning. The ability to shift attentional sets was impaired by lesions of the medial PFC (Birrell and Brown, 2000), while lesions of the orbital PFC induced perseverative responding when exemplars were reversed (Brown and Bowman, 2002).

The continuous performance test (CPT) has recently been developed for rodent translation of the human paradigm (Mar et al. 2012; Kim et al. 2015). Rats were presented with centrally located visual stimuli, a single target or a non-target

stimulus was presented across trials; rats were required to respond to the target stimulus and withhold from responding to non-target stimuli. Manipulations allowing for increased cognitive load include stimulus duration, inter-stimulus interval, target ratio, stimulus contrasts, or the addition of neighbouring distractors (Hvoslef-Eide *et al.*, 2015).

1.1.1.3 Models of decision making

Delay-discounting was originally developed using principles of operant behaviour. Animals are presented with a choice between two rewards, one reward is small but immediate, the other is large but delayed in delivery (Monterosso and Ainslie, 1999). Under delay-discounting conditions, impulsive choice is the preference for smaller, more immediate, rewards. Mar et al. (2011) reported dissociable effects in delay-discounting following lesions to the medial vs lateral orbital frontal cortex (OFC).

Along with delay-discounting, reinforcer devaluation is another model of decision making in the rat. There are two main types of reinforcer devaluation, Pavlovian or satiation. In the Pavlovian version, ingestion of a particular food is paired with illness induced by lithium chloride injections; illness reduces the value of the food reward, leading to decreased responding to the paired stimulus (Holland and Rescorla, 1975). Reduced responding is abolished following orbital PFC lesions in the rat (Gallagher, McMahan and Schoenbaum, 1999). In the satiation version of the task, first stimulus associations must be made between two stimuli and two rewards, following association one reward is made readily available, thereby decreasing its reward value; upon testing, monkeys will preferentially attend to the stimulus paired with the non-devalued reward (Izquierdo, Suda and Murray, 2004). Lesions of the orbital PFC abolish devaluation effects, with lesions of the dorsolateral and ventrolateral PFC showing normal devaluation effects (Baxter *et al.*, 2008, 2009), as do PrL lesions in the rat (Corbit and Balleine, 2003).

Reversal learning is a measure of the ability to adjust to changes in reward contingency. Although stimuli vary, the principle design of this task is the same across humans, monkeys and rodents. First, subjects must learn to discriminate between two stimuli, where one is associate with reward (CS+) and the other is not (CS-). Following successful acquisition of the pairing, the contingencies are reversed

(CS+ → CS-, and vice versa), and subjects must observe this change in contingencies and alter responding accordingly. Due to the translation of the reversal learning task across rodents, non-human primates, and humans, it allows for a further understanding of cognitive flexibility and behavioural inhibition.

1.1.2 Reversal learning

1.1.2.1 Types of reversal learning

Reversal learning is an essential paradigm for assessing cognitive function and flexibility, with reversal learning being disrupted in many neurological and psychiatric disorders (Cools, 2001; Ersche *et al.*, 2008, 2011; Leeson *et al.*, 2009; Remijnse *et al.*, 2013). Understanding cognitive flexibility is necessary to underpin the pathophysiology of neurological and psychiatric disorders, and to develop treatment options. There are a series of reversal learning paradigms that can be administered to rodents, non-human primates and humans in order to study the neuronal substrates associated with cognitive flexibility; while these paradigms are subtly different they all measure an adaptive response to the changing of response-outcome contingencies.

Classical reversal learning paradigms consist of training the subject to respond to either a stimulus or location that is always rewarded, while another is not. After demonstrating successful discrimination by reaching a predetermined level of performance (or criterion), the reward contingencies are reversed. This classical reversal learning paradigm can be employed across species, including mice (Thonnard, Callaerts-Vegh and D'Hooge, 2019), rats (King, Martin and Melville, 1974), monkeys (Butter, 1969) and humans (Lesley K Fellows and Farah, 2003). An advantage of this paradigm is its use across species leading to significant translational value; however, while the classical paradigm is suitable for testing all species it is not optimal in humans due to its simple nature and likely verbal mediation.

Reversal learning in rodents can be assessed through a variety of protocols, including mazes, levers, nose-poke portals, or touchscreens. Mazes, such as the T-maze, levers and portals are commonly used to measure spatial discrimination and reversal (Jentsch and Taylor, 2001; Palencia and Ragozzino, 2006); however, they

also can be paired with a combination of auditory or visual cues (Boulougouris, Dalley and Robbins, 2007; Castañé, Theobald and Robbins, 2010). A novel touchscreen paradigm has been developed for the rat, allowing for a serial visual reversal approach to testing (Alsiö *et al.*, 2015). The use of a touchscreen allows for full automation and a wide variety of visual stimuli, while controlling for spatial strategies (Izquierdo et al. 2006; Mar et al. 2013; Graybeal et al. 2014).

In non-human primates both touchscreens and a modified version of the Wisconsin General Testing Apparatus (WGTA) have been used to probe reversal learning (Roberts, 1996), allowing for spatial learning options where food bowls are baited with rewards (Jones and Mishkin, 1972; Stern and Passingham, 1995). Monkeys can be presented with a range of stimuli on a touchscreen, or cards , with correct selection leading to reward (Crofts *et al.*, 1999; Rygula *et al.*, 2010; Clarke *et al.*, 2011).

There are also a range of reversal learning paradigms for testing in humans. Such testing includes stimuli on a screen with various forms of responding (Lawrence *et al.*, 1999; Swainson *et al.*, 2000). Human testing paradigms are likely to be probabilistic, instead of deterministic, to slow the rate of learning (Cools *et al.*, 2002). Probabilistic reversal learning can also be used in non-human primates as a way to moderate rewards (Walton *et al.*, 2010; Costa *et al.*, 2015), and in rodents (Roberts *et al.*, 2019).

Reversal paradigms used in both non-human primates and rats are very similar in their design; however, the tasks differ in the number of reversals completed by the animal. In the standard WGTA version of reversal learning for macaques, animals often complete multiple serial reversals (Izquierdo, Suda and Murray, 2004), with more recent versions delivering multiple reversals within a single session. Marmosets also complete multiple reversals (Rygula *et al.*, 2010). Comparatively, using the traditional rat paradigm, only one reversal is completed (Schoenbaum *et al.*, 2002). Wisconsin Card Sort Test is a standard way of testing frontal lobe function in humans, and allows for both attentional set-shifting reversal testing (Roberts, 1996).

The study of reversal learning allows for a complex overview of multiple behaviour types, such as perseveration, response inhibition, new learning, and habit formation. In addition to the traditional measures of reversal learning more detailed analysis can be performed; this includes the study of the learning on a trial-by-trial basis, such as investigating a "win-stay, lose-shift" approach (Sala-Bayo *et al.*, submitted), as well as responses to negative and positive feedback through the use of probes (Alsiö *et al.*, 2019).

1.1.2.2 Neuroanatomy of reversal learning and cognitive flexibility

Two main distinct anatomical regions have been implicated in reversal learning, the cortical regions and the striatal regions.

Neuroimaging studies in humans have reported increased activity in the OFC (Kringelback and Rolls, 2003) and medial PFC during reversal learning (Remijnse *et al.*, 2005), and patients with damage to these regions demonstrate impaired reversal learning (Rolls *et al.*, 1994). Studies in non-human primates have also confirmed a role for the OFC in reversal learning; with frontal lobectomies, ablations, localized aspirations, and excitotoxic lesions impairing flexible responding and reversal learning performance (for reviews see Rudebeck and Murray, 2014; Hamilton and Brigman, 2015). Studies in rodents also confirm a role for the OFC in reversal learning; with excitotoxic lesions and inactivation impairing performance on visual, spatial and sensory reversal learning (for reviews, see Izquierdo et al. 2017; Izquierdo 2017). OFC lesion induced reversal learning impairments are abolished by accompanying lesions of the basolateral amygdala (BLA), while BLA lesions alone produce no effect on reversal learning (Stalnaker *et al.*, 2007)

In addition to the OFC, regions of the striatum have been implicated as having a role in reversal learning. As with the OFC, the dorsal and ventral striatum have been reported to be recruited though human imaging studies (Rogers *et al.*, 2000; Cools *et al.*, 2002). Studies in marmosets have reported that neurotoxic lesions of the dorsomedial striatum (DMS) impair various forms of reversal (Clarke, Robbins and Roberts, 2008), with lesions of the nucleus accumbens (NAc) altering performance on spatial, but not visual, reversal learning (Stern and Passingham, 1995). In the rat, DMS lesions impair performance on reversal (Ragozzino, 2007; Castañé, Theobald

and Robbins, 2010), while NAc shell lesions impair performance on a probabilistic reversal learning, with NAc core lesions affecting latency but not overall performance (Dalton, Phillips and Floresco, 2014).

Tracing studied in the rat have shown that the OFC projects into the striatum, accounting for the involvement of both regions in reversal learning (Schilman *et al.*, 2008).

1.1.2.3 Neurochemistry of reversal learning and cognitive flexibility

The most studied neurotransmitters systems contributing to reversal learning are 5-hydroxytryptamine (5-HT) and dopamine. Neurotransmitter investigations include a range of both systemic and local manipulations, producing variable effects depending on the region studied.

1.1.2.3.1 5-HT

5-HT receptors are found widely throughout the body, in both the central and peripheral nervous systems. 5-HT receptors can be divided into seven families of G protein coupled receptors (GPCRs), except for 5-HT_3 which is ligand gated, and acts via downstream second messenger cascades to produce either an excitatory or inhibitory response (Barnes and Sharp, 1999). Excitatory GPCR 5-HT receptors include, 5-HT_2, 5-HT_4, 5-HT_6 and 5-HT_7, whereas 5-HT_1 and 5-HT_4 are inhibitory. Excitatory GPCR 5-HT receptors are either $G_{q/11}$ – protein coupled, or G_s protein coupled and act by increasing cellular levels or IP_3 and DAG or increasing cellular levels of cAMP, respectively. Inhibitory 5-HT receptors are G_i/G_o-protein coupled and elicit their mechanisms by decreasing cellular levels of cAMP. The ligand gated 5-HT_3 receptor is a ligand gated sodium and potassium channel that depolarises the cell membrane. In addition to the seven families of 5-HT receptor, the 5-HT_1 and 5-HT_2 families consist of five and three subtypes, respectively. Due to the nature of the 5-HT receptor family they have been linked to many functions, including but not limited to memory, aggression, anxiety, impulsivity, nociception, heart rate, sexual behaviour, movement and gut motility (Wouters, Tulp and Bevan, 1988; Pitsikas, Brambilla and Borsini, 1994; Kennett *et al.*, 1997; Meneses and Hong, 1997; Parks *et al.*, 1998; McCreary, Bankson and Cunningham, 1999; Borman *et al.*, 2002; N. K. Popova and Amstislavskaya, 2002; Nina K Popova and Amstislavskaya, 2002; Bardin

et al., 2003; Smriga and Torii, 2003; Yasuno *et al.*, 2003; Winstanley *et al.*, 2005; Chojnacka-Wójcik, Kłodzińska and Tatarczyńska, 2005; de Boer and Koolhaas, 2005).

The role of 5-HT in reversal learning is poorly understood, with many results dependent on the type of manipulation used. Systemic 5-HT manipulations have been shown to produce generalised effects on reward learning; systemic treatment with parachhlorophenylalanine (PCPA) or parachloroamphetamine (PCA) to deplete brain 5-HT in rats produced impaired reversal learning, discrimination, and stimulus reward (Masaki *et al.*, 2006; Izquierdo *et al.*, 2012), therefore effects are not limited to reversal learning (Lapiz-Bluhm *et al.*, 2009). However, 5-HT tone in the OFC correlates with reversal learning performance in both the rat and vervet monkey, highlighting an important role for the neurotransmitter (Groman *et al.*, 2013; Barlow *et al.*, 2015). Trytophan depletion in humans also leads to impairments in learning and memory (Park *et al.*, 1994; Rogers *et al.*, 1999).

Citalopram, a 5-HT reuptake inhibitor, improved spatial reversal learning in rats following systemic administration, with animals requiring less trials to reach predetermined learning criterion and committing fewer incorrect responses. Conversely, systemic GBR12909, a dopamine reuptake inhibitor, produced a biphasic effect on reversal learning. High dose GBR12909 produced a significant impairment in reversal learning, with rats requiring more trials to reach criterion, whereas low dose GBR12909 significantly improved reversal performance in the same measure (Barlow *et al.*, 2015). Barlow et al also reported altered levels of 5-HT metabolite markers in the OFC in highly perseverative rats, with no differences in markers of 5-HT function in the DMS, confirming the finding of Clarke *et al.* (2005). Other studies of 5-HT reuptake inhibitors also report the improvement of reversal learning in both mice and rats (Brigman *et al.*, 2010; Brown *et al.*, 2012).

Much work has been performed into specific 5-HT receptor sub-types, producing differential effects. 5-HT_{2A} and 5-HT_{2C} have both been shown to alter spatial reversal learning (Boulougouris, Glennon and Robbins, 2008). Boulougouris et al showed that systemic administration of M100907, a 5-HT_{2A} antagonist, significantly impaired spatial reversal learning by increasing the number of errors and the

number trials to criterion; conversely, they also showed that systemic administration of SB-242,084, a 5-HT_{2C} antagonist, significantly improved performance in spatial reversal learning, by decreasing both the number of errors and the number of trials to criterion. Taken together these data suggest that 5-HT_{2A} and 5-HT_{2C} receptors have distinct roles in cognitive flexibility.

Boulougouris and Robbins (2010) also reported that 5-HT_{2C} mediated improvements of spatial reversal learning were specifically mediated by the OFC. Intracerebral infusions of SB-242,084 and M100907 into the OFC, medial PFC and NAc core produced dissociable effects on reversal learning by location and receptor subtype. 5-HT_{2A} antagonism produced no significant effect in any location, while 5-HT_{2C} mediated improvement was elicited by the OFC. Improvements in reversal performance through intra-OFC infusion of SB-242,084 have been replicated by Alsiö et al. (2015) in the current touchscreen serial reversal paradigm.

1.1.2.3.2 Dopamine

Dopamine receptors are prominent in the central nervous system and, similar to the 5-HT receptor, there are many subtypes. Dopamine receptors fall into two families dependent on their mechanism of action, D_1-like and D_2-like. D_1-like family consists of dopamine D_1 and D_5 receptors, whereas the D_2-like family contains D_2, D_3 and D_4 receptors. Dopamine D1-like receptors are GPCRs coupled to G_s and elicit their mechanism by increasing cellular cAMP. Dopamine D_2-like receptors are inhibitory GPCRs and act through G_i actions to lower cAMP (Sealfon and Olanow, 2000). There are two isoforms of the dopamine D_2 receptor, D_2Sh (short) and D_2Lh (long). D_2Lh functions as a classical post-synaptic receptor, whereas D_2Sh is found pre-synaptically and functions as a dopamine autoreceptor to regulate neurotransmitter levels in the synaptic cleft (Beaulieu and Gainetdinov, 2011). Much like 5-HT, dopamine receptors have a range of functions through the body, including spatial working memory, reward, cognition, motor, cardiac output, and diuresis (Sealfon and Olanow, 2000; Webster, 2001; Contreras *et al.*, 2002; Williams and Castner, 2006).

Dopamine mediates synaptic plasticity in regions of the brain associated with reversal learning, notably the striatum and cortex (Reynolds and Wickens, 2002;

Cagniard *et al.*, 2006; Calabresi *et al.*, 2007). Work by Schultz (2013) describes the role of dopamine in reward prediction error, when errors violate expected outcomes, to enable synaptic plasticity and behavioural learning.

Spatial reversal learning in rats is improved by optogenetic activation of dopaminergic neurons in the ventral tegmental area or substantia nigra pars compacta, suggesting a prominent role for dopamine in the striatum (Adamantidis *et al.*, 2011; Rossi *et al.*, 2013). Indeed, methylphenidate has been shown to increase striatal dopamine, resulting in improved reversal learning in humans (Clatworthy *et al.*, 2009).

Dopamine, not 5-HT, has been shown to regulate reversal learning in the marmoset caudate nucleus, homologous to the medial striatum (Clarke *et al.*, 2011). Using 5,7-dihydroxytryptamine (5,7-DHT) or 6-hydroxydopamine (6-OHDA) to selectively lesion 5-HT or dopamine innervations respectively, Clarke et al showed that dopamine depleted rats were significantly impaired on a serial reversal task, with 5-HT depletion producing no significant effect.

Similar to 5-HT, many studies have investigated the potential roles of the dopamine receptor subtypes in reversal learning. Dopamine D_1 receptor agonism in mice, via SKF81297, led to early reversal deficits in a touchscreen based reversal task (A Izquierdo *et al.*, 2006). In non-human primates, systemic dopamine D_1/D_5 receptor antagonism did not alter reversal learning (Lee *et al.*, 2007). However, dopamine D_2 receptor available has been shown to be important in mice, non-human primates and humans, with low receptor availability leading to poor reversal performance (Jocham *et al.*, 2009; Groman *et al.*, 2011; Laughlin *et al.*, 2011). Dopamine D_2 agonist (bromocriptine) administration led to impaired reversal learning performance, but improved short-term memory, in humans (Mehta *et al.*, 2001). In non-human primates, both stimulation and inhibition of dopamine D_2/D_3 receptors causes impairments on challenging versions of reversal learning (Smith, Neill and Costall, 1999; Lee *et al.*, 2007). In rodents, systemic administration of dopamine D_2/D_3 receptor agonists impaired spatial reversal learning; conversely, systemic dopamine D_2/D_3 receptor antagonism had no effect (Boulougouris, Castañé, and Robbins 2009).

The roles of 5-HT and dopamine in reversal learning seem to be anatomically distinct, with 5-HT mediating cognitive flexibility through actions in the OFC, whereas dopamine modulates reversal learning through phasic release in the striatum acting on the direct and indirect striatal output pathways (Frank and Claus, 2006; Yawata *et al.*, 2012; Klanker *et al.*, 2015, 2017).

1.2 The rat prefrontal cortex

As mentioned above, the PFC has been a focus of research in executive function for many decades due to the growing understanding that damage and dysfunction of these regions and associated circuitry are associated with a large number of neuropsychiatric disorders, including but not limited to OCD, ADHD, Tourette's, schizophrenia, and depression. Despite the non-human primate and rodent PFC regions being a fraction of the size of the human PFC, anatomically and functionally distinct sub-regions have been found (Dias, Robbins and Roberts, 1996, 1997; Bussey, Everitt and Robbins, 1997; Passetti, Chudasama and Robbins, 2002; Chudasama and Robbins, 2003; Chudasama *et al.*, 2003; Ghods-Sharifi, Haluk and Floresco, 2008; Rygula *et al.*, 2010; Mar *et al.*, 2011).

1.2.1 Anatomy of the rat prefrontal cortex

The volume of the rat cerebral cortex is approximately a thousand times smaller than that of the human brain (Uylings and van Eden, 1990); despite this, attempts have been made to characterise the rat PFC based on definitions and characteristics of the PFC. Based on Rose and Woolsey's definition of PFC as an area of the cortex that receives reciprocal projections from the mediodorsal nucleus of the thalamas (Rose and Woolsey, 1948), as well as other distinct criteria (Uylings, Groenewegen and Kolb, 2003), several area of the rat PFC have been identified (Figure 1.1).

The rat PFC can be divided into three areas. First, the medial frontal division; second, the ventral region; and third, the lateral region. The medial prefrontal cortex (mPFC), can be subdivided into the ventral region containing the medial orbital (MO), infralimbic (IL) and prelimbic (PrL) cortices, and the dorsal region containing the anterior cingulate (ACg) and precentral cortices (PrC). The mPFC composes the

majority of the medial wall of the hemispheres, anterior and dorsal to the corpus callosum. The ventral region consists of the ventral orbital (VO) and ventral lateral orbital (VLO) cortices. The lateral region of the PFC includes the lateral orbital (LO), and the dorsal and ventral agranular insula (AID, AIV). In addition to the divisions in the lateral plane, many studies have investigated the role of the OFC, consisting of the MO, VO, VLO, LO, dorsolateral orbital, agranular insular (AI) regions, at the dorsal bank of the olfactory bulb (Dalley, Cardinal and Robbins, 2004; Izquierdo, 2017).

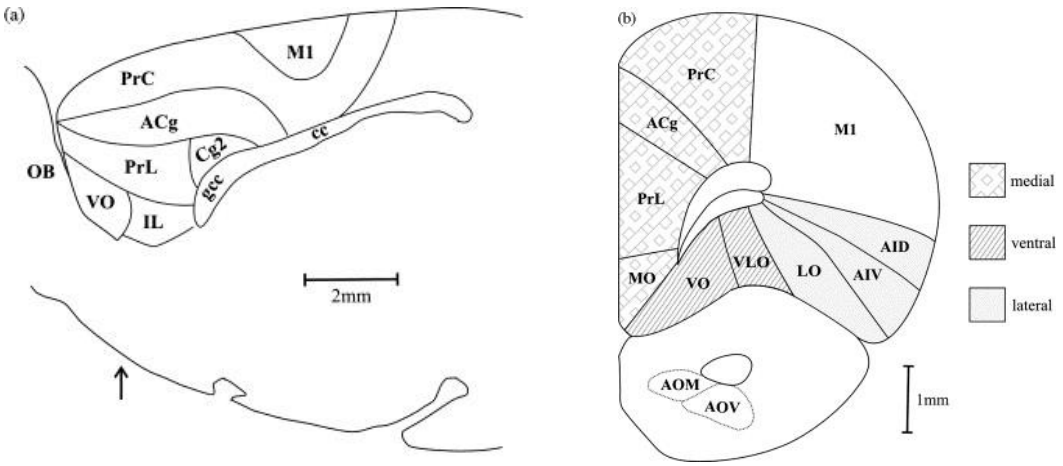


Figure 1.1: **Illustrative diagrams of the rat prefrontal cortex (taken from Dalley, Cardinal, and Robbins 2004). A) lateral view 0.9mm from the midline, B) coronal section, AP + 3.5mm approximately, depicted by the arrow on A. Different shadings representing the medial, ventral and lateral sub-divisions of the rat mPFC.**

The work in this thesis primarily focuses on the role of the OFC in reversal learning, integrating other mPFC regions as comparative controls.

1.2.2 The rat OFC and reversal learning

Over the past two decades, there has been a significant increase in the number of publications focused on the OFC, both in rats and other species, in attempts to further understand the complex functions of the OFC (Izquierdo, 2017). While these studies have provided valuable insights into the functions of the OFC and PFC as a whole, stereotaxic assessment of manipulations must be considered carefully. Table1.1 reports a brief summary of findings associated with the different sub-regions of the OFC in various reversal tasks.

General lesions of the OFC, combining lesions of the MO/VO/LO, with spread into the DLO and AI produce general overall impairments in reversal learning (Chudasama and Robbins, 2003; Boulougouris, Dalley and Robbins, 2007; Izquierdo *et al.*, 2013). While OFC lesions have been found to cause impairments in reversal learning in numerous studies, Izquierdo et al failed to show an increase in perseveration as reported by others. General lesions of the OFC cause an impairment in reversal learning, however region specific manipulation led to dissociable effects.

Few studies have selectively investigated the role of the mOFC in reversal behaviour, this could be due to the location in proximity to the midsagittal sinus, leading some to use an angled approach (Lopatina *et al.*, 2016). There is a diversity of findings within the mOFC, with NMDA lesions leading to accelerated reversal in a lever-delay discounting task (Mar et al. 2011), whereas local inactivation through baclofen/muscimol infusion caused an impairment on a probabilistic reversal learning task with increased perseveration (Dalton et al. 2016). Dalton et al found an impairment in discrimination learning following inactivation of the mOFC, this impairment in discrimination could be driving the impairment in reversal learning in this task as compared to the improvement in reversal learning seen by Mar et al. The differences in behaviour following mOFC manipulations could also be due to the different tasks, different lengths of training and the different methods of inactivation.

lOFC inactivation through various methods produced repeatable results in reversal learning. Both muscimol and muscimol/baclofen infusions led to impairment of

reversal leaning, with increased perseveration and no effects on discrimination learning (Mar *et al.*, 2011; Alsiö *et al.*, 2015; Dalton *et al.*, 2016). Impairments in reversal learning are standardised across multiple forms of reversal learning, including the novel touchscreen visual reversal task.

lOFC/AI NMDA lesions did not produce dissociable results from lOFC inactivation, with intact discrimination but subsequent impairments of reversal learning in an odour discrimination task (Schoenbaum, Chiba and Gallagher, 1999, 2000; Schoenbaum *et al.*, 2003).

Studies of set shifting and reversal have highlighted a role of the VLO in attentional tasks (McAlonan and Brown, 2003; Tait and Brown, 2007; Chase, Tait and Brown, 2012). Ibotenic acid VLO lesions led to retardation in acquisition of sets, requiring more trials to successfully shift, and impaired reversal learning. Lesions of the VLO led to a need for increase evidence before shifting behaviours, as measured by the number of trials, authors attribute this as a failure to link relevant cues to unexpected outcomes, eg non-reward.

Table 1.1: **A summary table of previous reversal studies in the rat OFC, the manipulations and tests used, and their findings.**				
Brain region	**Manipulation**	**Task**	**Findings**	**References**
MO/LO	NMDA lesions	Delay discounting	MO_- Increased preference for long delays, improved reversal LO- attenuated reversal learning	Mar et al., 2011
MO	Baclofen/Muscimol inactivation	Probabilistic discrimination and reversal learning	Impaired probabilistic discrimination, increased perseveration	Dalton et al., 2016
LO	Baclofen/Muscimol inactivation	Probabilistic discrimination and reversal learning	Intact probabilistic discrimination, increased perseveration	Dalton et al., 2016
LO	Baclofen/Muscimol inactivation	Serial visual reversal learning	Impaired reversal, increased perseveration	Alsiö et al., 2015
LO	Muscimol inactivation	Two- and four-choice discrimination and reversal learning	Intact discrimination learning, impaired two- and four-choice reversal learning, increased Intact discrimination learning, perseverative errors	Kim and Ragozzino, 2005
LO/AI	NMDA lesions	Odour discrimination and reversal learning	Intact discrimination learning, impaired reversal learning	Schoenbaum, Chiba, and Gallagher, 1999, 2000; Schoenbaum et al. 2003
VLO	Ibotenic acid lesions	Attentional set formation and shifts and reversal learning	Slower to acquire attention sets, more trials to shift, impaired reversal learning	McAlonan and Brown, 2003; Tait and Brown 2007; Chase, Tait, and Brown 2012
MO/VO/LO/ DLO/AI	Quinolinic acid lesions	Visual discrimination and reversal learning	Impaired autoshaping, increased perseveration in reversal learning	Chudasama and Robbins, 2003
MO/VO/LO/ DLO/AI	Quinolinic acid lesions	Spatial reversal learning	Early impairment, perseveration	Boulougouris, Dalley, and Robbins, 2007
MO/VO/LO/ DLO/AI	Ibotenic acid lesions	Visual discrimination and reversal learning	Intact discrimination learning, impaired reversal learning	Izquierdo et al., 2013

1.2.3 Connectivity of the rat PFC

Unlike temporal and posterior regions of neocortex, the PFC receives highly organised inputs from the basal ganglia via striatonigral and striatopallidal projections, and subsequently nigrothalamic and pallidothalamic projections that project to different areas of the PFC in a parallel segregated manor (Groenewegen and Berendse, 1994). In addition to thalamocortical connections, the PFC receives cortico-cortico inputs, from sensory cortical and posterior parietal areas; for example, the PFC also receives connections from subcortical structures, such as the hippocampus, amygdala, substantia nigra, ventral tegmental area (VTA), and lateral hypothalamus (Kolb, 1990; Groenewegen and Berendse, 1994). In addition to receiving connections from these areas, there are also reciprocal projections to these structures, as well as other areas. The PFC also targets and receives inputs from the main nuclei origins of the major cholinergic and neuromodulatory systems, including dopamine neurons in the VTA, 5-HT neurons in the raphé nuclei, noradrenaline containing neurons in the pontine central grey and acetylcholine neurons in the basal forebrain (Robbins, 2000).

Early anatomical studies highlighted several specialised circuits from the frontal cortex to the striatum, frontostriatal circuits (Alexander, DeLong and Strick, 1986). Since the early work of Alexander et al, these frontostriatal circuits have been further studied and expanded to include more limbic regions of the brain, including the hippocampus, anterior cingulate, and amygdala (Lawrence, Sahakian and Robbins, 1998; Phillips *et al.*, 2003). Frontostriatal circuits are thought to mediate cognitive and behavioural functions, as well as motor functions (Alexander, DeLong and Strick, 1986); with chemical neuromodulation eg monoamine systems facilitating these processes (Robbins, 2000; Tekin and Cummings, 2002). Frontostriatal circuits can be divided into cognitive, limbic, and motor projections (Figure 1.2) (Groenewegen *et al.*, 1999; Haber, 2003).

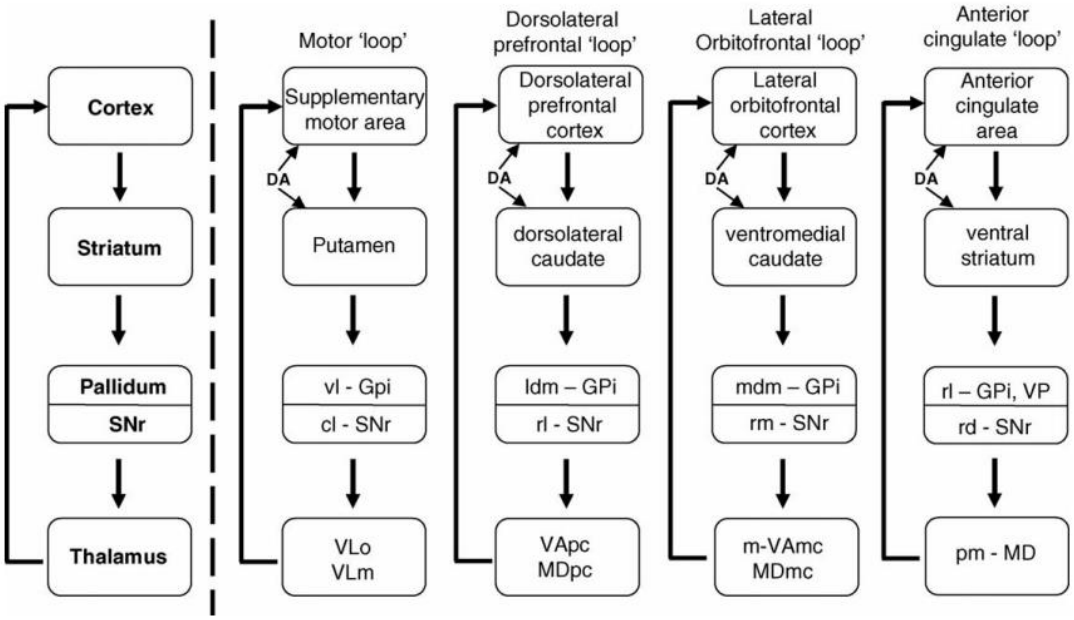


***Figure 1.2*: Parallel corticostriatal circuits connecting the frontal cortex and the striatum. Each circuit starts in different parts of the frontal cortex and projects to different areas of the striatum, before projecting back to the frontal cortex via the thalamus. Abbreviations: GPi, internal segment of globus pallidus; SNr, substantia nigra pars reticulata; VP, ventral pallidum; MD, medialis dorsalis; MDpc, medialis dorsalis pars parvocellularis; MDmc, medialis dorsalis pars magnocellularis; VAmc, ventralis anterior pars magnocellularis; VApc, ventralis anterior pars parvocellularis; VLo, ventralis lateralis pars oralis; VLm, ventralis lateralis pars medialis; cl, caudolateral; ldm, lateral dorsalmedial; mdm, medial dorsomedial; pm, posteromedial; rd, rostrodorsal; rl, rostrolateral; rm, rostromedial, Taken from Chudasama and Robbins (2006)**

Frontostriatal circuits and projections are thought to be important for cognitive and behavioural flexibility, and have been implicated in many human based studies (Morris *et al.*, 2016; Vaghi *et al.*, 2017). Using fMRI, Morris *et al* (2016) confirmed that the functional nature of the frontostriatal ciruits; mOFC and ventral striatum are recruited during goal-directed behaviour, lOFC and ventral striatum are recruited during probabilistic reversal learning, and dorsolateral PFC and ventral striatum are recruited during attentional shifting (Morris *et al.*, 2016). Altered frontostriatal circuit recruitment and activation have been identified in humans with OCD through the use of fMRI (Remijnse *et al.*, 2006, 2009). There have been numerous reviews on the role of frontostriatal circuits in OCD and other impulse control disorders (den Heuvel *et al.*, 2010; Burguière *et al.*, 2015; Morein-Zamir and

Robbins, 2015; Naaijen *et al.*, 2015; Thorsen *et al.*, 2015; Wood and Ahmari, 2015; Fettes, Schulze and Downar, 2017), and many groups agree that loss of cognitive and behavioural flexibility results from disruption in the frontostriatal circuits (Modell *et al.*, 1989; Graybiel and Rauch, 2000; Dvorkin *et al.*, 2010). For a detailed review on the similarities of human and primate anatomy and circuity in reward see Haber (Haber and Knutson, 2010).

A recent analysis of tracing studies in both the monkey and the rat have enabled circuit based homologies to be formed across species (Heilbronner *et al.*, 2016). Specifically, there are similar OFC-striatal projection organisation across monkeys and rats, with comparable efferent hubs. The MO in the rat and the mOFC in the monkey, both project to ventromedial regions of the striatum, whereas LO in the rat and central-lateral OFC in the monkey project to more central and lateral regions of the striatum in both species. Due to their similar projection patterns Heilbronner et al (2016) grouped rat LO and VO more closely together than the rat VO and MO, this grouping is consistent with previously reported behavioural effects in reversal learning (Table 1.1).

The rat MO, VO and PrL areas project to a strip along the medial wall of the striatum, inputs to the dorsal and lateral regions of the striatum are limited to the LO and DLO; whereas, the VO projects more medially than the LO, but not in the medial strip (figure 1.3)(Developed from Voorn et al. 2004, by H. Groenewegen, Personal Communication to T W Robbins). The anatomically distinct projections from the PFC suggest that as one moves medially to laterally in the rat OFC, there is more involvement of systems linked to sensory integration and less involvement of affective and motivational states (Hoover and Vertes, 2011; Heilbronner *et al.*, 2016).

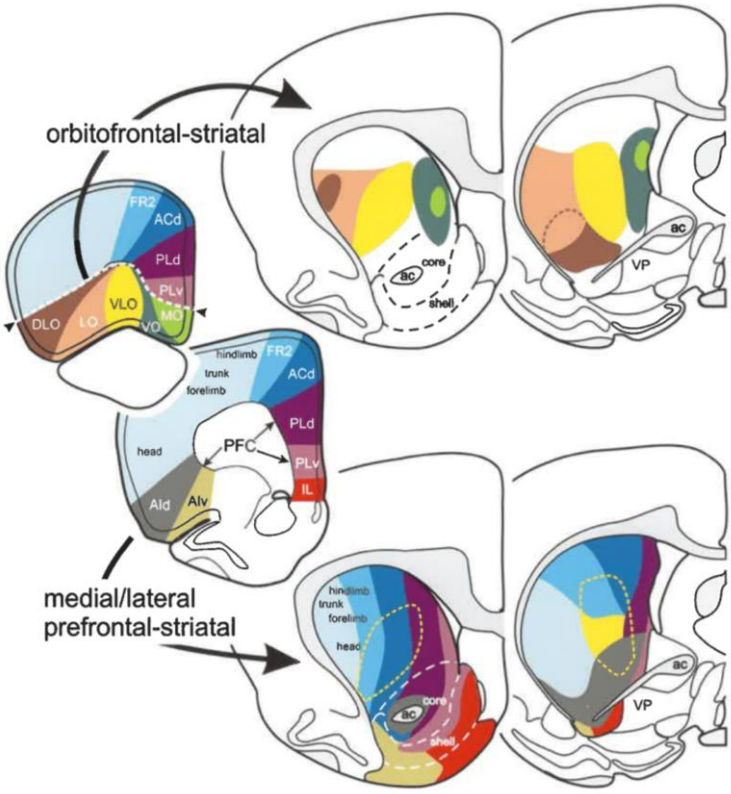


Figure 1.3: **A schematic representation of OFC (top) and PFC (bottom) projections to the striatum. (Developed from Voorn et al. 2004, by H. Groenewegen, Personal Communication to T W Robbins)**

1.3 The rat striatum

Although complex decision making is thought to rely on the neural networks of the PFC, limbic, and midbrain regions, efferents from these structures are known to converge within the striatum of the basal ganglia (McGeorge and Faull, 1989; Alexander and Crutcher, 1990; Haber, 2003; Pan, Mao and Dudman, 2010; Macpherson, Morita and Hikida, 2014). The striatum is hypothesised to integrate motivational, emotional, and cognitive information to guide behaviour of economical actions (Mogenson, Jones and Yim, 1980).

1.3.1 Anatomy of the striatum

The striatum is anatomically linked to the limbic system, cerebral cortex and thalamo-cortical motor system via parallel, functionally and structurally distinct, cortio-subcortical circuits (Gerfen and Young, 1988; Alexander and Crutcher, 1990; Dickinson and Balleine, 1994; Haber, 2003). The dorsomedial regions of the striatum receive afferents from the frontal and parietal cortices, whereas the dorsolateral regions receive afferents from the sensorimotor cortices. Conversely, the nucleus accumbens, of the ventral striatum, receives afferents from the mPFC and ACg cortices, as well as limbic structures, including the hippocampus and amygdala (Figure 1.3) (Alexander, DeLong and Strick, 1986; Haber, 2003). This regionally distinct afferent profile is hypothesised to convey dissociable functions of striatal subregions, allowing dynamic and adaptive control of behavioural and motor outputs (Mink, 1996; Nicola, 2007).

As well as being defined by their afferents, subregions of the striatum are also dissociable functionally.

1.3.2 Striatal mediated learning

The striatum has been heavily implicated in goal-directed learning, habit formation, and the switch between the two behaviours allowing for selection of economical outcomes. There have been numerous studies, including lesions, inactivation, and selective depletions in the dorsolateral striatum (DLS) and dorsomedial striatum (DMS) investigating their roles in goal-directed and habitual actions.

In 2004, Yin et al. showed that lesions of the DLS disrupted habit formation in instrumental learning, while preserving outcome expectancy. Using an interval based lever-reward paradigm, Yin et al were able to deduce that the DLS is necessary for habit formation. Lesions to the DMS had no effect on outcome devaluation, with animals performing at the same level as the sham controls; however, dorsolateral lesions lead to reduced lever press following devaluation, suggesting that a stimulus-response habit had not been formed. The DLS has also been implicated in sensitivity to action-outcome contingencies (Yin, Knowlton and Balleine, 2006). Inactivation of the DLS enhanced sensitivity to changes in action-outcome contingencies, tested through an omission task; rats that received muscimol

infusions into the DLS selectively reduced lever pressing compared to vehicle controls following omission contingency training. Yin et al concluded that DLS inactivation led to less compulsive responding under the new contingency, showing an enhanced sensitivity to action-consequence processing. Selective lesions to DLS dopamine neurons, using 6-hydroxydopamine (6-OHDA), have highlighted the role of striatal dopamine in habit formation (Faure *et al.*, 2005). DLS dopamine-depleted rats were sensitive to reward devaluation, whereas control rats were sensitive to goal devaluation but not reward devaluation. In this set of experiments, the control rats were able to form stimulus-response habits, whereas dopamine-depleted rats were not, suggesting a role for striatal dopamine in habit formation.

In 2005, Yin et al. reported that the posterior region of the DMS is necessary for acquisition and expression of action-consequence association in instrumental conditioning. Firstly, pre-training lesions of the posterior DMS abolished sensitivity to both contingency and outcome degradation, with anterior DMS lesions producing no significant effect. Secondly, pre-training and post-training lesions of the posterior DMS produced correlated reductions in sensitivity to both devaluation and degradation contingencies. Finally, the posterior DMS results were repeated with inactivation through muscimol infusion. Taken together, these three results highlight the importance of the posterior DMS compared to the anterior DMS in both acquisition and expression of action-outcome associations.

Gremel and Costa (2013) investigated the shift between goal-directed and habitual behaviours, and the neuronal ensembles responsible for the shift in responding. Using simultaneous *in vivo* recordings in the OFC, DMS and DLS during behavioural shifting it was revealed that the neuronal ensembles display different activities depending on behaviour. The OFC and DMS would become more engaged, and the DLS less, during goal-directed responding. The *in vivo* recordings confirm the previous lesion and inactivation data, that the DMS and DLS are involved in goal-directed and habitual responding, respectively.

The nucleus accumbens and its inputs from the basolateral amygdala, are implicated in facilitation of both Pavlovian and instrumental conditioning (for review, see Everitt, Dickinson and Robbins, 2001; Cardinal and Everitt, 2004). The nucleus

accumbens core has also been associated with compulsive checking behaviour. Dvorkin et al. (2010) showed that excitotoxic lesions of the NAc and OFC effected quinpirole-induced compulsive checking behaviour; NAc lesions affected the amount of checking behaviour, while OFC lesions affected staying away behaviours.

1.3.3 Striatal output pathways

The actions of the striatum encoding different types of behaviour are likely modulated by the striatal direct and indirect output pathways.

The primary cell type of the striatum, medium spiny neurons (MSNs), can be divided into two populations dependent on their releasable peptide, dopamine receptors, and projection targets (Gerfen and Young, 1988). Striatonigral neurons expressing dopamine D_1 receptors, dynorphin and Substance P form part of the direct pathway, whereas striatopallidal neurons expressing dopamine D_2 receptors and enkephalin form part of the indirect pathway (Figure 1.4)(Gerfen *et al.*, 1990; Bertran-Gonzalez *et al.*, 2010). Activation of striatonigral MSNs, through activation of dopamine D_1 receptors, induced long term potentiation of glutamatergic synapses within the direct pathway, thereby facilitating signalling (Grace *et al.*, 2007; Gerfen and Surmeier, 2011). Whereas activation of striatopallidal MSNs, through activation of dopamine D_2 receptors, induced long term depression of the neurons, thereby producing a blockade of the pathway (Steiner and Gerfen, 1998; Kreitzer and Malenka, 2007; Shen *et al.*, 2008).

Direct pathway striatonigral neurons inhibit the substantia nigra pars reticulata (SNr), and cease inhibition of the thalamus, promoting behaviour and motor activity. Conversely, indirect striatopallidal neurons inhibit the globus pallidus (GP), thereby disinhibiting the subthalamic nucleus (STN), and exciting the SNr leading to inhibiting of the thalamus and supressing behaviour and motor activity (Figure 1.4)(Macpherson, Morita and Hikida, 2014).

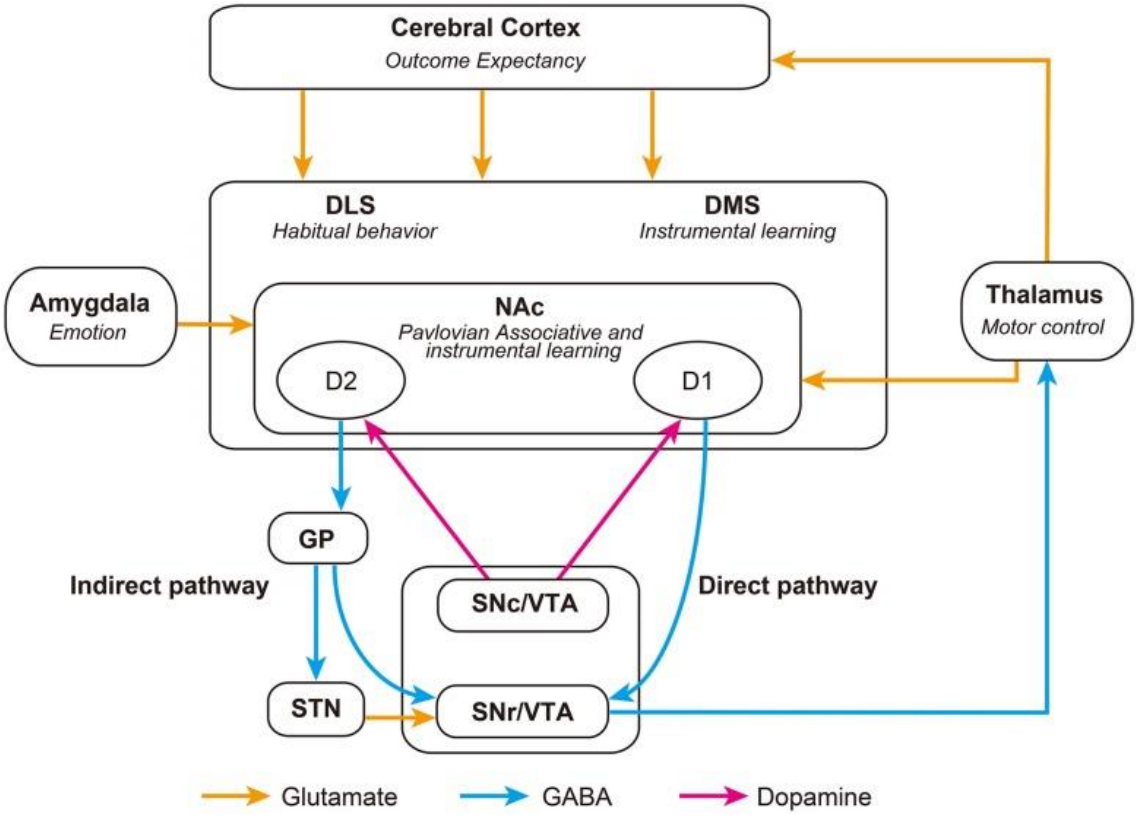


Figure 1.4: **A schematic representation of striatal direct and indirect pathways. Taken from Macpherson, Morita and Hikida (2014).**

The direct and indirect pathways have been implicated in both movement and goal-directed behaviours. Optogenetic excitation of the direct pathway led to reduced freezing and increased locomotor activity, whereas excitation of the indirect pathway induced a parkinsonian state with increased freezing and decreased locomotor initiations (Kravitz *et al.*, 2010). It has been proposed that pathway cooperative activity may be necessary for selection and initiation of actions (Cui *et al.*, 2013); concurrent activation of both the direct and indirect pathway striatal neurons, prior to directed movement initiation, suggests coordinated activity may act to integrate components needed for movement (Isomura *et al.*, 2013). In 2010, Hikida *et al.* reported that indirect and direct pathways are crucial for aversion-, and reward-learning, respectively. Subsequent investigation reported direct pathway

linked reward, and indirect pathway linked aversion, are dependent on activation of D_1- and inhibition of D_2 receptors in the nucleus accumbens, respectively (Hikida *et al.*, 2013).

As mentioned earlier in this chapter, reversal learning involves adaptation of behaviour to adjust to changes in reward contingency. For example, once a contingency is learned, by reaching predefined learning criterion, the reward contingencies are reversed, with the previous CS- becoming rewarded and vice versa. The rat must therefore adapt their behaviour, inhibit responding to the new CS-, and attend to the new CS+. Several processes underlie a successful reversal learning task, 1) detection of contingency reversal, 2) overcome learned non-reward to attend to the new CS+, 3) inhibit previously rewarded learned response, and 4) learn the new reward contingency; these processes can occur independently and concurrently.

Previous studies have highlighted the OFC and frontostriatal circuitry as neural correlates of reversal learning (see General Introduction 1.2.2). The role of the OFC in reversal learning is contradictory depending on the subregion involved, however this idea of opposing functions within the OFC has been elucidated from previous studies utilising different versions of the reversal learning paradigm. Inactivation of the mOFC led to an impairment in reversal on a probabilistic task, but improvements following lever reversal in a delay discounting task. lOFC lesion and inactivation led to comparable impaired performances in a serial reversal task, odour discrimination and reversal task, and a two- and four-choice reversal task. The interpretation of these contradictory results, both within and between different subregions of the OFC, across different tasks has led to non-unified theories surrounding the role of the OFC in reversal learning.

Dopamine and the striatum have also been implicated in reversal learning. Previous work in monkeys has reported the role of dopamine is localised to the striatum in reversal learning (Clarke *et al.*, 2011), using selective lesions to dissociate the mechanisms of dopamine from 5-HT. The role of the striatum, and dopamine in the striatum, is largely deduced from lesions and depletions in various sub-regions of the striatum. Using lesions allows for a study of the anatomy, as using dopamine

depletions allows for the study of dopamine in a region; however, little work has been done to understand the anatomical role of the indirect pathway in reversal learning.

The experiments described in this thesis focus on the neurochemical and neuroanatomical basis of reversal learning in the rat. Neurochemically, to examine the role of D_2/D_3 receptor antagonism in a novel touchscreen serial reversal learning task; Chapter 3 assessed the effects of systemic D_2/D_3 receptor modulation to validate the role of dopamine in the novel touchscreen serial reversal task, and assessed the effects of systemic antagonism against previous reversal studies performed across species. The effects of D_2/D_3 receptor antagonism were further investigated in Chapter 4, using intracerebral infusions into the striatum to elucidate a role for the contribution of the indirect pathway in differential anatomical locations. The neurochemical specificity of reversal learning was investigated in Chapter 5, using intracerebral infusions to antagonise dopamine D_1 receptors and 5-HT_{2C} receptors to investigate their role in serial reversal learning.

On neuroanatomical terms, one more study was undertaken to understand the dissociative effects of the OFC and their role in the novel touchscreen serial reversal learning task. Chapter 6 assessed the functional heterogeneity of the rat medial and lateral OFC, PrL and IL, using local inactivation to elucidate their roles in the novel touchscreen serial reversal learning task.

The functional neuronal circuitry of reversal learning was planned to be investigated using a chemogenetic approach, unfortunately these studies failed on technical grounds (see Appendix A).

Chapter 2: General Methods

This chapter will describe the general methodology common to the experiments in this thesis. Other methods will be detailed with each experiment if necessary.

2.1 Subjects

All subjects were male Lister-Hooded rats (Charles River, UK) weighing 300-350 g at the start of each experiment. Animals were housed in cages of four under a reversed light/dark cycle, lights on from 1900 to 0700. Testing took place between 0900 and 1300 five to seven days per week. Animals were put on a food restriction diet (13-19 g of Purina lab chow per day) to maintain approximately 85% of their free-feeding weight and were fed approximately one hour after testing. Water was available ad libitum. This research has been regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB), under the Home Office Project License 70/7548, and Home Office Personal Licence ICD446246.

2.2 Touchscreen Serial Reversal Task

2.2.1 Apparatus

The experiments used 30 cm x 39 cm x 29 cm operant chambers (Med Associates, Georgia, VT, USA), placed in 16 sound attenuating wooden boxes. The boxes were fitted with a fan for ventilation and to mask external noise. In each chamber, a central food magazine was connected to an external pellet dispenser, delivering 45mg sucrose pellets (TestDiet 5TUL; Sandown Scientific, Middlesex, UK) (Figure 2.1A). Each chamber also contained a house light near the ceiling directly above the food magazine. The opposite side of the chamber contained a touch-sensitive screen

(Figure 2.1B). Rats were always trained and tested in the same chamber to remove any inter-chamber variability. Chambers were wiped with water in between each use, and cleaned with disinfectant at the end of each day.

A  B 

Figure 2.1: **A. Photograph of the behavioural apparatus (operant chamber); showing the house light at the top of the chamber and the food magazine at the base. B. Photograph of the behavioural apparatus (touchscreen); showing the orientation of the touchscreen in the chamber. All experimental chambers had an identical setup. Rats were always trained and tested in the same chamber.**

2.2.2 Pretraining

2.2.2.1 Pretraining stage 1.

Rats were placed in the operant chamber as the experimenter initiated the program on the touchscreen, followed by closing the sound attenuating box. Rats responded to a single white box at the bottom centre of the screen through either a nose poke or the use of a paw, sucrose reward pellets were awarded on a 1:1 schedule following successful touching of the white box. Sessions were 60 minutes in duration and repeated until the rats reliably received 100 pellets in one session. The size of the white box was gradually reduced over three sessions until it measured 3 x 4 cm approximately (Figure 2.2, Stages 1-3). Rats progressed from Stage 1 to Stage 2 within three days, and spent one day on each of Stage 2 and Stage 3.

2.2.2.2 Pretraining stage 2.

Rats were placed in the chamber as in Pretraining stage 1. The program was increased in difficulty so that touching the white box to initiate a trial led to the presentation of a single stimulus (vertical or horizontal bars) to the right or left in a pseudo-random order. Responding to this stimulus was reinforced with a single reward pellet, whereas responding to the blank side was signalled as incorrect and led to the illumination of the house light for a 5 second time-out period. Failure to make a response led to the stimulus disappearing from the screen and a 5 second time-out. The stimulus presented was pseudo-randomly assigned as either vertical or horizontal, to ensure equal numbers of animals started on each stimuli, for a single session until the rat had reached ≥80% correct and alternated across days. After the rats had reached ≥80% correct responses on both individual stimuli, the position of the stimuli was raised by 5cm on the screen. After the rats had reached ≥80% correct responses on the both raised stimuli, visual discrimination testing was able to occur (Figure 2.2, Stages 4 and 5). All Pretaining sessions ended after 60 minutes or 100 rewards.

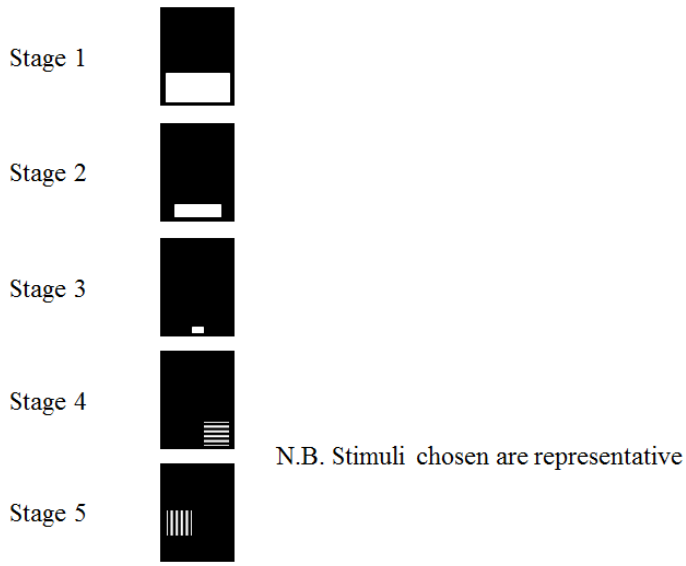


Figure 2.2: **Visual representation of the pretraining stages for the serial visual reversal task. Stage 1 consisted of a large white box that was rewarded on a fixed 1:1 ratio, Stages 2 and 3 consisted of the white box getting progressively smaller and Stage 4 shows the introduction of stimuli.**

2.3 Visual discrimination and serial reversal

Following successful pretraining the rats would undergo a visual discrimination. Rats would initiate a trial by touching the central white "start box" and be presented with both horizontal and vertical stimuli. One stimulus was associated with reward (CS+) and the other stimulus with a house-light 5 second time-out (CS-, Figure 2.3).

Rats were tested for one session daily. The session ended after 60 minutes, 250 trials, or 150 rewards, whichever occurred first. The inter-trial interval (ITI) was set to 5 seconds, and the limited hold (stimulus presentation time) was set to 10 seconds. Criterion for discrimination learning was set to 24 correct trials in a moving 30 trial bin. Once rats had successfully acquired the discrimination they were given a retention session using the same contingencies to confirm successful acquisition.

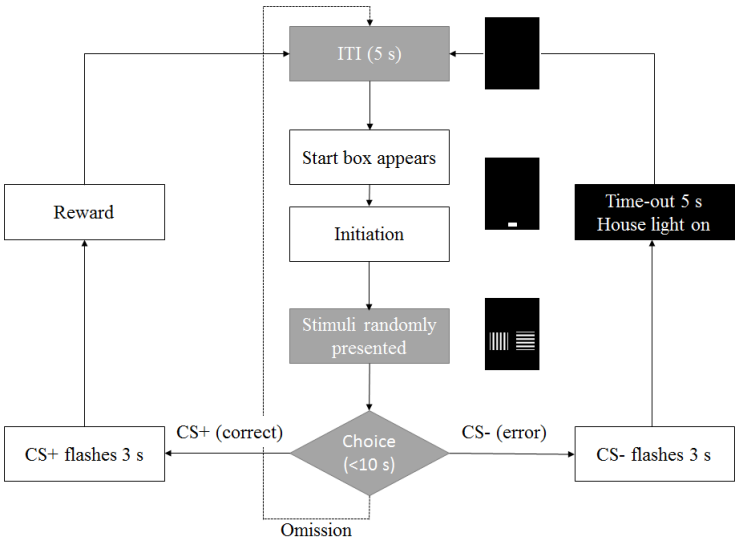


Figure 2.3: **Flowchart depiction of the serial visual reversal task. A start box is present in the bottom centre of the screen, upon trial initiation rats has 10 seconds to make a choice; if a correct choice was made rats received a reward pellet, if an incorrect choice was made then the house light would turn on and the box would be in a 5-second timeout, if no choice was made the box would enter a 5-second time out. Figure adapted from Alsiö et al., 2015.**

Following successful discrimination and the retention session, the contingencies were reversed. The rats now needed to respond to the previous CS- in order to receive rewards and reach reversal criterial (24 correct/30 trials). Retention

sessions were included before each reversal and after reaching criterion. Additional reversals were preformed successively until the rats were able to reach criterion within three sessions before testing occurred (Figure 2.4).

	CS+	CS-
Discrimination/Retention 1		
Reversal 1		
Retention 2		
Reversal 2		
Retention 3		
Reversal 3		
Retention 4		

Figure 2.4: **Table depicting an example of the stimulus-reward contingencies for the serial visual reversal task following pretaining. Figure adapted from Alsiö et al., 2015.**

2.4 Cannulation surgical technique

Animals were anaesthetised with 5% isoflurane in oxygen. Following induction of anaesthesia animals received subcutaneous injections of Baytril (10mg/kg, 100mg/ml, Bayer, Leverkusen, Germany) and Metacam (1mg/kg, 5mg/ml, Boehringer Ingelheim, Berkshire, UK) and were secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) fitted with atraumatic earbars (Figure 2.5), with maintenance isoflurane of 2-3%. The skull was exposed through a single incision down the midline and bregma and lambda were compared in depth to ensure a flat skull, adjusting the tooth bar as necessary. Using the cannula as a guide, working from bregma implantation coordinates were calculated and marked using a pencil. A dental drill was used to make holes into the skull above the implantation site. The dura mater was gently broken using the bent tip of a sterile needle, ensuring minimal damage to the underlying blood vessels and brain tissue. The cannulae (PlasticsOne, Roanoke, VA, USA) were lowered to the required depth and secured to the skull using three or four small metal screws and dental cement (Kemdent simplex rapid; Kemdent, Swindon, UK). Dummy injectors that ended flush

with the guide were inserted into the cannulae and protected with a small dust cap. All surgeries were performed using a microscope. All surgical co-ordinates were calculated using a stereotaxic atlas (Paxinos and Watson, 2006) using bregma as the origin. All dorsoventral measurements were taken from dura prior to its penetration.

All animals received oral pain relief for three days post-surgery, Metacam (1mg/kg, 1.5mg/ml, Boehringer Ingelheim). Animals were allowed to recover for seven days before training resumed. Following surgery, the subjects were single housed to protect the cannulation site.

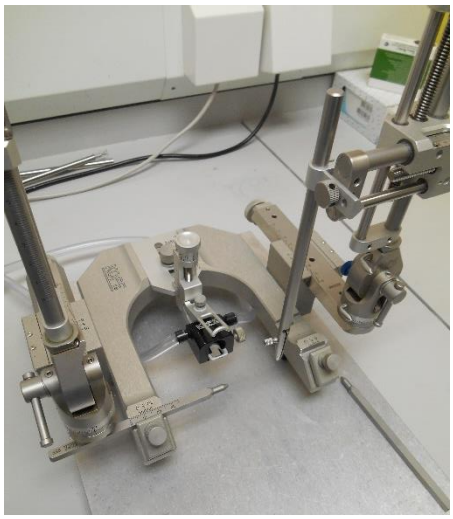


Figure 2.5: **Photograph showing a Kopf stereotaxic frame used for all intracranial surgeries to ensure correct location of cannulae implantation.**

2.5 Histology

Upon completion of behavioural testing, all animals were given a lethal dose of sodium pentobarbitone (Euthatal, 200mg/ml; Merial, UK) and transcardially perfused with 0.01M PBS (Gibco, Fisher Scientific; Loughborough, UK) for two minutes followed by 4% paraformaldehyde for three minutes. Following perfusion, the brains were removed, post-fixed in 4% paraformaldehyde overnight, and cyroprotected in 30% sucrose (w/v) in 0.01M PBS until fully dehydrated. Brains were frozen and coronal slices 60 μm were cut using a freezing sledge microtome.

Sections were mounted on double-subbed glass slides and stained with Cresyl Violet. The sections were then used to verify cannulae placement; the location of the injector-tip was mapped onto the standardised rat brain atlas (Paxinos and Watson, 2006).

2.6 Statistical analysis

All experiments employed a within-subject design, unless otherwise stated. Data from all days was combined for each reversal. Trial outcome was coded as perseverative, random, or learning, depending on performance within a moving 30 trial bin, based on a binomial distribution of probabilities (Jones and Mishkin, 1972). Any 30-trial bin with significant bias (<11 correct) toward the previously rewarded stimulus (new CS-) was labelled perseverative, whereas a 30-trial bin with significant bias (>19 correct) towards the new correct stimuli was labelled as learning; bins with 11<correct<19 were labelled as random. Bins were coded based on performance irrespective of wherever they occurred within a reversal session.

The primary dependent variables for the serial reversal task were number of trials and incorrect responses. Omissions, latencies to respond following trial initiation and latencies to collect rewards were analysed as secondary variables. Validation of statistical analysis and power calculations were performed using n=88 rats (Appendix B).

Data for trials and incorrect response were normalised using a square-root transformation unless otherwise stated. Following transformation, data were analysed using a repeated measures ANOVA; violations of sphericity were corrected accordingly (Girden, 1992). When significant interactions were found, further analysis was performed of Simple Main Effects or Simple Main Interactions, as appropriate.

Omission data were analysed using Friedman's one-way ANOVA by ranks on each separate phase of the reversal paradigm unless otherwise stated. When significant differences were found, post-hoc Wilcoxon Signed Rank tests were performed within each experimental phase comparing to Vehicle.

Latencies were analysed using mean log transformed values for the different doses for each subject using a repeated measures ANOVA or Students' paired *t*-test as appropriate, unless otherwise stated.

Animals that lost their cannulae, or had incorrect placements were excluded from all analysis.

Chapter 3: SYSTEMIC DOPAMINE D_2/D_3 RECEPTOR ANTAGONISM SPECIFIC AFFECTS IN REVERSAL LEARNING, BUT NOT NEW LEARNING

3.1 Introduction

As discussed in Chapter 1, dopamine has been shown to play a major role in motivated behaviour, along with reward learning and motor functions; however not as much is known about the role of dopamine in cognitive flexibility.

Ridley et al reported in 1981 that systemic administration of haloperidol, an antipsychotic with high affinity for the dopamine D_2 receptor as an antagonist, impaired the ability to alter behaviour in response to contingency shifts in a marmoset reversal learning task. Marmosets were subjected to a simple object discrimination reversal task, and the effects of amphetamine and haloperidol on behavioural flexibility were assessed. Amphetamine induced high rates of perseverative responding, with worse than chance performance in the early stages of reversal; whereas haloperidol caused a mild, non-perseverative impairment of reversal learning. Ridley et al also showed that the effects of amphetamine were blocked through pre-treatment of haloperidol, deducing that these effects were dopamine mediated.

Experimentally induced dysregulation of central dopamine, through either stress-level cortisol-induced disruption or phencyclidine (Jentsch *et al.*, 1997; Lyons *et al.*, 2000), has been shown to impair response inhibition in an object retrieval task. This may suggest that dopamine systems regulate adaptive responding, and therefore a role in reversal learning might be inferred.

More recently, Kruzich and Grandy (2004) used a dopamine D_2 receptor knock-out mouse in an odour discrimination and reversal learning task. Dopamine D_2 receptor knock-out mice took significantly more trials, and committed significantly more

errors, to acquire an odour based discrimination task. D_2 receptor knock-out mice also required significantly more trials and produced more errors following contingency reversal when compared to wild type mice; reversal learning measures also showed that while knock-out mice produced significantly more errors of commission, there was no difference in errors of omission. These data suggest that the errors were perseveration rather than extinction, based.

In 2006 it was shown that eticlopride, a dopamine D_2/D_3 receptor antagonist, impaired the ability of rats to alter performance in a maze-based set-shifting task (Floresco *et al.*, 2006). Microinfusion of eticlopride into the medial prefrontal cortex led to a significant impairment in shifting in response to a visual-cue discrimination strategy, and also produced a significant increase in the number of perseverative errors. Similar results were seen with PD-168,007, a D_4 receptor agonist, while L-745,860, a D_4 receptor antagonist, elicited a significant improvement in set-shifting. Infusions of D_1 or D_2 agonists had no effects. These results show the importance of dopamine in the mediation of executive function in the prefrontal cortex, with multiple receptor subtypes playing an essential role in set-shifting and behavioural flexibility.

Lee et al. (2007) showed that dopamine D_2/D_3 receptors have a specific role in the reversal of learned visual discrimination in the vervet monkey. Systemic raclopride at doses ranging from 0.001-0.03 mg/kg had no significant effect on the number of trials or errors in a reversal task with no retention sessions. A dose of 0.03mg/kg raclopride did lead to a significant increase in the number of reversal errors when the task was altered to include a pre-reversal retention session suggesting that within-session reversal learning would be sensitive to deficits. Whilst there was no significant effect on the number of perseverative errors, there was a trend for an increase. 0.03mg/kg raclopride also significantly impaired performance during reversal sessions when compared to new learning, with a significant increase in the number of trials taken to reach criterion, the number of errors of commission and the number of perseverative errors. No effects were found with a dopamine D_1/D_5 receptor antagonist.

Conversely, Boulougouris, Castañé, and Robbins (2009) reported the effects of numerous dopaminergic agents in a rat model spatial reversal learning. Systemic administration of quinpirole, a dopamine D_2/D_3 receptor agonist lead to a significant impairment in reversal learning, increasing both the number of trials and the number of incorrect responses. Raclopride and nafadotride, a selective D_3 receptor antagonist had no effect when administered alone; however, raclopride was able to block the quinpirole induced deficits.

The studies of Lee et al. and Boulougouris et al. report somewhat different effects when raclopride is administered to the vervet monkey or rats in a reversal task. Additionally, there were also differences between the tasks employed, Lee et al. used a visual reversal, whereas Boulougouris et al. investigated dopaminergic agents with a spatial reversal task. Lee et al. only saw the effects of raclopride administration following exposure during the pre-reversal retention session; however, this was not reported by Boulougouris et al. in rats.

Using the touchscreen serial visual reversal learning task I will be able to fully investigate the cross-species translation of Lee's results, and provide additional information such as response latencies and a breakdown of the types of errors and where they occur – omission or commission, perseveration or random phase of the task. Investigation and validation of a raclopride effect in this task will allow for further work in an attempt to localise any raclopride effects (see Chapter 4).

Following the work of Lee et al. in the monkey, Ridley et al and Boulougouris et al, it is clear that raclopride and the dopamine system are having an effect on reversal learning across different species. I predicted that systemic injection of raclopride will lead to a mild generalised impairment on the touchscreen serial visual learning task in the rat. I also predicted that a dose of raclopride that does not cause impairment during a serial reversal task conducted between sessions will lead to impairment in reversal following receipt of drug during a retention phase prior to reversal, with no effect on new learning, as reported by Lee et al.

3.2 Methods

3.2.1 Subjects

Sixteen male Lister-Hooded rats (Charlies River, UK) were trained on a touchscreen discrimination and serial reversal learning task (see Chapter 2 for details). These rats were used for Experiments 1-3.

3.2.2 Behavioural procedure

Rats were trained on the touchscreen serial reversal task as previously described in Chapter 2.

Serial Reversal Learning Task: The serial reversal learning task is described in detail in Chapter 2.

Novel Stimuli Visual Discrimination: Animals were exposed to a novel set of stimuli under the same conditions as the original visual discrimination.

Each rat continued on the new stimuli pair until criterion was reached. Once this criterion was reached, this novel visual discrimination was complete and the animal progressed onto the novel stimuli reversal learning task.

Novel Stimuli Reversal Learning Task: The novel reversal learning task used the novel visual discrimination stimuli and continued as a new reversal task (Table 3.1).

	CS+	CS-
Discrimination/Retention 1		
Reversal 1		
Retention 2		
Reversal 2		
Retention 3		
Reversal 3		
Retention 4 etc		
Novel Stimuli Visual Discrimination		
Novel Stimuli Retention		
Novel Stimuli Reversal 1		
Novel Stimuli Retention 2		

Table 3.1: **Table depicting the stimulus-reward contingencies for the serial visual reversal task, followed by the novel visual discrimination and reversal tasks. Figure adapted from Alsiö *et al.*, 2015.**

3.2.3 Drugs

The D_2/D_3 receptor antagonist raclopride (Tocris Bioscience, Bristol, UK) was tested on the serial reversal learning, novel stimuli visual discrimination, and novel stimuli reversal learning tasks.

Serial Reversal Task: Prior to drug administration, animals were counterbalanced across the drug doses and a vehicle control, matched for their performance during the final training stages of the serial visual reversal learning task. Each animal received i.p. injections of vehicle and raclopride (0.01, 0.03, 0.1mg/kg, from Boulougouris, Castañé, and Robbins (2009)) on separate reversals on repeated sessions until criterion was reached. All animals received all doses in a within-subject design.

Novel stimuli visual discrimination and reversal task: Prior to drug administration, animals were counterbalanced between vehicle and drug, matched for their

performance in a baseline drug-free reversal. Each animal received i.p. injections of either vehicle or 0.01mg/kg raclopride before every session, including retention sessions. Each animal only received vehicle or drug in a between-subject design.

All drugs were administered 20 minutes prior to the start of the task in a designated procedure area. During the 20-minute period prior to testing, animals were returned to their home cage in a designated holding room.

Raclopride was dissolved in physiological saline. All injections were given in a volume of 1ml/kg.

3.2.4 Statistical analysis

The main measures of the animals' ability to learn the reversals and visual discrimination were: (i) the number of trials to criterion and (ii) the number of incorrect responses to criterion (errors of commission). Additional secondary measures recorded for each trial were (iii) the number of omissions (errors of omission), (iv) the latency to respond to the stimuli, (v) the latency to collect the reward (seconds).

3.2.4.1 Serial reversal task

Data for each primary variable were analysed using a two-way repeated measures ANOVA consisting of two within-subject factors (Dose and Phase). When significant interactions between Dose and Phase were detected, further analysis was performed of Simple Main Effects or Simple Main Interactions, as appropriate. Analysis was followed by post-hoc Sidak's corrected pair-wise comparisons to vehicle.

Omission data were analysed using Friedman's one-way ANOVA by ranks on each separate phase of the reversal paradigm. When significant differences were found, Wilcoxon Signed Rank tests were performed within each experimental phase comparing to Vehicle.

For all comparisons, significant difference was assumed at $p < 0.05$.

3.2.4.2 Novel stimuli visual discrimination and reversal

Data for each primary variable were analysed using a two-way ANOVA consisting of one within-subject factor (Phase) and one between-subjects factor (Dose). When significant interactions between Dose and Phase were found, further analysis was using an independent samples *t*-test.

Omission data were analysed using the Mann-Whitney U test on each separate phase of the visual discrimination and reversal paradigm.

For all comparisons, significant difference was assumed at $p < 0.05$.

3.3 Results

3.3.1 Experiment 1: Effects of systemic D_2/D_3 receptor antagonism on a serial visual reversal learning task

0.1mg/kg raclopride was excluded from the analysis as rats treated with this dose were unable to perform the task (data not shown, no trials initiated in the one hour testing period). Three animals were excluded from the analysis following illness and the incomplete Latin Square design; for the serial visual reversal learning task analysis n = 13.

3.3.1.1 Trials to criterion

Figure 3.1 shows the number of trials to criterion for each dose of raclopride. A two-way repeated measures ANOVA revealed a significant main effect of Dose ($F_{2,24}$ = 5.862, p = 0.009, figure 3.1A), and a significant main effect of Phase ($F_{2,24}$ = 14.616, p < 0.001); however, there was no significant Dose x Phase interaction ($F_{4,48}$ = 0.451, p = 0.77, figure 3.1B).

0.03mg/kg raclopride led to a significant increase in the total number of trials when compared to vehicle (p = 0.012, Figure 3.1A).

3.3.1.2 Incorrect responses

Figure 3.2 shows the number of incorrect responses to criterion following systemic raclopride. A two-way repeated measures ANOVA revealed a significant main effect of Dose ($F_{2,24}$ = 5.852, p = 0.008, figure 3.2A) and a significant main effect of Phase ($F_{2,24}$ = 15.89, p < 0.001); however, there was no significant Dose x Phase interaction ($F_{4,48}$ = 0.67, p = 0.61, Figure 3.2B).

0.03mg/kg raclopride led to a significant increase in the number of incorrect responses compared to vehicle (p = 0.017, Figure 3.2A).

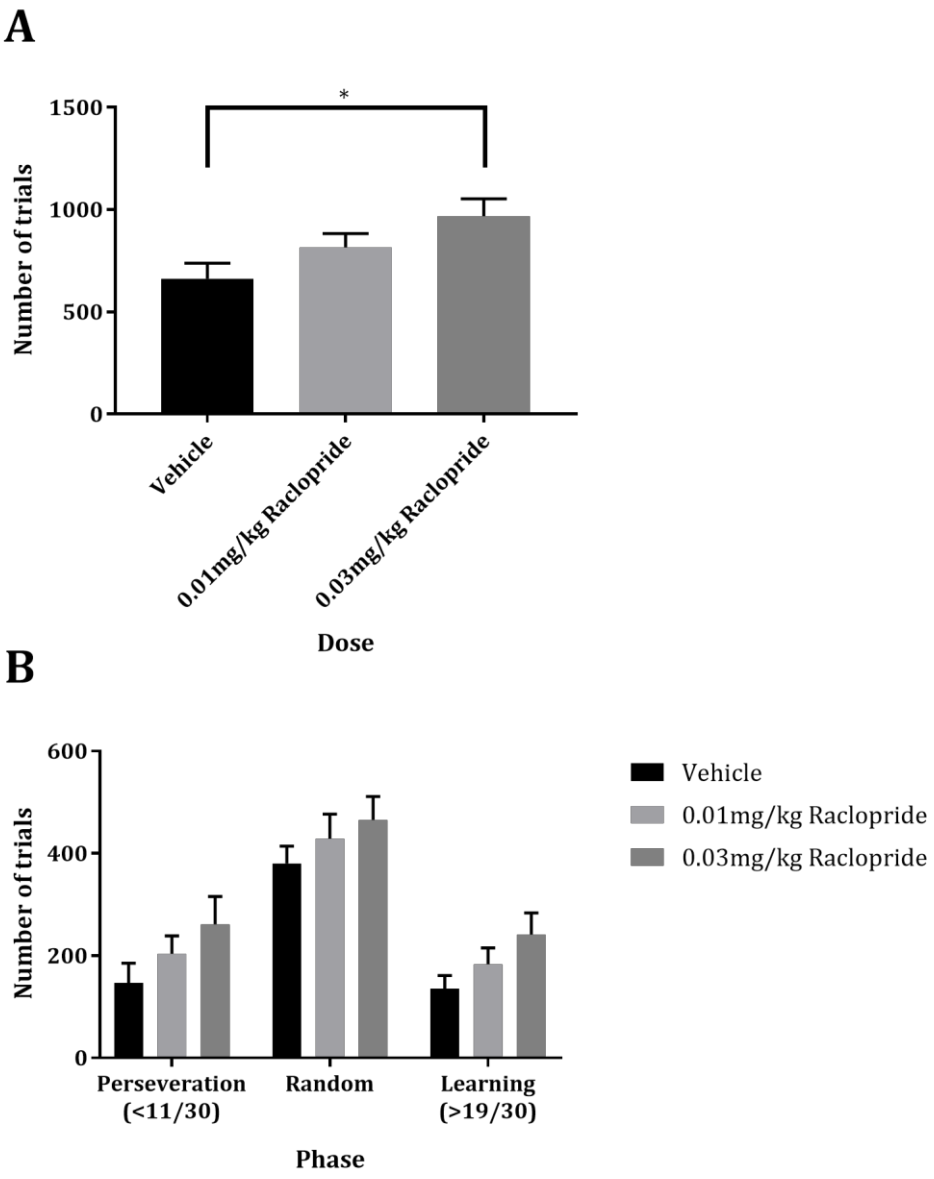


Figure 3.1: **Histograms showing the number of trials taken to criterion following repeated systemic injections of vehicle, 0.01mg/kg and 0.03 mg/kg raclopride (A), and the number of trials per phase of reversal (B). There was a significant increase in the total number of trials following injection of 0.03 mg/kg raclopride compared to vehicle ($p = 0.012$). Data are represented as mean ± SEM. Asterisks denote significant differences (pairwise comparisons: *, $p < 0.05$) from vehicle controls.**

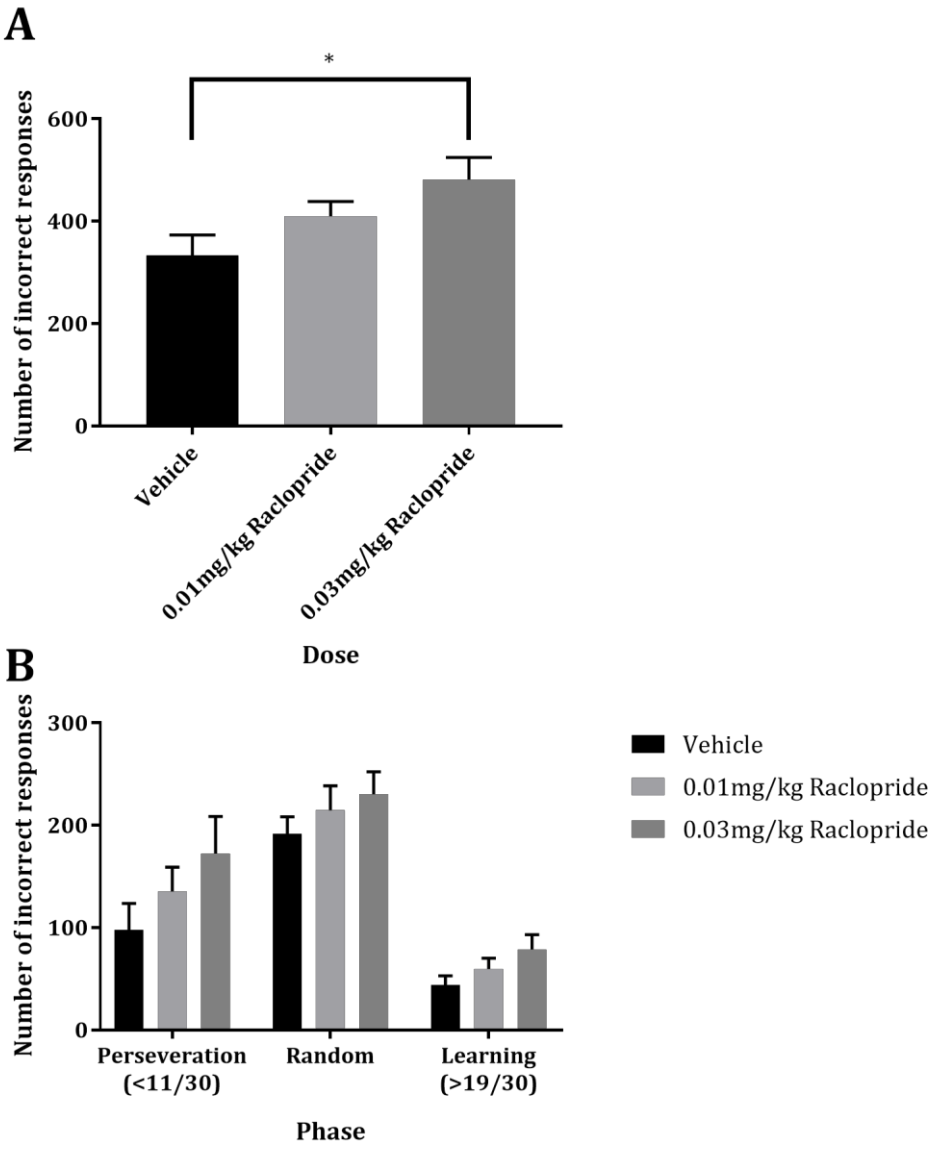


Figure 3.2: **Histograms showing the number of incorrect response to criterion following repeated systemic injections of vehicle, 0.01mg/kg and 0.03 mg/kg raclopride (A), and the number of incorrect responses per phase of reversal (B). There was a significant increase in the total number of incorrect responses following injection of 0.03 mg/kg raclopride compared to vehicle (p = 0.017). Data are represented as mean ± SEM. Asterisks denote significant differences (pairwise comparisons: *, $p < 0.05$) from vehicle controls.**

3.3.1.3 Omissions

A Freidman's ANOVA-by-ranks revealed a significant difference in the distribution of omissions under D_2/D_3 receptor antagonism in both the perseveration and

random phases of the serial reversal task ($\chi^2(2) = 6.381$, $p = 0.041$ and $\chi^2(2) = 6.00$, $p = 0.050$, respectively, Figure 3.3).

Wilcoxon Signed Rank comparisons to the control groups within each phase showed 0.01mg/kg raclopride caused a significant increase in the number of omissions during the random phase of the reversal learning paradigm ($Z = -2.456$, $p = 0.014$, Figure 3.3).

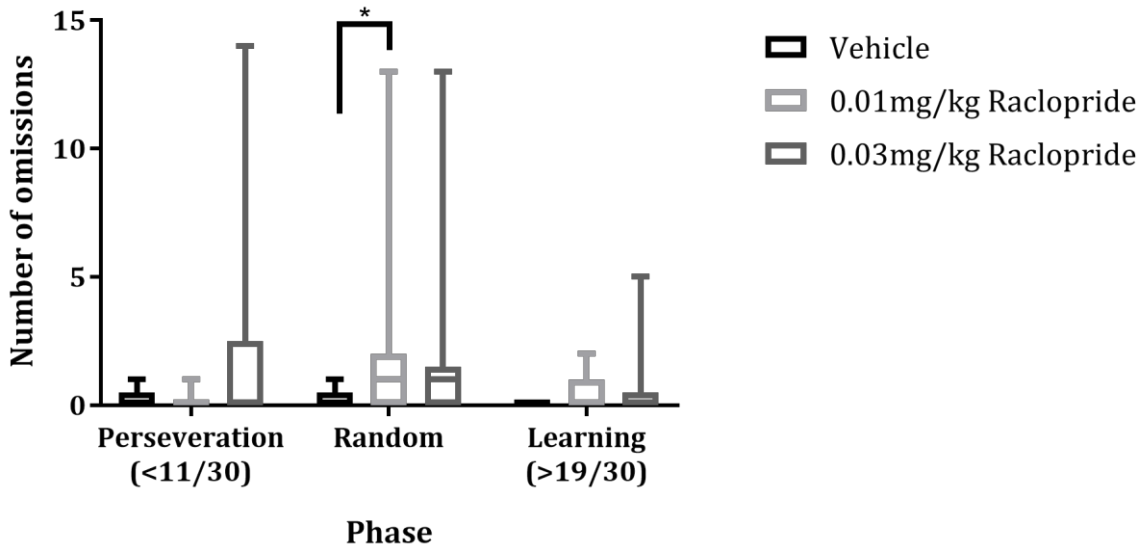


Figure 3.3: **Box and whisker plots showing the number of omissions per phase of the serial reversal learning paradigm following injection of vehicle, 0.01 mg/kg and 0.03 mg/kg raclopride. There was a significant increase in the number of omissions during the random phase following 0.01mg/kg raclopride (Wilcoxon Signed Rank, $p = 0.014$). Data are represented as median values ± minimum and maximum. Asterisks denote significant differences (pairwise comparisons: *, $p < 0.05$) from vehicle controls.**

Dopamine D_2/D_3 receptor antagonism had no significant effects on the number of omissions in the learning phase.

3.3.1.4 Response and Retrieval Latencies

Table 3.1 shows the mean response and retrieval latencies following administration of systemic raclopride. There was significant effect of raclopride on response latency

($F_{2,24}$ = 6.541, p = 0.0054). Pairwise comparisons to control revealed a significant slowing following injection of 0.03mg/kg raclopride (p = 0.003).

Systemic raclopride has no effect on collection latency ($F_{2,24}$ = 2.138, p = 0.1441).

	Response latency (s)	**Retrieval latency (s)**
Vehicle	0.84 ± 0.097	1.12 ± 0.13
0.01 mg/kg raclopride	0.88 ± 0.079	1.17 ± 0.11
0.03 mg/kg raclopride	0.94 ± 0.098 * *	1.34 ± 0.15

Table 3.1: **A table of the response and reward retrieval latencies. Systemic raclopride had a significant effect on response latency ($F_{2,24}$ = 6.541, p = 0.0054), with 0.03mg/kg leading to significant slowing compared to vehicle controls (p = 0.003, pairwise comparison). Data are represented as the mean values ± SEM (seconds). Asterisks denote significant differences (pairwise comparisons: **, p < 0.01) from vehicle controls.**

Although retrieval latencies were not statistically significant different from baseline performance, there was a tendency to perform more slowly at the highest dose of raclopride.

3.3.1.5 Summary of experiment 1

In summary, raclopride produced a dose-related deficit in reversal learning with significant effects at the high dose of 0.03mk/kg. The dose-dependent deficit in reversal learning was also conveyed in the response latency, with 0.03mg/kg causing significant slowing. On the other hand, 0.01mg/kg produced a significant increase in omissions in the random phase.

3.3.2 Experiment 2: The effects of systemic D_2/D_3 receptor antagonism on new learning in a visual discrimination and subsequent retention task

To study the effects of systemic raclopride on new learning the dose of 0.01mg/kg as used in Experiment 1, was employed as this dose did not significantly affect basic reversal behaviours.

3.3.2.1 Trials to criterion

Figure 3.4 shows the number of trials taken to attain criterion on a novel visual discrimination task. A mixed-design ANOVA showed a significant effect of Phase ($F_{1,11}$ = 5.307, p = 0.042); however, there was no significant effect of Drug ($F_{1,11}$ = 2.203, p = 0.17, and $F_{1,11}$ = 0.245, p = 0.63, Figure 3.4A) and no significant Drug x Phase interaction ($F_{1,11}$ = 2.228, p = 0.16, Figure 3.4B).

3.3.2.2 Incorrect responses

Figure 3.5 shows the effects of 0.01 mg/kg raclopride on the number of errors to criterion on the novel visual discrimination task. A mixed-design ANOVA showed a significant effect of Phase ($F_{1,11}$ = 34.088, p < 0.001); however, there was no significant effect of Drug and no significant Drug x Phase interaction ($F_{1,11}$ = 0.297, p = 0.597, Figure 3.5A and $F_{1,11}$ = 1.055, p = 0.326, Figure 3.5B, respectively).

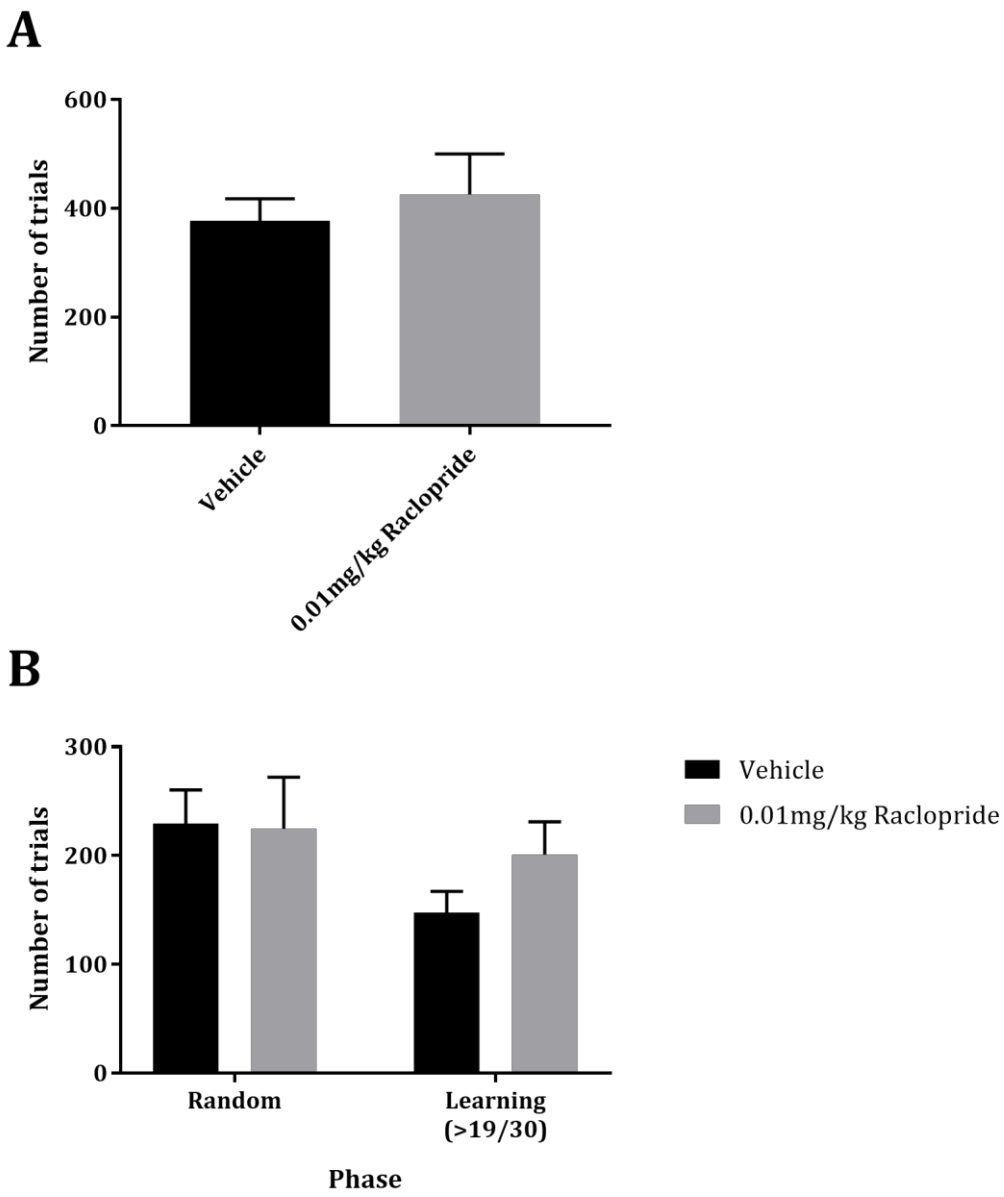


Figure 3.4: **Histograms showing the number of trials taken to criterion following injection of vehicle or 0.01mg/kg raclopride (A), and the number of trials per phase of visual discrimination (B). There was no significant difference in the number of trials taken to reach criterion after raclopride. Data are represented as mean ± SEM.**

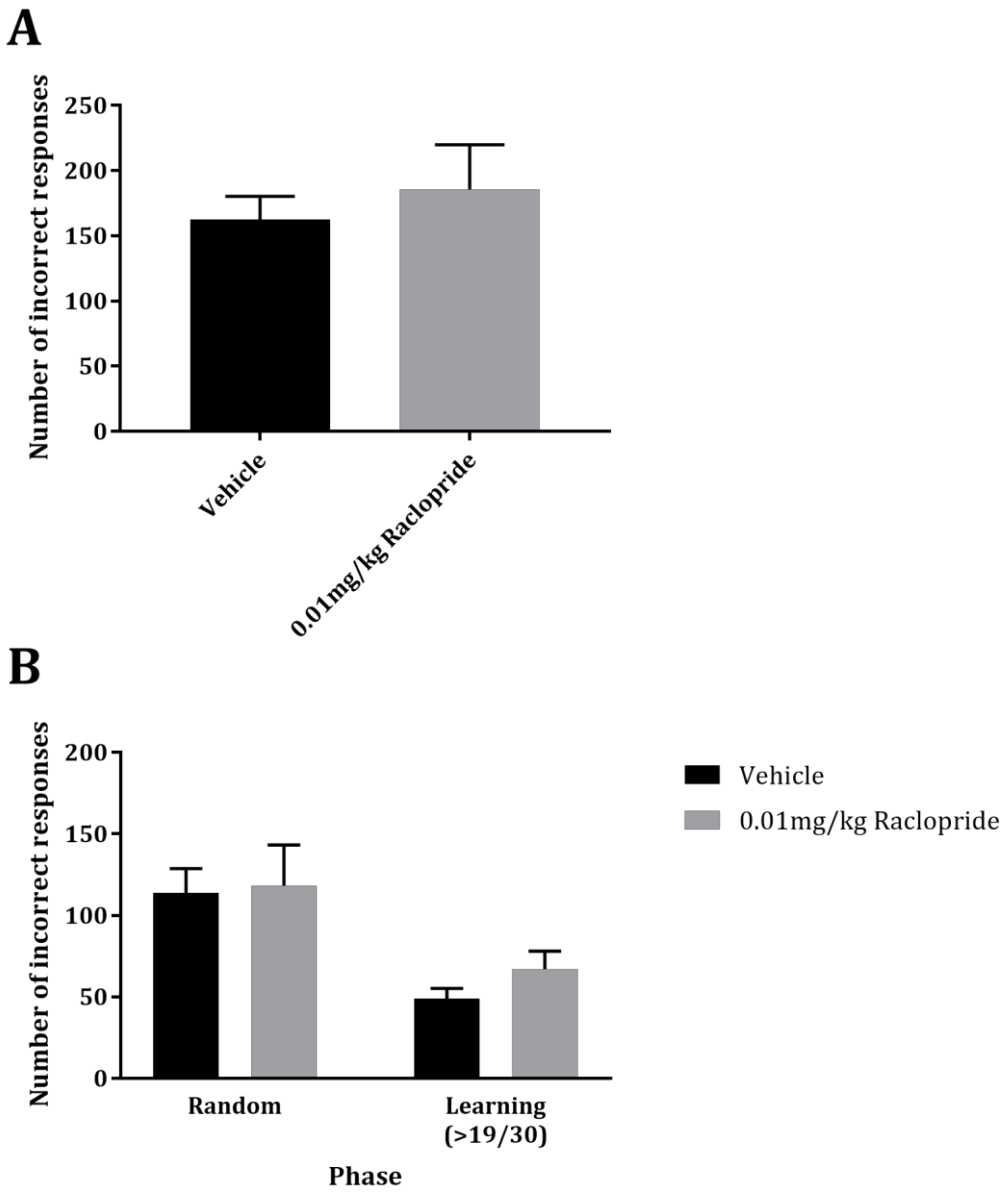


Figure 3.5: **Histograms showing the number of incorrect responses taken to criterion following injection of vehicle or 0.01mg/kg raclopride (A), and the number of incorrect responses per phase of visual discrimination (B). There was no significant difference in the number of errors to reach criterion after raclopride. Data are represented as mean ± SEM.**

3.3.2.3 Omissions

Mann-Whitney U tests revealed that raclopride had no significant effect on the number of omissions within the random or learning phases of the novel stimulus

discrimination paradigm (U = 10.5, p = 0.138 and U = 18.0, p = 0.731, respectively, Figure 3.6).

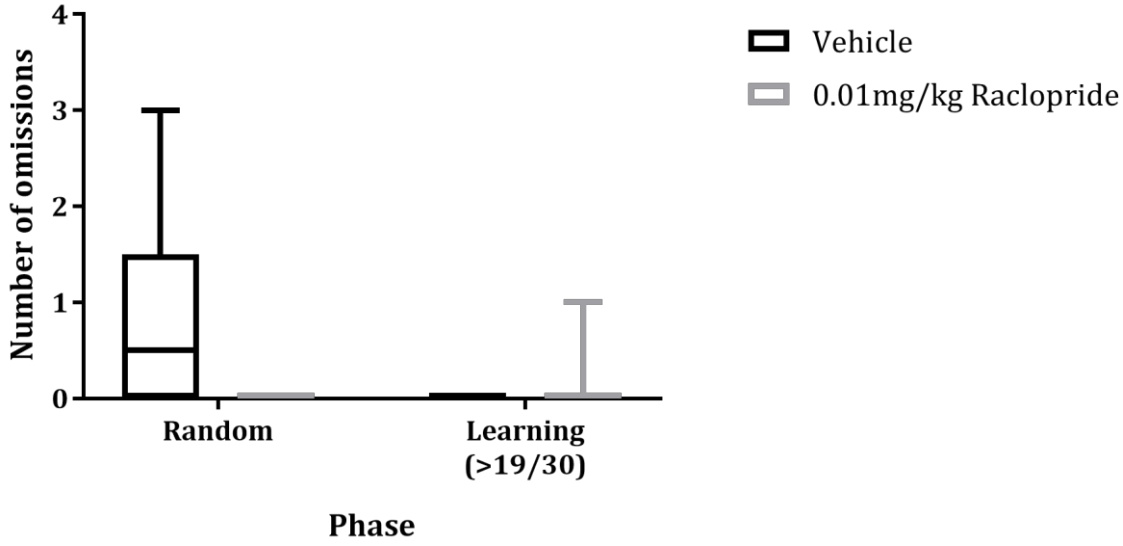


Figure 3.6: **Box and whisker plots showing the number of omissions per phase of the novel visual discrimination task. There was no significant difference in the number of omission after raclopride. Data are represented as median values ± minimum and maximum.**

3.3.2.4 Response and Retrieval Latencies

Table 3.2 shows the response and retrieval latencies during the novel visual discrimination task. Systemic raclopride had no significant effect on either response or retrieval latency ($t(12)$ = 0.07, p = 0.95, and ($t(11)$ = 0.16, p = 0.88, respectively).

	Response latency (s)	**Retrieval latency (s)**
Vehicle	0.84 ± 0.07	1.40 ± 0.30
0.01mk/kg raclopride	0.90 ± 0.16	1.19 ± 0.07

Table 3.2: **A table of the response and reward retrieval latencies. Systemic raclopride had a no significant effect on response or retrieval latencies. Data are represented as the mean values ± SEM (seconds).**

3.3.2.5 Retention

Following the novel visual discrimination, on the next session the rats underwent retention testing (Figure 3.7).

Systemic raclopride had no significant effect on the number of trials to criterion during the retention session ($t(11)$ = -1.845, p = 0.092, Figure 3.7A). However, there was a significant increase in the number of errors on retention following injection of raclopride ($t(11)$ = -3.170, p = 0.009, Figure 3.7B).

An independent-samples Mann-Whitney U test revealed no significant difference in the number of omissions during retention between drug and vehicle controls (U = 20.5, p = 0.945, Figure 3.7C).

3.3.2.6 Summary of experiment 2

In summary, 0.01mg/kg raclopride has no effects on new learning. 0.01mg/kg raclopride significantly impaired retention of a novel visual discrimination, leading to an increase in the number of incorrect responses to reach criterion.

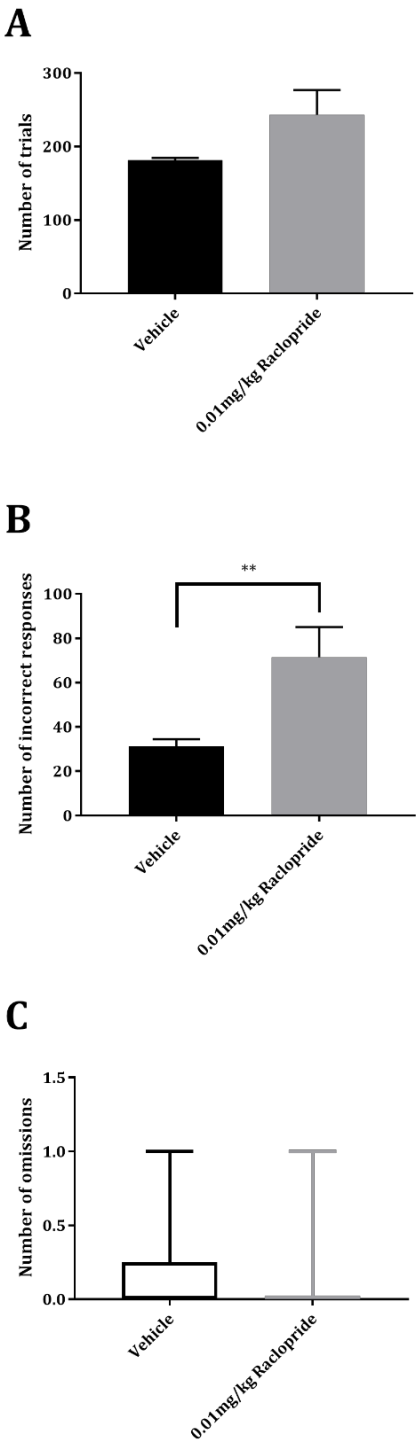


Figure 3.7: **Histograms and box and whisker plots showing the number of trials (A), incorrect responses (B), and omissions (C) during the retention session following reaching criterion the novel visual discrimination task with 0.01mg/kg raclopride. There was a significant increase in the number of incorrect responses ($t(11) = -3.170$, $p = 0.009$, (B)). Data are represented as mean ± SEM or median values ± minimum and maximum. Asterisks denote significant differences (pairwise comparisons: **, $p < 0.01$) from vehicle controls.**

3.3.3 Experiment 3: Effects of systemic D_2/D_3 receptor antagonism on a novel visual reversal learning task

3.3.3.1 Trials to criterion

Injection of 0.01 mg/kg raclopride led to a significant increase in the number of trials taken to reach criterion during a novel visual reversal task (Figure 3.8). A mixed-design ANOVA highlighted both a significant main effect of Phase ($F_{1,11}$ = 25.806, p < 0.001), and a significant Drug x Phase interaction ($F_{1,11}$ = 6.009, p = 0.008, Figure 3.8B); however, there was no main effect of Drug ($F_{1,11}$ = 2.528, p = 0.14, Figure 3.8A).

Injection of 0.01 mg/kg raclopride led to a significant increase in the number of trials during the perseveration phase of the novel reversal task (independent *t*-test, *t(11)* = -3.552, p = 0.005).

3.3.3.2 Incorrect responses

Figure 3.9 shows a significant increase in the number of incorrect responses following systemic injection of 0.01mg/kg raclopride. A mixed-design ANOVA revealed a significant main effect of Phase ($F_{1,11}$ = 28.027, p < 0.001), and a significant Drug x Phase interaction ($F_{1,11}$ = 6.666, p = 0.005, Figure 3.9B). There was no main effect of Drug ($F_{1,11}$ = 3.617, p = 0.084, Figure 3.9A).

Injection of 0.01 mg/kg raclopride led to a significant increase in the number of incorrect responses during the perseveration phase of the novel reversal task (independent *t*-test, *t(11)* = -3.490, p = 0.005).

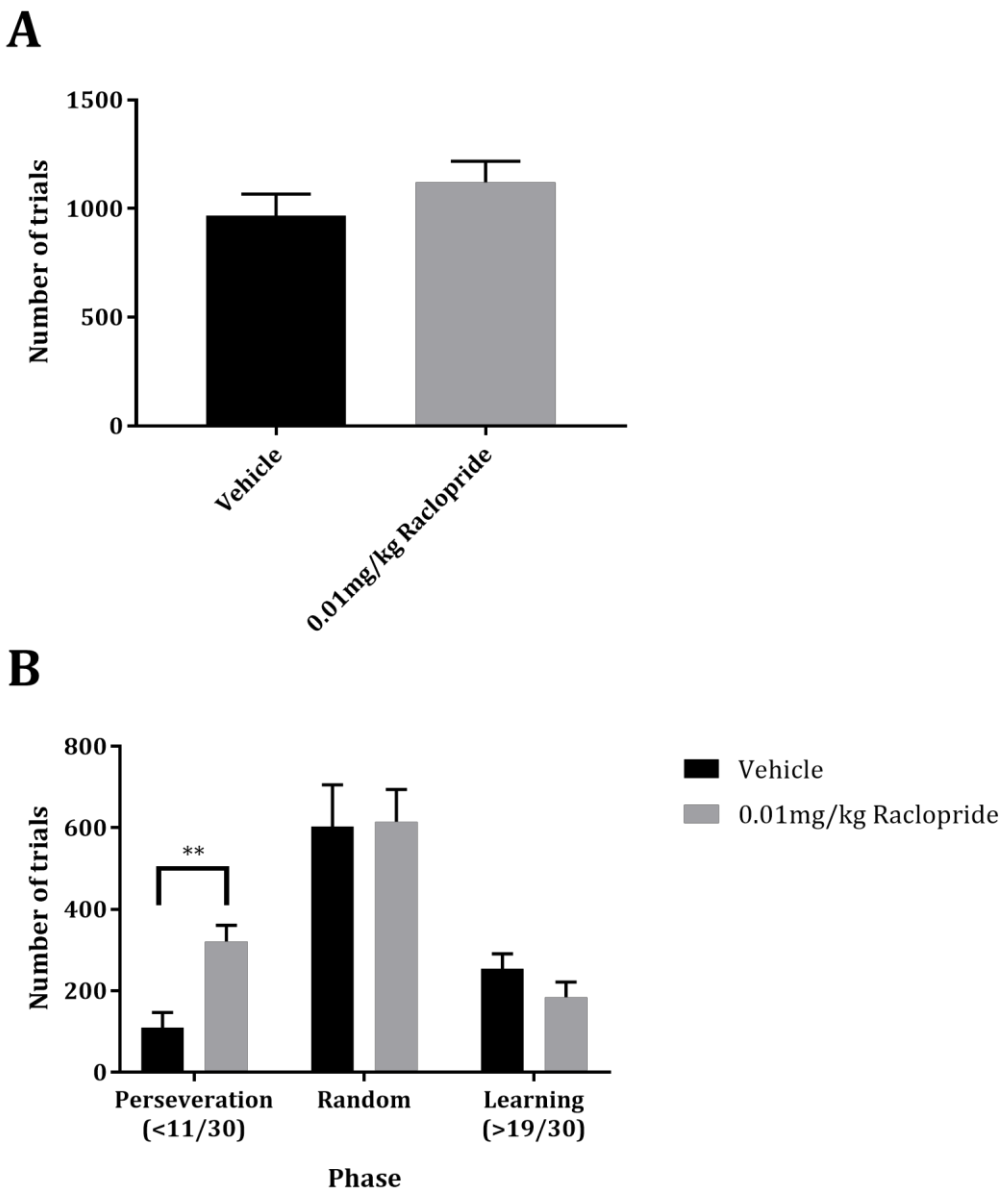


Figure 3.8: **Histograms showing the number of trials taken to criterion (A) per phase of the novel stimuli reversal task (B). A two-way ANOVA revealed a significant Drug x Phase interaction ($F_{1,11}$ = 6.009, p = 0.008). 0.01mg/kg raclopride caused a significant increase in the number of trials during the perseveration phase of the reversal (*$t(11) = -3.552$, $p = 0.005$*). Data are represented as mean ± SEM. Asterisks denote significant differences (pairwise comparisons: **, $p < 0.01$) from vehicle controls.**

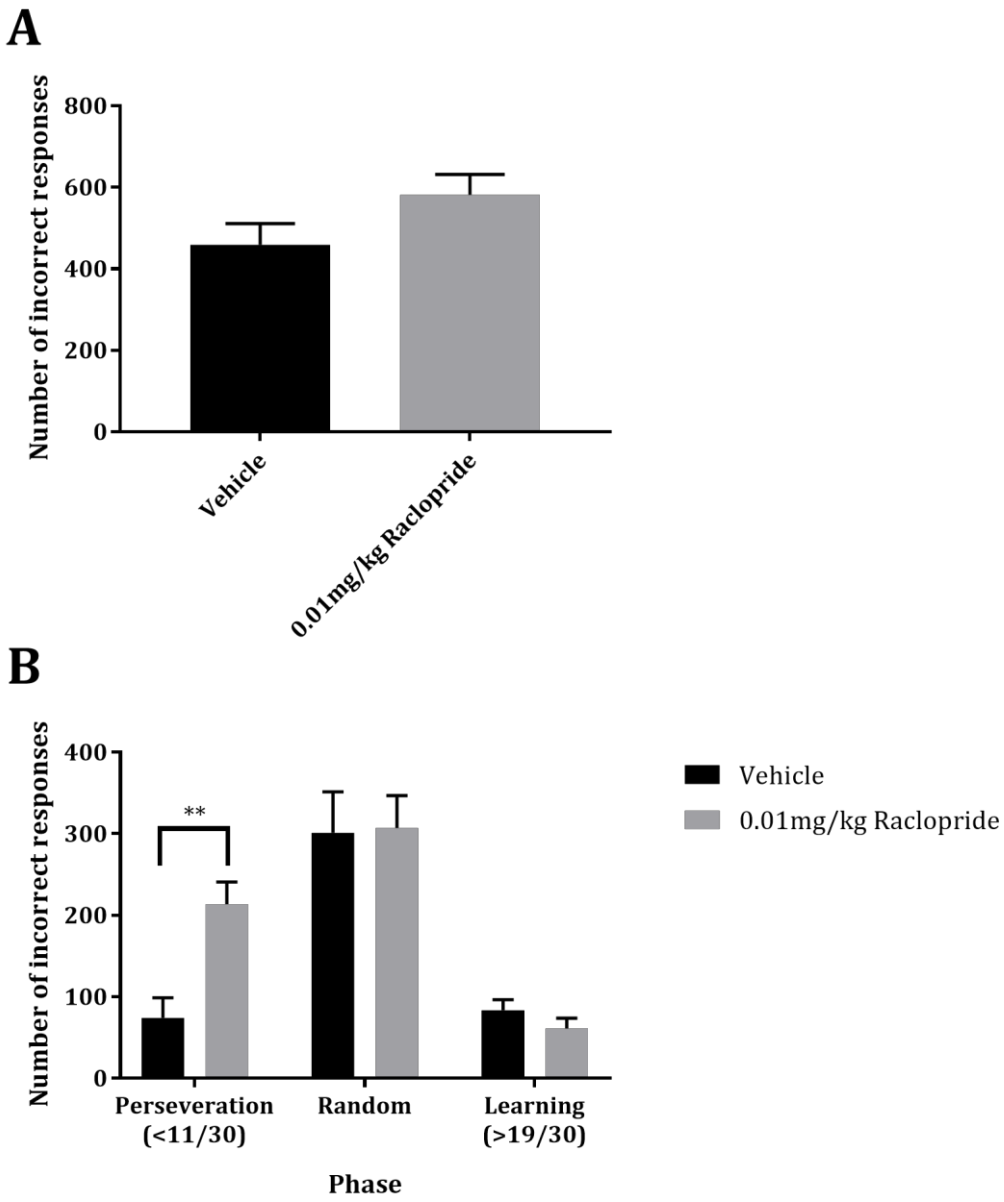


Figure 3.9: **Histograms showing the number of incorrect responses to criterion (A) per phase of the novel stimuli reversal task (B). A two-way ANOVA revealed a significant Drug x Phase interaction ($F_{1,11}$ = 6.666, p = 0.005). 0.01mg/kg raclopride caused a significant increase in the number of incorrect responses during the perseveration phase of the reversal (*t(11) = -3.490, p = 0.005*). Data are represented as mean ± SEM. Asterisks denote significant differences (pairwise comparisons: **, p < 0.01) from vehicle controls.**

3.3.3.3 Omissions

Mann-Whitney U tests within each phase revealed that raclopride had no significant effect on the number of omissions during the perseveration phase, random phase,

or learning phase of the novel stimulus reversal paradigm (U = 21.0, p = 1.0; U = 12.5, p = 0.234; and U = 10.5, p = 0.138, respectively, Figure 3.10).

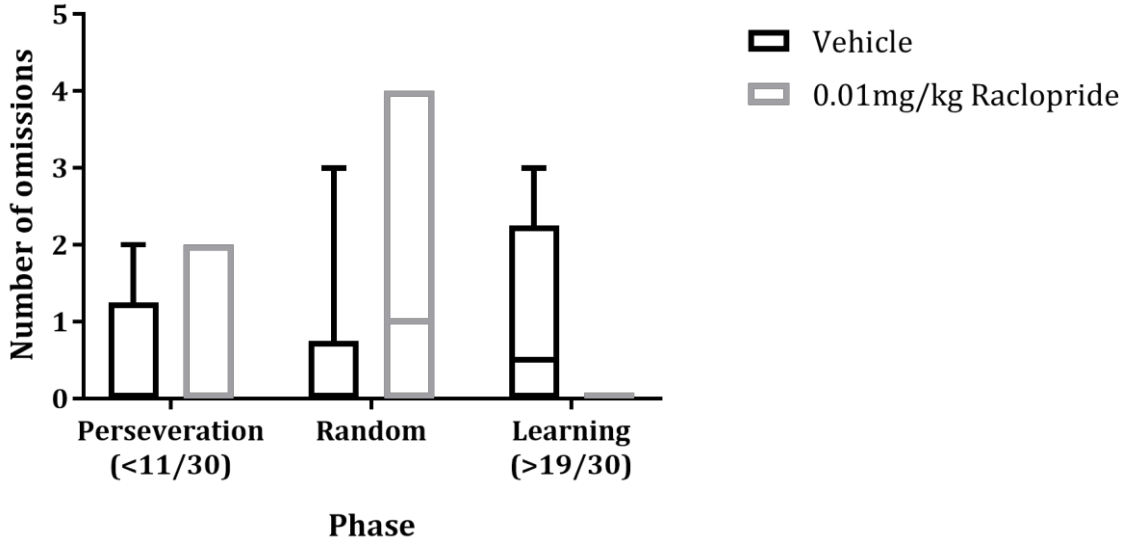


Figure 3.10: **Box and whisker plots showing the number of omissions per phase of the novel stimulus reversal task. There was no significant difference in the number of omission after raclopride. Data are represented as median values ± minimum and maximum.**

3.3.3.4 Response and Retrieval latencies

Table 3.3 reports the effects of 0.01mg/kg raclopride on response and retrieval latency during the novel stimulus reversal task. 0.01mg/kg raclopride has no significant effect on either response or retrieval latency ($t(12)$ = 0.30, p = 0.77, and $t(12)$ = 0.29, p = 0.78, respectively).

	Response latency (s)	**Retrieval latency (s)**
Vehicle	0.89 ± 0.10	1.13 ± 0.23
0.01mk/kg raclopride	0.97 ± 0.14	1.48 ± 0.33

Table 3.3: **A table of the response and retrieval latencies following systemic administration of 0.01mg/kg raclopride on the novel stimulus reversal task.**

3.3.3.5 Summary of experiment 3

In summary, 0.01mg/kg raclopride led to significant phase-specific deficits in novel stimuli reversal learning. 0.01mg/kg raclopride produced a significant increase in both the number of trials and incorrect responses during the perseveration phase of the reversal, these effects were not carried over to the random or learning phases. Deficits in performance were limited to the number of trials and the number of incorrect responses, with no effects on omissions or response or retrieval latencies.

3.4 Discussion

Systemic dopamine D_2/D_3 antagonism caused a mild, generalised impairment on the serial visual reversal task. 0.03 mg/kg raclopride led to a significant increase both in the number of trials to criterion and the number of incorrect responses; this effect was not specific to any one phase of the task. 1 mg/kg raclopride led to significant impairment and an inability to perform the task, it is unclear if this effect was motor or motivational in nature.

Raclopride, at a low dose (0.01 mg/kg), had no significant effect on new learning to a pre-set criterion, on the novel stimulus discrimination learning task. However, this dose did significantly impair retrieval of the previously learnt discrimination, with a significant increase in the number of trials, and the number of incorrect responses during a retention session.

Subsequent to this drugged retention session, 0.01mg kg raclopride also caused a significant decrease in cognitive flexibility, with rats requiring more trials and committing more incorrect responses under the visual reversal paradigm; moreover, this effect was limited to the perseveration phase of the reversal task.

These results taken together show that dopamine D_2/D_3 receptors play a specific role in cognitive and behavioural flexibility under certain test conditions. Motivational factors seem largely to be ruled out as magazine entry latencies were not generally prolonged and errors of omission, although sometimes increased by the drug, were generally very low. Raclopride causing a generalised impairment on performance on the rodent reversal task corresponds with work by Ridley et at (1981). Ridley et al showed that systemic haloperidol, a dopamine $D_{2/3}$ receptor antagonist, had similar effects in a marmoset visual reversal task.

Lee et al (2007) reported similar results with raclopride in the vervet monkey using a visual reversal task. Both studies show an effect of raclopride depending on whether or not a retention session was included beforehand. Lee et al showed no effect of raclopride during a reversal task with no retention sessions, this result is comparable to the present serial reversal task, as rats were not drugged during their prior retention session. Raclopride given during a retention session in both studies subsequently led to a significant impairment in reversal performance following the

drug, showing the importance of dopamine signalling during this stage and the subsequent reversal. Lee et al hypothesised three potential reasons for this effect following retention, including alterations in synaptic dopamine levels, striatal 'priming', and short-term vs long-term memory; however, these were reliant on reversal occurring immediately following retention during the same session, whereas in the present case the retention session was performed 24 hours prior to reversal. Raclopride did not affect vervet performance within the retention part of the task on the Lee et al protocol, however raclopride did lead to a significant increase in the number of errors in the rat touchscreen visual discrimination paradigm.

In the present study, 0.01 mg/kg of raclopride caused only marginal effects on reversal learning initially, although deficits were found at the higher dose of 0.03mg/kg, which also produced more signs of motivational or motor impairment. The lower dose (0.01mg/kg) also produced only marginal evidence for impairments on a new visual discrimination learning task. However, there was a substantial deficit in retention of this discrimination on the next test session, although criterion performance was eventually reached in all of the drugged rats. Nevertheless, on the next (reversal) session the drug produced considerable perseveration to the previously rewarded CS+, indicating that the drugged animals continued to respond to the formerly reinforced stimulus (regardless of where it was presented on the touch-screen). This pattern of findings suggests that the prior experience in the discrimination task under the influence of the drug renders the stimulus-reward associations to be encoded in a form that is less flexible to subsequent changed contingencies, again when under the influence of this drug state, - hence producing perseveration, potentially as a consequence of its actions in the indirect striatal output pathway to prevent the influence of signals of non-reward.

An alternative explanation of the pattern of findings shown here depends on the chronicity of repeated $D_{2/3}$ antagonist treatment: clearly the rats in Experiment 3 had had much more experience with raclopride than they had experienced by the time of Experiment 1. By the time they exhibited increases in perseverative responding in Experiment 3 many of the rats had cumulatively experienced several doses of 0.01mg/kg. A possible argument against this account is that omissions were

no higher in Experiment 3 than they had been in Experiment 1, and there were no effects on response or retrieval latencies in the latter experiments.

Overall, these experiments were able to emulate the studies of Ridley et al and Lee et al in non-human primates and produce a similar pattern of results in a rat touchscreen serial visual reversal task, supporting a possible role of dopamine in reversal learning and supporting a general role of dopamine in reversal performance across species.

Raclopride produced a mild, generalised effect on serial reversal learning when injected systemically, this could be due to opposing roles on D_2/D_3 receptors anatomically; therefore, the following chapters will report on local infusions of raclopride and other neurotransmitter modulators into different brain region, especially the prefrontal cortex and striatum which contain relatively high numbers of D_2 receptors.

Chapter 4: LOCAL D_2/D_3 RECEPTOR ANTAGONISM WITHIN DIFFERENT STRIATAL SUB-REGIONS PRODUCES DISSOCIABLE AND OPPOSING EFFECTS ON REVERSAL LEARNING

4.1 Introduction

Chapter 3 reported effects of systemic D_2/D_3 receptor antagonism on the touchscreen serial reversal paradigm, as well as new learning and subsequent reversal of novel stimuli. To understand the anatomical effects of D_2/D_3 antagonism, raclopride needed to be administered locally into different brain regions.

Investigating the role of dopamine in the striatum was justified as dopamine, not 5-hydroxytryptamine (5-HT), has been shown to regulate reversal learning in the marmoset caudate nucleus, homologous to the medial striatum (Clarke *et al.*, 2011). Using 5,7-dihydroxytryptamine (5,7-DHT) or 6-hydroxydopamine (6-OHDA) to selectively lesion 5-HT or dopamine innervations respectively, Clarke et al showed that dopamine depleted monkeys were significantly impaired on a serial reversal task, with 5-HT depletion producing no significant effect.

Dopamine has also been shown to play a role in reversal learning in the DMS in rats (O'Neill and Brown, 2007). Dopamine was depleted in the DMS using 6-OHDA; following dopamine depletion rats performed a significantly higher number of trials following reversals compared to controls, indicating an impairment in reversal learning. An impairment in reversal learning following dopamine depletion in the DMS suggests that dopamine transmission is critical for cognitive flexibility and response shifting.

Using a response and visual cue discrimination learning task, Ragozzino et al. (2002) showed that the DMS is involved in the ability to learn and maintain a new

discrimination strategy. Inactivation of the DMS did not impair the acquisition of either response or visual cue discrimination, but inactivation did impair performance when shifting from one learned strategy to the other. The impairment in performance was not perseverative in responding, but an inability to maintain the new strategy.

In 2010, Castañé, Theobald, and Robbins reported the effects of selective lesions of the dorsomedial striatum, and their effects on a serial spatial reversal learning task in rats. Lesions to the DMS and DLS produced changes in latency on a previously acquired spatial discrimination, whereas lesions to the nucleus accumbens core and shell did not affect retention performance. Conversely, core, shell and DLS lesions did not affect reversal performance, while DMS lesions led to an increase in the number of errors to reach criterion.

The role of the nucleus accumbens in reversal learning is unclear. Lesions of the NAc have been reported to impair initial discrimination in a reversal task (Taghzouti *et al.*, 1985), and the subsequent reversal; interpretation of such results is difficult as the latter finding could be impacted by the former. With subsequent studies reporting that lesions of the NAc did not affect spatial reversal tasks in rats (Castañé, Theobald and Robbins, 2010), or visual reversal tasks monkeys (Stern and Passingham, 1995).

Haluk and Floresco (2009) reported the effects of nucleus accumbens core D_1 and D_2 receptor manipulations on a strategy set-shifting task. It was reported that D_1, not D_2, antagonism impaired set-shifting, through an inability to maintain the new strategy. Conversely, D_2, not D_1, receptor agonists also impaired set-shifting, through perseveration. However, D_2 receptor agonist only impaired strategy reversal learning without disrupting initial learning. These data show a specific role for dopamine receptor sub-types in the NAc core.

Following the findings of O'Neill and Brown, Ragozzino et al, and Castañé et al, I hypothesised that local infusion of raclopride, a dopamine D_2/D_3 receptor antagonist, will produce differential effects across the touchscreen serial reversal task. I hypothesised that infusion of raclopride into the posterior DMS will increase responding during the random phase (ie new learning phase), while infusion into

the DLS will increase perseverative responding during the early phase, due to its putative role in habit formation (Yin et al 2004; see General Introduction). These hypotheses justify the use of *a priori* comparisons within these phases, although I also adopted an overall ANOVA approach to compare the effects of the drug at different phases of reversal learning across the striatal regions. On the basis of the previous literature, I did not make any specific predictions on the drug effects within the anterior DMS or NAc.

4.2 Methods

4.2.1 Subjects

Fifty-four male Lister-Hooded rats (Charles River, UK), split across four cohorts, were trained on a touchscreen discrimination and serial reversal learning task (see Chapter 2 for details). Table 4.1 presents the number of animals allocated to each experiment, the number of rats excluded from each experiment, and the final number of rats in each group.

Exp.	Drug	Number of rats	Number of rats excluded	Final *N*
1	Raclopride – posterior DMS	18	2 – dysfunctional 1 – cannula misplacement	15
2	Raclopride – anterior DLS	22	3– sickness 2- dysfunctional 6- cannula misplacement	11
3	Raclopride – anterior DMS	8		8
4	Raclopride – nucleus accumbens core	8	1 – dysfunctional 1 - sickness 1 - cannula misplacement	5
	Total	**56**	**Total animals used for analysis**	**39**

Table 4.1: **Summary of the cohorts of animals used in each experiment.**

Of the excluded animals, eight were cannulated into incorrect coordinates, six of these were in the anterior DLS group and have been analysed as a separate cohort, two lost their cannula during the testing period and two animals started experiencing seizures. The five animals classed a dysfunctional were unable to be included for various reasons, including failure to relearn the task following surgery, or an injector snapping in the cannula during an infusion.

4.2.2 Behavioural procedure

Rats were trained on the touchscreen serial reversal task as previously described in Chapter 2.

Serial Reversal Learning Task: The serial reversal learning task is described in detail in Chapter 2.

4.2.3 Surgical cannulation

Rats underwent surgical cannulation as previously described in Chapter 2. Briefly, rats were anaesthetized and secured in a stereotaxic frame with atraumatic earbars. PlasticsOne cannulae (22-GA) were inserted in the pDMS (AP -0.4, ML ±2.6, DV -2.4), aDLS (AP +1.2, ML ±3.5, DV -2.4), or aDMS/NAc (AP +1.2, ML ±1.9, DV -1.9) and secured with screws and dental cement. All surgical co-ordinates were calculated using a stereotaxic atlas (Paxinos and Watson, 2006) using bregma as the origin. All dorsoventral measurements were taken from dura.

4.2.4 Drugs

Raclopride (Tocris Bioscience, Bristol, UK) was dissolved in physiological saline to concentrations of 0.1 and 1.0 μg/μl (final infusion concentration 0.05 and 0.5 μg/side). Solutions were stored as aliquots at -20°C.

Serial Reversal Task: Prior to drug administration, animals were counterbalanced across the drug doses, matched for their performance during the final training stages of the serial visual reversal learning task. Each animal received intracranial infusions of vehicle and raclopride on separate reversals until criterion was reached.

4.2.5 Microinfusions

After recovering from surgery (≥7 days), animals received a baseline reversal to reintroduce the animals to the task. During this baseline reversal, animals were assessed to determine suitability for testing, including errors to criterion, stable performance, and ensuring no presence of a side bias. During this reversal, animals were habituated to the infusion procedure, receiving mock and vehicle infusions

during the latter stages of the reversal. Injectors from PlasticsOne (28-GA) were extended 2.0 mm below the guide for pDMS and aDLS, 2.5 mm below the guide for aDMS, or 5.0 mm below the guide for NAc infusions. Infusions were performed at a rate of 0.5 µl over 2 minutes. The injector was left in place for 1 minute before and after the infusion. During the infusion procedure, animals were allowed to freely move on the lap of the experimenter or gently restrained. Following the infusion animals were returned to their home cage and placed in the experimental chamber 8 minutes after the start of the infusion procedure.

4.2.6 Statistical analysis

The main measures of the animals' ability to learn the reversals and visual discrimination were: (i) the number of trials to criterion and (ii) the number of incorrect responses to criterion (errors of commission). Additional secondary measures recorded for each trial were (iii) the number of omissions (errors of omission), (iv) the latency to respond to the stimuli, (v) the latency to collect the reward.

Data for each primary variable were analysed using a repeated measures mixed model ANOVA consisting of one between-subject factor (Region) and two within-subject factors (Dose and Phase). When significant interactions were found, further analysis was performed of Simple Main Effects or Simple Main Interactions, as appropriate. Analysis was followed by post-hoc Sidak's corrected pair-wise comparisons to vehicle.

Omission data were analysed using Friedman's test on each separate phase of the reversal paradigm. When significances were found, Wilcoxon Signed Rank tests were performed within each experimental phase comparing to Vehicle.

For all comparisons, significant difference was assumed at $p < 0.05$.

4.3 Results

4.3.1 Histological results

Figure 4.1 shows a schematic reconstruction of the position of injector tips in the pDMS, aDLS, aDMS, and NAc. Animals were excluded from data analysis if the cannula position was not correct. Positions were correct as per the defined regions by Paxinos and Watson (2006), or excluded from further analysis (N=6).

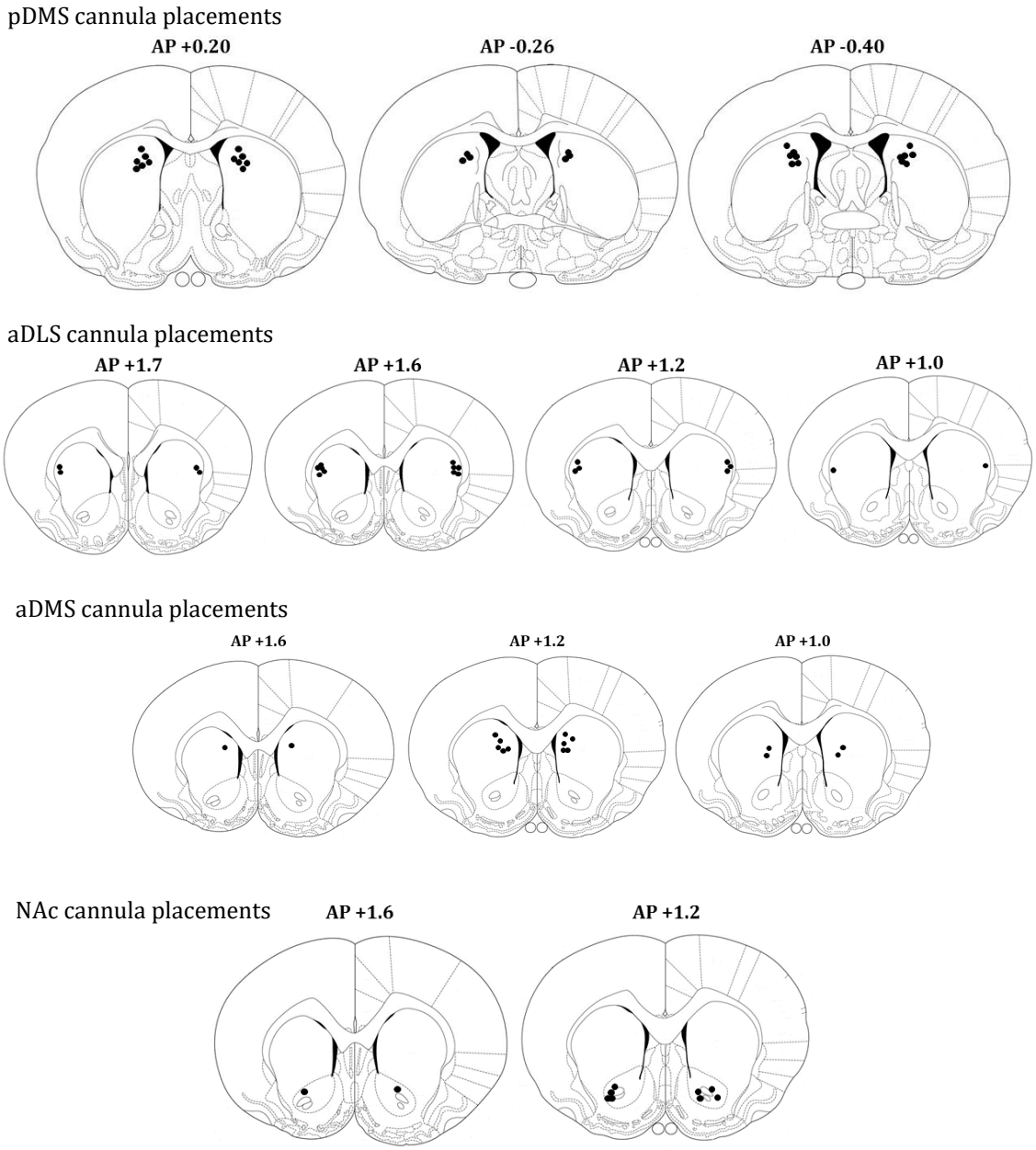
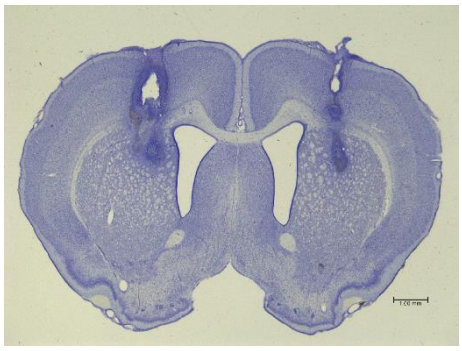


Figure 4.1: **Schematic diagrams showing the injection sites in the pDMS, aDLS, aDMS, and NAc. Reconstructions from Paxinos and Watson (2006).**

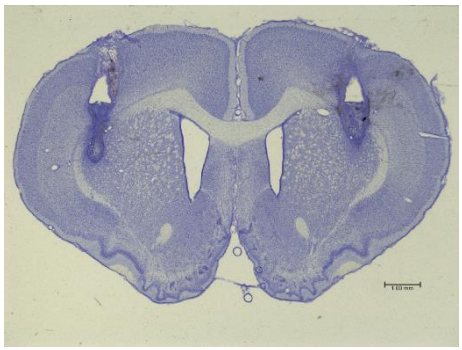
Figure 4.2 shows photomicrographs of coronal sections taken from representative rats to show cannula and injector placement.

A – pDMS



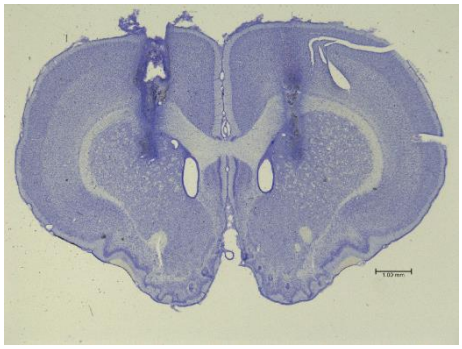
AP – 0.26

B - aDLS



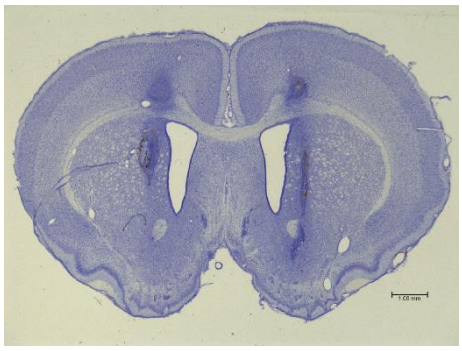
AP +1.2

C – aDMS



AP +1.6

D - NAc



AP 1.2

Figure 4.2: **Photomicrographs of coronal sections taken from representative rats: (A) pDMS, (B) aDLS, (C) aDMS, and (D) NAc.**

4.3.2 Behavioural results

4.3.2.1 Trials

A three-way repeated measures mixed design ANOVA showed a significant main effect of Phase ($F_{2,70} = 46.748, p < 0.001$).

There was also a significant Dose x Region interaction ($F_{6,70} = 2.25, p = 0.048$, Figure 4.3), however there were no other significant two-way interactions.

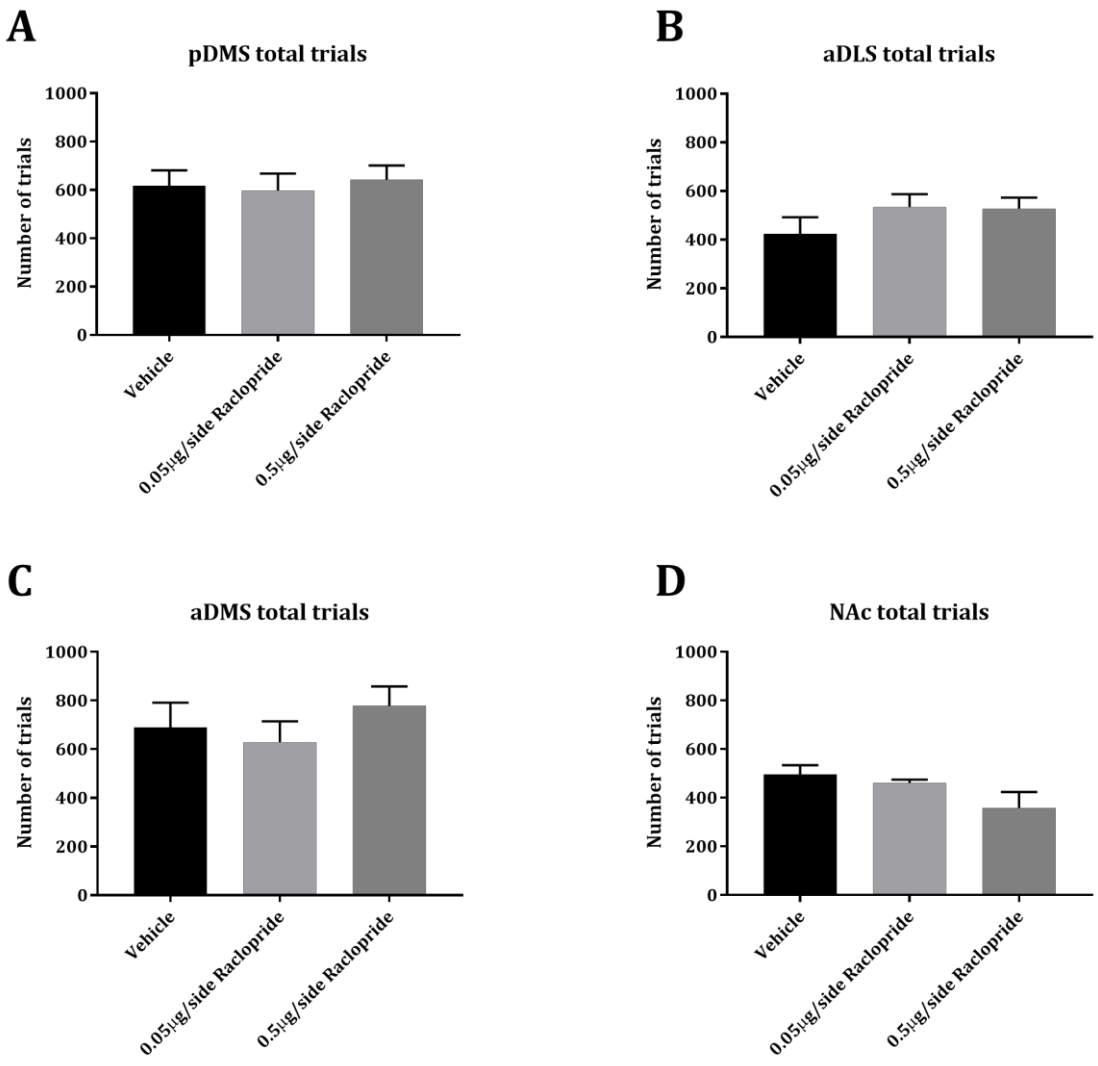


Figure 4.3: **Histograms showing the number of trials to criterion following infusion of 0.05µg and 0.5µg Raclopride per hemisphere into the posterior DMS (A), anterior DLS (B), anterior DMS (C), and Nucleus accumbens core (D). Data are represented as mean values ± SEM. A three-way ANOVA revealed a significant Dose x Region interaction ($F_{6,70} = 2.25, p = 0.048$).**

Figure 4.3A shows the number of trials taken to reach criterion on the touchscreen reversal task following infusion of raclopride into the posterior DMS. A test of simple main effects showed that there was no significant effect of Dose on the number of trials to criterion ($F_{2,72}$ = 0.187, p = 0.829).

Figure 4.3B shows the number of trials to criterion following infusion of raclopride into the anterior DLS. A test of simple main effects showed that there was no significant effect of Dose ($F_{2,72}$ = 1.44, p = 0.243).

A test of simple main effects showed that there was no significant effect of Dose on the number of trials taken to reach criterion following infusion of raclopride into the anterior DMS ($F_{2,72}$ = 0.819, p = 0.444).

Figure 4.3D shows the number of trials taken to criterion following infusion of raclopride into the nucleus accumbens core. A test of simple main effects revealed that there was no significant effect of Dose in the number of trials required ($F_{2,72}$ = 0.822, p = 0.444).

The three-way ANOVA showed there was no significant Dose x Region x Phase three-way interaction ($F_{4,70}$ = 1.704, p = 0.072, Figure 4.4).

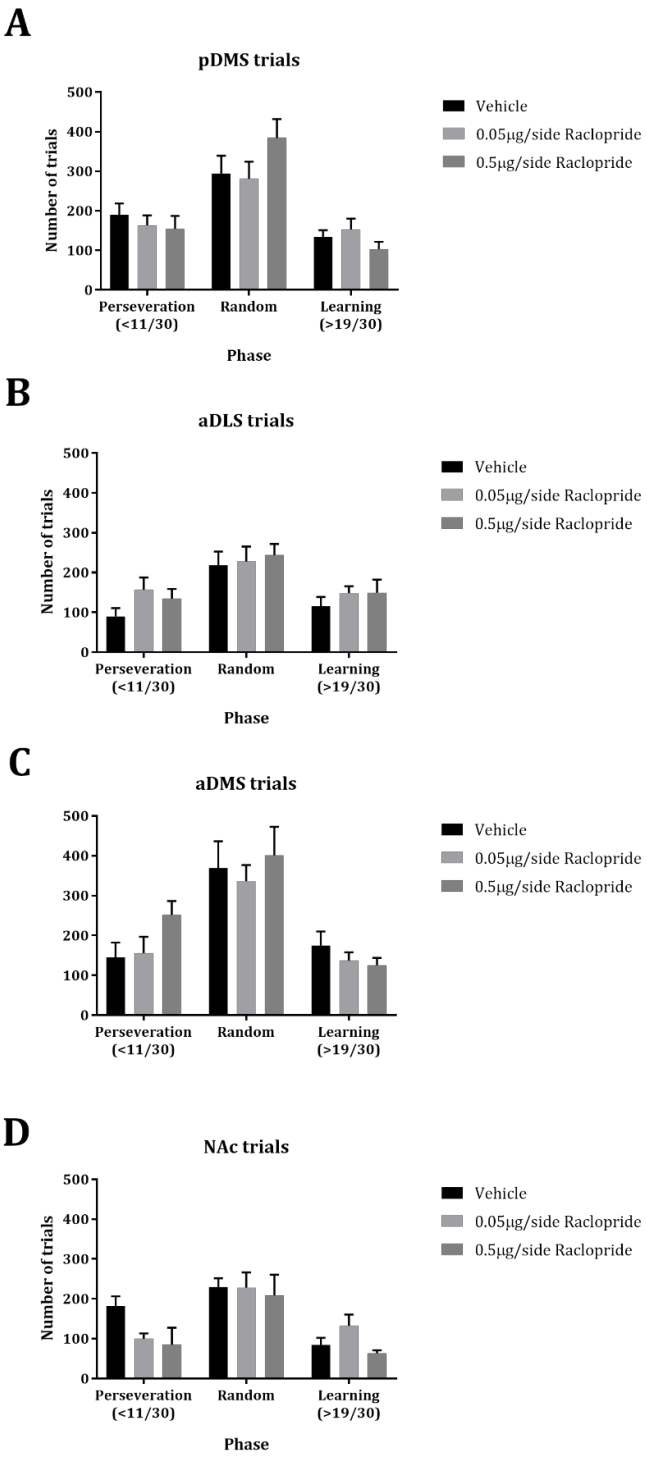


Figure 4.4: **Histograms showing the number of trials to criterion through the perseveration, random, and learning phases, following infusion of 0.05µg and 0.5µg Raclopride per hemisphere into the posterior DMS (A), anterior DLS (B), anterior DMS (C), and nucleus accumbens core (D). Data are represented as mean values ± SEM. There was no significant Dose x Phase x Location three-way interaction ($F_{4,70}$ = 1.704, p = 0.072).**

4.3.2.2 Incorrect responses

A three-way repeated measures mixed design ANOVA showed a significant main effect of Phase ($F_{2,70}$ = 64.807, p < 0.001).

There was also a significant Dose x Region interaction ($F_{6,70}$ = 2.46, p = 0.032), however there were no other significant two-way interactions.

The three-way ANOVA showed there was a significant Dose x Region x Phase three-way interaction ($F_{4,70}$ = 1.828, p = 0.049, figures 4.5-4.8).

An initial attempt to dissect the complex nature of the three-way interaction was to interpret the data for Dose x Phase within each Region.

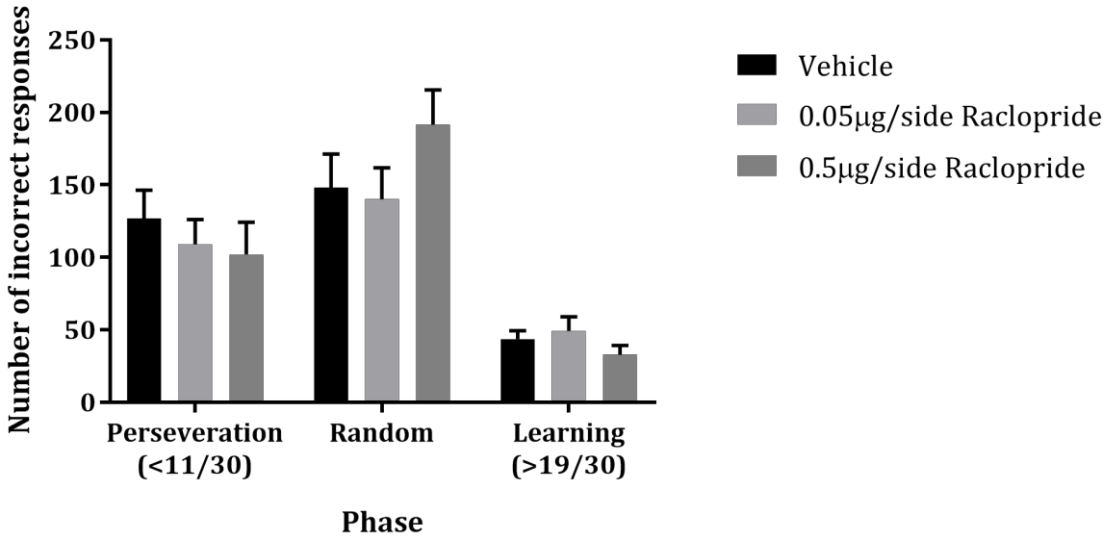


Figure 4.5: **Histograms showing the number of incorrect responses through the perseveration, random and learning phases following infusion of raclopride into the posterior DMS. Data are represented as mean ± SEM. A test of simple effects revealed a significant simple interaction of Dose x Phase ($F_{4,140}$ = 2.87, p = 0.02), however there were no significant pairwise comparisons.**

Figure 4.5 shows the number of incorrect responses to criterion per phase of the touchscreen serial reversal task following infusion of raclopride in the posterior DMS. A test of simple interactions revealed that there was a significant interaction of Dose x Phase within the posterior DMS ($F_{4,140}$ = 2.87, p = 0.02).

Further investigation did not reveal any significant effect of Dose within each Phase ($F_{2,140}$ = 1.959, p = 0.14, $F_{2,140}$ = 3.00, p = 0.053, and $F_{2,140}$ = 0.933, p = 0.396, for Perseveration, Random and Learning phases respectively).

Inspection of Figure 4.5 suggests that the major contributor to the signficant Dose x Phase Interaction is the contrast between the effects of the high dose in the perseveration and random phases, in comparison to the effects of the vehicle. However, it is evident that the high dose is trending to a significant impairment in the random phase. Indeed, the results of the pre-planned comparison, as rationalised in the introduction, also reveal a trend to signficant impairment within the random phase ($F_{2,28}$ = 2.031, p = 0.150).

There were no simple interaction effects of Dose x Phase in the anterior DLS, posterior DMS, or NAc ($F_{4,140}$ = 0.78, p = 0.78, $F_{4,140}$ = 2.24, p = 0.068, and $F_{4,140}$ = 1.24, p = 0.29, respectively, Figures 4.6-4.8). Therefore, to further dissect the three-way interaction the effects Dose x Location interactions were investigated for each Phase.

A test of simple interaction effects revealed a significant Dose x Location interaction within the Perseveration phase ($F_{6,209}$ = 5.06, p < 0.001); however, there were no significant Dose x Location interactions in the Random or Learning phases ($F_{6,209}$ = 0.49, p = 0.81 and $F_{6,209}$ = 0.50, p = 0.81, respectively).

A test of simple effects revealed no significant effect of Dose within the Perseveration phase in the posterior DMS ($F_{2,209}$ = 2.06, p = 0.13), this result is consistent with the previous analysis.

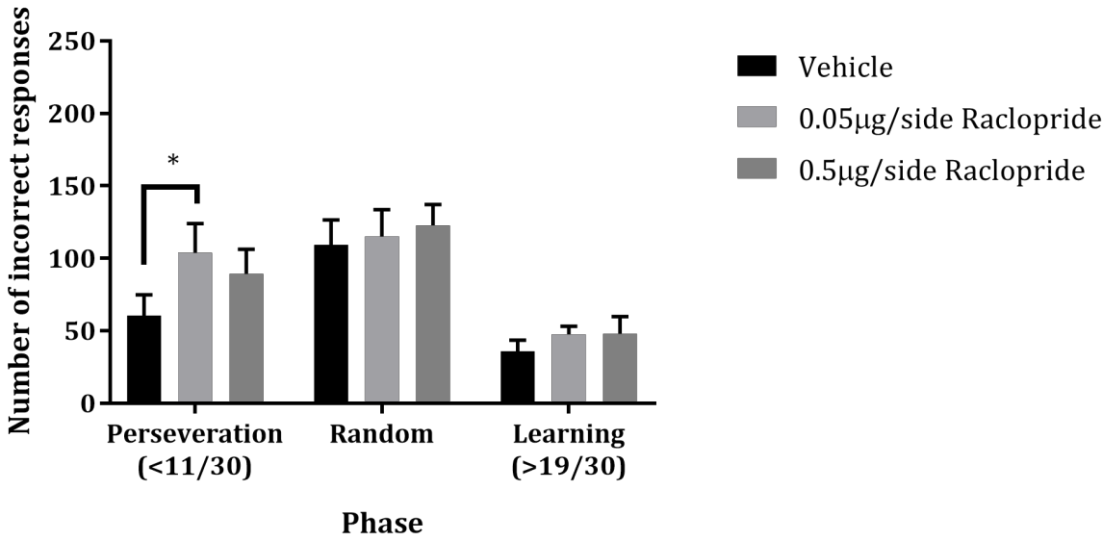


***Figure 4.6*: Histograms showing the number of incorrect responses per phase following infusion of raclopride into the anterior DLS. Data are represented as mean ± SEM. A test of simple effects revealed a significant simple effect of Dose within the Perseveration phase ($F_{2,209}$ = 3,87, p = 0.02). 0.05µg raclopride led to a significant increase in the number of incorrect responses within the perseveration phase compared to vehicle controls (p = 0.032). Asterisks denote significant differences (pairwise comparison: *, $p < 0.05$) from vehicle controls.**

Figure 4.6 shows the number of incorrect responses per phase of the reversal task following local infusion of raclopride into the anterior DLS. A test of simple effects revealed a significant effect of Dose within the perseveration phase ($F_{2,209}$ = 3.87, p = 0.02), and a significant increase in the number of incorrect responses following infusion of 0.05µg raclopride into the anterior DLS (p = 0.032).

Figure 4.7 shows the number of incorrect responses to criterion through each phase of the reversal task following infusion of raclopride into the anterior DMS. Raclopride led to a significant increase in the number of incorrect responses within the perseveration phase ($F_{2,209}$ = 5.48, p = 0.005); however, there were no significant pairwise comparisons to the vehicle controls.

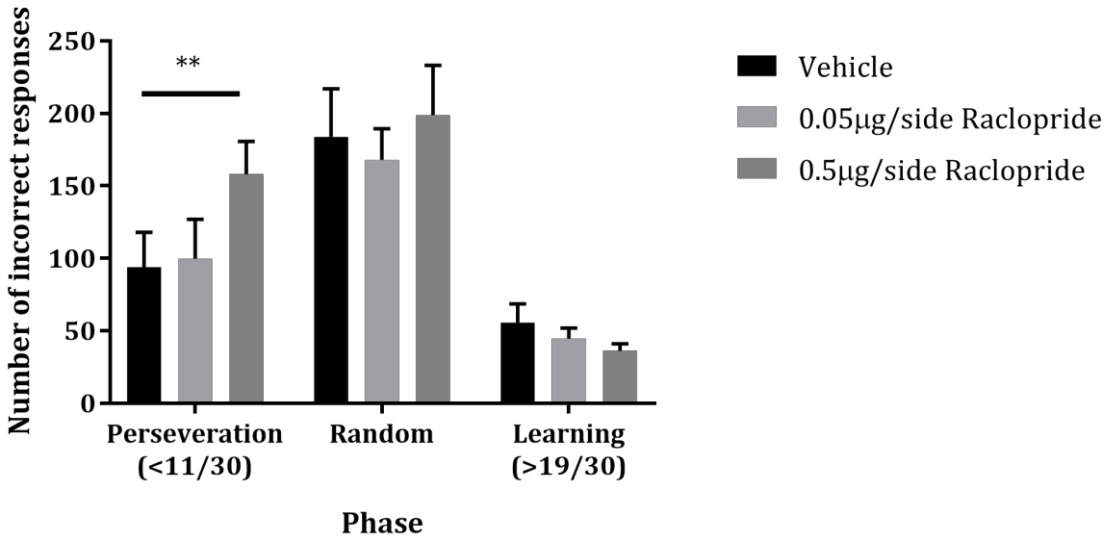


***Figure 4.7*: Histograms showing the number of incorrect responses per phase following infusion of raclopride into the anterior DMS. Data are represented as mean ± SEM. A test of simple effects revealed a significant simple effect of Dose within the perseveration phase ($F_{2,209}$ = 5.49, p = 0.005). Asterisks denote significant effects (**, p < 0.01).**

Figure 4.8 shows the number of incorrect responses per phase of the reversal task following local infusion of raclopride into the nucleus accumbens core. Raclopride led to a significant reduction in the number of perseverative incorrect responses ($F_{2,209}$ = 4.00, p = 0.02); however, there were no significant pairwise differences from the vehicle control.

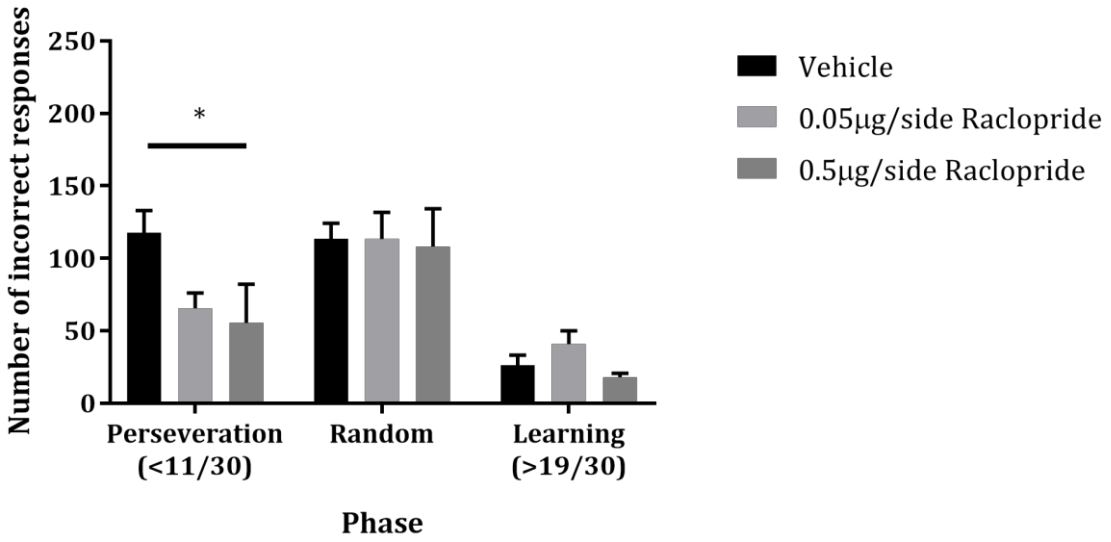


Figure 4.8: **Histograms showing the number of incorrect responses per phase following infusion of raclopride into the NAc. Data are represented as mean ± SEM. A test of simple effects revealed a significant simple effect of Dose within the perseverative phase ($F_{2,209}$= 4.00, p = 0.02). Asterisks denote significant effects (*, $p < 0.05$)**

It should be noted that these analyses were performed and reported using the Welch-Satterthwaite correction (Howell, 2013); however, all results remained significant with the more conservative analysis and degrees of freedom.

4.3.2.3 Omissions

Friedman's test, performed within each Phase and each Location, revealed that local infusion of raclopride had no significant effect on the number of omissions (Figure 4.9).

Figure 4.9A shows the number of omissions following infusion of raclopride into the posterior DMS. Friedman's test revealed there was no significant effect of raclopride in the perseveration ($\chi^2(2) = 2.6$, $p = 0.273$), random ($\chi^2(2) = 0.195$, $p = 0.907$), or learning phase ($\chi^2(2) = 2.375$, $p = 0.305$).

Figure 4.9B shows the number of omissions following local infusion of raclopride into the anterior DLS. Friedman's test revealed that there was no signficant effect of

raclopride during the perseveration, random or learning phases ($\chi^2(2) = 1.83$, $p = 0.40$, $\chi^2(2) = 0.875$, $p = 0.646$, and $\chi^2(2) = 1.077$, $p = 0.584$, respectively).

Figure 4.9C shows the number of omissions following raclopride infusion into the anterior DMS. Raclopride infusion had no significant effect on the number of omissions in the perseveration ($\chi^2(2) = 2.39$, $p = 0.30$), random ($\chi^2(2) = 1.04$, $p = 0.595$), or learning phase ($\chi^2(2) = 3.571$, $p = 0.168$).

Figure 4.9D shows the number of omissions following local infusion of raclopride into the nucleus accumbens core. Friedman's test revealed that there was no signficant effect of raclopride during the perseveration, random or learning phases ($\chi^2(2) = 2.92$, $p = 0.23$, $\chi^2(2) = 0.429$, $p = 0.807$, and $\chi^2(2) = 3.00$, $p = 0.223$, respectively).

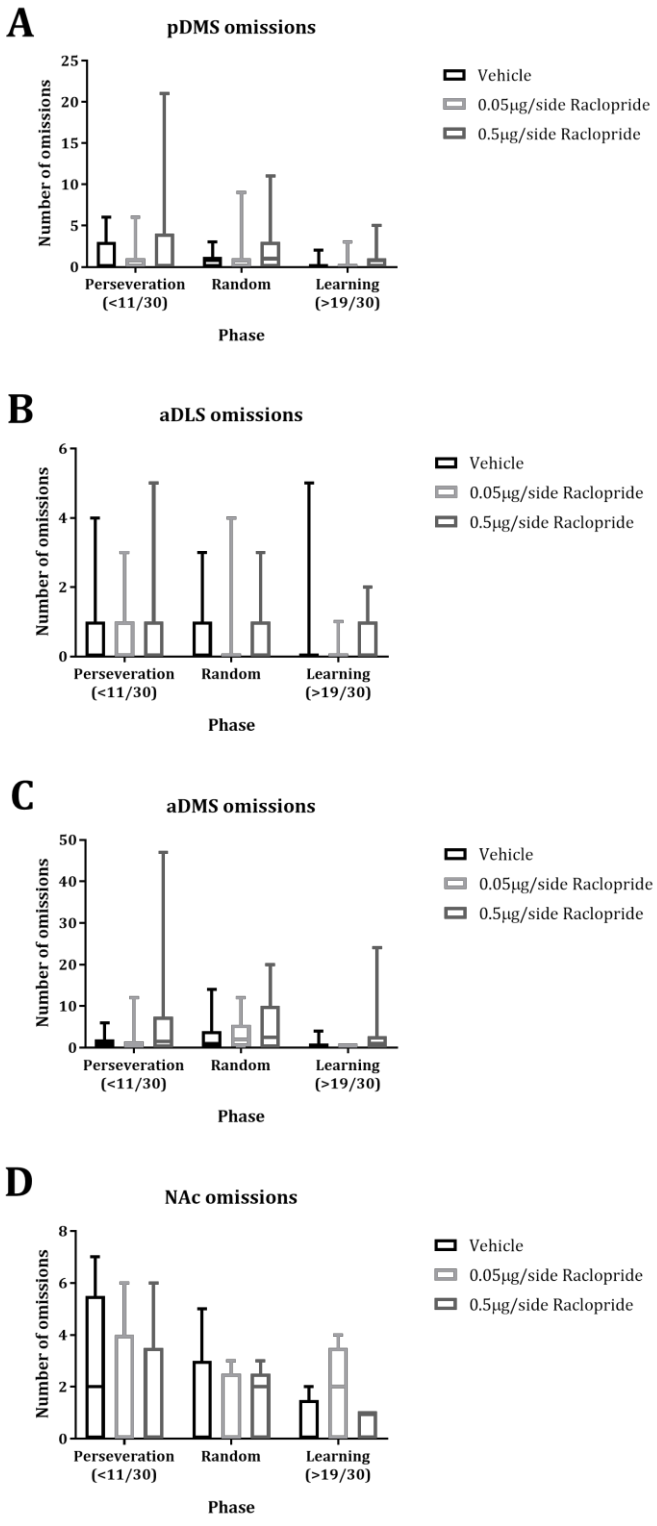


Figure 4.9: **Box and whisker plots showing the number of omissions for phase of the reversal task following local infusion of raclopride into the (A) pDMS, (B) aDLS, (C) aDMS, or (D) NAc. Data are represented as median values ± minimum and maximum. No significant effects were found.**

4.3.2.4 Response and collection latencies

Table 4.2 shows the response and collection latencies following infusion of raclopride into the posterior DMS, anterior DLS, anterior DMS and nucleus accumbens core.

There were no significant differences in the response latencies; however, there was a significant slowing in collection latencies with 0.5µg/hemisphere infusions of raclopride ($F_{2,28} = 6.828$, $p = 0.004$, pairwise comparisons $p = 0.013$).

Region	Dose	Response	Collection
pDMS	Vehicle	0.975 ± 0.188	1.021 ± 0.308
	0.05µg/hemisphere	0.969 ± 0.187	1.192 ± 0.432
	0.5µg/hemisphere	1.096 ± 0.310	1.354 ± 0.537 *
aDLS	Vehicle	1.008 ± 0.204	1.049 ± 0.413
	0.05µg/hemisphere	0.950 ± 0.129	1.097 ± 0.605
	0.5µg/hemisphere	1.065 ± 0.175	1.109 ± 0.353
aDMS	Vehicle	1.133 ± 0.318	1.203 ± 0.485
	0.05µg/hemisphere	1.221 ± 0.419	1.436 ± 0.782
	0.5µg/hemisphere	1.266 ± 0.389	1.713 ± 0.785 *
NAc	Vehicle	1.125 ± 0.215	1.370 ± 0.461
	0.05µg/hemisphere	1.281 ± 0.300	1.363 ± 0.602
	0.5µg/hemisphere	1.218 ± 0.170	1.302 ± 0.585

Table 4.2: **The response and collection latencies following raclopride infusion into the posterior pDMS, aDLS, aDMS, or NAc . Data are represented as the mean value for each dose ± SEM (seconds). There were no significant effects in response latency. Asterisks denote significant differences (Sidak corrected comparison: *, $p < 0.05$) from vehicle controls.**

There was a significant slowing effect of Dose on collection latency, and a trend to slowing on response latency ($F_{2,14}$ = 7.565, p = 0.006 and $F_{2,14}$ = 3.146, p = 0.074, respectively). Further investigation within of the collection latencies revealed a significant slowing following 0.5µg/hemisphere compared to vehicle control (p = 0.048).

There were no significant effects on either response or retrieval latency following infusion of raclopride into the anterior DLS or NAc (Table 4.2).

4.3.2.5 Analysis of incorrect placements

Due to the high number of misplaced cannulae in the aDLS group (6), these data were analysed to show any anatomical specific findings.

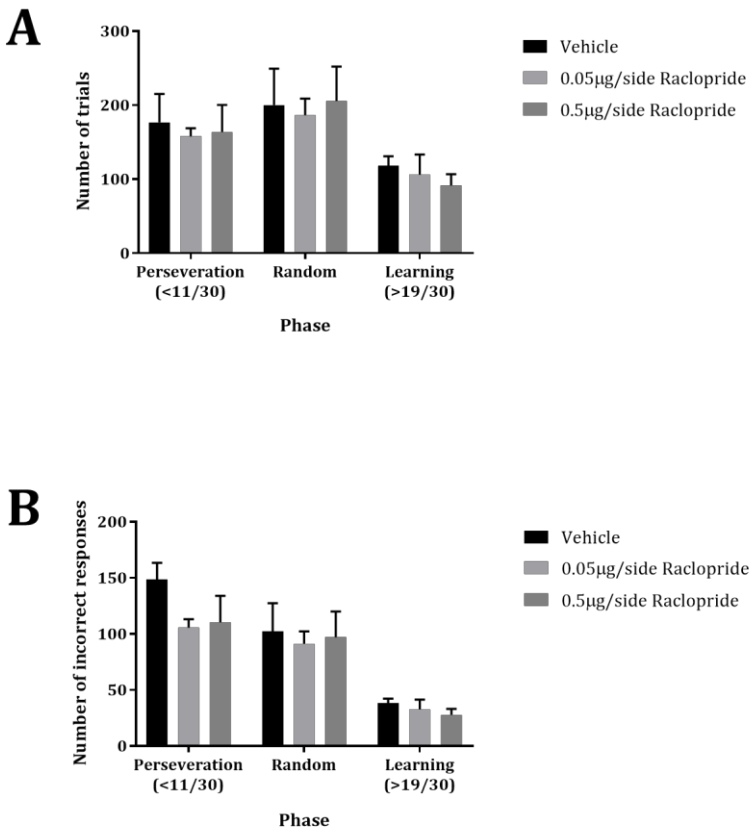


Figure 4.10: **Histograms showing the number of trials (A) and incorrect responses (B) for each phase of the reversal task following local infusion of raclopride into the incorrect aDLS locations. Data are represented as mean ± SEM. No significant effects were found.**

A two-way repeated measures ANOVA in the number of trials showed that there was no significant effect of raclopride infusion or Dose x Phase interaction ($F_{2,10} = 0.03$, $p = 0.97$ and $F_{4,20} = 0.106$, $p = 0.98$, respectively); however there was a significant effect of Phase ($F_{2,10} = 10.84$, $p = 0.003$).

Similarly, a two-way repeated measures ANOVA in the number of incorrect responses showed that there was no significant effect of raclopride infusion or Dose x Phase interaction ($F_{2,10} = 0.13$, $p = 0.88$ and $F_{4,20} = 0.06$, $p = 0.99$, respectively); however there was a significant effect of Phase ($F_{2,10} = 30.51$, $p < 0.001$).

These results were as expected due to the nature of the misplaced cannula locations. Due to the target location being in the lateral region, misplaced cannula were only infusing into one hemisphere and missing the other as the infusion sites were shifted in the medial/lateral plane.

Omissions and latencies were not analysed.

4.3.2.6 Summary of behavioural results

	Trials	**Incorrect responses**	**Omissions**	**Response latency**	**Collection latency**
pDMS	n.s.	n.s. trend to ↑ in random phase	n.s.	n.s.	* selective high dose slowing
aDLS	n.s.	*↑ 0.05µg perseveration phase	n.s.	n.s.	n.s
aDMS	n.s.	**↑ Non-selective dose dependent perseveration phase	n.s.	Trend to Non-selective dose dependent slowing	* selective high dose slowing
NAc	n.s	*↓ Non-selective dose dependent perseveration phase	n.s	n.s.	n.s

Table 4.3: **A summary of results for each brain region following Raclopride infusion when compared to vehicle. n.s. denotes no significant difference at any dose when compared to vehicle controls. Pairwise comparisons are reported where present; non-selective effects of Dose are also reported. Asterisks denote significant effects (*, $p < 0.05$; **, $p < 0.01$) from vehicle controls.**

4.4 Discussion

Dopamine D_2/D_3 receptor antagonism within the striatum led to dissociable effects in a serial visual reversal learning task. Major effects of D_2/D_3 receptor antagonists were confined to the number of incorrect responses of the reversal paradigm, with no effects on the number of trials. D_2/D_3 receptor antagonism in the nucleus accumbens core caused a dose-dependent decrease in perseveration; however, infusions into the anterior DMS led to a dose-dependent increase in perseveration, and 0.05μg was able increase perseverative errors in the anterior DLS. Conversely, a dopamine D_2/D_3 receptor antagonist infused into the posterior DMS tended to increase the number of incorrect responses within the Random phase. These results show a divide between the dorsal and ventral striatum, with impairments following infusion into the dorsal striatum and improvements in the ventral striatum. This finding is consistent with previous studies indicating that dopaminergic function in the dorsal striatum is necessary for reversal learning in non-human primates (Clarke *et al.*, 2011; Groman *et al.*, 2011); conversely, that excess dopaminergic activity in the nucleus accumbens core impairs reversal performance in Parkinson's Disease patients (Cools *et al.*, 2007; Dagher and Robbins, 2009).

The DLS has been implicated in habit formation, through lesions, inactivation and selective lesions of dopamine neurons (Yin, Knowlton and Balleine, 2004, 2006; Faure *et al.*, 2005). 0.05μg raclopride into the anterior DLS led to an increase in the number of perseverative errors compared to controls; increased responding to the previous CS+ (now CS-) following antagonism of the D_2/D_3 receptor indicates an inability to overcome a previously learned stimulus pairing and a habitual response. Low dose raclopride in the anterior DLS caused an increase in the number of perseverative errors, however the high dose produced no significant difference compared to vehicle control. This implies that dopamine signalling may have a biphasic effect in the anterior DLS during reversal learning similar to those found with a dopamine reuptake inhibitor (Barlow *et al.*, 2015), and also locomotor activity (Eilam and Szechtman, 1989). Biphasic effects of dopamine have previously been attributed to presynaptic D_2 receptors responsible for neuronal firing and dopamine release (Aghajanian and Bunney, 1977; De Mei *et al.*, 2009).

Dopamine D_2/D_3 receptor antagonism in the anterior DMS caused a non-selective dose dependent increase in the number of perseverative errors in the touchscreen visual reversal learning task. Previously, lesions of the anterior DMS were found to have no significant effects on acquisition and expression of action-outcome associations, contradicting the findings of this experiment (Yin *et al.*, 2005); however, Castañé, Theobald and Robbins (2010) reported significant impairments in spatial reversal learning following DMS lesions. The dose dependent increase in the number of perseverative errors corresponds to findings of Castañé, Theobald and Robbins, suggesting that lesion impairments in the anterior DMS are facilitated by dopamine signalling and the D_2/D_3 receptor. The contradictory findings between reversal learning and action-outcome associations highlight the importance of the reversal learning as a complex measure of cognitive and behavioural flexibility.

Dopamine D_2/D_3 receptor antagonism in the nucleus accumbens core was able to elicit a non-selective dose dependent reduction in perseverative incorrect responding, suggesting an increase in reward sensitivity and cognitive flexibility. Dopamine D_2 receptors in the nucleus accumbens core have previously been reported to play a role in aversive behaviour, whereas D_1 receptors are responsible for reward learning (Hikida *et al.*, 2013). The nucleus accumbens core has also been reported to promote approach to reward-associated stimuli (Dalton, Phillips and Floresco, 2014). Taken together, D_2/D_3 receptor antagonism could increase reward sensitivity through increased D_1 signalling and output of the direct pathway to promote reward-associated responding and decreasing perseveration.

The DMS has been implicated in the retention of decision making strategies (Ragozzino, 2007), correlating with the trend to increase in incorrect responses during the random phase following dopamine D_2/D_3 receptor antagonism in the posterior DMS in the touchscreen visual reversal learning task. Ragozzino (2007) reported the role of the DMS in executing new strategies following inactivation, however DMS coordinates were more anterior than those in this experiment for the posterior DMS. Ferguson *et al.*, (2013) reported similar strategy retention effects in posterior DMS regions, corresponding with the findings in this experiment. Following transient increases in $G_{i/o}$ signalling in striatonigral posterior DMS neurons, Ferguson *et al.* reported impaired retention of decision making strategies;

this experiment suggests an increase in $G_{i/o}$ signalling in striatonigral produces similar behavioural phenotypes as dopamine D_2/D_3 receptor antagonism on striatopallidal neurons.

Dopamine D_2/D_3 receptor antagonism in both the anterior and posterior DMS led to a slowing of retrieval latency, with 0.5μg/hemisphere producing a significant impairment in both regions. Systemic D_2/D_3 receptor antagonism caused a significant slowing in magazine entry during the reversal learning task (Chapter 3), this impairment can now probably be attributed, at least in part, to manipulations in the DMS. The effect of dopaminergic manipulations to cause magazine entry slowing is likely due to the role of dopamine (along with other monoamines) on the motor cortex and control of motor function (Vitrac and Benoit-Marand, 2017), along with alterations in signalling in the indirect output pathway.

While attempts have been made to attribute effects of drug infusions to their intended targets, this is difficult without further validation on the spread of raclopride in their brain regions. Further investigation of raclopride spread within these target regions could include the use of 3H-raclopride; however, comparing the effects of infusion into the aDLS and the aDMS we see dissociable effects between the two regions, specifically effects on collection latency. The injection sites for the aDLS and aDMS are separated by 1.6mm, therefore we can determine that there is limited spread of raclopride into neighbouring regions in this instance.

Understanding the role of dopamine signalling during reversal learning is difficult to dissect; this is due to phasic and regionally distinct release of dopamine during different phases of the reversal task and the signalling of positive and negative feedback (Klanker *et al.*, 2015, 2017), along with the expression of dopamine D_2 receptors on both pre- and post-synaptic neurons, as well as on striatal GABAergic interneurons (Delle Donne, Sesack and Pickel, 1996; De Mei *et al.*, 2009). Therefore, while attempts have been made to further the understanding of dopamine signalling in the rat striatum during the touchscreen serial visual reversal learning task, the abundance of dopamine D_2 receptors in the striatum suggests that more experiments need to be performed. Nonetheless, this experiment has shown dissociable and opposing effects of dopamine D_2/D_3 receptor manipulations in the

touchscreen visual reversal learning task in the rat. The neurochemical specificity of these findings in the striatum will be investigated in the next Chapter. The striatum receives afferents from the frontal lobe, therefore top-down signalling could influence the role of dopamine in the striatum during reversal learning and this will be investigated in the Chapter 6.

Chapter 5: DOPAMINE D_1 RECEPTOR AND 5-HT_{2C} RECEPTOR ANTAGONISM HAVE NO EFFECT IN STRIATAL SUB-REGIONS

5.1 Introduction

Chapters 3 and 4 reported effects of dopamine D_2/D_3 manipulations on the touchscreen serial reversal paradigm. Intracerebral dopamine D_2/D_3 manipulations have highlighted areas within the striatum that are sensitive to dopaminergic manipulation; in order to investigate the neurochemical specificity of these regions other receptor antagonists need to be utilised.

Chapter 4 reported the effects of dopamine D_2/D_3 antagonism in the pDMS, aDLS, aDMS and NAc, therefore it is important to investigate the role of D_1 receptors in these striatal sub-regions to demonstrate receptor specific results. Lee et al. (2007) showed that systemic raclopride, but not SCH-23390, affected reversal performance with an in-session retention session, suggesting receptor specificity.

Systemic dopamine D_1 receptor antagonism, through SCH-23390, has been shown to significantly decrease the number of trials and increase response latency in a probabilistic reversal learning task in rats (Verharen, Adan and Vanderschuren, 2019). Conversely, SKF82958, a dopamine D_1 receptor agonist, reduced the number of completed trials while decreasing response latency, along with a decrease in learning rate. Infusion of SCH-23390 into the ventral striatum had no significant effects on the number of trials completed or their latencies, while a significant increase in the number of reversals was observed. SKF82958 infusion into the ventral striatum had no significant effects on measures of task performance. Neither SCH-23390 or SKF82958 had any effects following infusion into the DLS or DMS.

Dopamine D_1 receptor antagonism in the nucleus accumbens impaired set-shifting in rats (Haluk and Floresco, 2009), with dopamine D_2 receptor antagonism having no significant effects. Conversely, D_2 receptor agonism through quinpirole also

impaired set-shifting, but D_1 receptor agonism did not. Izquierdo et al. (2006) also reported effects of dopamine D_1 receptor agonism in rodents; systemic SKF81297 significantly impaired performance in the early phase of a touchscreen-based reversal learning task, as well as a spatial working memory task.

As we have seen results from dopamine receptor manipulations, it is important to investigate the neurochemical specificity of these results. The role of 5-HT receptors in reversal learning is poorly understood, however 5-HT_{2A} and 5-HT_{2C} have both been shown to alter spatial reversal learning (Boulougouris, Glennon and Robbins, 2008). Boulougouris et al showed that systemic administration of M100907, a 5-HT_{2A} antagonist, significantly impaired spatial reversal learning by increasing the number of errors and the number trials to criterion; conversely, they also showed that systemic administration of SB-242,084, a 5-HT_{2C} antagonist, significantly improved performance in spatial reversal learning, by decreasing both the number of errors and the number of trials to criterion. Taken together these data suggest that 5-HT_{2A} and 5-HT_{2C} receptors have distinct roles in cognitive flexibility.

Tucci et al. (2013) reported that *meta*-chlorophenylpiperazine (mCPP), a 5-HT agonist, attenuates quinpirole-induced checking in rats. Rats were co-injected with quinpirole and mCPP or quinpirole alone, administration of mCPP significantly reduced the vigour of checking and increased rest after a bout of checking compared to vehicle controls. In 2015, Tucci et al. further investigated the mechanism of action of mCPP attentuation of quinpirole-induced checking. Ritanserin, a selective 5-$HT2_{A/C}$ recepter antagonist, did not inhibit mCPP attentuation of checking behaviours, suggecting that another receptor subtype is responsible for mCPP mediated activity.

Dopamine, not 5-HT, has been shown to regulate reversal learning in the marmoset caudate nucleus, homologous to the medial striatum (Clarke et al. 2011). Using 5,7-dihydroxytryptamine (5,7-DHT) or 6-hydroxydopamine (6-OHDA) to selectively lesion 5-HT or dopamine innervations respectively, Clarke et al showed that dopamine depleted rats were significantly impaired on a serial reversal task, with 5-HT depletion producing no significant effect.

Boulougouris and Robbins (2010) reported that 5-HT_{2C} receptor antagonism induced improvements of spatial reversal learning were specifically mediated by the orbitofrontal cortex (OFC). Intracerebral infusions of SB-242,084 and M100907 into the OFC, medial prefrontal cortex and nucleus accumbens core produced dissociable effects on reversal learning by location and receptor subtype. 5-HT_{2A} antagonism produced no significant effect in any location, while 5-HT_{2C} mediated improvement was elicited by the OFC.

Improvement in reversal performance has been replicated by Alsiö et al. (2015) in the current touchscreen serial reversal paradigm.

Following the studies of by Clarke and Boulougouris, I predicted that 5-HT_{2C} antagonism, in either the DMS or nucleus accumbens core, would produce no significant differences from controls.

5.2 Methods

5.2.1 Subjects

Thirty male Lister-Hooded rats (Charles River, UK), split across four cohorts, were trained on a touchscreen discrimination and serial reversal learning task (see Chapter 2 for details). Table 5.1 presents the number of animals allocated to each experiment, the number of rats excluded from each experiment, and the final number of rats in each group.

Exp.	Drug	Number of rats	Number of rats excluded	Final *N*
1	SCH-23390 – anterior DMS	8	2 - sickness	6
2	SCH-23390 – nucleus accumbens core	8	1 – dysfunctional 1 - sickness 1 - cannula misplacement	5
3	SB-242,084 – anterior DMS	6		6
4	SB-242,084 – nucleus accumbens core	8		8
	Total	**30**	**Total animals used for analysis**	**25**

Table 5.1: **Summary of the cohorts of animals used in each experiment.**

5.2.2 Behavioural procedure

Rats were trained on the touchscreen serial reversal task as previously described in Chapter 2.

Serial Reversal Learning Task: The serial reversal learning task is described in detail in Chapter 2.

5.2.3 Surgical cannulation

Rats underwent surgical cannulation as previously described in Chapter 2. Briefly, rats were anaesthetized and secured in a stereotaxic frame with atraumatic earbars. PlasticsOne cannulae (22-GA) were inserted in the aDMS/NAc (AP +1.2, ML ±1.9, DV -1.9) and secured with screws and dental cement. All surgical co-ordinates were calculated using a stereotaxic atlas (Paxinos and Watson, 2006) using bregma as the origin. All dorsoventral measurements were taken from dura.

5.2.4 Drugs

SCH-23390 (Sigma-Aldrich, UK) was dissolved in physiological saline to a concentration 0.2 μg/μl (final infusion concentration 0.1 μg/side). Solutions were stored as aliquots at -20°C.

SB-242,084 (Eli Lilly, IN, USA) was dissolved in PEG400 (Fisher Scientific, UK) at 20% of the final volume, this was then made up by 10% (w/v) hydroxypropyl-beta-cyclodextrin (Sigma-Aldrich, UK) in physiological saline, to concentrations of 2.0 and 6.0 μg/μl (final infusion concentration 1.0 and 3.0 μg/side). Solutions were stored as aliquots at -80°C.

Serial Reversal Task: Prior to drug administration, animals were counterbalanced across the drug doses, matched for their performance during the final training stages of the serial visual reversal learning task. Each animal received intracranial infusions of vehicle and SCH-23390 or SB-242,084 on separate reversals until criterion was reached.

5.2.5 Microinfusions

After recovering from surgery (≥7 days), animals received a baseline reversal to reintroduce the animals to the task. During this baseline reversal, animals were assessed to determine suitability for testing, including errors to criterion, stable performance, and ensuring no presence of a side bias. During this reversal, animals

were habituated to the infusion procedure, receiving mock and vehicle infusions during the latter stages of the reversal. Injectors from PlasticsOne (28-GA) were extended 2.5 mm below the guide for aDMS, or 5.0 mm below the guide for NAc infusions. Infusions were performed at a rate of 0.5 µl over 2 minutes. The injector was left in place for 1 minute before and after the infusion. During the infusion procedure, animals were allowed to freely move on the lap of the experimenter or gently restrained. Following the infusion animals were returned to their home cage and placed in the experimental chamber 8 minutes after the start of the infusion procedure.

5.2.6 Statistical analysis

The main measures of the animals' ability to learn the reversals and visual discrimination were: (i) the number of trials to criterion and (ii) the number of incorrect responses to criterion (errors of commission). Additional secondary measures recorded for each trial were (iii) the number of omissions (errors of omission), (iv) the latency to respond to the stimuli, (v) the latency to collect the reward.

Data for each primary variable were analysed using a repeated measures mixed model ANOVA consisting of one between-subject factor (Region) and two within-subject factors (Dose and Phase). When significant interactions were found, further analysis was performed of Simple Main Effects or Simple Main Interactions, as appropriate. Analysis was followed by post-hoc Sidak's corrected pair-wise comparisons to vehicle.

Omission data were analysed using Friedman's test on each separate phase of the reversal paradigm. When significances were found, Wilcoxon Signed Rank tests were performed within each experimental phase comparing to Vehicle.

For all comparisons, significant difference was assumed at $p < 0.05$.

5.3 Results

5.3.1 Histological results

Figure 5.1 shows a schematic reconstruction of the position of injector tips in the aDMS, and NAc. Animals were excluded from data analysis if the cannula position was not correct. Positions were correct as per the defined regions by Paxinos and Watson (2006), or excluded from further analysis (N=1).

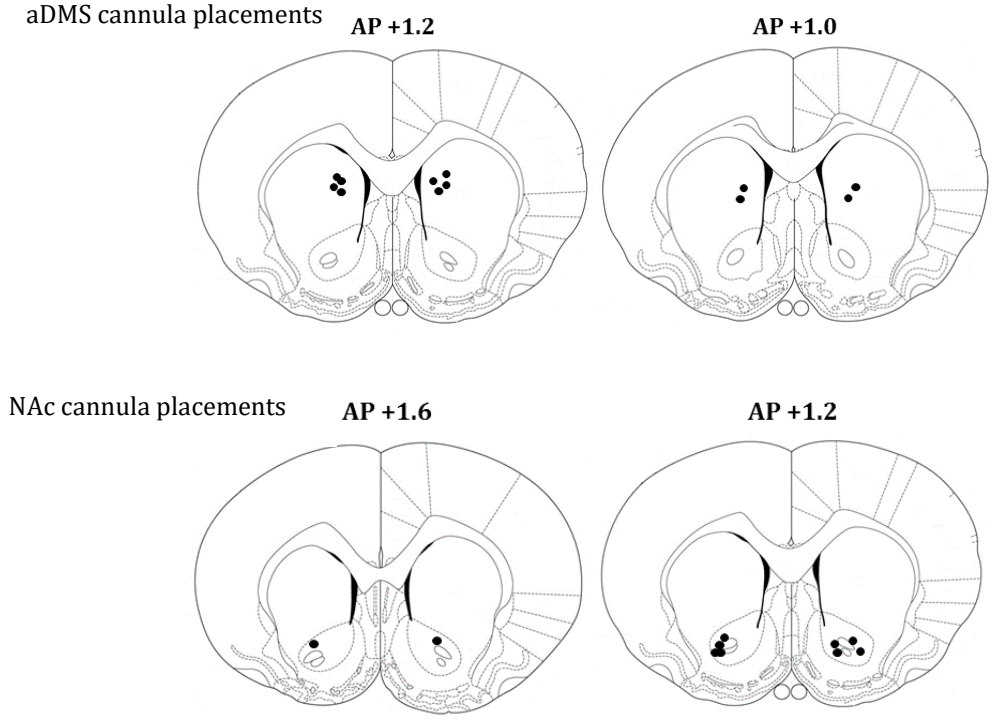
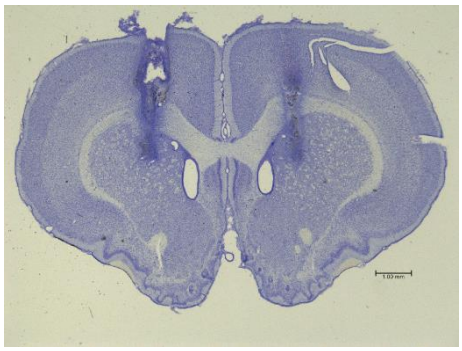


Figure 5.1: **Schematic diagrams showing the injection sites in the aDMS and NAc. Reconstructions from Paxinos and Watson (2006).**

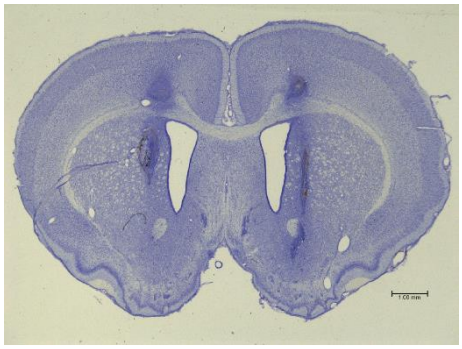
Figure 5.2 shows photomicrographs of coronal sections taken from representative rats to show cannula and injector placement. Following staining with cresyl violet, infusion locations were analysed and recorded.

A – aDMS



AP +1.6

B - NAc



AP +1.2

Figure 5.2: **Photomicrographs of coronal sections taken from representative rats: (A) aDMS and (B) NAc.**

5.3.2 Behavioural results

5.3.2.1 Experiment 1: The effects of local dopamine D_1 receptor antagonist, SCH-23390, in the anterior DMS and Nucleus Accumbens core

5.3.2.1.1 Trials

A three-way repeated measures mixed design ANOVA revealed that there was a significant main effect of Phase ($F_{2,18}$ = 5.105, p = 0.018); however, there were no significant main effects of Dose or Location ($F_{1,9}$ = 0.130, p = 0.727, and $F_{1,9}$ = 1.300, p = 0.294, respectively, Figure 5.3A and 5.3C).

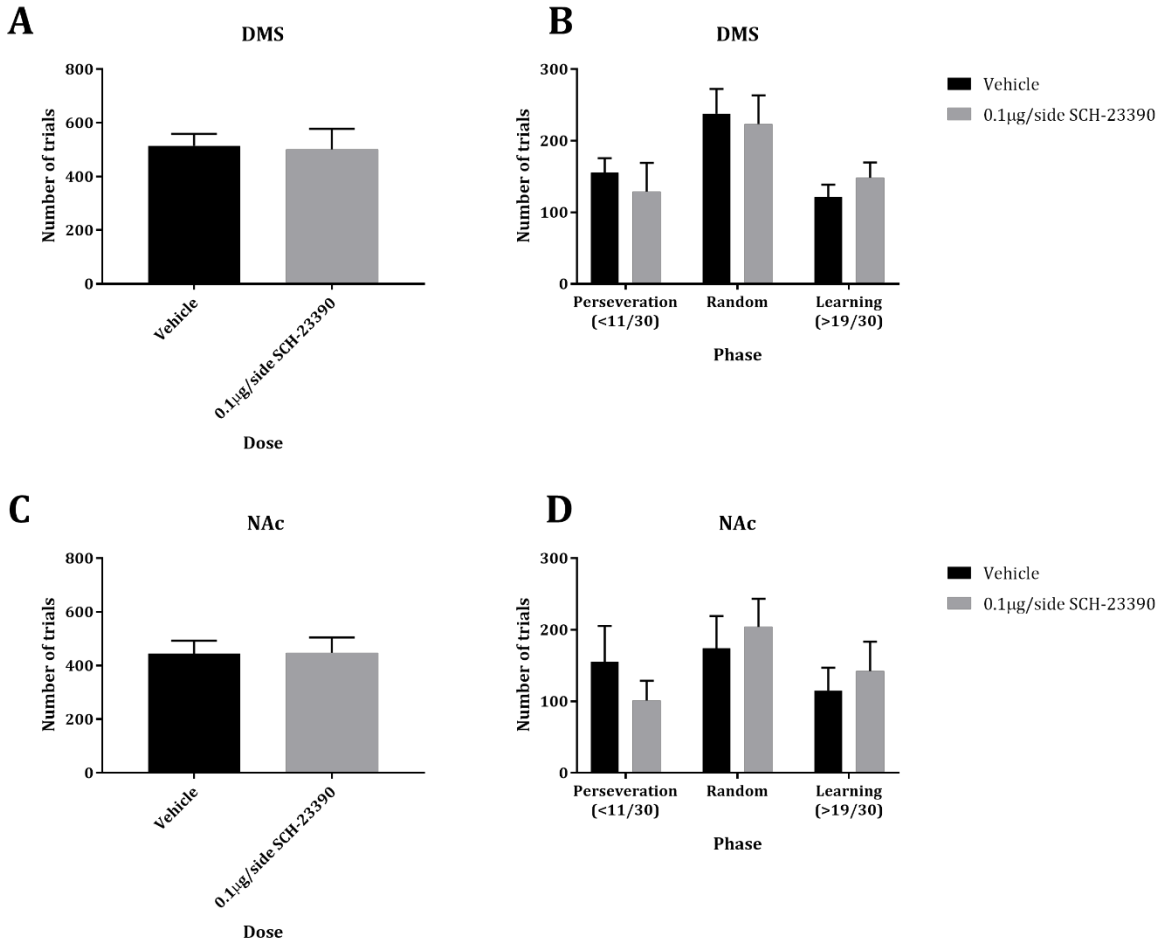


Figure 5.3: **Histograms showing the total number of trials to criterion following infusion of 0.1µg SCH-23390 per hemisphere into the DMS (A), and Nucleus accumbens core (C), and the number of trials per phase (B and D). No significant differences were found. Data are represented as mean values ± SEM.**

There were also no significant two-way interactions of Phase x Location ($F_{2,18}$ = 0.082, p = 0.922), Dose x Phase ($F_{2,18}$ = 1.351, p = 0.284), or Dose x Location ($F_{1,9}$ = 0.08, p = 0.784).

The three-way ANOVA showed that there was no significant three-way interaction of Dose x Phase x Location ($F_{2,18}$ = 0.131, p = 0.878, Figure 5.3B and 5.3D).

5.3.2.1.2 Incorrect responses

A three-way repeated measures mixed design ANOVA revealed that there was a significant main effect of Phase ($F_{2,18}$ = 9.114, p = 0.002); however, there were no significant main effects of Dose or Location ($F_{1,9}$ = 0.336, p = 0.576, and $F_{1,9}$ = 1.293, p = 0.295, respectively, Figure 5.4A and 5.4C).

There were also no significant two-way interactions of Phase x Location ($F_{2,18}$ = 0.064, p = 0.938), Dose x Phase ($F_{2,18}$ = 1.963, p = 0.169), or Dose x Location ($F_{1,9}$ = 0.08, p = 0.784).

The three-way ANOVA showed that there was no significant three-way interaction of Dose x Phase x Location ($F_{2,18}$ = 0.158, p = 0.855, Figure 5.3B and 5.3D).

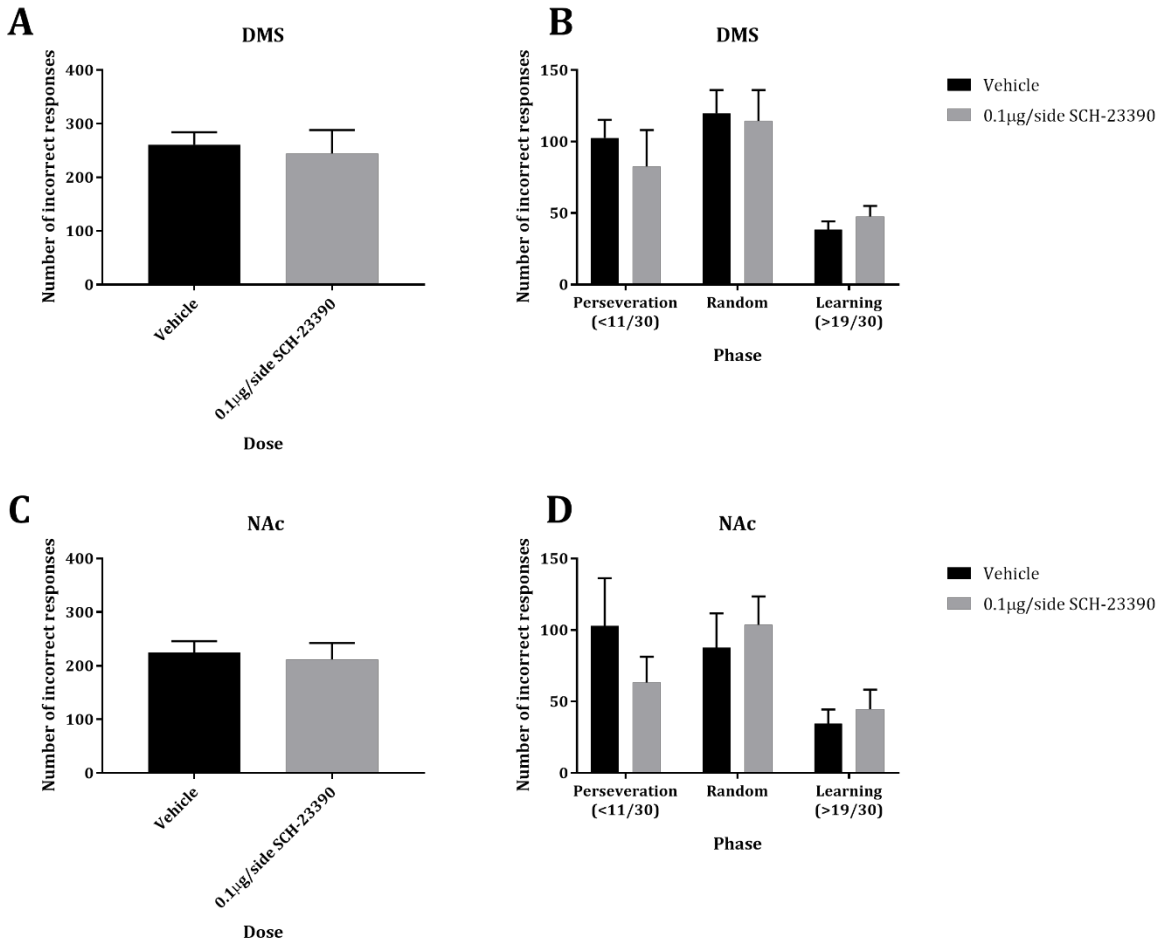


***Figure 5.4*: Histograms showing the total number of incorrect responses to criterion following infusion of 0.1µg SCH-23390 per hemisphere into the DMS (A), and Nucleus accumbens core (C), and the number of incorrect responses per phase (B and D). No significant differences were found. Data are represented as mean values ± SEM.**

5.3.2.1.3 Omissions

Figure 5.4 shows the number of omissions following infusion of SCH-23390 into the anterior DMS (Figure 5.4A) and the nucleus accumbens core (Figure 5.4B).

Wilcoxon signed-ranks tests showed that infusion of SCH-23390 into the DMS did not elicit a significant change in the number of omissions compared to vehicle controls in the perseveration, random, or learning phase of the serial reversal task (Z = 0.0, p = 1.0; Z = -0.447, p = 0.665; and Z = -1.00, p = 0.317, respectively).

Infusion of SCH-23390 into the nucleus accumbens core did not produce any significant effect on the number of omissions compared to vehicle controls on any phase of the reversal task (Perseveration: Z = -0.816, p = 0.414; Random: Z = -0.535, p = 0.593; and Learning: Z = -0.447, p = 0.655).

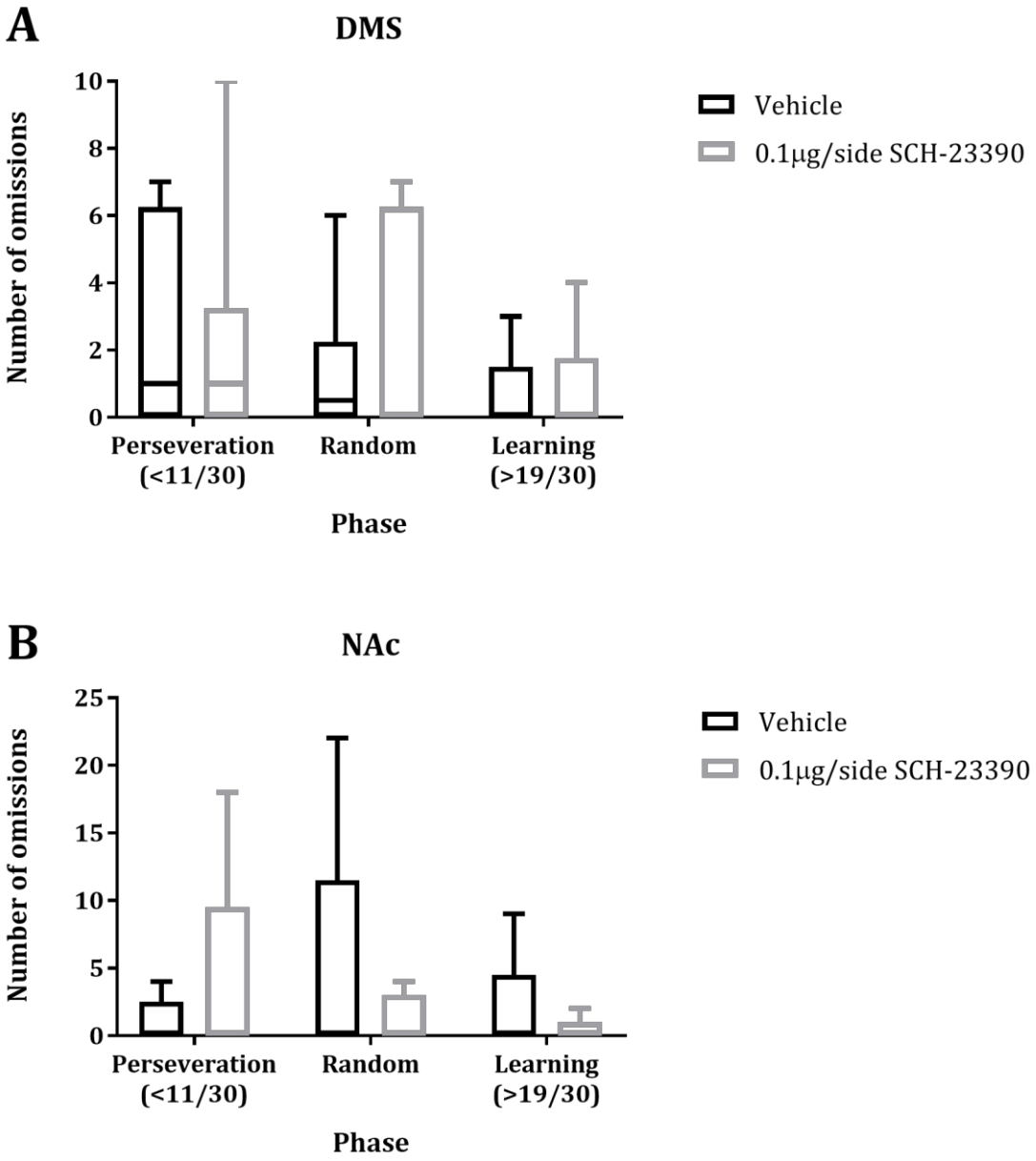


Figure 5.4: **Box and whisker plots showing the number of omissions for phase of the reversal task following local infusion of SCH-23390 into the (A) DMS or (B) NAc. Data are represented as median values ± minimum and maximum. No significant effects were found.**

5.3.2.1.4 Latencies

Table 5.2 shows the response and retrieval latencies following SCH-23390 infusion into the anterior DMS. Paired t-tests revealed that SCH-23390 did not elicit a significant effect on either response or retrieval latency (*t(5)* = 0.158, *p* = 0.881, and *t(5)* = -2.544, *p* = 0.052 respectively).

Region	Dose	Response	Retrieval
DMS	Vehicle	1.174 ± 0.102	1.613 ± 0.445
	0.1µg/side SCH-23390	1.165 ± 0.119	2.037 ± 0.432
NAc	Vehicle	1.212 ± 0.198	1.107 ± 0.108
	0.1µg/side SCH-23390	1.414 ± 0.169	1.716 ± 0.440

Table 5.2: **A table showing the response and reward retrieval latencies following infusion of SCH-23390 into the DMS or NAc. No significant differences were found. Data are represented as the mean values ± SEM (seconds).**

Table 5.2 shows the average response and retrieval latencies following D1 receptor antagonism in the NAc. Infusion of SCH-23390 did not elicit a significant effect on response or retrieval latency (*t(4)* = -2.243, *p* = 0.088, and *t(4)* = -1.426, *p* = 0.227, respectively).

5.3.2.2 Experiment 2: The effects of local 5HT$_{2C}$ receptor antagonist, SB-242,084, in the anterior DMS and Nucleus Accumbens core

5.3.2.2.1 Trials

A three-way repeated measures ANOVA revealed that there was a significant main effect of Phase ($F_{2,24}$ = 10.393, *p* = 0.001); however, there were no significant main effects of Dose or Location ($F_{2, 24}$ = 1.523, *p* = 0.238 and $F_{1,12}$ = 0.075, *p* = 0.789, respectively, Figure 5.5A and 5.5C).

There were also no significant two-way interactions of Dose x Location ($F_{2,24}$ = 0.827, p = 0.449), Phase x Location ($F_{2,24}$ = 0.751, p = 0.483), or Dose x Phase ($F_{4,48}$= 1.284, p = 0.290).

The three-way ANOVA showed that there was no significant three-way interaction of Dose x Phase x Location ($F_{4,48}$ = 0.596, p = 0.667, Figure 5.5B and 5.5D).

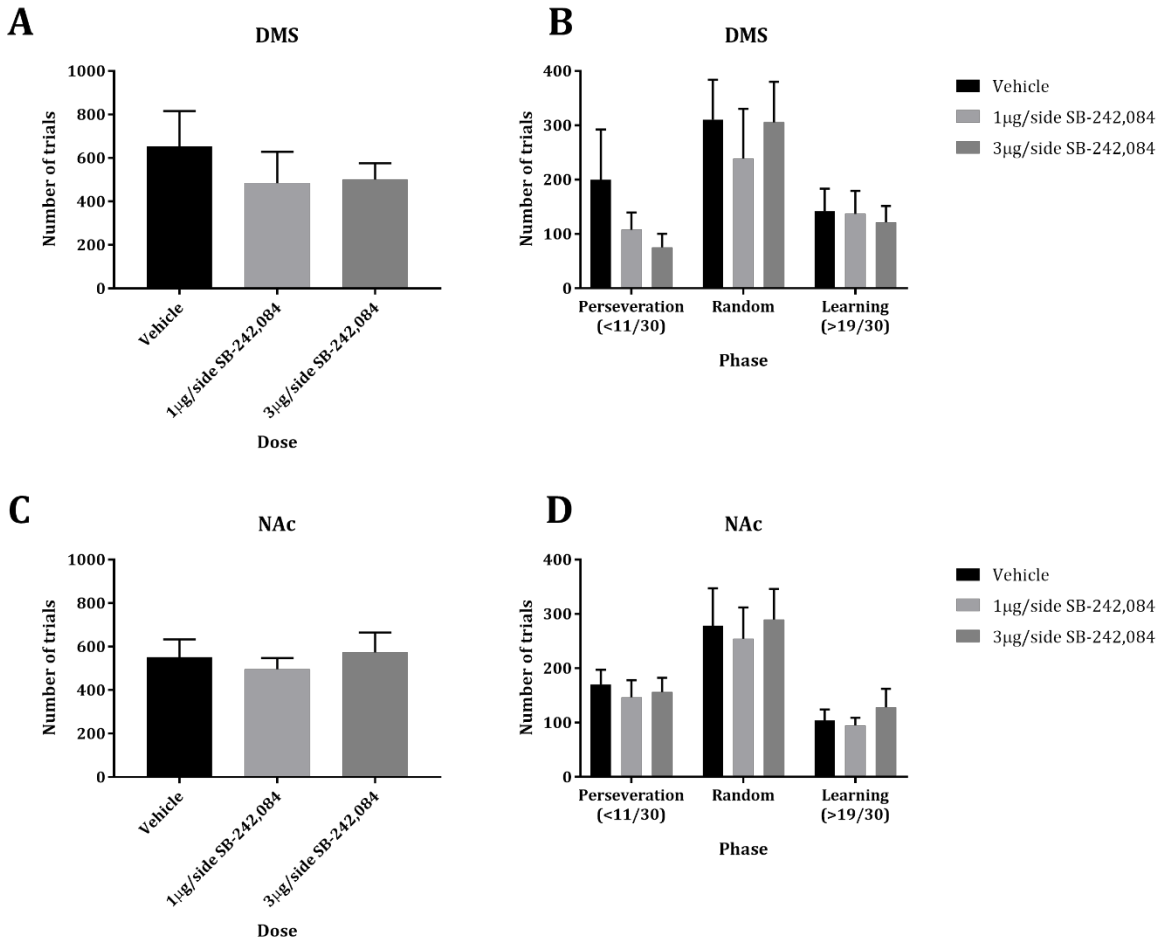


Figure 5.5: **Histograms showing the total number of trials to criterion following infusion of 1µg and 3 µg SB-242,084 per hemisphere into the DMS (A), and Nucleus accumbens core (C), and the number of trials per phase (B and D). No significant differences were found. Data are represented as mean values ± SEM.**

5.3.2.2.2 Incorrect responses

A three-way repeated measures mixed design ANOVA revealed that there was a significant main effect of Phase ($F_{2,24}$ = 14.939, p < 0.001); however, there were no significant main effects of Dose or Location ($F_{2,24}$ = 1.881, p = 0.174, and $F_{1,12}$= 0.125, p = 0.730, respectively, Figure 5.6A and 5.6C).

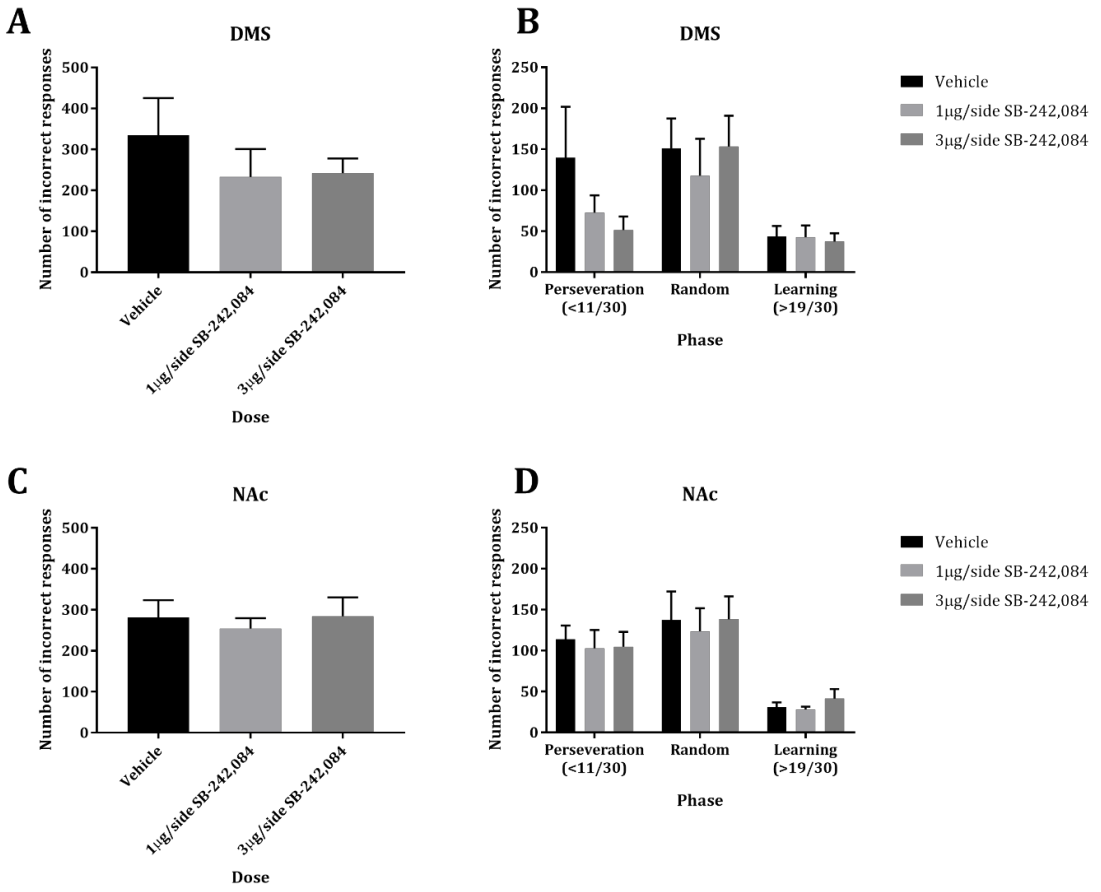


Figure 5.6: **Histograms showing the total number of incorrect responses to criterion following infusion of 1µg and 3 µg SB-242,084 per hemisphere into the DMS (A), and Nucleus accumbens core (C), and the number of incorrect responses per phase (B and D). No significant differences were found. Data are represented as mean values ± SEM.**

There were also no significant two-way interactions of Dose x Location ($F_{2,24}$ = 1.023, p = 0.375), Phase x Location ($F_{2,24}$ = 0.779, p = 0.470), or Dose x Phase ($F_{4,48}$= 1.691, p = 0.168).

The three-way ANOVA showed that there was no significant three-way interaction of Dose x Phase x Location ($F_{4,48} = 0.795$, $p = 0.534$, Figure 5.6B and 5.6D).

5.3.2.2.3 Omissions

Friedman's test, performed within each Phase and each Location, revealed that local infusion of SB-242,084 had no significant effect on the number of omissions (Figure 5.7).

Figure 5.7A shows the number of omissions following infusion of SB-242,084 into the posterior DMS. Friedman's test revealed there was no significant effect of SB-242,084 in the perseveration ($\chi^2(2) = 5.2$, $p = 0.074$), random ($\chi^2(2) = 1.33$, $p = 0.513$), or learning phase ($\chi^2(2) = 0.133$, $p = 0.936$).

Figure 5.7B shows the number of omissions following infusion of SB-242,084 into the nucleus accumbens core. Friedman's test revealed there was no significant effect of SB-242,084 in the perseveration ($\chi^2(2) = 0.125$, $p = 0.939$), random ($\chi^2(2) = 0.333$, $p = 0.846$), or learning phase ($\chi^2(2) = 2.00$, $p = 0.368$).

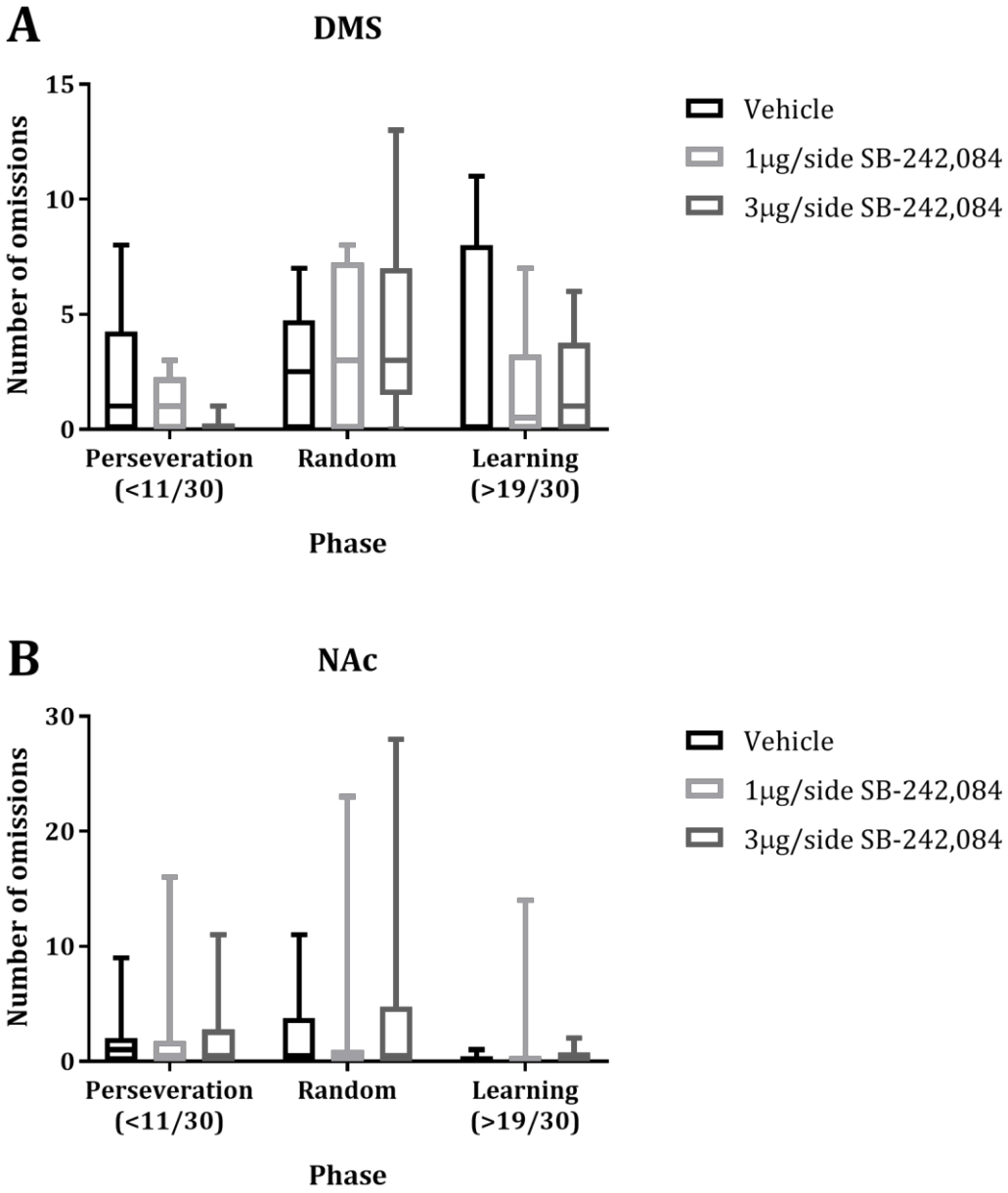


Figure 5.7: **Box and whisker plots showing the number of omissions for phase of the reversal task following local infusion of SB-242,084 into the (A) DMS or (B) NAc. Data are represented as median values ± minimum and maximum. No significant effects were found.**

5.3.2.2.4 Latencies

Table 5.3 shows the response and retrieval latencies following infusion of SB-242,084 into the anterior DMS. One-way repeated measures ANOVAs showed that

there was no significant effect of dose on either response or retrieval latency ($F_{2,10}$ = 0.3678, p = 0.063, and $F_{2,10}$ = 2.312, p = 0.150, respectively).

Region	Dose	Response	Retrieval
DMS	Vehicle	1.258 ± 0.119	1.293 ± 0.169
	1µg/side SB-242,084	1.363 ± 0.129	1.339 ± 0.203
	3µg/side SB-242,084	1.121 ± 0.092	1.128 ± 0.162
NAc	Vehicle	1.038 ± 0.140	1.224 ± 0.218
	1µg/side SB-242,084	1.053 ± 0.141	1.131 ± 0.180
	3µg/side SB-242,084	1.143 ± 0.194	1.176 ± 0.194

Table 5.3: **A table showing the response and reward retrieval latencies following infusion of SB-242,084 into the DMS or NAc. No significant differences were found. Data are represented as the mean values ± SEM (seconds).**

One-way ANOVAs revealed that infusion of SB-242,084 into the nucleus accumbens core did not produce a significant effect of dose on either response or retrieval latency ($F_{2,14}$ = 0.667, p = 0.529, and $F_{2,14}$ = 0.080, p = 0.923, respectively, Table 5.3).

5.3.2.3 Summary of behavioural results

	Trials		**Incorrect responses**		**Omissions**		**Response latency**		**Retrieval latency**	
	SCH	SB	SCH	SB	SCH	SB	SCH	SB	SCH	SB
DMS	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NAc	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 5.4: **A summary of results for each brain region following infusions of SCH-23390 (SCH) or SB-242,084 (SB) when compared to vehicle. n.s. denotes no significant difference at any dose when compared to vehicle controls.**

5.4 Discussion

Dopamine D_1 receptor and 5-HT_{2C} receptor antagonism in the posterior dorsomedial striatum and nucleus accumbens core had no significant effects on the touchscreen serial visual reversal task at the concentrations used in these experiments.

Dopamine manipulations in the posterior striatum have been shown to affect reversal learning (see Chapter 4); however, D_1 receptor antagonism has had no significant effects in the regions investigated here.

Haluk and Floresco (2009) reported an increase in trials to criterion and the number of errors in a set-shifting tasking following infusion of SCH-23390 into the nucleus accumbens; however, they saw no effects following infusion of eticlopride. These results do not compare with those found in these studies. The effective concentrations of SCH-23390 used by Haluk and Floresco were higher than those used in this experiment, 1.0 μg compared to 0.1 μg. They also reported no significant effect when using 0.1 μg SCH-23390. Haluk and Floresco also reported no effects following D_2 receptor antagonism using eticlopride; this contradicts the findings of Chapter 4 where raclopride produced a reduction in perseveration and improved performance on this task. Taken together these differences in results could be due to differences in the task; however, it is also possible that the effective concentration of SCH-23390 was not reached in this experiment. Indeed, further work has since been performed, finding that 1.0 μg SCH-23390 produces no significant effects following infusion into the nucleus accumbens core; however, infusion into the nucleus accumbens shell has been found to produce a significant reduction in perseveration and errors to criterion (Sala-Bayo et al., submitted).

5-HT manipulations in the OFC have been shown to affect reversal learning performance in both the marmoset and rat (Clarke *et al.*, 2004; Alsiö *et al.*, 2015). Clarke et al showed that 5-HT depletion in the frontal cortex, while dopamine depletion in the caudate nucleus, affects serial reversal learning performance in marmosets. Alsio et al reported the effects of SB-242,084 in the lateral OFC on this task, showing that 5-HT_{2C} antagonism reduced early errors but increased late errors.

5-HT_{2A} receptor antagonism, with M100907, in the dorsomedial striatum has been reported to alleviate reversal learning impairments in the BTBR mouse model of autism, along with attenuating abnormal grooming behaviours. Conversely, infusions into the orbitofrontal cortex increased perseveration and potentiated grooming (Amodeo *et al.*, 2017). This would suggest that while 5-HT may play a role in the striatum during reversal learning, it is activing through the 5-HT_{2A} receptor subtype, not 5-HT_{2C}.

Whilst dopamine D_1 receptor antagonism in the DMS or nucleus accumbens core has previously been shown to have no effect on reversal learning, and reproduced in this study, there were several limitations to both of these studies. First, the SCH-23390 dose was not ideal based on work by Haluk and Floresco (2009) and SB-242,084 concentrations were those used by Alsio et al. (2015) in the frontal lobe and therefore cannot be guaranteed to be effective in the striatum. Additionally; histology was unable to be performed on the rats receiving 5-HT_{2C} manipulations and therefore we do not know if these infusions were into the correct location. Overall the numbers of animals used were quite low, especially following exclusion in the dopamine D_1 receptor antagonist experiment, and therefore these results cannot be presumed to be reliable and should be interpreted as more of a pilot experiment with further investigation being necessary.

Chapter 6: Dissociable and Opposing Effects Of Medial Prefrontal and Orbitofrontal Cortex Inactivation On Reversal Learning

6.1 Introduction

Chapter 4 reported effects of striatal manipulations on the touchscreen reversal learning paradigm. The striatum receives afferents from the frontal cortex and so it can be hypothesised that this projection will also be involved in reversal learning. As discussed in Chapter 1, the frontal cortex is involved in executive function which can include control of behaviour during reversal learning.

The rat brain has been shown to have homologous circuits with the brains of both humans (Balleine and O'Doherty, 2010) and non-human primates (Heilbronner *et al.*, 2016), in terms of anatomical connectivity and the functional substrates of goal and habit based actions by corticostriatal systems (Haber and Knutson, 2010).

Humans and non-human primates have been shown to have the same organisational principles in projections from the ventral prefrontal cortex (vPFC) (Jbabdi *et al.*, 2013). By comparing tracing measurements in the macaque to MRI tractography in both macaques and humans, Jbabdi et al showed that the principles of vPFC projection organization were preserved between macaques and humans. Lehman et al (2011) showed through tracing studies that vPFC projection trajectories are largely governed by organisational rules, with regional differences in projections from different vPFC areas. The medial/lateral vPFC axis dictates both the route taken by efferent projections, and their position within a precise tract. Medial vPFC region projects more ventrally than the more lateral regions.

The rat striatum receives afferents from the prefrontal cortex, which can be mapped onto different regions (Voorn *et al.*, 2004), with similar circuit based homologies to primates potentially enabling translation of findings between rodent models, non-

human primates, and human pathology (Heilbronner *et al.*, 2016). Rat striatal neurons have activity that is correlated with that of the medial frontal cortex, with disruption of striatal activity following medial frontal cortex inactivation (Emmons *et al.*, 2017).

Previous work has shown that the medial and lateral OFC can have opposing functions. Using lesions, Fuchs et al showed that mOFC lesioned rats exhibited attenuated cocaine-primed reinstatement, whereas lateral OFC lesioned rats exhibited a more perseverative cocaine-primed reinstatement (Fuchs *et al.*, 2004). Using inactivations, Fuchs also showed that lateral OFC inactivation impaired cue-induced reinstatement, while other inactivations failed to alter behaviour (Fuchs *et al.*, 2004). Using lesions, Mar et al showed that medial OFC lesioned rats exhibited a significant increase in delay-discounting, while lateral OFC lesioning lead to a decrease in delay-discounting (Mar *et al.*, 2011). More recently, optogenetics have been used to highlight the opposing functions of the medial and lateral OFC. Repeated overstimulation of the ventromedial OFC lead to a significant increase in grooming in the mouse (Ahmari *et al.*, 2013), this effect was able to be reversed by fluoxetine treatment. Whereas, stimulation of the lateral OFC was able to alleviate excessive grooming and return normal grooming behaviours in a *Sapap3* knockout mouse model of OCD (Burguière *et al.*, 2013); stimulation of the lateral OFC was able to elicit a behavioural response, overcoming the genetic predisposition of grooming.

In humans, the lateral frontopolar prefrontal cortex has been shown to play a role in exploration through several fMRI and EEG studies (Daw *et al.*, 2006; Yoshida and Ishii, 2006; Badre *et al.*, 2012; Cavanagh *et al.*, 2012). However, the dorsomedial prefrontal cortex (dmPFC) has been shown to monitor the ability to switch from exploitation to exploration behaviours (Kolling *et al.*, 2012; Donoso, Collins and Koechlin, 2014), with neurons in the dmPFC exhibiting coding activity as animals switch from exploitation to exploration (Durstewitz *et al.*, 2010; Hayden, Pearson and Platt, 2011; Karlsson, Tervo and Karpova, 2012). The balance between the medial and lateral frontal cortex in explore vs exploit behaviours may allow us to understand the motivational responses observed in this touchscreen serial reversal paradigm. Inactivation of the medial OFC region could lead to an increase in exploration facilitated by the lateral OFC; by inactivating the exploitation of the

previously learned reward, the rat is able to switch to the now correct stimuli and produce significantly less incorrect responses. Conversely, inactivation of the lateral OFC leads to a loss of exploration and thus a significant increase in the number of incorrect responses through uncontrolled exploitation of the previously correct stimuli. The balance between explore and exploit is vital for normal behaviours, for the understanding of reliable reward and the ability to switch under uncertainty (Domenech and Koechlin, 2015).

The aim of the experiments in this chapter was to gain understanding of dissociable functions of the different regions of the orbitofrontal cortex in a touchscreen serial reversal paradigm. Previous works using touchscreen visual reversal paradigms have been shown to be sensitive to OFC lesions (Bussey, Everitt and Robbins, 1997; Chudasama and Robbins, 2003; Graybeal *et al.*, 2011). This chapter aims to further this research by using intracranial infusions of baclofen/muscimol to temporarily inactivate the region of interest; the benefit of using the intracranial infusion approach is that it allows for a within subject design, while the serial reversal paradigm allows for a quick testing protocol.

I hypothesise that intracranial infusions of baclofen/muscimol, leading to region activation, will highlight opposing functions between the medial and lateral OFC given apparent dissociations in humans with OCD (Menzies *et al.*, 2008; Milad and Rauch, 2012; Fettes, Schulze and Downar, 2017), rodent optogenetics (Ahmari *et al.*, 2013; Burguière *et al.*, 2014), and lesions and inactivations (Mar *et al.*, 2011; Alsiö *et al.*, 2015). Previous work has shown that rats are impaired on this task following lateral OFC inactivation (Alsiö *et al.*, 2015), therefore I hypothesise that medial OFC inactivation will improve performance on the touchscreen serial visual reversal paradigm.

6.2 Methods

6.2.1 Subjects

Fifty-four male Lister-Hooded rats (Charles River, UK), split across four cohorts, were trained on a touchscreen discrimination and serial reversal learning task (see Chapter 2 for details). Table 6.1 presents the number of animals allocated to each experiment, the number of rats excluded from each experiment, and the final number of rats in each group.

Exp.	Drug	Number of rats	Number of rats excluded	Final *N*
1	Baclofen/Muscimol - Prelimbic	14	1 - dysfunctional 1 - sickness 1 - died in surgery	11
2	Baclofen/Muscimol - Infralimbic	11	1 - dysfunctional 1 - unable to undergo surgery 1 - cannula misplacement	8
3	Baclofen/Muscimol - lOFC	13	1 – dysfunctional	12
4	Baclofen/Muscimol - mOFC	16	2 – cannula misplacement	14
	Total	**54**	**Total animals used for analysis**	**45**

Table 6.1: **Summary of the cohorts of animals used in each experiment.**

Of the excluded animals, three were cannulated into incorrect coordinates, one died during surgery, one was unable to be anaesthetised using either isoflurane or ketamine and therefore could not undergo surgery, one lost its cannula during the testing period, and one animal started experiencing seizures. The three animals classed as dysfunctional were unable to be included for various reasons, including failure to relearn the task following surgery, increased aggression and inability to be handled, or an injector snapping in the cannula during an infusion.

6.2.2 Behavioural procedure

Rats were trained on the touchscreen serial reversal task as previously described in Chapter 2.

Serial Reversal Learning Task: The serial reversal learning task is described in detail in Chapter 2.

6.2.3 Surgical cannulation

Rats underwent surgical cannulation as previously described in Chapter 2. Briefly, rats were anaesthetized and secured in a stereotaxic frame with atraumatic earbars. PlasticsOne cannulae (21-GA) were inserted in the PrL or IL (AP +2.7, ML ±0.75, DV -1.0), lOFC (AP +3.7, ML ±2.5, DV -1.7), and mOFC (AP +4.2, ML ±0.6, DV -1.4) and secured with screws and dental cement. All surgical co-ordinates were calculated using a stereotaxic atlas (Paxinos and Watson, 2006) using bregma as the origin. All dorsoventral measurements were taken from dura.

6.2.4 Drugs

Baclofen hydrochloride and muscimol hydrobromide (Sigma-Aldrich) were prepared separately by dissolving in saline, the two separate solutions were then combined to form a cocktail with each drug at the final concentration of 1.0 mM (Zeeb, Floresco and Winstanley, 2010; Alsiö *et al.*, 2015).

Serial Reversal Task: Prior to drug administration, animals were counterbalanced across the drug doses, matched for their performance during the final training stages of the serial visual reversal learning task. Each animal received intracranial infusions of vehicle and the baclofen/muscimol cocktail on separate reversals until criterion was reached.

6.2.5 Microinfusions

After recovering from surgery (≥7 days), animals received a baseline reversal to reintroduce the animals to the task. During this baseline reversal, animals were assessed to determine suitability for testing, including incorrect responses to criterion, stable performance, and ensuring no presence of a side bias. During this reversal, animals were habituated to the infusion procedure, receiving mock and vehicle infusions during the latter stages of the reversal. Injectors from PlasticsOne (28-GA) were extended 2.5 mm below the guide for PrL 3.5 mm below the guide for IL, or 2 mm below the guide for lOFC and mOFC infusions. Infusions were performed at a rate of 0.5 µl over 2 minutes. The injector was left in place for 1 minute before and after the infusion. During the infusion procedure, animals were allowed to freely move on the lap of the experimenter or gently restrained. Following the infusion animals were returned to their home cage and placed in the experimental chamber 8 minutes after the start of the infusion procedure.

6.2.6 Statistical analysis

The main measures of the animals' ability to learn the reversals and visual discrimination were: (i) the number of trials to criterion and (ii) the number of incorrect responses to criterion (errors of commission). Additional secondary measures recorded for each trial were (iii) the number of omissions (errors of omission), (iv) the latency to respond to the stimuli, (v) the latency to collect the reward.

Data for each primary variable were analysed using a repeated measures mixed model ANOVA consisting of one between-subject factor (Region) and two within-subject factors (Inactivation and Phase). When significant interactions were found, further analysis was performed of Simple Main Effects or Simple Main Interactions, as appropriate. Analysis was followed by post-hoc Student's paired *t*-test.

Omission data were analysed using Wilcoxon Signed Ranks tests on each separate phase of the reversal paradigm.

For all comparisons, significant difference was assumed at $p < 0.05$.

6.3 Results

6.3.1 Histological results

Figure 6.1 shows a schematic reconstruction of the position of injector tips in the prelimbic, infralimbic, lOFC and mOFC. Animals were excluded from data analysis if the cannula position was not correct. Positions were correct as per the defined regions by Paxinos and Watson (2006), or excluded from further analysis (N=3).

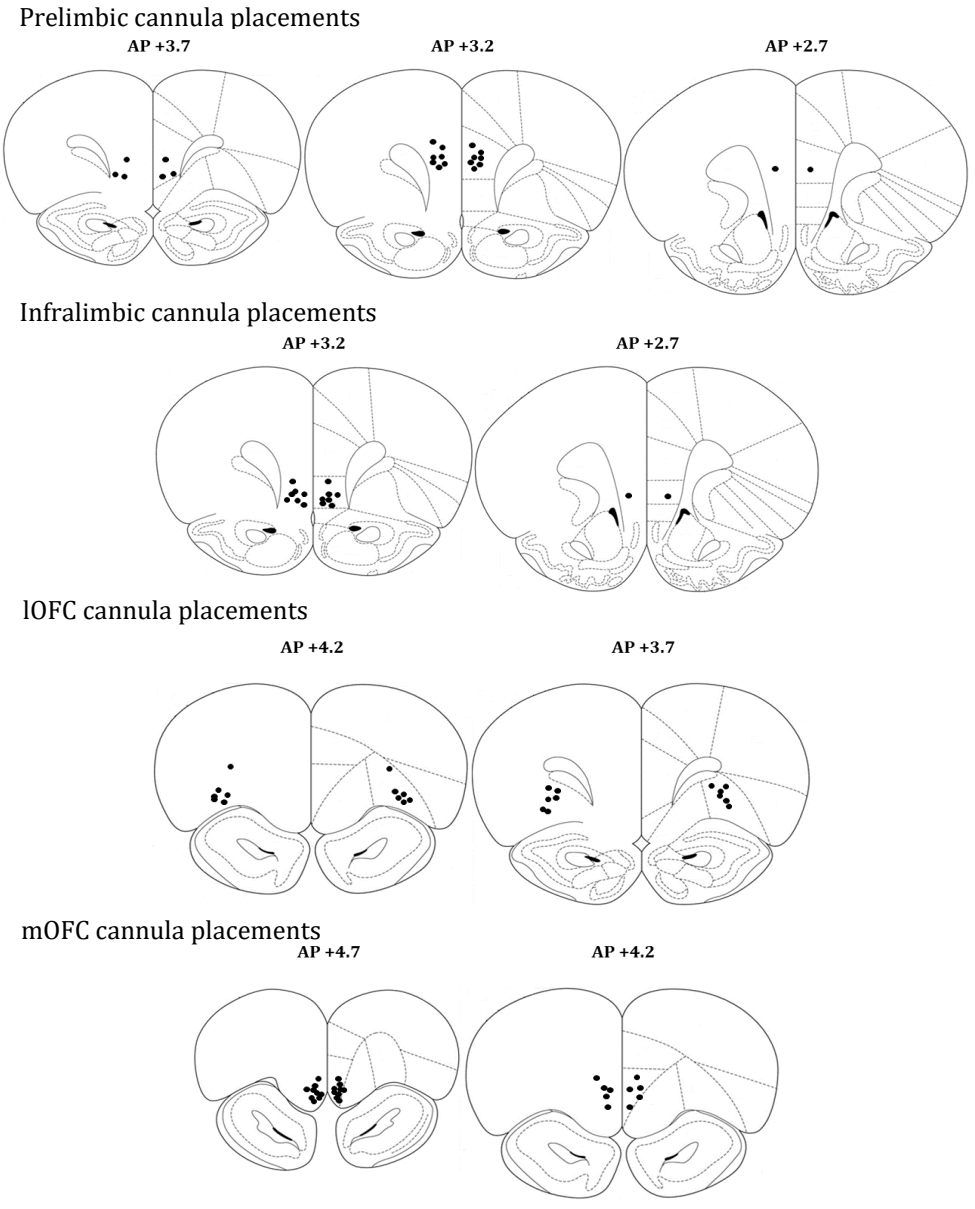
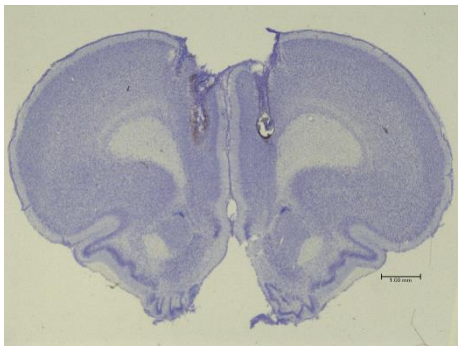


Figure 6.1: **Schematic diagrams showing the injection sites in the prelimbic cortex, infralimbic cortex, lOFC and mOFC. Reconstructions from Paxinos and Watson (2006).**

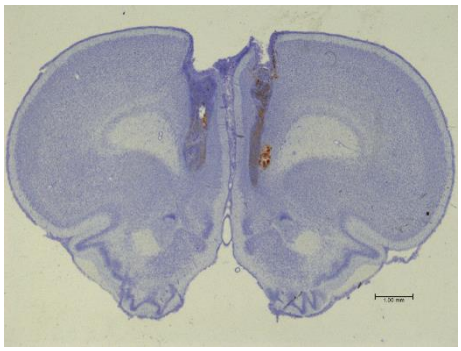
Figure 6.2 shows photomicrographs of coronal sections taken from representative rats to show cannula and injector placement.

A – PrL



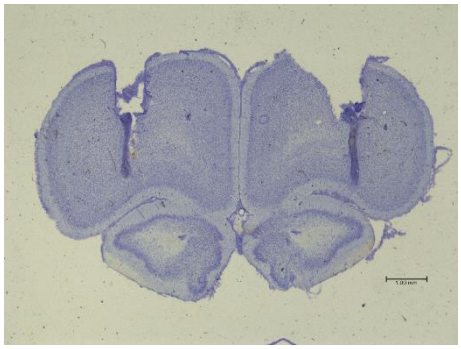
AP +2.7

B – IL



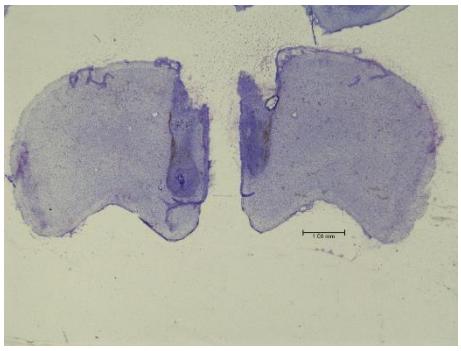
AP +2.7

C – lOFC



AP +3.7

D – mOFC



AP +4.2

Figure 6.2: **Photomicrographs of coronal sections taken from representative rats: (A) prelimbic cortex, (B) infralimbic cortex, (C) lateral OFC, (D) medial OFC.**

6.3.2 Behavioural results

6.3.2.1 Trials to criterion

A three-way repeated measures mixed design ANOVA showed significant main effects of Inactivation and Phase ($F_{1,41}$ = 6.005, p = 0.019 and $F_{2,82}$ = 25.77, p < 0.001, respectively).

There was also a significant Inactivation x Region interaction ($F_{3,41}$ = 4.134, p = 0.012, Figure 6.3), however there were no other significant two-way interactions.

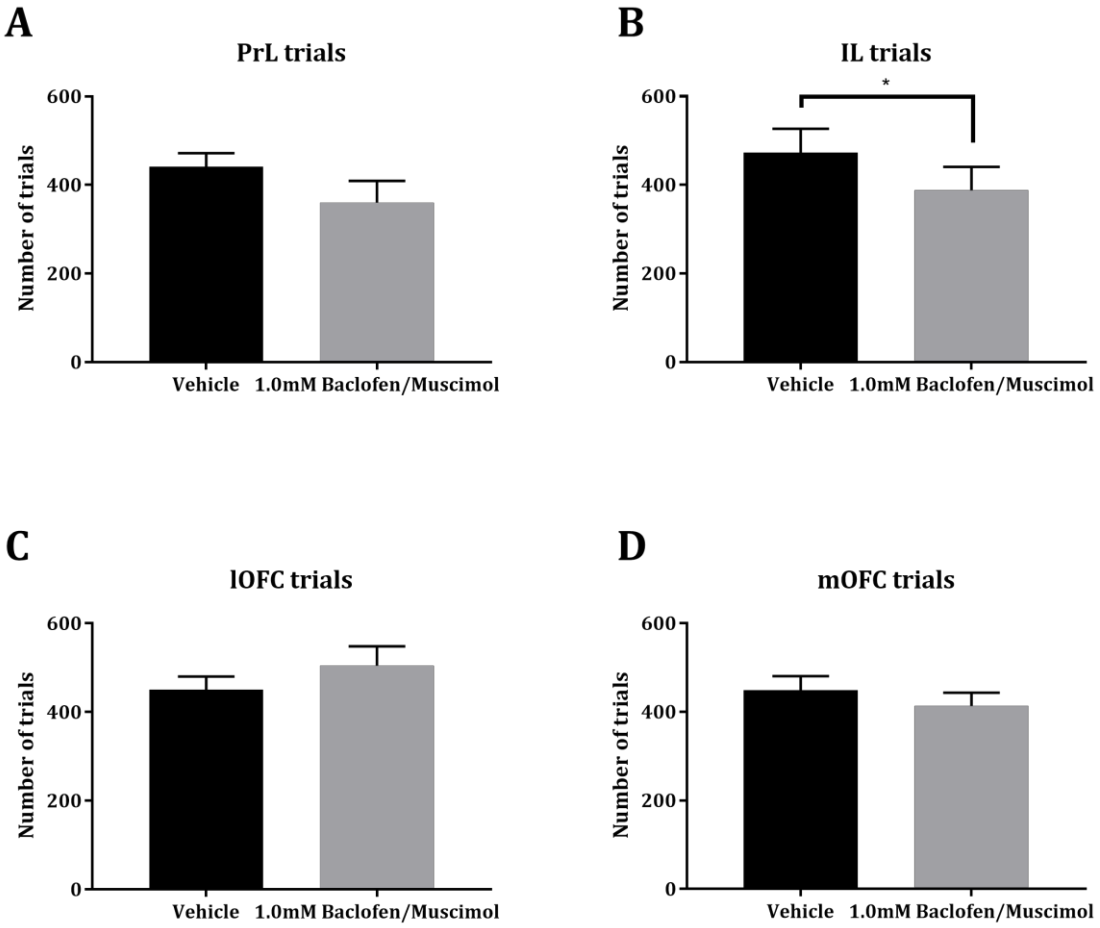


Figure 6.3: **Histograms showing the number of trials to criterion following infusion on baclofen/muscimol into the prelimbic cortex (A), infralimbic cortex (B), lateral OFC (C), and medial OFC (D). Data are represented as mean values ± SEM. There was a significant Inactivation x Region interaction ($F_{3,41}$ = 4.134, p = 0.012), and a significant effect of Inactivation in the infralimbic cortex (B). Asterisks denote significant differences (paired *t*-test: *, p < 0.05) from vehicle controls.**

Figure 6.3A shows the number of trials taken to reach criterion of the touchscreen reversal task following infusion of baclofen/muscimol into the PrL. A test of simple main effects showed that there was no significant effect of Inactivation on the number of trials ($t(10)$ = 2.173, p = 0.055).

Figure 6.3B shows the number of trials to criterion of reversal, following infusion of baclofen/muscimol into the IL. A Student's paired *t*-test showed that there was a significant reduction in the number of trials to criterion following inactivation ($t(7)$ = 2.528, p = 0.04).

Figure 6.3C shows the number of trials to criterion following infusion into the lOFC. A test of simple main effects showed that there was no significant effect of Inactivation on the number of trials ($t(11)$ = -1.468, p = 0.17).

Figure 6.3D shows the number of trials to criterion following infusion with vehicle or baclofen/muscimol into the mOFC. A test of simple main effects showed that there was no significant effect of Inactivation on the number of trials ($t(13)$ = 0.989, p = 0.341).

The three-way ANOVA showed there was no significant three-way interaction ($F_{6,82}$ = 2.165, p = 0.055, Figure 6.4).

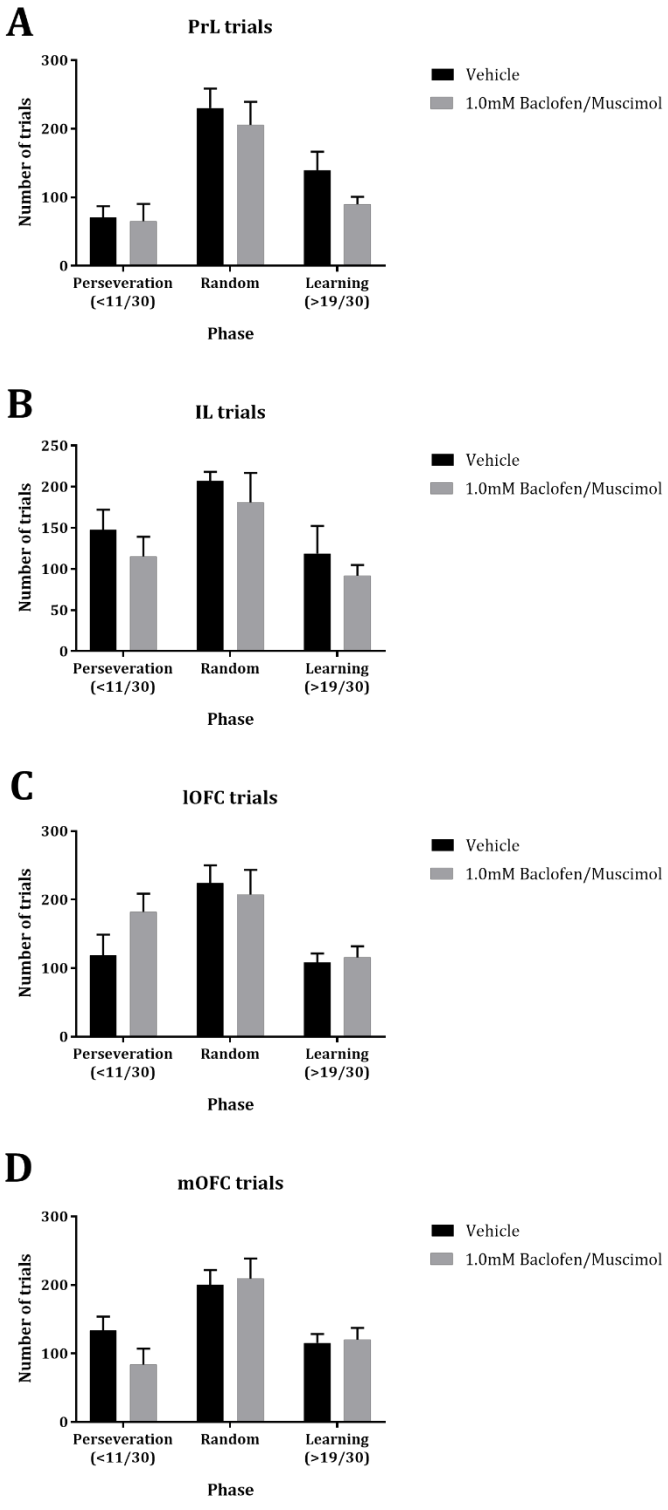


Figure 6.4: **Histograms showing the number of trials to criterion through the perseveration, random and learning phases, following infusion of baclofen/muscimol into the prelimbic (A), infralimbic (B), lOFC (C), and mOFC (D). Data are represented as mean values ± SEM. There was no significant three-way interaction ($F_{6,82} = 2.165$, $p = 0.055$)**

6.3.2.2 Incorrect responses

A three-way repeated measures mixed design ANOVA produced a significant main effect of Inactivation ($F_{1,41}$ = 5.787, p = 0.021), and a significant main effect of Phase ($F_{2,82}$ = 33.979, p < 0.001).

There was a significant Inactivation x Region interaction ($F_{3,41}$ = 4.546, p = 0.008), however there was no significant Phase x Region interaction or Inactivation x Phase interaction ($F_{6,82}$ = 1.826, p = 0.104 and $F_{2,82}$ = 0.063, p = 0.939, respectively).

The three-way mixed design ANOVA revealed a significant three-way interaction of Region x Inactivation x Phase ($F_{6,82}$ = 2.676, p = 0.02, Figures 6.5-6.8).

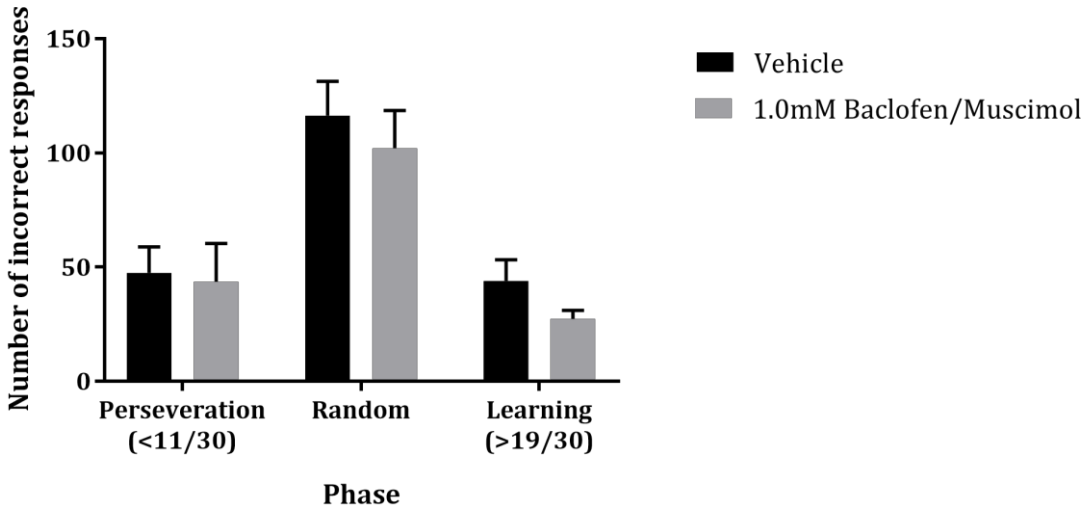


Figure 6.5: **Histograms showing the number of incorrect responses to criterion through the perseveration, random and learning phases following infusion of baclofen/muscimol into the PrL. Data are represented as mean values ± SEM. There was no significant simple interaction effect of Inactivation x Phase ($F_{2,82}$ = 0.034, p = 0.97).**

Figure 6.5 shows the number of incorrect responses to criterion per phase of the reversal task following infusion of baclofen/muscimol into the PrL. A test of the simple interaction effects did not produce a significant Inactivation x Phase interaction ($F_{2,82}$ = 0.034, p = 0.97).

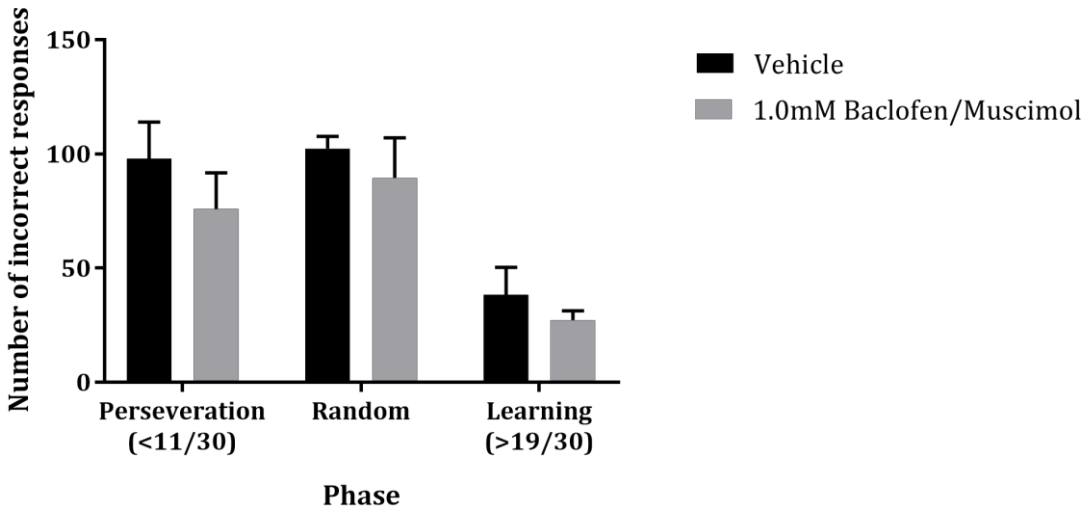


Figure 6.6: **Histograms showing the number of incorrect responses to criterion through the perseveration, random and learning phases following infusion of baclofen/muscimol into the IL. Data are represented as mean values ± SEM. There was no significant simple interaction effect of Inactivation x Phase ($F_{2,82}$ = 0.185, p = 0.83).**

Figure 6.6 shows the number of incorrect responses per phase of the reversal task, following local infusion of baclofen/muscimol into the IL. A test of the simple interaction effects did not produce a significant Inactivation x Phase interaction ($F_{2,82}$ = 0.185, p = 0.83).

Figure 6.7 shows the number of incorrect responses following baclofen/muscimol infusion into the lOFC. A test of the simple interaction effects showed a significant Inactivation x Phase interaction ($F_{2,82}$ = 3.98, p = 0.022).

A paired *t*-test revealed that there was a significant increase in the number of incorrect responses during the perseveration phase ($t(11)$ = -3.42, p = 0.006) following baclofen/muscimol infusion into lOFC.

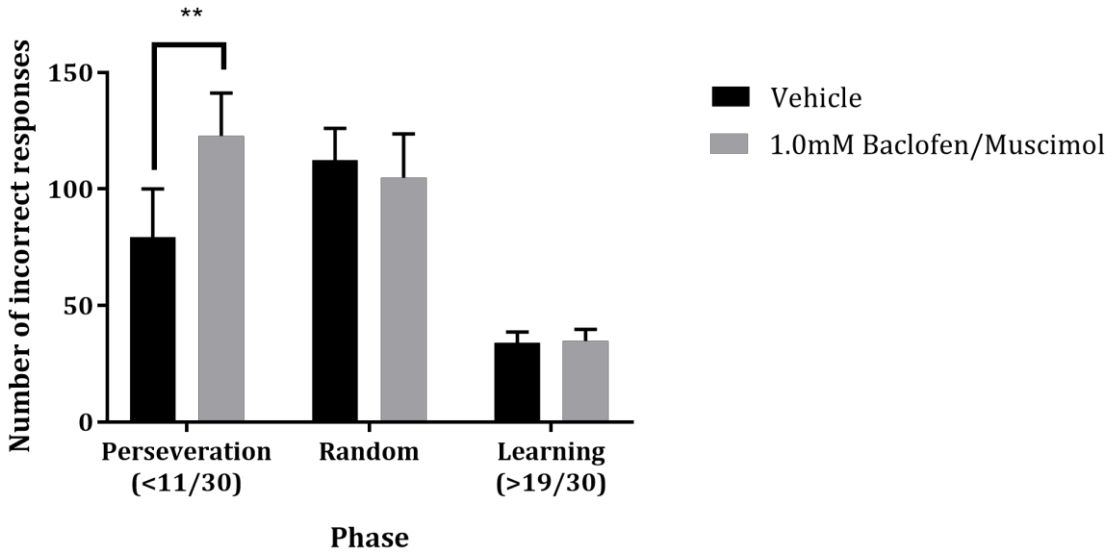


***Figure 6.7*: Histograms showing the number of incorrect responses to criterion through the perseveration, random and learning phases following infusion of baclofen/muscimol into the lOFC. Data are represented as mean values ± SEM. There was a simple interaction effect of Inactivation x Phase ($F_{2,82}$ = 3.98, p = 0.022). Asterisks denote significant differences (paired *t*-test: **, p < 0.01) from vehicle controls.**

Figure 6.8 shows the number of incorrect responses per phase of the touchscreen reversal task following infusion of baclofen/muscimol into the mOFC. A test of the simple interaction effects showed a significant Inactivation x Phase interaction ($F_{2,82}$ = 6.23, p = 0.003).

A paired *t*-test revealed that there was a significant reduction in the number of incorrect responses during the perseveration phase (paired *t* test, *t(8)* = 2.51; p = 0.026).

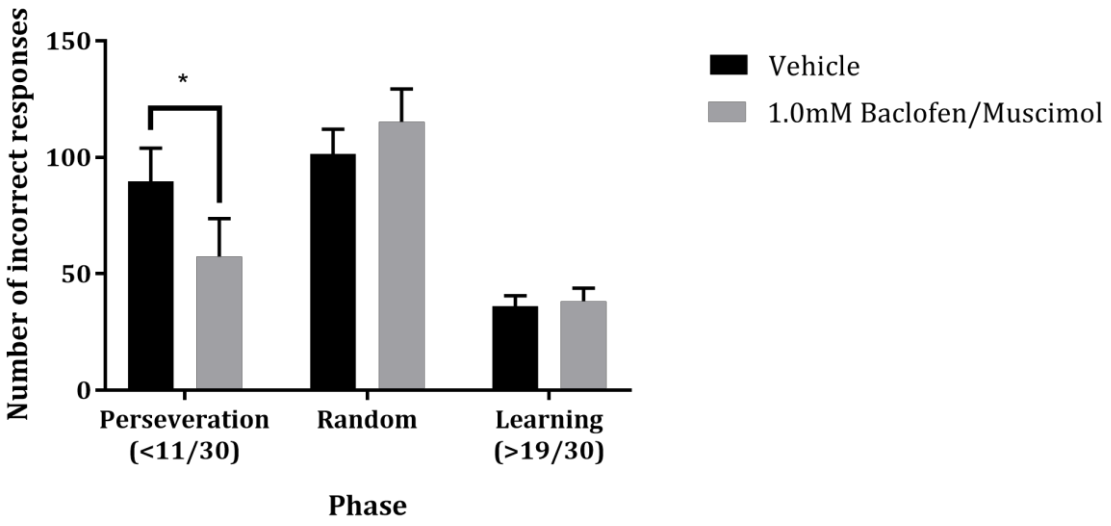


***Figure 6.8*: Histograms showing the number of incorrect responses to criterion through the perseveration, random and learning phases following infusion of baclofen/muscimol into the mOFC. Data are represented as mean values ± SEM. There was a significant simple interaction effect of Inactivation x Phase ($F_{2,82}$ = 6.23, p = 0.003). Asterisks denote significant differences (paired *t*-test: *, $p < 0.05$) from vehicle controls.**

6.3.2.3 Omissions

Figure 6.9 shows the number of omissions during each phase of the touchscreen reversal learning test following infusion of baclofen/muscimol into the PrL. There was a significant increase in the number of omissions during the random phase of the reversal learning paradigm following inactivation of the PrL (Z = -2.214, p = 0.027), there were no effects on the perseveration or learning phases (Z = -0.447, p = 0.665, and Z = 0.0, p = 1.0, respectively).

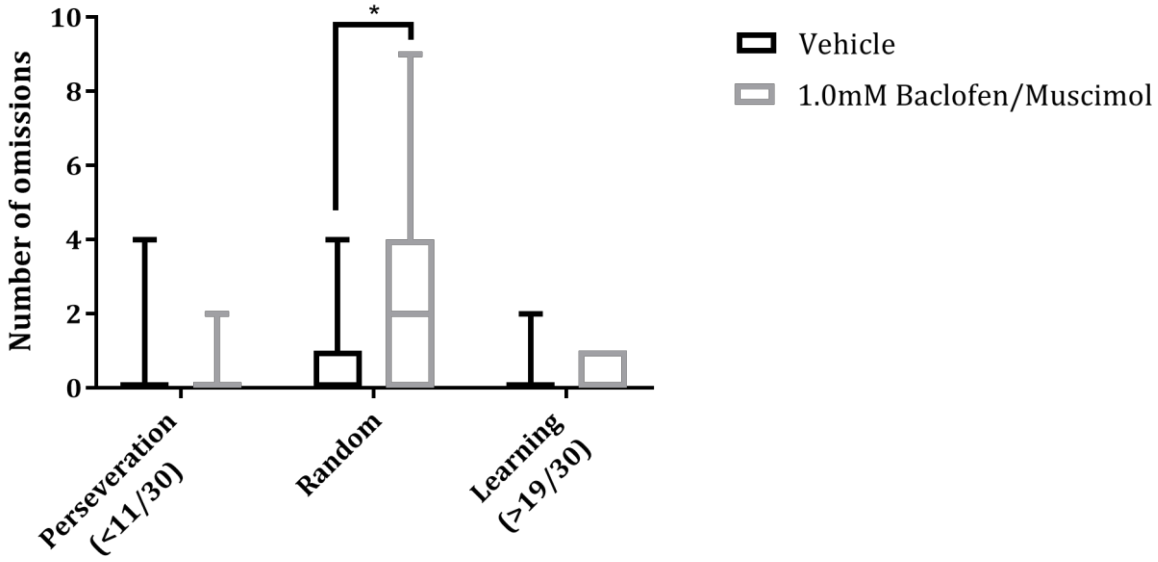


Figure 6.9: **Box and whisker plots showing the number of omissions to criterion through the perseveration, random and learning phases following infusion of baclofen/muscimol into the PrL. Data are represented as mean values ± SEM. There was a significant increase in the number of omissions during the random phase (Z = -2.214, p = 0.027). Asterisks denote significant differences (Wilcoxon Signed Rank test: *, $p < 0.05$) from vehicle controls. Data are represented as median values ± minimum and maximum**

Figure 6.10 shows the number of omissions following local infusion of baclofen/muscimol into the IL. There was a significant increase in the number of omissions during the perseveration phase (Z = -2.121, p = 0.034) following inactivation; however, there were no effects on the number of omissions in the random or learning phases (Z = -0.755, p = 0.45, and Z = -1.342, p = 0.180, respectively).

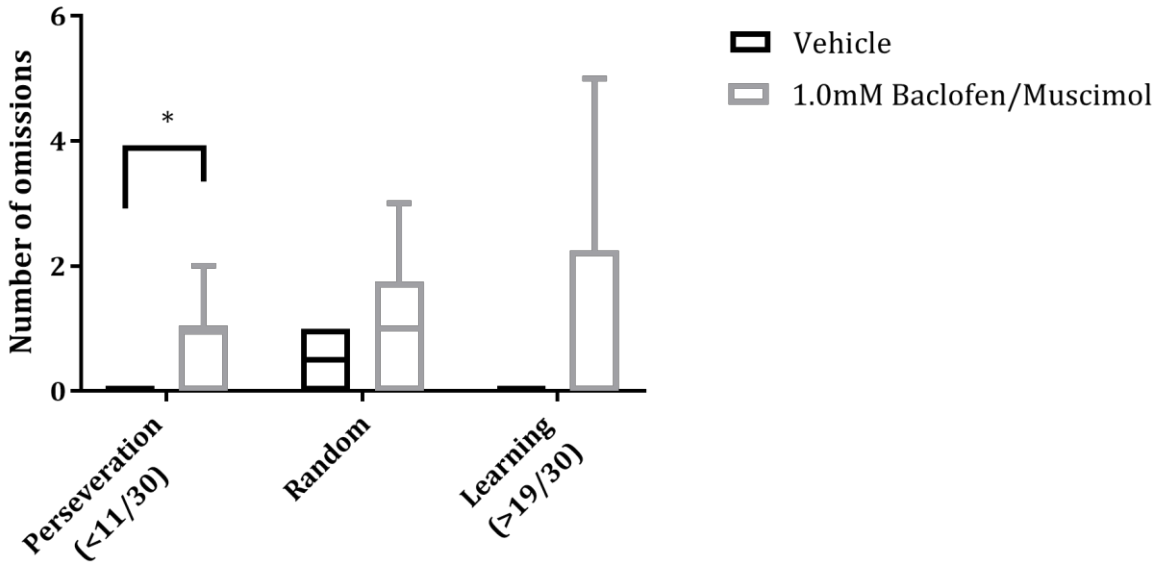


Figure 6.10: **Box and whisker plots showing the number of omissions to criterion through the perseveration, random and learning phases following infusion of baclofen/muscimol into the IL. There was a significant increase in the number of omissions during the perseveration phase (Z = -2.121, p = 0.034). Asterisks denote significant differences (Wilcoxon Signed Rank test: *, $p < 0.05$) from vehicle controls. Data are represented as median values ± minimum and maximum .**

Figure 6.11 shows the number of omissions following the infusion of baclofen/muscimol into the lOFC. There was a significant increase in the number of omissions during the perseveration phase following inactivation (Z = -2.371, p = 0.018), however; there were no effects within the random or learning phases (Z = -0.211, p = 0.833, and Z = -1.289, p = 0.198, respectively).

Figure 6.12 shows the number of omissions following local infusion of baclofen/muscimol into the mOFC. Wilcoxon Signed Rank tests showed that there were no effects of mOFC inactivation on the number of omissions in any phase of the reversal (Perseveration: Z = -0.141, p = 0.888, Random: Z = -0.816, p = 0.414, and Learning: Z = -1.089, p = 0.276).

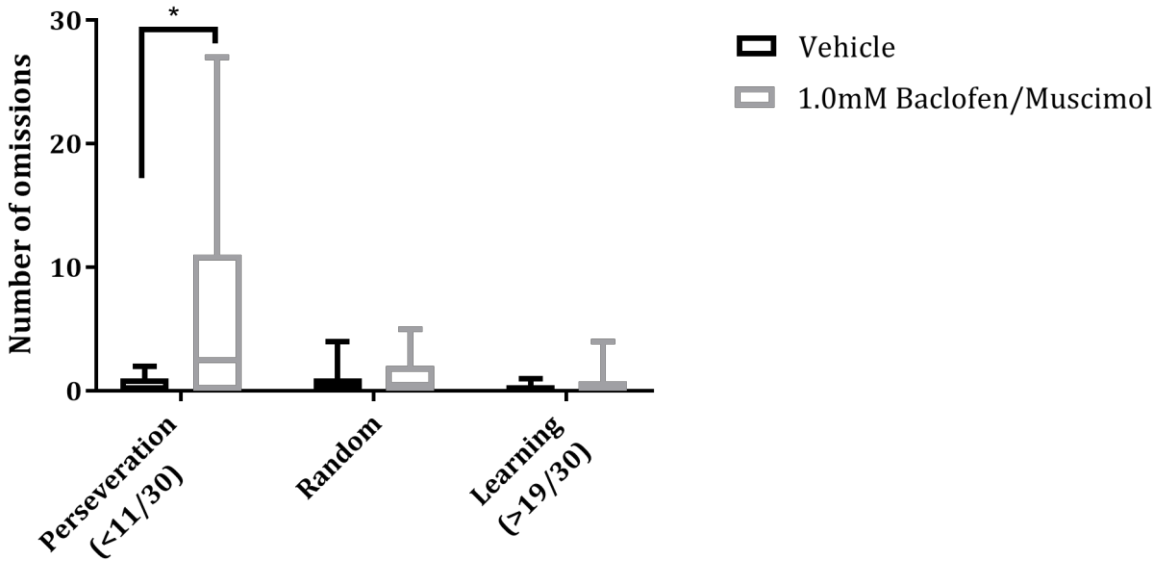


Figure 6.11: **Box and whisker plots showing the number of omissions to criterion through the perseveration, random and learning phases following infusion of baclofen/muscimol into the lOFC. There was a significant increase in the number of omissions in the perseveration phase (Z = -2.371, p = 0.018). Asterisks denote significant differences (Wilcoxon-Signed Rank test: *, $p < 0.05$) from vehicle controls. Data are represented as median values ± minimum and maximum.**

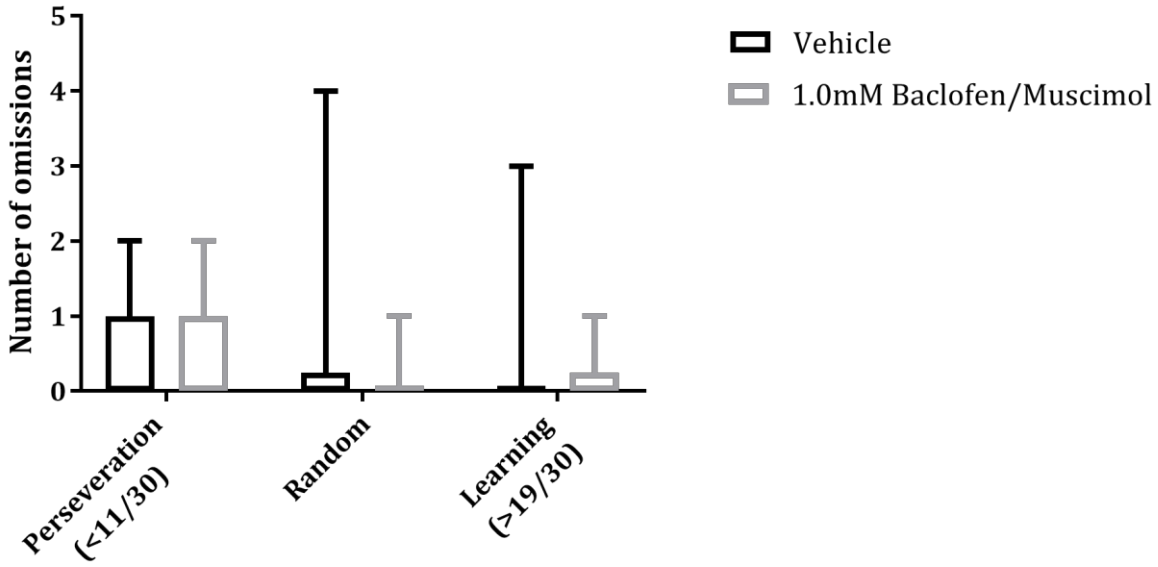


Figure 6.12: **Box and whisker plots showing the number of omissions to criterion through the perseveration, random and learning phases following infusion of baclofen/muscimol into the mOFC. There were no significant differences within any phase. Data are represented as median values ± minimum and maximum.**

6.3.2.4 Response and collection latencies

There were no significant effects of baclofen/muscimol infusion into the PrL or IL on either response of reward retrieval latencies (Table 6.2).

Table 6.2 shows the response and collection latencies following infusion of baclofen/muscimol into the lOFC. There were no significant differences in response latencies between vehicle and baclofen/muscimol groups (paired *t* test, $t(11) = 1.19$, $p = 0.26$), however, there was a significant effect on reward retrieval times (paired *t* test, $t(11) = 2.41$, $p = 0.03$).

Region	Dose	Response	Retrieval
PrL	Vehicle	0.96 ± 0.05	1.18 ± 0.14
	1mM baclofen/muscimol	0.92 ± 0.06	1.47 ± 0.19
IL	Vehicle	0.89 ± 0.04	1.12 ± 0.09
	1mM baclofen/muscimol	1.02 ± 0.13	1.45 ± 0.20
lOFC	Vehicle	0.91 ± 0.07	1.44 ± 0.14
	1mM baclofen/muscimol	1.00 ± 0.08	1.62 ± 0.14 *
mOFC	Vehicle	0.98 ± 0.05	1.13 ± 0.09
	1mM baclofen/muscimol	0.98 ± 0.06	0.84 ± 0.05 ***

Table 6.2: **A table showing the response and reward retrieval latencies following inaction of the PrL, IL, lOFC and mOFC. Asterisks denote significant differences (paired *t*-test: *, $p < 0.05$, ***, $p < 0.001$) from vehicle controls. Data are represented as the mean values ± SEM (seconds).**

Table 6.2 shows the response and collection latencies following infusion of baclofen/muscimol into the mOFC. There were no significant differences in response latencies between vehicle and baclofen/muscimol groups (paired *t* test, $t(13) = 0.03$, $p = 0.98$), however, there was a significant effect on reward retrieval times (paired *t* test, $t(13) = 4.281$, $p = 0.0009$).

6.3.2.5 Summary of behavioural results

	Trials	**Incorrect responses**	**Omissions**	**Response latency**	**Retrieval latency**
PrL	n.s.	n.s.	↑ * Increase in random phase	n.s.	n.s.
IL	↓ * Dose dependent decrease	n.s.	↑ * Increase in perseveration phase	n.s.	n.s.
lOFC	n.s.	↑ * Increase in perseveration phase	↑ * Increase in perseveration phase	n.s.	↑ * General slowing
mOFC	n.s.	↓ * Decrease in perseveration phase	n.s.	n.s.	↓ *** General quickening

Table 6.3: **A summary of results for each brain region following inactivation with baclofen/muscimol when compared to vehicle. n.s. denotes no significant difference from vehicle controls. Asterisks denote significant differences (paired *t*-test: *, p <0.05, **, *p* < 0.01, ***, *p* < 0.001) from vehicle controls.**

6.4 Discussion

6.4.1 Effects of mOFC and lOFC inactivation on Serial Visual Reversal Learning

The effects of inactivation of both the mOFC and lOFC were confined to the perseveration phase of the reversal learning paradigm. Inactivation of the mOFC, following infusion of baclofen/muscimol, led to a decrease in perseveration; whereas inactivation of the lOFC elicited an increase in perseveration. Inactivation in these two regions had no significant effect on learning, as there was no significant difference in the number of trials to criterion in either group. Along with an increase in the number of incorrect responses, lOFC inactivation also significant increased omissions.

mOFC inactivation improved performance on the serial visual reversal learning task in the phases, decreasing perseveration. Few studies have previously investigated the mOFC in reversal learning, having either reported no significant effect (Dalton *et al.*, 2016) or an increase in perseveration in an instrumental spatial reversal task (Gourley *et al.*, 2010). The differences in these results could be explained by the use of a spatial design rather than a serial visual touchscreen reversal task, that requires more training.

lOFC inactivation led to an increase in both errors of commission and errors of omission; this impairment in reversal learning following lOFC inactivation is consistent with previous studies in rats (Kim and Ragozzino, 2005; Ragozzino, 2007; Alsiö *et al.*, 2015), lesions in rodents (Chudasama and Robbins, 2003; McAlonan and Brown, 2003; Boulougouris, Dalley and Robbins, 2007; Riceberg and Shapiro, 2012) and monkeys (Dias, Robbins and Roberts, 1996; Clarke, Robbins and Roberts, 2008), and damage in humans (Rahman *et al.*, 1999; O'Doherty *et al.*, 2001; L. K. Fellows and Farah, 2003).

As discussed in the General Introduction (Chapter 1), recent studies have shown population specific neuronal ensembles in the OFC, DMS and DLS responsible for goal-directed or habitual behaviours (Gremel and Costa, 2013). The lOFC has also been suggested to regulate habitual vs goal-directed behaviours via the striatum (see Chapter 1). I have also shown that manipulations of the anterior DLS increase

incorrect responses during the perseveration phase in this task (see Chapter 4). Therefore, it could be postulated that the lateral OFC is exerting its actions through the aDLS to mediate, in part, a balance between habitual and goal-directed learning. Similarly, the mOFC could be acting through the nucleus accumbens.

The contrasting effects of inactivation of the medial and lateral OFC have been previously observed in other situations following lesions of the two regions. For example, Mar et al (2011) reported opposing functions of the medial and lateral OFC in a delay-discounting task; they reported that lOFC lesions led to increases in delay-discounting, hypothetically through increased impulsive choice and disruption of associative learning processes, whereas mOFC lesions may have reduced delay-discounting by enhancing the sensitivity to reward values.

A decrease in retrieval latency following mOFC inactivation could also be due to an increase in exploring behaviour in conjunction with an increased sensitivity to rewards. Following a correct response, the rat must retrieve a reward pellet prior to initiation of the next trial, while a decrease in incorrect responses could be a measure of cognitive exploration, a decrease in retrieval latency could be a measure of physical exploration around the testing chamber. Conversely, lOFC inactivation led to a general slowing, this is likely due to disruption of striatal afferents encoding the habit of reward retrieval in the lateral striatum. Thus, inactivation of the medial or lateral OFC may affect the balance of the explore vs exploit pathways as a hypothesised role of the human mOFC (Domenech and Koechlin, 2015).

6.4.2 Effects of mPFC inactivation on Serial Visual Reversal Learning

Inactivation of the IL cortex actively reduced the number of trials required to reach criterion for learning on the reversal task; conversely inactivation increased the number of omissions in the random phase with no significant effects on the number of incorrect responses. While inactivation of the PrL cortex led to a significant increase in the number of omissions, this was also localised to the random phase of the task, with no other effects on trials or incorrect responses. The reduction in trials following IL inactivation suggests a general improvement in performance and an increase sensitivity to reward contingencies; this is supported by previous reports

of increased sensitivity to reward value following reinforcer devaluation extinction testing (Killcross and Coutureau, 2003).

Chudasama and Robbins (2003) have reported dissociable effects of IL and OFC lesions on both discrimination reversal learning and the 5-choice serial reaction time task (Chudasama *et al.*, 2003). In the latter case, IL lesions led to a significant increase in premature responses and an increase in omissions when presented with a long variable inter-trial interval, however there was a significant decrease in omissions when presented with a short inter-trial interval; this variability in omission response rate could be replicated significant increase in omissions in the perseveration phase in this visual reversal learning paradigm. The 5-choice serial reaction time data implicate the IL cortex in preventing impulsive responding. Inactivation of the IL cortex would lead to generalised to omissions in this task; while there was a general increase in omissions in this task, the increase was only significant in the perseveration phase.

Chudasama and Robbins (2003) also showed that IL lesions led to an increase in incorrect responses and an increase in the number of sessions to complete a discrimination learning task; however, these data contradict the findings of this experiment. The differences in these results could be explained by the different training and testing of each task, as well as differences between lesions and inactivation. As part of the Chudasama training and testing protocol rats were exposed to correction procedures, these correction procedures were then analysed separately in the overall analysis. Also, the rats only underwent two training reversals prior to testing; as part of the serial reversal learning task animals undergo multiple training reversal to reach a testing standard, the additional training prior to testing in the serial reversal task could abolish the IL lesion impairment effects seen by Chudasama and Robbins.

PrL lesions produce a non-selective devaluation effect, reducing general performance in extinction (Corbit and Balleine, 2003). PrL inactivation also produce impaired decision-making on a rat gambling task (Zeeb *et al.*, 2015). During the early phases of the serial reversal learning task, rats are not receiving rewards due to a high proportion of incorrect responses. During the trials in the perseveration phase,

rats can undergo extinction due to a lack of reinforcing feedback. A non-selective devaluation of action and general reduction in performance would lead to an increase in omissions and an inability to make optimal decisions. The significant increase in omissions in the random phase of the reversal learning task following inactivation of the PrL cortex could be due to extinction during the perseveration phase, and an inability to make and maintain an optimum decision-making strategy.

PrL and IL lesions have been shown to have dissociable effects in spatial reversal learning (Ashwell and Ito, 2014). The findings of this experiment compliment those found in a spatial reversal learning; Ashwell and Ito reported IL lesions led to superior performance of spatial context-dependent discrimination and reversal learning, this finding corresponds to the decreased number of trials to reach learning criterion following IL inactivation. Both experiments report no significant effects of PrL lesion on overall reversal performance.

Although animals were randomised based on performance prior to surgery and injector insertion, there was a marked difference in the baseline performance between the different cannulation locations under vehicle infusion (not significant). This difference in post-surgery could be due to the mechanical effects of infusion in each location, leading to a behavioural effect independent of the drug being infused. To minimise the effects of infusions on behaviour, a within-subject design between the different cannulation locations was used, therefore if there was a mechanical effect of infusion that this effect was present under both vehicle and inactivation infusions. Animals were also placed in their home cages following infusion, this allowed for any mechanical activation to subside, and for the drug to take effect before starting the testing protocol.

This experiment has shown the dissociable, and to some extent opponent, involvement of frontal regions of the rat brain in a touchscreen serial reversal learning task.

Chapter 7: General Discussion

The development of the novel touchscreen serial reversal learning task has allowed for further understanding of the role of the indirect pathway in different anatomical regions, and the top-down control of the PFC on the striatum during tests of behavioural flexibility. The task enabled the analysis of different stages of the reversal learning paradigm to dissociate the type of trial or incorrect response, similar to the method used by Jones and Mishkin (1972); the separation of phases allows for analysis of perseveration and new learning, and how manipulations can effect these processes individually or together. The reversal task, while being relatively simple in its design, is a complex measure of cognitive flexibility and reward contingency updating, with several processes underlying the task, 1) detection of contingency reversal, 2) inhibition of a prepotent response, 3) overcoming learned non-reward to attend to the new CS+, and 4) learning of a new reward contingency. All four processes must occur for a successful reversal, where failures in one or more process leading to cognitive inflexibility and altered performance. The experiments in this thesis report that a number of regions of the rat brain are recruited during reversal learning.

The experiments in this thesis focused on the neurochemical and neuroanatomical basis of reversal learning in the rat in a touchscreen serial reversal learning task. Specifically, they address (i) the effects of systemic dopamine D_2/D_3 receptor antagonism on the serial reversal task, as well as novel visual discrimination, retention of new learning, and subsequent reversal; (ii) the role of D_2/D_3 receptors within distinct anatomical regions of the striatum on the serial reversal paradigm, (iii) the neurochemical specificity of reversal learning in the striatum, and (ix) the role of the rat OFC in reversal learning following inactivation. These findings validate the touchscreen serial reversal learning task as a complex measure of cognitive function, requiring anatomically distinct regions to perform the task.

7.1 Overview of experimental results

The role of dopamine D_2 receptors in reversal learning and novel discrimination has previously been studied in the monkey, with specific roles being identified following systemic modulation (see Chapter 1 and Chapter 3). In view of the role of dopamine D_2 receptors in a visual reversal learning task in the monkey, similarities were investigated in the rat touchscreen task. Chapter 3 investigated the role of systemic dopamine D_2/D_3 receptor antagonism on the serial visual reversal task, as well as novel visual discrimination and subsequent reversal. Systemic dopamine D_2/D_3 receptor antagonism, through administration of raclopride, impaired both retention of novel discrimination and subsequent reversal. Systemic raclopride produced a dose-related impairment in reversal learning, with 0.03mg/kg producing more trials and incorrect responses than vehicle controls, 0.03mg/kg also led to a slowing in response latency. Low dose raclopride (0.01mg/kg) produced equivocal behavioural flexibility in the serial reversal task to vehicle controls. Low dose raclopride (0.01mg/kg) had no marked effects on new learning of novel discrimination, however produced substantial impairments on retention testing of the learned discrimination. The impaired of retention of novel discrimination carried forwards into marked deficits in subsequent reversal learning of the novel discrimination, suggesting learning under the influence of raclopride encoded the association in a form that is less flexible to changing contingencies. The findings in Chapter 3 accord with those reported by Ridley, Haystead, and Baker (1981) and Lee et al. (2007) on the role of dopamine and the D_2 receptor in non-human primates during reversal learning; these finding provide evidence that the roles of dopamine and its receptors are conserved across species in their effects on reversal learning and cognitive flexibility.

The specific role of striatal dopamine D_2/D_3 receptors was further assessed in Chapter 4, with local intracranial infusions of raclopride into the anterior DLS, posterior DMS, anterior DMS and nucleus accumbens core. Low dose raclopride (0.05μg/hemisphere) infusion into the anterior DLS led to a significant increase in perseverative responding to the previously rewarded CS+ (now CS-), this increase in perseverative incorrect responding was also observed in a dose-dependent manner following raclopride infusion into the anterior DMS. Conversely, raclopride

infusion into the nucleus accumbens core caused a dose-dependent decrease in perseveration, suggesting opponency between the dorsal and ventral striatum. Raclopride infusions into the posterior DMS also tended to decrease perseverative responding, while increasing the number of incorrect responses during the random (new learning) phase, suggesting the animals were able to exert inhibitory control but not learn a new choice strategy. Infusions into the anterior and posterior DMS had motoric effects, slowing magazine entry latency. The opponent results following local infusion of raclopride into the striatum account, in part, for the non-specific effects of systemic administration and highlight that optimum dopamine signalling at the D_2/D_3 receptor is essential for reversal learning performance. The findings of Chapter 4 expand the our current understanding of the anatomical basis of the indirect pathway in reversal learning; previous reports of intracerebral dopamine manipulations have investigated effects on set-shifting (Floresco *et al.*, 2006; Haluk and Floresco, 2009), and reversal learning in the nucleus accumbens (Calaminus and Hauber, 2007; Haluk and Floresco, 2009), while DMS dopamine manipulations have been investigated through dopamine depletion in rat and marmoset (O'Neill and Brown, 2007; Clarke *et al.*, 2011). Local dopamine D_2/D_3 receptor antagonism has allowed for investigation of the indirect pathway in anatomically distinct regions, not previously studied through lesions or neurochemical depletion.

Chapter 4 investigated the role of dopamine D_2 receptors in sub-regions of the striatum. The neurochemical specificity of the claims made in Chapter 4 was investigated in Chapter 5, where the role of D_1 and 5-HT_{2C} receptors in the same sub-regions was also probed. Infusions of 0.1µg/hemisphere SCH-23390, a dopamine D_1 receptor antagonist, into the anterior DMS and nucleus accumbens core did not produce any significant effects on serial reversal learning; similarly, 1 and 3µg/hemisphere SB-242,084, a 5-HT_{2C} receptor antagonist, infused into the same striatal sub-regions did not produce any significant effects. Higher concentrations of SCH-23390 have since been shown to also produce no significant effects on serial reversal learning (Sala-Bayo et al., submitted); therefore, we can deduce D_2 receptor specific effects for dopamine manipulations in the anterior DMS and nucleus accumbens core. 5-HT has been shown to play a role in the performance of reversal learning in the OFC in both the rat and marmoset (Clarke *et al.*, 2004; Alsiö *et al.*,

2015), with antagonism of 5-HT_{2C} receptors being shown affect reversal learning. Conversely, 5-HT_{2A} antagonism in the striatum has been reported to improve reversal learning performance. Taken together this suggests a role for 5-HT_{2C} receptors in the OFC and 5-HT_{2A} receptors in the striatum.

The contribution of top-down control of the OFC and mPFC to striatal regions during reversal learning was assessed in Chapter 6. Using local inactivation, the contributions of the PrL, IL, lOFC, and mOFC regions of the rat prefrontal cortex in reversal learning performance were assessed. Inactivation of the PrL had no effect on overall reversal performance, however this did generate an increase in the number of omissions during the random (new learning) phase, whereas inactivation of the IL caused an increase in the number of omissions during the perseveration phase, paired with a general improvement in performance and a reduction in the number of trials required to attain the criterion for reversal learning. lOFC inactivation led to significant impairments in reversal learning, with increases in perseverative errors and omissions, along with a general slowing of magazine entry. Conversely, mOFC inactivation caused a significant improvement in reversal learning performance, with a decreased number of perseverative errors and a general quickening of magazine entry; with neither lOFC or mOFC inactivation eliciting an effect on the number of trials required to complete the reversal learning task. Therefore, subregions of the mPFC and OFC play dissociable roles in governing cognitive flexibility and performance in reversal learning. Chapter 6 provides a comprehensive study of the role of different anatomical regions of the PFC in reversal learning in the rat; previous findings have investigated dissociable functions within the OFC and mPFC, however this anatomical and functional opponency has not been investigated in reversal learning and has been implied through other measures of cognitive flexibility and contingency revaluation. The findings in Chapter 6 contribute to the understanding of the function of the rodent PFC, as well as the complexity of the reversal learning task.

7.2 Anatomical perspectives of reversal learning

Distinct projections from the PFC to the striatum have been reported (Alexander, DeLong and Strick, 1986; Lawrence, Sahakian and Robbins, 1998; Phillips *et al.*,

2003; Voorn *et al.*, 2004; Heilbronner *et al.*, 2016); these frontostriatal circuits and projections are thought to be important for cognitive and behavioural flexibility (Modell *et al.*, 1989; Graybiel and Rauch, 2000; Dvorkin *et al.*, 2010), and have been implicated in human studies (Morris *et al.*, 2016; Vaghi *et al.*, 2017), including OCD and other disorders such as schizophrenia (Leeson *et al.*, 2009) and Huntingdon's disease (Sprengelmeyer, Lange and Hömberg, 1995).

The findings of Chapters 4 and 6 highlight distinct dissociable effects of different brain regions within both the PFC and striatum during reversal learning. We know that lesions to the lateral OFC produce impairments in reversal learning (Iversen and Mishkin, 1970; Chudasama and Robbins, 2003), inactivation of the lateral OFC through infusion of GABA agonists can also produce deficits in reversal learning through increasing perseveration; impairments in reversal learning and increasing perseverative behaviour following lesion or inactivation of the lateral OFC is conserved across species. This perseverative impairment was also produced following local dopamine D_2/D_3 receptor antagonism in the anterior DLS; in contrast to the findings of Castañé, Theobald and Robbins (2010) where DLS lesions produced no effect on reversal learning performance. As reported in Chapter 1, the lateral OFC projects to the DLS, suggesting that the lateral OFC – DLS projection is necessary for cognitive flexibility. Low dose dopamine D_2/D_3 receptor antagonism will increase presynaptic release of dopamine into the anterior DLS through D_2 autoreceptors, increasing synaptic dopamine signalling through postsynaptic D_2 receptors and leading to a net inhibition, producing a similar effect as local inactivation of the lOFC through GABA agonists. The DLS has also been implicated in stimulus-response habit learning (Yin, Knowlton and Balleine, 2004) and so it is plausible that prepotent responses occurring as a result of extensive training are under cortical inhibitory control via this projection.

At first sight, the reduction in incorrect responses in perseveration following inactivation of the medial OFC is a surprising result, and could call into question the role of GABA agonists deactivating a structure, as it appears to enhance performance in this case. It is assumed that GABA agonists lead to the inactivation of brain regions, as this method is used generally to inactivate structures, and analogous improvements in flexibility have been previously reported following lesions to the

medial OFC (Mar *et al.*, 2011). Presumably the medial OFC is involved in enhancement of exploitation behaviours, maintaining behaviour that has been deemed to be preferable; through inactivation of the medial OFC the rat is able to utilise exploration methods to rapidly learn the new association within the reversal paradigm, obtaining maximum reward (Durstewitz *et al.*, 2010; Karlsson, Tervo and Karpova, 2012; Donoso, Collins and Koechlin, 2014; Domenech and Koechlin, 2015).

The result of reduced perseveration following medial OFC inactivation was mirrored following dopamine D_2/D_3 receptor antagonism in the nucleus accumbens, suggesting an increase in motivation and improved learning. The suggestion that the medial OFC improvements are mediated by an increase in exploitation would also apply to the nucleus accumbens, suggesting there is a system involved in the mediation of 'exploitation'. The circuitry between the OFC and the nucleus accumbens is classically named as the "reward pathway" and mediated by dopamine (Olds and Milner, 1954; Kringelbach and Berridge, 2010), perhaps a more suitable name for this pathway would be the "exploitation pathway"; as inactivation of the medial OFC, as well as dopamine D_2/D_3 receptor antagonism in the nucleus accumbens, led to decreased perseveration, and improved performance.

Dopamine D_2/D_3 receptor antagonism in the anterior DMS caused an impairment in reversal learning and an increase in perseverative responding (opposite to the effects found in the posterior DMS and nucleus accumbens) however similar impairments of reversal learning have also been reported by Clarke *et al.* (2011) following dopamine depletion, however these impairments in reversal performance were not perseveration. Depleting dopamine in the DMS led to generalised impairments in reversal behaviour, and an increase in the number of trial required to criterion; conversely, dopamine D_2/D_3 receptor antagonism in the anterior DMS led to an increase in perseverative responding while D_1 signalling in the same region is still intact. Taken together these results would implicate a role for D_2/D_3 receptor signalling through the indirect pathway in the early perseverative phase of reversal learning, whereas potentially D_1 signalling through the direct pathway mediating the latter stages of reversal learning, hence not being impaired in this experiment. In the anterior DMS the medial OFC projections are located in a more ventral tract, thereby not promoting reductions in perseveration as seen elsewhere; the anterior

DMS likely receives afferents from the more ventral OFC, whose actions have been more closely linked to those of the lateral OFC compared to the medial OFC (Izquierdo, 2017).

The posterior DMS has been more linked with goal-directed behaviours (Yin *et al.*, 2005) and instrumental conditioning, where responses produce reward, through the use of lesions and inactivation. Dopamine D_2/D_3 receptor inactivation in the posterior DMS produced a differential response pattern between the perseverative and random (new learning) phases, indicating an interaction and balance between a habit system and a new instrumental goal directed system. Dopamine D_2/D_3 receptor antagonism reduced perseverative errors, improving performance, however an increase in errors in the random (new learning) phase suggests that while the rats were able to actively prevent responding to the prepotent stimulus, learning of the new reward contingency did not occur (Ragozzino, 2007). The improvement in perseverative responding could be controlled through projections from the mPFC, as inactivation of the IL caused a general improvement in reversal learning, requiring less trials to obtain criterion for learned reversal.

The PrL and IL regions have been reported to have dissociable effects in spatial reversal learning following lesions (Ashwell and Ito, 2014), with IL lesions causing superior performance and PrL lesions producing no effects. As with the medial and lateral OFC, temporary inactivation of these regions has produced complementary results in a visual reversal learning task. While inactivation led to an improvement or no effect on overall performance in the IL and PrL respectively, there were also increases in the number of omissions following inactivation of each region. IL inactivation caused a general increase in omissions, that was significant in the perseveration phase, this corresponds to findings of Chudasama *et al.* (2003) where IL lesions led to an increase in omissions in the 5-choice serial reaction time task. The increase in PrL omissions in the random phase are more likely due to an extinction effect. The PrL has been shown to be sensitive to extinction and reward devaluation following lesion studies (Corbit and Balleine, 2003), while inactivation has also been shown to impair decision making (Zeeb *et al.*, 2015); the combination of decision impairment and mild extinction following a reduction in reward during

the perseveration phase likely account for the increase in omissions in the random (new learning) phase following PrL inactivation.

Previous studies have found differences in responding between the anterior and posterior DMS (Yin *et al.*, 2005) in instrumental conditioning, those differences in goal directed function are carried over into reversal learning, with opposing effects found following dopamine D_2/D_3 receptor antagonism in each region. To further understand the difference in roles of the anterior and posterior DMS in reversal learning, simple inactivation could be carried out, along with the investigation of more neurotransmitters and their receptors.

Combining the results from Chapters 4 and 6 highlight the importance of frontostriatal circuits for cognitive flexibility, with similar results following manipulations of each distinct anatomical region. This thesis has provided a basis for the investigation of different frontostriatal circuits in reversal learning and cognitive flexibility in the rat. The findings in this thesis investigated each anatomical region individually, implying a functional connection between the regions through the indirect pathway, further investigations into the role of the circuits and projections would provide further insight into the balance of frontostriatal circuits in reversal learning.

7.3 Impact of experiment findings

The findings of Chapter 4 highlight the role of dopamine signalling in striatal sub-regions during serial reversal learning. While it was previously known that dopamine plays a role in these regions, I was able to report a dissociable effect of sub-region based on different phases of the reversal task.

Chapter 6 highlights the functional dissociation between the medial and lateral OFC in serial reversal learning. Understanding the dissociation between OFC sub-regions and their somewhat opposing effects following inactivation can further the understanding of the role played by each region. In addition to this, when taken together with previous knowledge concerning frontostriatal circuitry, it highlights a functional connection between distinct brain regions that mediate serial reversal learning and cognitive flexibility.

7.4 Limitations of experimental findings

Despite the demonstration of regionally specific, often dose-dependent effects (of raclopride) on reversal learning, neuronal circuit specificity could not be demonstrated for the effects reported. Raclopride is a selective antagonist at dopamine D_2 receptors, but it also unavoidably antagonises D_3 receptors that are also present at striatal sites, especially in the nucleus accumbens (Schwartz *et al.*, 1994). However, there are few drugs available that block D_2 receptors selectively, although I might have tested agents such as the relatively selective D_3 receptor antagonist nafadotride to compare the effects of D_2/D_3 antagonism vs D_3 antagonism to dissociate receptor effects (Sautel *et al.*, 1995).

Although attempts were made to investigate the neurochemical specificity of results shown, studies using intracranial infusions of SCH-23390 and SB-242,084 into striatal sub-regions fell below the calculated power required (Appendix B). In addition, histology was unable to be obtained for SB-242,084 rats. Therefore, while the results of these two studies show no significant effects of dopamine D_1 and 5-HT_{2C} antagonism in the anterior DMS and nucleus accumbens core, in line further work (Sala-Bayo et al., submitted), they should not be viewed as standalone studies.

DREADDs were used in an attempt to replicate the findings of Chapters 4 and 6 using a chemogenetic approach (Appendix A), that could then be furthered to include pathway specific manipulations. However, the DREADDs experiments proved inclusive with no significant findings. It is not known the cause of their negative results as functional testing was unable to be performed to ensure DREADD expression at the infusion site. In addition to this, CNO concentration was not measured in the brain to ensure adequate DREADD activation was achieved.

As discussed in Chapter 6, following surgical cannulation there were marked differences in vehicle controls, although not significant. To minimise the risk of statistical error, a conservative within-subject approach was adopted to ensure accurate interpretation. The difference in performance under vehicle control could be as a result of damage dorsal to the region of interest following cannulation surgery; however, if that were the case vehicle responding would be equal between groups with the same cannulation site (eg PrL and IL, or aDMS and NAc) which is

not the case. Differences in vehicle control performance could be in part have been due to the physical effects of the infusion procedure, causing mechanical stimulation of the region of interest.

7.5 Conclusions and further directions

Continued focus on the role of the OFC in reversal learning and understand cognitive flexibility will be essential for possible translational aspects of this research; for example, in seeking new drugs to enhance learning and executive control for mental health disorders.

The discovery of opponent functions within the OFC and its projections to the striatum have theoretical implications for our understanding of how the basal ganglia normally operate. The data in this thesis show how an apparently relatively simple task such as reversal learning in rodents has a complex set of control mechanisms in the brain that presumably, by checks and balances in component processes, usually serve to optimise performance.

Further elucidation of the interactions among these frontostriatal circuits will require circuit based studies using new technologies. It would be interesting to investigate (i) the effects of over-activation of the lOFC and mOFC, to understand if their opposing action is limited to inactivity, and (ii) to test the anatomical circuitry using DREADDs technology to target specific projections from the OFC and manipulate the pathways in an excitatory or inhibitory manner.

In conclusion, the findings reported in this thesis expand on our knowledge of the role of the PFC in reversal learning, particularly the lOFC and mOFC, and the role of striatal dopamine in cognitive flexibility.

REFERENCES

Adamantidis, A. R. *et al.* (2011) 'Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(30), pp. 10829–35. doi: 10.1523/JNEUROSCI.2246-11.2011.

Aghajanian, G. K. and Bunney, B. S. (1977) 'Dopamine "autoreceptors" pharmacological characterization by microiontophoretic single cell recording studies.', *Naunyn-Schmiedeberg's archives of pharmacology*, 297(1), pp. 1–7.

Ahmari, S. E. *et al.* (2013) 'Repeated cortico-striatal stimulation generates persistent OCD-like behavior.', *Science (New York, N.Y.)*, 340(6137), pp. 1234–9. doi: 10.1126/science.1234733.

Alexander, G. E. and Crutcher, M. D. (1990) 'Functional architecture of basal ganglia circuits: neural substrates of parallel processing.', *Trends in neurosciences*, 13(7), pp. 266–71.

Alexander, G. E., DeLong, M. R. and Strick, P. L. (1986) 'Parallel organization of functionally segregated circuits linking basal ganglia and cortex', *Annual Review of Neuroscience*, pp. 357–381.

Alsiö, J. *et al.* (2015) 'The role of 5-HT2C receptors in touchscreen visual reversal learning in the rat: a cross-site study.', *Psychopharmacology*. doi: 10.1007/s00213-015-3963-5.

Alsiö, J. *et al.* (2019) 'Dopamine D2-like receptor stimulation blocks negative feedback in visual and spatial reversal learning in the rat: behavioural and computational evidence.', *Psychopharmacology*, 236(8), pp. 2307–2323. doi: 10.1007/s00213-019-05296-y.

Amodeo, D. A. *et al.* (2017) '5HT2A receptor blockade in dorsomedial striatum reduces repetitive behaviors in BTBR mice.', *Genes, brain, and behavior*. NIH Public Access, 16(3), pp. 342–351. doi: 10.1111/gbb.12343.

Armbruster, B. N. *et al.* (2007) 'Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand.', *Proceedings of the National Academy of Sciences of the United States of America*, 104(12), pp. 5163–8. doi: 10.1073/pnas.0700293104.

Ashwell, R. and Ito, R. (2014) 'Excitotoxic lesions of the infralimbic, but not prelimbic cortex facilitate reversal of appetitive discriminative context conditioning: the role of the infralimbic cortex in context generalization', *Frontiers in Behavioral Neuroscience*, 8, p. 63. doi: 10.3389/fnbeh.2014.00063.

Badre, D. *et al.* (2012) 'Rostrolateral Prefrontal Cortex and Individual Differences in Uncertainty-Driven Exploration', *Neuron*, 73(3), pp. 595–607. doi: 10.1016/j.neuron.2011.12.025.

Balleine, B. W. and O'Doherty, J. P. (2010) 'Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 35(1), pp. 48–69. doi: 10.1038/npp.2009.131.

Bardin, L. *et al.* (2003) 'Profound, non-opioid analgesia produced by the high-efficacy 5-HT(1A) agonist F 13640 in the formalin model of tonic nociceptive pain.', *Pharmacology*, 67(4), pp. 182–94. doi: 10.1159/000068404.

Barlow, R. L. *et al.* (2015) 'Markers of Serotonergic Function in the Orbitofrontal Cortex and Dorsal Raphé Nucleus Predict Individual Variation in Spatial-Discrimination Serial Reversal Learning', *Neuropsychopharmacology*, 40(7), pp. 1619–1630. doi: 10.1038/npp.2014.335.

Barnes, N. M. and Sharp, T. (1999) 'A review of central 5-HT receptors and their function', *Neuropharmacology*. Elsevier Ltd, 38(8), pp. 1083–1152. doi: 10.1016/S0028-3908(99)00010-6.

Bauer, R. H. and Fuster, J. M. (1976) 'Delayed-matching and delayed-response deficit from cooling dorsolateral prefrontal cortex in monkeys.', *Journal of comparative and physiological psychology*, 90(3), pp. 293–302.

Baxter, M. G. *et al.* (2008) 'Dorsolateral prefrontal lesions do not impair tests of scene learning and decision-making that require frontal-temporal interaction.', *The European journal of neuroscience*, 28(3), pp. 491–9. doi: 10.1111/j.1460-9568.2008.06353.x.

Baxter, M. G. *et al.* (2009) 'Ventrolateral prefrontal cortex is required for performance of a strategy implementation task but not reinforcer devaluation effects in rhesus monkeys.', *The European journal of neuroscience*, 29(10), pp. 2049–59. doi: 10.1111/j.1460-9568.2009.06740.x.

Beaulieu, J.-M. and Gainetdinov, R. R. (2011) 'The physiology, signaling, and pharmacology of dopamine receptors.', *Pharmacological reviews*, 63(1), pp. 182–217. doi: 10.1124/pr.110.002642.

Bender, D., Holschbach, M. and Stöcklin, G. (1994) 'Synthesis of n.c.a. carbon-11 labelled clozapine and its major metabolite clozapine-N-oxide and comparison of their biodistribution in mice.', *Nuclear medicine and biology*, 21(7), pp. 921–5.

Bertran-Gonzalez, J. *et al.* (2010) 'What is the degree of segregation between striatonigral and striatopallidal projections?', *Frontiers in Neuroanatomy*, 4. doi: 10.3389/fnana.2010.00136.

Birrell, J. M. and Brown, V. J. (2000) 'Medial frontal cortex mediates perceptual attentional set shifting in the rat.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 20(11), pp. 4320–4.

de Boer, S. F. and Koolhaas, J. M. (2005) '5-HT1A and 5-HT1B receptor agonists and aggression: a pharmacological challenge of the serotonin deficiency hypothesis.', *European journal of pharmacology*, 526(1–3), pp. 125–39. doi: 10.1016/j.ejphar.2005.09.065.

Borman, R. A. *et al.* (2002) '5-HT2B receptors play a key role in mediating the excitatory effects of 5-HT in human colon in vitro', *British Journal of Pharmacology*,

135(5), pp. 1144–1151. doi: 10.1038/sj.bjp.0704571.

Boulougouris, V., Castañé, A. and Robbins, T. W. (2009) 'Dopamine D2/D3 receptor agonist quinpirole impairs spatial reversal learning in rats: investigation of D3 receptor involvement in persistent behavior.', *Psychopharmacology*. Springer-Verlag, 202(4), pp. 611–20. doi: 10.1007/s00213-008-1341-2.

Boulougouris, V., Dalley, J. W. and Robbins, T. W. (2007) 'Effects of orbitofrontal, infralimbic and prelimbic cortical lesions on serial spatial reversal learning in the rat', *Behavioural Brain Research*. Elsevier, 179(2), pp. 219–228. doi: 10.1016/J.BBR.2007.02.005.

Boulougouris, V., Glennon, J. C. and Robbins, T. W. (2008) 'Dissociable effects of selective 5-HT2A and 5-HT2C receptor antagonists on serial spatial reversal learning in rats.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 33(8), pp. 2007–19. doi: 10.1038/sj.npp.1301584.

Boulougouris, V. and Robbins, T. W. (2010) 'Enhancement of spatial reversal learning by 5-HT2C receptor antagonism is neuroanatomically specific.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(3), pp. 930–8. doi: 10.1523/JNEUROSCI.4312-09.2010.

Brigman, J. L. *et al.* (2010) 'Pharmacological or genetic inactivation of the serotonin transporter improves reversal learning in mice.', *Cerebral cortex (New York, N.Y. : 1991)*, 20(8), pp. 1955–63. doi: 10.1093/cercor/bhp266.

Brown, H. D. *et al.* (2012) 'The selective serotonin reuptake inhibitor, escitalopram, enhances inhibition of prepotent responding and spatial reversal learning.', *Journal of psychopharmacology (Oxford, England)*, 26(11), pp. 1443–55. doi: 10.1177/0269881111430749.

Brown, V. J. and Bowman, E. M. (2002) 'Rodent models of prefrontal cortical function.', *Trends in neurosciences*, 25(7), pp. 340–3.

Burguière, E. *et al.* (2013) 'Optogenetic stimulation of lateral orbitofronto-striatal pathway suppresses compulsive behaviors.', *Science (New York, N.Y.)*, 340(6137), pp. 1243–6. doi: 10.1126/science.1232380.

Burguière, E. *et al.* (2014) 'Striatal circuits, habits, and implications for obsessive-compulsive disorder.', *Current opinion in neurobiology*, 30C, pp. 59–65. doi: 10.1016/j.conb.2014.08.008.

Burguière, E. *et al.* (2015) 'Striatal circuits, habits, and implications for obsessive–compulsive disorder', *Current Opinion in Neurobiology*, 30, pp. 59–65. doi: 10.1016/j.conb.2014.08.008.

Bussey, T. J. *et al.* (2012) 'New translational assays for preclinical modelling of cognition in schizophrenia: The touchscreen testing method for mice and rats', *Neuropharmacology*, 62(3), pp. 1191–1203. doi: 10.1016/j.neuropharm.2011.04.011.

Bussey, T. J., Everitt, B. J. and Robbins, T. W. (1997) 'Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel Pavlovian autoshaping procedure for the rat: implications for the neurobiology of

emotion.', *Behavioral neuroscience*. United States, 111(5), pp. 908–919.

Butter, C. M. (1969) *Perseveration in Extinction and in Discrimination Reversal Tasks Following Selective Frontal Ablations in Macaca Mulatta'*. Pergamoll Press.

Cagniard, B. *et al.* (2006) 'Dopamine scales performance in the absence of new learning.', *Neuron*, 51(5), pp. 541–7. doi: 10.1016/j.neuron.2006.07.026.

Calabresi, P. *et al.* (2007) 'Dopamine-mediated regulation of corticostriatal synaptic plasticity', *Trends in Neurosciences*, pp. 211–219. doi: 10.1016/j.tins.2007.03.001.

Calaminus, C. and Hauber, W. (2007) 'Intact discrimination reversal learning but slowed responding to reward-predictive cues after dopamine D1 and D2 receptor blockade in the nucleus accumbens of rats', *Psychopharmacology*, 191(3), pp. 551–566. doi: 10.1007/s00213-006-0532-y.

Cardinal, R. N. and Everitt, B. J. (2004) 'Neural and psychological mechanisms underlying appetitive learning: links to drug addiction.', *Current opinion in neurobiology*, 14(2), pp. 156–62. doi: 10.1016/j.conb.2004.03.004.

Carli, M. *et al.* (1983) 'Effects of lesions to ascending noradrenergic neurones on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal.', *Behavioural brain research*, 9(3), pp. 361–80.

Castañé, A., Theobald, D. E. H. and Robbins, T. W. (2010) 'Selective lesions of the dorsomedial striatum impair serial spatial reversal learning in rats.', *Behavioural brain research*, 210(1), pp. 74–83. doi: 10.1016/j.bbr.2010.02.017.

Cavanagh, J. F. *et al.* (2012) 'Frontal Theta Reflects Uncertainty and Unexpectedness during Exploration and Exploitation', *Cerebral Cortex*, 22(11), pp. 2575–2586. doi: 10.1093/cercor/bhr332.

Chase, E. A., Tait, D. S. and Brown, V. J. (2012) 'Lesions of the orbital prefrontal cortex impair the formation of attentional set in rats.', *The European journal of neuroscience*, 36(3), pp. 2368–75. doi: 10.1111/j.1460-9568.2012.08141.x.

Chojnacka-Wójcik, E., Kłodzińska, A. and Tatarczyńska, E. (2005) 'The anxiolytic-like effect of 5-HT1B receptor ligands in rats: a possible mechanism of action.', *The Journal of pharmacy and pharmacology*, 57(2), pp. 253–7. doi: 10.1211/0022357055399.

Chudasama, Y. *et al.* (2003) 'Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity', *Behavioural Brain Research*, 146(1–2), pp. 105–119. doi: 10.1016/j.bbr.2003.09.020.

Chudasama, Y. (2011) 'Animal models of prefrontal-executive function.', *Behavioral Neuroscience*, 125(3), pp. 327–343. doi: 10.1037/a0023766.

Chudasama, Y., Bussey, T. J. and Muir, J. L. (2001) 'Effects of selective thalamic and prelimbic cortex lesions on two types of visual discrimination and reversal learning.', *The European journal of neuroscience*, 14(6), pp. 1009–20.

Chudasama, Y. and Robbins, T. W. (2003) 'Dissociable Contributions of the

Orbitofrontal and Infralimbic Cortex to Pavlovian Autoshaping and Discrimination Reversal Learning: Further Evidence for the Functional Heterogeneity of the Rodent Frontal Cortex', *Journal of Neuroscience*, 23(25).

Chudasama, Y. and Robbins, T. W. (2004) 'Psychopharmacological approaches to modulating attention in the five-choice serial reaction time task: implications for schizophrenia', *Psychopharmacology*, 174(1), pp. 86–98. doi: 10.1007/s00213-004-1805-y.

Chudasama, Y. and Robbins, T. W. W. (2006) 'Functions of frontostriatal systems in cognition: Comparative neuropsychopharmacological studies in rats, monkeys and humans', *Biological Psychology*, 73(1), pp. 19–38. doi: 10.1016/j.biopsycho.2006.01.005.

Clarke, H. F. *et al.* (2004) 'Cognitive inflexibility after prefrontal serotonin depletion.', *Science (New York, N.Y.)*, 304(5672), pp. 878–80. doi: 10.1126/science.1094987.

Clarke, H. F. *et al.* (2011) 'Dopamine, but not serotonin, regulates reversal learning in the marmoset caudate nucleus.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(11), pp. 4290–7. doi: 10.1523/JNEUROSCI.5066-10.2011.

Clarke, H. F., Robbins, T. W. and Roberts, A. C. (2008) 'Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 28(43), pp. 10972–82. doi: 10.1523/JNEUROSCI.1521-08.2008.

Clatworthy, P. L. *et al.* (2009) 'Dopamine release in dissociable striatal subregions predicts the different effects of oral methylphenidate on reversal learning and spatial working memory.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(15), pp. 4690–6. doi: 10.1523/JNEUROSCI.3266-08.2009.

Cohen, J. (1988) *Statistical Power Analysis for the Behavioral Sciences*. New York.

Collins, P. *et al.* (1998) 'Perseveration and strategy in a novel spatial self-ordered sequencing task for nonhuman primates: Effects of excitotoxic lesions and dopamine depletions of the prefrontal cortex', *Journal of Cognitive Neuroscience*, 10(3), pp. 332–354. doi: 10.1162/089892998562771.

Contreras, F. *et al.* (2002) 'Dopamine, hypertension and obesity.', *Journal of human hypertension*, 16 Suppl 1, pp. S13-7. doi: 10.1038/sj.jhh.1001334.

Cools, R. (2001) 'Enhanced or Impaired Cognitive Function in Parkinson's Disease as a Function of Dopaminergic Medication and Task Demands', *Cerebral Cortex*. Oxford University Press (OUP), 11(12), pp. 1136–1143. doi: 10.1093/cercor/11.12.1136.

Cools, R. *et al.* (2002) 'Defining the neural mechanisms of probabilistic reversal learning using event-related functional magnetic resonance imaging.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(11), pp. 4563–7. doi: 20026435.

Cools, R. *et al.* (2007) 'L-DOPA disrupts activity in the nucleus accumbens during reversal learning in Parkinson's disease.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 32(1), pp. 180–9. doi: 10.1038/sj.npp.1301153.

Corbit, L. H. and Balleine, B. W. (2003) 'The role of prelimbic cortex in instrumental conditioning.', *Behavioural brain research*, 146(1–2), pp. 145–57.

Costa, V. D. *et al.* (2015) 'Reversal learning and dopamine: a bayesian perspective.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 35(6), pp. 2407–16. doi: 10.1523/JNEUROSCI.1989-14.2015.

Crofts, H. S. *et al.* (1999) 'Home cage presentation of complex discrimination tasks to marmosets and rhesus monkeys', *Laboratory Animals*. Royal Society of Medicine Press Ltd, 33(3), pp. 207–214. doi: 10.1258/002367799780578174.

Cui, G. *et al.* (2013) 'Concurrent activation of striatal direct and indirect pathways during action initiation.', *Nature*, 494(7436), pp. 238–42. doi: 10.1038/nature11846.

Dagher, A. and Robbins, T. W. (2009) 'Personality, addiction, dopamine: insights from Parkinson's disease.', *Neuron*, 61(4), pp. 502–10. doi: 10.1016/j.neuron.2009.01.031.

Dalley, J. W., Cardinal, R. N. and Robbins, T. W. (2004) 'Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates', *Neuroscience & Biobehavioral Reviews*. Pergamon, 28(7), pp. 771–784.

Dalton, G. L. *et al.* (2016) 'Multifaceted Contributions by Different Regions of the Orbitofrontal and Medial Prefrontal Cortex to Probabilistic Reversal Learning.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 36(6), pp. 1996–2006. doi: 10.1523/JNEUROSCI.3366-15.2016.

Dalton, G. L., Phillips, A. G. and Floresco, S. B. (2014) 'Preferential Involvement by Nucleus Accumbens Shell in Mediating Probabilistic Learning and Reversal Shifts', *Journal of Neuroscience*, 34(13), pp. 4618–4626. doi: 10.1523/JNEUROSCI.5058-13.2014.

Daw, N. D. *et al.* (2006) 'Cortical substrates for exploratory decisions in humans', *Nature*, 441(7095), pp. 876–879. doi: 10.1038/nature04766.

Delle Donne, K. T., Sesack, S. R. and Pickel, V. M. (1996) 'Ultrastructural immunocytochemical localization of neurotensin and the dopamine D2 receptor in the rat nucleus accumbens', *The Journal of Comparative Neurology*, 371(4), pp. 552–566. doi: 10.1002/(SICI)1096-9861(19960805)371:4<552::AID-CNE5>3.0.CO;2-3.

Dias, R., Robbins, T. W. and Roberts, A. C. (1996) 'Dissociation in prefrontal cortex of affective and attentional shifts.', *Nature*, 380(6569), pp. 69–72. doi: 10.1038/380069a0.

Dias, R., Robbins, T. W. and Roberts, A. C. (1997) 'Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: restriction to novel situations and independence from "on-line" processing.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 17(23), pp. 9285–97.

Dickinson, A. and Balleine, B. (1994) 'Motivational control of goal-directed action', *Animal Learning & Behavior*. Springer-Verlag, 22(1), pp. 1–18. doi: 10.3758/bf03199951.

Domenech, P. and Koechlin, E. (2015) 'Executive control and decision-making in the prefrontal cortex', *Current Opinion in Behavioral Sciences*, 1, pp. 101–106. doi: 10.1016/j.cobeha.2014.10.007.

Donoso, M., Collins, A. G. E. and Koechlin, E. (2014) 'Foundations of human reasoning in the prefrontal cortex', *Science*, 344(6191).

Dudchenko, P. A. (2004) 'An overview of the tasks used to test working memory in rodents', *Neuroscience & Biobehavioral Reviews*, 28(7), pp. 699–709. doi: 10.1016/j.neubiorev.2004.09.002.

Durstewitz, D. *et al.* (2010) 'Abrupt Transitions between Prefrontal Neural Ensemble States Accompany Behavioral Transitions during Rule Learning', *Neuron*, 66(3), pp. 438–448. doi: 10.1016/j.neuron.2010.03.029.

Dvorkin, A. *et al.* (2010) 'Features of compulsive checking behavior mediated by nucleus accumbens and orbital frontal cortex.', *The European journal of neuroscience*, 32(9), pp. 1552–63. doi: 10.1111/j.1460-9568.2010.07398.x.

Eilam, D. and Szechtman, H. (1989) 'Biphasic effect of D-2 agonist quinpirole on locomotion and movements.', *European journal of pharmacology*, 161(2–3), pp. 151–7.

Emmons, E. B. *et al.* (2017) 'Rodent Medial Frontal Control of Temporal Processing in the Dorsomedial Striatum', *Journal of Neuroscience*, 37(36).

Ersche, K. D. *et al.* (2008) 'Chronic cocaine but not chronic amphetamine use is associated with perseverative responding in humans', *Psychopharmacology*, 197(3), pp. 421–431. doi: 10.1007/s00213-007-1051-1.

Ersche, K. D. *et al.* (2011) 'Response perseveration in stimulant dependence is associated with striatal dysfunction and can be ameliorated by a D(2/3) receptor agonist.', *Biological psychiatry*, 70(8), pp. 754–62. doi: 10.1016/j.biopsych.2011.06.033.

Everitt, B. J., Dickinson, A. and Robbins, T. W. (2001) 'The neuropsychological basis of addictive behaviour.', *Brain research. Brain research reviews*, 36(2–3), pp. 129–38.

Faure, A. *et al.* (2005) 'Lesion to the Nigrostriatal Dopamine System Disrupts Stimulus-Response Habit Formation', *Journal of Neuroscience*, 25(11), pp. 2771–2780. doi: 10.1523/JNEUROSCI.3894-04.2005.

Fellows, Lesley K and Farah, M. J. (2003) 'Ventromedial frontal cortex mediates affective shifting in humans: evidence from a reversal learning paradigm.', *Brain : a journal of neurology*, 126(Pt 8), pp. 1830–7. doi: 10.1093/brain/awg180.

Fellows, L. K. and Farah, M. J. (2003) 'Ventromedial frontal cortex mediates affective shifting in humans: evidence from a reversal learning paradigm', *Brain*, 126(8), pp. 1830–1837. doi: 10.1093/brain/awg180.

Ferguson, S. M. *et al.* (2011) 'Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization.', *Nature neuroscience*, 14(1), pp. 22–4.

doi: 10.1038/nn.2703.

Ferguson, S. M. *et al.* (2013) 'Direct-pathway striatal neurons regulate the retention of decision-making strategies.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*. Society for Neuroscience, 33(28), pp. 11668–76. doi: 10.1523/JNEUROSCI.4783-12.2013.

Fettes, P., Schulze, L. and Downar, J. (2017) 'Cortico-Striatal-Thalamic Loop Circuits of the Orbitofrontal Cortex: Promising Therapeutic Targets in Psychiatric Illness.', *Frontiers in systems neuroscience*, 11, p. 25. doi: 10.3389/fnsys.2017.00025.

Floresco, S. B. *et al.* (2006) 'Multiple Dopamine Receptor Subtypes in the Medial Prefrontal Cortex of the Rat Regulate Set-Shifting', *Neuropsychopharmacology*, 31(2), pp. 297–309. doi: 10.1038/sj.npp.1300825.

Frank, M. J. and Claus, E. D. (2006) 'Anatomy of a decision: Striato-orbitofrontal interactions in reinforcement learning, decision making, and reversal.', *Psychological Review*, 113(2), pp. 300–326. doi: 10.1037/0033-295X.113.2.300.

Fuchs, R. A. *et al.* (2004) 'Differential Involvement of Orbitofrontal Cortex Subregions in Conditioned Cue-Induced and Cocaine-Primed Reinstatement of Cocaine Seeking in Rats', *Journal of Neuroscience*, 24(29), pp. 6600–6610. doi: 10.1523/JNEUROSCI.1924-04.2004.

Fuster, J. M. (1985) 'Temporal organization of behaviour', *Human Neurobiology*, 4(2), pp. 57–60.

Fuster, J. M. (1988) 'Prefrontal Cortex', in *Comparative Neuroscience and Neurobiology*. Boston, MA: Birkhäuser Boston, pp. 107–109. doi: 10.1007/978-1-4899-6776-3_43.

Fuster, J. M. (1997) *The prefrontal cortex : anatomy, physiology, and neuropsychology of the frontal lobe*. Lippincott-Raven.

Gallagher, M., McMahan, R. W. and Schoenbaum, G. (1999) 'Orbitofrontal cortex and representation of incentive value in associative learning.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 19(15), pp. 6610–4.

Gerfen, C. R. *et al.* (1990) 'D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons.', *Science (New York, N.Y.)*, 250(4986), pp. 1429–32.

Gerfen, C. R. and Surmeier, D. J. (2011) 'Modulation of Striatal Projection Systems by Dopamine', *Annual Review of Neuroscience*, 34(1), pp. 441–466. doi: 10.1146/annurev-neuro-061010-113641.

Gerfen, C. R. and Young, W. S. (1988) 'Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study.', *Brain research*, 460(1), pp. 161–7.

Gether, U. (2000) 'Uncovering molecular mechanisms involved in activation of G protein-coupled receptors.', *Endocrine reviews*, 21(1), pp. 90–113. doi: 10.1210/edrv.21.1.0390.

Ghods-Sharifi, S., Haluk, D. M. and Floresco, S. B. (2008) 'Differential effects of

inactivation of the orbitofrontal cortex on strategy set-shifting and reversal learning.', *Neurobiology of learning and memory*, 89(4), pp. 567–73. doi: 10.1016/j.nlm.2007.10.007.

Girden, E. (1992) *ANOVA: Repeated Measures*. Newbury Park, CA: SAGE Publications, Inc. doi: 10.4135/9781412983419.

Gomez, J. L. *et al.* (2017) 'Chemogenetics revealed: DREADD occupancy and activation via converted clozapine', *Science*, 357(6350), pp. 503–507. doi: 10.1126/science.aan2475.

Gourley, S. L. *et al.* (2010) 'Dissociable regulation of instrumental action within mouse prefrontal cortex', *European Journal of Neuroscience*, 32(10), pp. 1726–1734. doi: 10.1111/j.1460-9568.2010.07438.x.

Grace, A. A. *et al.* (2007) 'Regulation of firing of dopaminergic neurons and control of goal-directed behaviors', *Trends in Neurosciences*, 30(5), pp. 220–227. doi: 10.1016/j.tins.2007.03.003.

Graybeal, C. *et al.* (2011) 'Paradoxical reversal learning enhancement by stress or prefrontal cortical damage: rescue with BDNF.', *Nature neuroscience*, 14(12), pp. 1507–9. doi: 10.1038/nn.2954.

Graybeal, C. *et al.* (2014) 'Strains and stressors: an analysis of touchscreen learning in genetically diverse mouse strains', *PLoS ONE*, 9.

Graybiel, A. M. and Rauch, S. L. (2000) 'Toward a neurobiology of obsessive-compulsive disorder.', *Neuron*, 28(2), pp. 343–7.

Gremel, C. M. and Costa, R. M. (2013) 'Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions.', *Nature communications*, 4, p. 2264. doi: 10.1038/ncomms3264.

Groenewegen, H. J. *et al.* (1999) 'Convergence and segregation of ventral striatal inputs and outputs', in *Annals of the New York Academy of Sciences*. New York Academy of Sciences, pp. 49–63. doi: 10.1111/j.1749-6632.1999.tb09260.x.

Groenewegen, H. J. and Berendse, H. W. (1994) 'Anatomical Relationships Between the Prefrontal Cortex and the Basal Ganglia in the Rat', in, pp. 51–77. doi: 10.1007/978-3-642-85007-3_4.

Groman, S. M. *et al.* (2011) 'Dorsal striatal D2-like receptor availability covaries with sensitivity to positive reinforcement during discrimination learning.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(20), pp. 7291–9. doi: 10.1523/JNEUROSCI.0363-11.2011.

Groman, S. M. *et al.* (2013) 'Monoamine levels within the orbitofrontal cortex and putamen interact to predict reversal learning performance.', *Biological psychiatry*, 73(8), pp. 756–62. doi: 10.1016/j.biopsych.2012.12.002.

Haber, S. N. (2003) 'The primate basal ganglia: parallel and integrative networks.', *Journal of chemical neuroanatomy*, 26(4), pp. 317–30.

Haber, S. N. and Knutson, B. (2010) 'The Reward Circuit: Linking Primate Anatomy and Human Imaging', *Neuropsychopharmacology*. Nature Publishing Group, 35(1), pp. 4–26. doi: 10.1038/npp.2009.129.

Haluk, D. M. and Floresco, S. B. (2009) 'Ventral Striatal Dopamine Modulation of Different Forms of Behavioral Flexibility', *Neuropsychopharmacology*. Nature Publishing Group, 34(8), pp. 2041–2052. doi: 10.1038/npp.2009.21.

Hamilton, D. A. and Brigman, J. L. (2015) 'Behavioral flexibility in rats and mice: contributions of distinct frontocortical regions.', *Genes, brain, and behavior*, 14(1), pp. 4–21. doi: 10.1111/gbb.12191.

Hayden, B. Y., Pearson, J. M. and Platt, M. L. (2011) 'Neuronal basis of sequential foraging decisions in a patchy environment', *Nature Neuroscience*, 14(7), pp. 933–939. doi: 10.1038/nn.2856.

Heilbronner, S. R. *et al.* (2016) 'Circuit-Based Corticostriatal Homologies Between Rat and Primate', *Biological Psychiatry*, 80(7), pp. 509–521. doi: 10.1016/j.biopsych.2016.05.012.

den Heuvel, O. A. van *et al.* (2010) 'Frontal–striatal abnormalities underlying behaviours in the compulsive–impulsive spectrum', *Journal of the Neurological Sciences*, 289(1–2), pp. 55–59. doi: 10.1016/j.jns.2009.08.043.

Hikida, T. *et al.* (2010) 'Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior.', *Neuron*, 66(6), pp. 896–907. doi: 10.1016/j.neuron.2010.05.011.

Hikida, T. *et al.* (2013) 'Pathway-specific modulation of nucleus accumbens in reward and aversive behavior via selective transmitter receptors', *Proceedings of the National Academy of Sciences*, 110(1), pp. 342–347. doi: 10.1073/pnas.1220358110.

Holland, P. C. and Rescorla, R. A. (1975) 'Second-order conditioning with food unconditioned stimulus.', *Journal of comparative and physiological psychology*, 88(1), pp. 459–67.

Hoover, W. B. and Vertes, R. P. (2011) 'Projections of the medial orbital and ventral orbital cortex in the rat', *The Journal of Comparative Neurology*, 519(18), pp. 3766–3801. doi: 10.1002/cne.22733.

Howell, D. C. (2013) *Statistical methods for psychology*. Wadsworth Cengage Learning.

Hvoslef-Eide, M. *et al.* (2015) 'Cognitive Translation Using the Rodent Touchscreen Testing Approach', in *Current topics in behavioral neurosciences*, pp. 423–447. doi: 10.1007/7854_2015_5007.

Isomura, Y. *et al.* (2013) 'Reward-modulated motor information in identified striatum neurons.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 33(25), pp. 10209–20. doi: 10.1523/JNEUROSCI.0381-13.2013.

Iversen, S. D. and Mishkin, M. (1970) 'Perseverative interference in monkeys following selective lesions of the inferior prefrontal convexity.', *Experimental brain research*, 11(4), pp. 376–86.

Izquierdo, A *et al.* (2006) 'Genetic and dopaminergic modulation of reversal learning in a touchscreen-based operant procedure for mice', *Behavioural Brain Research*. Elsevier, 171(2), pp. 181–188.

Izquierdo, Alicia *et al.* (2006) 'Genetic and dopaminergic modulation of reversal

learning in a touchscreen-based operant procedure for mice', *Behavioural Brain Research*. Elsevier, 171(2), pp. 181–188. doi: 10.1016/J.BBR.2006.03.029.

Izquierdo, A. *et al.* (2012) 'Impaired reward learning and intact motivation after serotonin depletion in rats.', *Behavioural brain research*, 233(2), pp. 494–9. doi: 10.1016/j.bbr.2012.05.032.

Izquierdo, A. *et al.* (2013) 'Basolateral Amygdala Lesions Facilitate Reward Choices after Negative Feedback in Rats', *Journal of Neuroscience*, 33(9), pp. 4105–4109. doi: 10.1523/JNEUROSCI.4942-12.2013.

Izquierdo, A. (2017) 'Functional Heterogeneity within Rat Orbitofrontal Cortex in Reward Learning and Decision Making.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 37(44), pp. 10529–10540. doi: 10.1523/JNEUROSCI.1678-17.2017.

Izquierdo, A. *et al.* (2017) 'The neural basis of reversal learning: An updated perspective', *Neuroscience*, 345, pp. 12–26. doi: 10.1016/j.neuroscience.2016.03.021.

Izquierdo, A., Suda, R. K. and Murray, E. A. (2004) 'Bilateral Orbital Prefrontal Cortex Lesions in Rhesus Monkeys Disrupt Choices Guided by Both Reward Value and Reward Contingency', *Journal of Neuroscience*, 24(34), pp. 7540–7548. doi: 10.1523/JNEUROSCI.1921-04.2004.

Jacobsen, C. F. (1936) 'Studies of cerebral function in primates. I. The functions of the frontal association areas in monkeys.', *Comparative Psychology Monographs*, 13, 3, pp. 1–60.

Jbabdi, S. *et al.* (2013) 'Human and Monkey Ventral Prefrontal Fibers Use the Same Organizational Principles to Reach Their Targets: Tracing versus Tractography', *Journal of Neuroscience*, 33(7).

Jentsch, J. D. *et al.* (1997) 'Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine.', *Science (New York, N.Y.)*. American Association for the Advancement of Science, 277(5328), pp. 953–5. doi: 10.1126/SCIENCE.277.5328.953.

Jentsch, J. and Taylor, J. R. (2001) 'Impaired Inhibition of Conditioned Responses Produced by Subchronic Administration of Phencyclidine to Rats', *Neuropsychopharmacology*. Nature Publishing Group, 24(1), pp. 66–74. doi: 10.1016/S0893-133X(00)00174-3.

Jocham, G. *et al.* (2009) 'Dopamine DRD2 polymorphism alters reversal learning and associated neural activity.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(12), pp. 3695–704. doi: 10.1523/JNEUROSCI.5195-08.2009.

Jones, B. and Mishkin, M. (1972) 'Limbic lesions and the problem of stimulus—Reinforcement associations', *Experimental Neurology*, 36(2), pp. 362–377. doi: 10.1016/0014-4886(72)90030-1.

Jones, E. G., Huntley, G. W. and Benson, D. L. (1994) 'Alpha calcium/calmodulin-dependent protein kinase II selectively expressed in a subpopulation of excitatory neurons in monkey sensory-motor cortex: comparison with GAD-67 expression.',

The Journal of neuroscience : the official journal of the Society for Neuroscience, 14(2), pp. 611–29.

Karlsson, M. P., Tervo, D. G. R. and Karpova, A. Y. (2012) 'Network Resets in Medial Prefrontal Cortex Mark the Onset of Behavioral Uncertainty', *Science*, 338(6103), pp. 135–139. doi: 10.1126/science.1226518.

Kennett, G. A. *et al.* (1997) 'SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist.', *Neuropharmacology*, 36(4–5), pp. 609–20. doi: 10.1016/s0028-3908(97)00038-5.

Killcross, S. and Coutureau, E. (2003) 'Coordination of actions and habits in the medial prefrontal cortex of rats.', *Cerebral cortex (New York, N.Y. : 1991)*, 13(4), pp. 400–8.

Kim, C. H. *et al.* (2015) 'The continuous performance test (rCPT) for mice: a novel operant touchscreen test of attentional function', *Psychopharmacology*, 232(21–22), pp. 3947–3966. doi: 10.1007/s00213-015-4081-0.

Kim, J. and Ragozzino, M. E. (2005) 'The involvement of the orbitofrontal cortex in learning under changing task contingencies', *Neurobiology of Learning and Memory*, 83(2), pp. 125–133. doi: 10.1016/j.nlm.2004.10.003.

King, A. R., Martin, I. L. and Melville, K. A. (1974) 'Reversal learning enhanced by lysergic acid diethylamide (LSD): concomitant rise in brain 5-hydroxytryptamine levels.', *British journal of pharmacology*, 52(3), pp. 419–26. doi: 10.1111/j.1476-5381.1974.tb08611.x.

Klanker, M. *et al.* (2015) 'Phasic dopamine release induced by positive feedback predicts individual differences in reversal learning', *Neurobiology of Learning and Memory*, 125, pp. 135–145. doi: 10.1016/j.nlm.2015.08.011.

Klanker, M. *et al.* (2017) 'Regionally distinct phasic dopamine release patterns in the striatum during reversal learning', *Neuroscience*, 345, pp. 110–123. doi: 10.1016/j.neuroscience.2016.05.011.

Kolb, B. (1990) 'Prefrontal cortex', in *The cerebral cortex of the rat*, pp. 437–458.

Kolling, N. *et al.* (2012) 'Neural mechanisms of foraging.', *Science (New York, N.Y.)*. Europe PMC Funders, 336(6077), pp. 95–8. doi: 10.1126/science.1216930.

Kravitz, A. V. *et al.* (2010) 'Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry', *Nature*. Nature Publishing Group, 466(7306), pp. 622–626. doi: 10.1038/nature09159.

Kreitzer, A. C. and Malenka, R. C. (2007) 'Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models.', *Nature*, 445(7128), pp. 643–7. doi: 10.1038/nature05506.

Kringelbach, M. L. and Berridge, K. C. (2010) 'The Functional Neuroanatomy of Pleasure and Happiness', *Discovery Medicine*, 9(49), pp. 579–587.

Kringelback, M. L. and Rolls, E. T. (2003) 'Neural correlates of rapid reversal learning in a simple model of human social interaction', *NeuroImage*. Academic Press, 20(2), pp. 1371–1383. doi: 10.1016/S1053-8119(03)00393-8.

Kruzich, P. J. and Grandy, D. K. (2004) 'Dopamine D2 receptors mediate two-odor

discrimination and reversal learning in C57BL/6 mice.', *BMC Neuroscience*, 5(1), p. 12. doi: 10.1186/1471-2202-5-12.

Kügler, S., Kilic, E. and Bähr, M. (2003) 'Human synapsin 1 gene promoter confers highly neuron-specific long-term transgene expression from an adenoviral vector in the adult rat brain depending on the transduced area', *Gene Therapy*, 10(4), pp. 337–347. doi: 10.1038/sj.gt.3301905.

Lapiz-Bluhm, M. D. S. *et al.* (2009) 'Chronic intermittent cold stress and serotonin depletion induce deficits of reversal learning in an attentional set-shifting test in rats.', *Psychopharmacology*, 202(1–3), pp. 329–41. doi: 10.1007/s00213-008-1224-6.

Laughlin, R. E. *et al.* (2011) 'Genetic dissection of behavioral flexibility: reversal learning in mice.', *Biological psychiatry*, 69(11), pp. 1109–16. doi: 10.1016/j.biopsych.2011.01.014.

Lawrence, A. D. *et al.* (1999) 'Discrimination, reversal, and shift learning in Huntington's disease: Mechanisms of impaired response selection', *Neuropsychologia*. Elsevier Ltd, 37(12), pp. 1359–1374. doi: 10.1016/S0028-3932(99)00035-4.

Lawrence, A. D., Sahakian, B. J. and Robbins, T. W. (1998) 'Cognitive functions and corticostriatal circuits: insights from Huntington's disease', *Trends in Cognitive Sciences*, 2(10), pp. 379–388. doi: 10.1016/S1364-6613(98)01231-5.

Lee, B. *et al.* (2007) 'Dopamine D2/D3 receptors play a specific role in the reversal of a learned visual discrimination in monkeys.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 32(10), pp. 2125–34. doi: 10.1038/sj.npp.1301337.

Lee, D. K., George, S. R. and O'Dowd, B. F. (2003) 'Continued discovery of ligands for G protein-coupled receptors', *Life Sciences*, 74(2–3), pp. 293–297. doi: 10.1016/j.lfs.2003.09.016.

Leeson, V. C. *et al.* (2009) 'Discrimination Learning, Reversal, and Set-Shifting in First-Episode Schizophrenia: Stability Over Six Years and Specific Associations with Medication Type and Disorganization Syndrome', *Biological Psychiatry*, 66(6), pp. 586–593. doi: 10.1016/j.biopsych.2009.05.016.

Lehman, J. F. *et al.* (2011) 'Rules Ventral Prefrontal Cortical Axons Use to Reach Their Targets: Implications for Diffusion Tensor Imaging Tractography and Deep Brain Stimulation for Psychiatric Illness', *Journal of Neuroscience*, 31(28).

Levy, R. and Goldman-Rakic, P. S. (2000) 'Segregation of working memory functions within the dorsolateral prefrontal cortex', *Experimental Brain Research*, 133(1), pp. 23–32. doi: 10.1007/s002210000397.

Liu, X. B. and Jones, E. G. (1996) 'Localization of alpha type II calcium calmodulin-dependent protein kinase at glutamatergic but not gamma-aminobutyric acid (GABAergic) synapses in thalamus and cerebral cortex.', *Proceedings of the National Academy of Sciences*, 93(14), pp. 7332–7336. doi: 10.1073/pnas.93.14.7332.

Lopatina, N. *et al.* (2016) 'Medial Orbitofrontal Neurons Preferentially Signal Cues Predicting Changes in Reward during Unblocking.', *The Journal of neuroscience : the*

official journal of the Society for Neuroscience, 36(32), pp. 8416–24. doi: 10.1523/JNEUROSCI.1101-16.2016.

Lopez, A. J. *et al.* (2016) 'Promoter-Specific Effects of DREADD Modulation on Hippocampal Synaptic Plasticity and Memory Formation', *Journal of Neuroscience*, 36(12), pp. 3588–3599. doi: 10.1523/JNEUROSCI.3682-15.2016.

Lyons, D. M. *et al.* (2000) 'Stress-level cortisol treatment impairs inhibitory control of behavior in monkeys.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 20(20), pp. 7816–21.

Macpherson, T., Morita, M. and Hikida, T. (2014) 'Striatal direct and indirect pathways control decision-making behavior', *Frontiers in Psychology*, 5. doi: 10.3389/fpsyg.2014.01301.

Manes, F. *et al.* (2002) 'Decision-making processes following damage to the prefrontal cortex.', *Brain : a journal of neurology*, 125(Pt 3), pp. 624–39.

Mar, A. *et al.* (2012) 'Assessment of Behavioural Flexibility and Executive Function Using Novel Touch Screen Paradigms'.

Mar, A. C. *et al.* (2011) 'Dissociable effects of lesions to orbitofrontal cortex subregions on impulsive choice in the rat.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31(17), pp. 6398–404. doi: 10.1523/JNEUROSCI.6620-10.2011.

Mar, A. C. *et al.* (2013) 'The touchscreen operant platform for assessing executive function in rats and mice.', *Nature protocols*, 8(10), pp. 1985–2005. doi: 10.1038/nprot.2013.123.

Masaki, D. *et al.* (2006) 'Relationship between limbic and cortical 5-HT neurotransmission and acquisition and reversal learning in a go/no-go task in rats.', *Psychopharmacology*, 189(2), pp. 249–58. doi: 10.1007/s00213-006-0559-0.

McAlonan, K. and Brown, V. J. (2003) 'Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat.', *Behavioural brain research*, 146(1–2), pp. 97–103.

McCreary, A. C., Bankson, M. G. and Cunningham, K. A. (1999) 'Pharmacological studies of the acute and chronic effects of (+)-3, 4-methylenedioxymethamphetamine on locomotor activity: role of 5-hydroxytryptamine(1A) and 5-hydroxytryptamine(1B/1D) receptors.', *The Journal of pharmacology and experimental therapeutics*, 290(3), pp. 965–73.

McGeorge, A. J. and Faull, R. L. (1989) 'The organization of the projection from the cerebral cortex to the striatum in the rat.', *Neuroscience*, 29(3), pp. 503–37.

Mehta, M. A. *et al.* (2001) 'Improved short-term spatial memory but impaired reversal learning following the dopamine D(2) agonist bromocriptine in human volunteers.', *Psychopharmacology*, 159(1), pp. 10–20. doi: 10.1007/s002130100851.

De Mei, C. *et al.* (2009) 'Getting specialized: presynaptic and postsynaptic dopamine D2 receptors.', *Current opinion in pharmacology*. NIH Public Access, 9(1), pp. 53–8. doi: 10.1016/j.coph.2008.12.002.

Meneses, A. and Hong, E. (1997) 'Effects of 5-HT4 receptor agonists and antagonists in learning.', *Pharmacology, biochemistry, and behavior*, 56(3), pp. 347–51. doi: 10.1016/s0091-3057(96)00224-9.

Menzies, L. *et al.* (2008) 'Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: the orbitofronto-striatal model revisited.', *Neuroscience and biobehavioral reviews*, 32(3), pp. 525–49. doi: 10.1016/j.neubiorev.2007.09.005.

Milad, M. R. and Rauch, S. L. (2012) 'Obsessive-compulsive disorder: beyond segregated cortico-striatal pathways', *Trends in Cognitive Sciences*, 16(1), pp. 43–51. doi: 10.1016/j.tics.2011.11.003.

Mink, J. W. (1996) 'The basal ganglia: focused selection and inhibition of competing motor programs.', *Progress in neurobiology*, 50(4), pp. 381–425.

Modell, J. G. *et al.* (1989) 'Neurophysiologic dysfunction in basal ganglia/limbic striatal and thalamocortical circuits as a pathogenetic mechanism of obsessive-compulsive disorder.', *The Journal of neuropsychiatry and clinical neurosciences*, 1(1), pp. 27–36.

Mogenson, G. J., Jones, D. L. and Yim, C. Y. (1980) 'From motivation to action: functional interface between the limbic system and the motor system.', *Progress in neurobiology*, 14(2–3), pp. 69–97.

Monterosso, J. and Ainslie, G. (1999) 'Beyond discounting: possible experimental models of impulse control.', *Psychopharmacology*, 146(4), pp. 339–47.

Morein-Zamir, S. and Robbins, T. W. (2015) 'Fronto-striatal circuits in response-inhibition: Relevance to addiction', *Brain Research*, 1628, pp. 117–129. doi: 10.1016/j.brainres.2014.09.012.

Morris, L. S. *et al.* (2016) 'Fronto-striatal organization: Defining functional and microstructural substrates of behavioural flexibility', *Cortex*, 74, pp. 118–133. doi: 10.1016/j.cortex.2015.11.004.

Naaijen, J. *et al.* (2015) 'Fronto-striatal glutamatergic compounds in compulsive and impulsive syndromes: a review of magnetic resonance spectroscopy studies.', *Neuroscience and biobehavioral reviews*, 52, pp. 74–88. doi: 10.1016/j.neubiorev.2015.02.009.

Nicola, S. M. (2007) 'The nucleus accumbens as part of a basal ganglia action selection circuit', *Psychopharmacology*, 191(3), pp. 521–550. doi: 10.1007/s00213-006-0510-4.

O'Doherty, J. *et al.* (2001) 'Abstract reward and punishment representations in the human orbitofrontal cortex', *Nature Neuroscience*, 4(1), pp. 95–102. doi: 10.1038/82959.

O'Neill, M. and Brown, V. J. (2007) 'The effect of striatal dopamine depletion and the adenosine A2A antagonist KW-6002 on reversal learning in rats', *Neurobiology of Learning and Memory*, 88(1), pp. 75–81. doi: 10.1016/j.nlm.2007.03.003.

Olds, J. and Milner, P. (1954) 'Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain.', *Journal of comparative and physiological psychology*, 47(6), pp. 419–27.

Olton, D. S. and Samuelson, R. J. (1976) 'Remembrance of places passed: Spatial memory in rats.', *Journal of Experimental Psychology: Animal Behavior Processes*, 2(2), pp. 97–116. doi: 10.1037/0097-7403.2.2.97.

Owen, A. M. *et al.* (1990) 'Planning and spatial working memory following frontal lobe lesions in man.', *Neuropsychologia*, 28(10), pp. 1021–34. doi: 10.1016/0028-3932(90)90137-d.

Palencia, C. and Ragozzino, M. (2006) 'The effect of N-methyl-d-aspartate receptor blockade on acetylcholine efflux in the dorsomedial striatum during response reversal learning', *Neuroscience*. Pergamon, 143(3), pp. 671–678. doi: 10.1016/J.NEUROSCIENCE.2006.08.024.

Pan, W. X., Mao, T. and Dudman, J. T. (2010) 'Inputs to the dorsal striatum of the mouse reflect the parallel circuit architecture of the forebrain.', *Frontiers in neuroanatomy*, 4, p. 147. doi: 10.3389/fnana.2010.00147.

Park, S. B. *et al.* (1994) 'Tryptophan depletion in normal volunteers produces selective impairments in learning and memory.', *Neuropharmacology*, 33(3–4), pp. 575–88. doi: 10.1016/0028-3908(94)90089-2.

Parks, C. L. *et al.* (1998) 'Increased anxiety of mice lacking the serotonin1A receptor', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 95(18), pp. 10734–10739. doi: 10.1073/pnas.95.18.10734.

Passetti, F., Chudasama, Y. and Robbins, T. W. (2002) 'The frontal cortex of the rat and visual attentional performance: dissociable functions of distinct medial prefrontal subregions.', *Cerebral cortex (New York, N.Y. : 1991)*, 12(12), pp. 1254–68.

Passingham, R. E. (1985) 'Memory of monkeys (Macaca mulatta) with lesions in prefrontal cortex.', *Behavioral Neuroscience*, 99(1), pp. 3–21. doi: 10.1037/0735-7044.99.1.3.

Paxinos, G. and Watson, C. (2006) *The rat brain in stereotaxic coordinates.* Academic Press.

Petrides, M. and Milner, B. (1982) 'Deficits on subject-ordered tasks after frontal- and temporal-lobe lesions in man', *Neuropsychologia*, 20(3), pp. 249–262. doi: 10.1016/0028-3932(82)90100-2.

Phillips, M. L. *et al.* (2003) 'Neurobiology of emotion perception I: the neural basis of normal emotion perception', *Biological Psychiatry*, 54(5), pp. 504–514. doi: 10.1016/S0006-3223(03)00168-9.

Pitsikas, N., Brambilla, A. and Borsini, F. (1994) 'Effect of DAU 6215, a novel 5-HT3 receptor antagonist, on scopolamine-induced amnesia in the rat in a spatial learning task.', *Pharmacology, biochemistry, and behavior*, 47(1), pp. 95–9. doi: 10.1016/0091-3057(94)90116-3.

Popova, Nina K and Amstislavskaya, T. G. (2002) '5-HT2A and 5-HT2C serotonin receptors differentially modulate mouse sexual arousal and the hypothalamo-pituitary-testicular response to the presence of a female.', *Neuroendocrinology*, 76(1), pp. 28–34. doi: 10.1159/000063681.

Popova, N. K. and Amstislavskaya, T. G. (2002) 'Involvement of the 5-HT1A and 5-

HT1B serotonergic receptor subtypes in sexual arousal in male mice', in *Psychoneuroendocrinology*, pp. 609–618. doi: 10.1016/S0306-4530(01)00097-X.

Preuss, T. M. (1995) 'Do Rats Have Prefrontal Cortex? The Rose-Woolsey-Akert Program Reconsidered', *Journal of Cognitive Neuroscience*, 7(1), pp. 1–24. doi: 10.1162/jocn.1995.7.1.1.

Ragozzino, M. E. *et al.* (2002) 'Role of the dorsomedial striatum in behavioral flexibility for response and visual cue discrimination learning.', *Behavioral neuroscience*. NIH Public Access, 116(1), pp. 105–15.

Ragozzino, M. E. (2007) 'The Contribution of the Medial Prefrontal Cortex, Orbitofrontal Cortex, and Dorsomedial Striatum to Behavioral Flexibility', *Annals of the New York Academy of Sciences*, 1121(1), pp. 355–375. doi: 10.1196/annals.1401.013.

Rahman, S. *et al.* (1999) 'Specific cognitive deficits in mild frontal variant frontotemporal dementia', *Brain*, 122(8), pp. 1469–1493. doi: 10.1093/brain/122.8.1469.

Remijnse, P. *et al.* (2005) 'Neural correlates of a reversal learning task with an affectively neutral baseline: An event-related fMRI study', *NeuroImage*, 26(2), pp. 609–618. doi: 10.1016/j.neuroimage.2005.02.009.

Remijnse, P. L. *et al.* (2006) 'Reduced Orbitofrontal-Striatal Activity on a Reversal Learning Task in Obsessive-Compulsive Disorder', *Archives of General Psychiatry*, 63(11), p. 1225. doi: 10.1001/archpsyc.63.11.1225.

Remijnse, P. L. *et al.* (2009) 'Differential frontal–striatal and paralimbic activity during reversal learning in major depressive disorder and obsessive–compulsive disorder', *Psychological Medicine*, 39(09), p. 1503. doi: 10.1017/S0033291708005072.

Remijnse, P. L. *et al.* (2013) 'Cognitive Inflexibility in Obsessive-Compulsive Disorder and Major Depression Is Associated with Distinct Neural Correlates', *PLoS ONE*, 8(4). doi: 10.1371/journal.pone.0059600.

Reynolds, J. N. J. and Wickens, J. R. (2002) 'Dopamine-dependent plasticity of corticostriatal synapses', *Neural Networks*. Elsevier Ltd, 15(4–6), pp. 507–521. doi: 10.1016/S0893-6080(02)00045-X.

Riceberg, J. S. and Shapiro, M. L. (2012) 'Reward stability determines the contribution of orbitofrontal cortex to adaptive behavior.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 32(46), pp. 16402–9. doi: 10.1523/JNEUROSCI.0776-12.2012.

Ridley, R., Haystead, T. and Baker, H. (1981) 'An analysis of visual object reversal learning in the marmoset after amphetamine and haloperidol', *Pharmacology Biochemistry and Behavior*. Elsevier, 14(3), pp. 345–351. doi: 10.1016/0091-3057(81)90401-9.

Robbins, T. W. *et al.* (1996) 'Dissociating Executive Functions of the Prefrontal Cortex [and Discussion]', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 351(1346), pp. 1463–1471. doi: 10.1098/rstb.1996.0131.

Robbins, T. W. (1998) 'Homology in behavioural pharmacology: an approach to

animal models of human cognition.', *Behavioural pharmacology*, 9(7), pp. 509–19.

Robbins, T. W. (2000) 'Chemical neuromodulation of frontal-executive functions in humans and other animals', in *Executive Control and the Frontal Lobe: Current Issues*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 130–138. doi: 10.1007/978-3-642-59794-7_14.

Roberts, A. C. (1996) 'Comparison of cognitive function in human and non-human primates', *Cognitive Brain Research*. Elsevier B.V., 3(3–4), pp. 319–327. doi: 10.1016/0926-6410(96)00017-1.

Roberts, B. Z. *et al.* (2019) 'Oxytocin improves probabilistic reversal learning but not effortful motivation in Brown Norway rats', *Neuropharmacology*. Elsevier Ltd, 150, pp. 15–26. doi: 10.1016/j.neuropharm.2019.02.028.

Rogan, S. C. and Roth, B. L. (2011) 'Remote control of neuronal signaling.', *Pharmacological reviews*, 63(2), pp. 291–315. doi: 10.1124/pr.110.003020.

Rogers, R. *et al.* (1999) 'Tryptophan depletion impairs stimulus-reward learning while methylphenidate disrupts attentional control in healthy young adults: implications for the monoaminergic basis of impulsive behaviour.', *Psychopharmacology*, 146(4), pp. 482–491.

Rogers, R. D. *et al.* (2000) 'Contrasting cortical and subcortical activations produced by attentional-set shifting and reversal learning in humans.', *Journal of cognitive neuroscience*, 12(1), pp. 142–62.

Rolls, E. T. *et al.* (1994) 'Emotion-related learning in patients with social and emotional changes associated with frontal lobe damage', *Journal of Neurology Neurosurgery and Psychiatry*, 57(12), pp. 1518–1524.

Rose, J. E. and Woolsey, C. N. (1948) 'The orbitofrontal cortex and its connections with the mediodorsal nucleus in rabbit, sheep and cat.', *Research publications - Association for Research in Nervous and Mental Disease*, 27 (1 vol.), pp. 210–32.

Rossi, M. A. *et al.* (2013) 'Operant self-stimulation of dopamine neurons in the substantia nigra.', *PloS one*, 8(6), p. e65799. doi: 10.1371/journal.pone.0065799.

Roth, B. L. (2016) 'DREADDs for Neuroscientists', *Neuron*. Cell Press, 89(4), pp. 683–694. doi: 10.1016/J.NEURON.2016.01.040.

Rudebeck, P. H. and Murray, E. A. (2014) 'The Orbitofrontal Oracle: Cortical Mechanisms for the Prediction and Evaluation of Specific Behavioral Outcomes', *Neuron*, 84(6), pp. 1143–1156. doi: 10.1016/j.neuron.2014.10.049.

Rygula, R. *et al.* (2010) 'Differential Contributions of the Primate Ventrolateral Prefrontal and Orbitofrontal Cortex to Serial Reversal Learning', *Journal of Neuroscience*, 30(43), pp. 14552–14559. doi: 10.1523/JNEUROSCI.2631-10.2010.

Sala-Bayo, J. *et al.* (submitted) 'Dorsal and ventral striatal dopamine D1 and D2 receptors differentially modulate distinct phases of reversal learning'.

Sautel, F. *et al.* (1995) 'Nafadotride, a potent preferential dopamine D3 receptor antagonist, activates locomotion in rodents.', *The Journal of pharmacology and experimental therapeutics*, 275(3), pp. 1239–46.

Schilman, E. A. *et al.* (2008) 'The orbital cortex in rats topographically projects to

central parts of the caudate–putamen complex', *Neuroscience Letters*, 432(1), pp. 40–45. doi: 10.1016/j.neulet.2007.12.024.

Schoenbaum, G. *et al.* (2002) 'Orbitofrontal lesions in rats impair reversal but not acquisition of go, no-go odor discriminations.', *Neuroreport*, 13(6), pp. 885–90.

Schoenbaum, G. *et al.* (2003) 'Lesions of orbitofrontal cortex and basolateral amygdala complex disrupt acquisition of odor-guided discriminations and reversals.', *Learning & memory (Cold Spring Harbor, N.Y.)*, 10(2), pp. 129–40. doi: 10.1101/lm.55203.

Schoenbaum, G., Chiba, A. A. and Gallagher, M. (1999) 'Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 19(5), pp. 1876–84.

Schoenbaum, G., Chiba, A. A. and Gallagher, M. (2000) 'Changes in functional connectivity in orbitofrontal cortex and basolateral amygdala during learning and reversal training.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 20(13), pp. 5179–89.

Schultz, W. (2013) 'Updating dopamine reward signals.', *Current opinion in neurobiology*, 23(2), pp. 229–38. doi: 10.1016/j.conb.2012.11.012.

Schwartz, J.-C. *et al.* (1994) 'The dopamine D3 receptor in nucleus accumbens: selective cellular localisation, function and regulation', *European Neuropsychopharmacology*. Elsevier, 4(3), pp. 190–191. doi: 10.1016/0924-977X(94)90030-2.

Sealfon, S. C. and Olanow, C. W. (2000) 'Dopamine receptors: From structure to behavior', *Trends in Neurosciences*. Elsevier Ltd. doi: 10.1016/S1471-1931(00)00025-2.

Shen, W. *et al.* (2008) 'Dichotomous dopaminergic control of striatal synaptic plasticity.', *Science (New York, N.Y.)*, 321(5890), pp. 848–51. doi: 10.1126/science.1160575.

Smith, A. G., Neill, J. C. and Costall, B. (1999) 'The dopamine D3/D2 receptor agonist 7-OH-DPAT induces cognitive impairment in the marmoset.', *Pharmacology, biochemistry, and behavior*, 63(2), pp. 201–11.

Smriga, M. and Torii, K. (2003) 'L-Lysine acts like a partial serotonin receptor 4 antagonist and inhibits serotonin-mediated intestinal pathologies and anxiety in rats.', *Proceedings of the National Academy of Sciences of the United States of America*, 100(26), pp. 15370–5. doi: 10.1073/pnas.2436556100.

Sprengelmeyer, R., Lange, H. and Hömberg, V. (1995) 'The pattern of attentional deficits in Huntington's disease.', *Brain : a journal of neurology*, 118 (Pt 1), pp. 145–52.

Stalnaker, T. A. *et al.* (2007) 'Basolateral amygdala lesions abolish orbitofrontal-dependent reversal impairments.', *Neuron*, 54(1), pp. 51–8. doi: 10.1016/j.neuron.2007.02.014.

Steiner, H. and Gerfen, C. R. (1998) 'Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior.', *Experimental brain research*,

123(1–2), pp. 60–76.

Stern, C. E. and Passingham, R. E. (1995) 'The nucleus accumbens in monkeys (Macaca fascicularis). III. Reversal learning.', *Experimental brain research*, 106(2), pp. 239–47.

Swainson, R. *et al.* (2000) 'Probabilistic learning and reversal deficits in patients with Parkinson's disease or frontal or temporal lobe lesions: Possible adverse effects of dopaminergic medication', *Neuropsychologia*, 38(5), pp. 596–612. doi: 10.1016/S0028-3932(99)00103-7.

Taghzouti, K. *et al.* (1985) 'Alternation behavior, spatial discrimination, and reversal disturbances following 6-hydroxydopamine lesions in the nucleus accumbens of the rat.', *Behavioral and neural biology*, 44(3), pp. 354–63.

Tait, D. S. and Brown, V. J. (2007) 'Difficulty overcoming learned non-reward during reversal learning in rats with ibotenic acid lesions of orbital prefrontal cortex.', *Annals of the New York Academy of Sciences*, 1121(1), pp. 407–20. doi: 10.1196/annals.1401.010.

Talpos, J. C. *et al.* (2010) 'Trial-unique, delayed nonmatching-to-location (TUNL): A novel, highly hippocampus-dependent automated touchscreen test of location memory and pattern separation', *Neurobiology of Learning and Memory*, 94(3), pp. 341–352. doi: 10.1016/j.nlm.2010.07.006.

Tekin, S. and Cummings, J. L. (2002) 'Frontal-subcortical neuronal circuits and clinical neuropsychiatry: An update', *Journal of Psychosomatic Research*. Elsevier Inc., 53(2), pp. 647–654. doi: 10.1016/S0022-3999(02)00428-2.

Thonnard, D., Callaerts-Vegh, Z. and D'Hooge, R. (2019) 'Differential effects of post-training scopolamine on spatial and non-spatial learning tasks in mice.', *Brain research bulletin*, 152, pp. 52–62. doi: 10.1016/j.brainresbull.2019.07.012.

Thorsen, A. L. *et al.* (2015) 'Neuroimaging of psychotherapy for obsessive-compulsive disorder: A systematic review.', *Psychiatry research*, 233(3), pp. 306–13. doi: 10.1016/j.pscychresns.2015.05.004.

Tucci, M. C. *et al.* (2013) 'Effects of the serotonergic agonist mCPP on male rats in the quinpirole sensitization model of obsessive-compulsive disorder (OCD).', *Psychopharmacology*, 227(2), pp. 277–85. doi: 10.1007/s00213-013-2976-1.

Uylings, H. B. and van Eden, C. G. (1990) 'Qualitative and quantitative comparison of the prefrontal cortex in rat and in primates, including humans.', *Progress in brain research*, 85, pp. 31–62.

Uylings, H. B. M., Groenewegen, H. J. and Kolb, B. (2003) 'Do rats have a prefrontal cortex?', *Behavioural brain research*, 146(1–2), pp. 3–17.

Vaghi, M. M. *et al.* (2017) 'Specific Frontostriatal Circuits for Impaired Cognitive Flexibility and Goal-Directed Planning in Obsessive-Compulsive Disorder: Evidence From Resting-State Functional Connectivity', *Biological Psychiatry*, 81(8), pp. 708–717. doi: 10.1016/j.biopsych.2016.08.009.

Verharen, J. P. H., Adan, R. A. H. and Vanderschuren, L. J. M. J. (2019) 'Differential contributions of striatal dopamine D1 and D2 receptors to component processes of value-based decision making', *Neuropsychopharmacology*. Nature Publishing Group,

pp. 1–10. doi: 10.1038/s41386-019-0454-0.

Vitrac, C. and Benoit-Marand, M. (2017) 'Monoaminergic Modulation of Motor Cortex Function', *Frontiers in Neural Circuits*, 11, p. 72. doi: 10.3389/fncir.2017.00072.

Voorn, P. *et al.* (2004) 'Putting a spin on the dorsal-ventral divide of the striatum.', *Trends in neurosciences*, 27(8), pp. 468–74. doi: 10.1016/j.tins.2004.06.006.

Walton, M. E. *et al.* (2010) 'Separable learning systems in the macaque brain an... [Neuron. 2010] - PubMed result', *Neuron*, 65(6), pp. 927–39. doi: 10.1016/j.neuron.2010.02.027.

Webster, R. A. (2001) *Neurotransmitters, Drugs and Brain Function*, *Neurotransmitters, Drugs and Brain Function*. Wiley. doi: 10.1002/0470846577.

Weiner, D. M. *et al.* (2004) 'The role of M1 muscarinic receptor agonism of N-desmethylclozapine in the unique clinical effects of clozapine.', *Psychopharmacology*, 177(1–2), pp. 207–16. doi: 10.1007/s00213-004-1940-5.

Wess, J. (2004) 'Muscarinic acetylcholine receptor knockout mice: novel phenotypes and clinical implications.', *Annual review of pharmacology and toxicology*, 44, pp. 423–50. doi: 10.1146/annurev.pharmtox.44.101802.121622.

Wettschureck, N. and Offermanns, S. (2005) 'Mammalian G proteins and their cell type specific functions.', *Physiological reviews*, 85(4), pp. 1159–204. doi: 10.1152/physrev.00003.2005.

Williams, G. V and Castner, S. A. (2006) 'Under the curve: critical issues for elucidating D1 receptor function in working memory.', *Neuroscience*, 139(1), pp. 263–76. doi: 10.1016/j.neuroscience.2005.09.028.

Winstanley, C. A. *et al.* (2005) 'Interactions between serotonin and dopamine in the control of impulsive choice in rats: therapeutic implications for impulse control disorders.', *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 30(4), pp. 669–82. doi: 10.1038/sj.npp.1300610.

Wise, S. P. (2008) 'Forward frontal fields: phylogeny and fundamental function.', *Trends in neurosciences*, 31(12), pp. 599–608. doi: 10.1016/j.tins.2008.08.008.

Wood, J. and Ahmari, S. E. (2015) 'A Framework for Understanding the Emerging Role of Corticolimbic-Ventral Striatal Networks in OCD-Associated Repetitive Behaviors.', *Frontiers in systems neuroscience*, 9, p. 171. doi: 10.3389/fnsys.2015.00171.

Wouters, W., Tulp, M. T. and Bevan, P. (1988) 'Flesinoxan lowers blood pressure and heart rate in cats via 5-HT1A receptors.', *European journal of pharmacology*, 149(3), pp. 213–23. doi: 10.1016/0014-2999(88)90651-6.

Yasuno, F. *et al.* (2003) 'Inhibitory effect of hippocampal 5-HT1A receptors on human explicit memory.', *The American journal of psychiatry*, 160(2), pp. 334–40. doi: 10.1176/appi.ajp.160.2.334.

Yawata, S. *et al.* (2012) 'Pathway-specific control of reward learning and its flexibility via selective dopamine receptors in the nucleus accumbens', *Proceedings of the National Academy of Sciences*, 109(31), pp. 12764–12769. doi:

10.1073/pnas.1210797109.

Yin, H. H. *et al.* (2005) 'The role of the dorsomedial striatum in instrumental conditioning.', *The European journal of neuroscience*. Blackwell Science Ltd, 22(2), pp. 513–23. doi: 10.1111/j.1460-9568.2005.04218.x.

Yin, H. H., Knowlton, B. J. and Balleine, B. W. (2004) 'Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning.', *The European journal of neuroscience*. Blackwell Science Ltd, 19(1), pp. 181–9. doi: 10.1111/j.1460-9568.2004.03095.x.

Yin, H. H., Knowlton, B. J. and Balleine, B. W. (2006) 'Inactivation of dorsolateral striatum enhances sensitivity to changes in the action–outcome contingency in instrumental conditioning', *Behavioural Brain Research*. Elsevier, 166(2), pp. 189–196. doi: 10.1016/J.BBR.2005.07.012.

Yoshida, W. and Ishii, S. (2006) 'Resolution of Uncertainty in Prefrontal Cortex', *Neuron*, 50(5), pp. 781–789. doi: 10.1016/j.neuron.2006.05.006.

Zeeb, F. D. *et al.* (2015) 'Inactivation of the prelimbic or infralimbic cortex impairs decision-making in the rat gambling task', *Psychopharmacology*, 232(24), pp. 4481–4491. doi: 10.1007/s00213-015-4075-y.

Zeeb, F. D., Floresco, S. B. and Winstanley, C. A. (2010) 'Contributions of the orbitofrontal cortex to impulsive choice: interactions with basal levels of impulsivity, dopamine signalling, and reward-related cues', *Psychopharmacology*, 211(1), pp. 87–98. doi: 10.1007/s00213-010-1871-2.

Appendix A. THE EFFECTS OF DREADD INFUSIONS INTO THE ORBITOFRONTAL CORTEX OR STRIATUM

A.1. Introduction

G protein-coupled receptors (GPCRs) are a diverse family of receptors that have a large range of functions, whose primary function is to transduce stimuli into intracellular signals (Lee, George and O'Dowd, 2003). GPCRs are among the most diverse and largest protein families within the mammalian genome. They consist of seven transmembrane helices, with an extracellular N-terminus and an intracellular C-terminus (Gether, 2000). GPCRs transduce their signal via intracellular coupling of a G protein, the structure of one GPCR member has been solved.

The binding of an agonist to the receptor causes a conformational change in the receptor and activation of the G protein, this G protein will have different affects depending on the class of its α-subunit. There are four classes of G protein, $G\alpha_s$, $G\alpha_{i/o}$, $G_{q/11}$, and $G\alpha_{12/13}$ (Wettschureck and Offermanns, 2005). Upon activation the Gα and Gβγ subunits dissociate leading to downstream signalling. The $G\alpha_s$ and $G\alpha_{i/o}$ pathways utilise adenylate cyclase (AC) as an effector, $G\alpha_s$ acts as a stimulator while $G\alpha_{i/o}$ inhibits the functions of AC. The $G_{q/11}$ subunit acts through phospholipase C-β, which leads to calcium release within from the endoplasmic reticulum. The $G\alpha_{12/13}$ subunit acts to activate Rho, a small GTPase (Wettschureck and Offermanns, 2005).

Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are mutated versions of GPCRs that are able to be selectively activated or inactivated. The muscarinic cholinergic receptor family is comprised of five receptors, M_1-M_5. M_1, M_3, and M_5 receptors are coupled to G_q, while M_2 and M_4 receptors are coupled with G_i (Wess, 2004). Clozapine is a weak partial agonist at muscarinic receptors, and clozapine-N-oxide (CNO) is a structurally similar designer ligand that is known to be inert at endogenous targets (Bender, Holschbach and Stöcklin, 1994; Weiner *et al.*, 2004). The mutation of two amino acid residues in highly conserved

transmembrane domains is sufficient to cause insensitivity to the endogenous ligand, ACh, and sensitivity to CNO (Figure 8.1)(Weiner *et al.*, 2004; Armbruster *et al.*, 2007). These mutations are able to generate a whole family of DREADDs by mutating analogous domains.

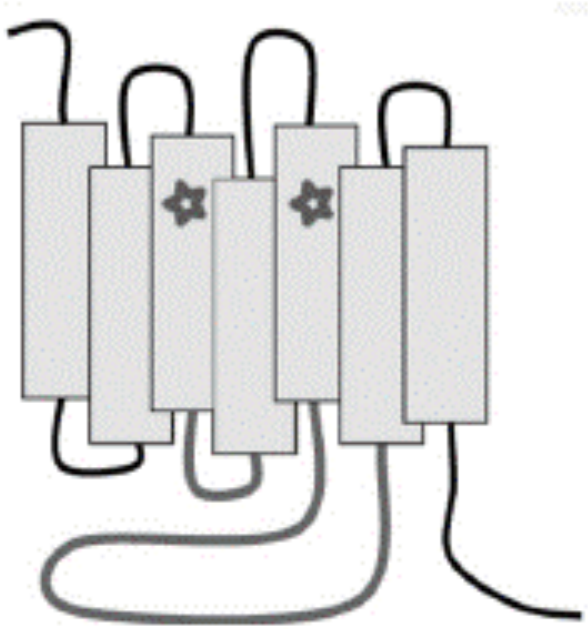


Figure 8.1: **Taken from Rogan et al (Rogan and Roth, 2011). DREADDs are formed by mutations in the third and fifth transmembrane regions of the muscarinic receptor.**

DREADDs can be selectively expressed in cell populations by the use of viral vectors, such as adeno-associated virus (AAV) and canine adenovirus (CAV). The use of different promoter sequences allows the DREADDs to be expressed in different cell populations. One example of this was published recently (Ferguson *et al.*, 2011), transgenes were expressed under the control of prodynorphin (pDYN) or proenkephalin (pENK) promoter sequences; this allowed selectively allow for expression of the transgene on either the striatoniagral or striatopallidal medium spiny neurons, respectively.

The use of DREADDs in research allows interrogation of the role of specific projections by the use of retrograde Cre recombinase. The study of specific projections allows for the investigation of cross-talk between regions of the brain,

whereas before lesions and pharmacological actions were the only tools that were previously available.

By using a DREADD approach we hoped to be able to specifically manipulate a region of interest and measure the effects of the increase of decrease in activity on reversal behaviours.

Due to effects on reversal learning behaviours following pharmacological manipulations of both the striatum and the orbitofrontal cortex (Chapters 4 and 6), we hoped to replicate these findings using a chemogenetic approach.

A.2. Methods

Animals were trained as previously described in General Methods (Chapter2); following training procedures animals underwent viral transfusion surgery.

A.2.1 Viral transfusion surgery

Animals were prepared and secured in a stereotaxic frame as with cannulation surgery (Chapter 2.4). The brain was accessed as with cannulation surgery, ensuring flat skull and small holes drilled above the desired infusion sites. Dura mater was broken, ensuring minimal damage to the underlying tissue structures. A stainless steel bevelled injection needle (31GA), attached via fine bore polythene tubing (0.28mm inner diameter; Portex, UK) to a 10μL Hamilton precision syringe, was inserted and the virus was infused. Following infusion, the injector was left in place for a period of time to allow the virus to spread. Infusions were carried out using a Harvard infusion pump (Harvard Apparatus Ltd, UK).

Viral vectors were diluted in filtered PBS and infused bilaterally at stereotaxic coordinates, based on Paxinos and Watson (2006). Other methods are detailed in each experiment as necessary.

Animals were allowed to recover for 1 week, after this time training resumed while the virus expressed.

A.2.2 Drugs

Clozapine-N-Oxide (CNO) (Sequoia Research, Torrance, CA, USA) was pre-dissolved in Dimethyl Sulfoxide (DMSO, Sigma, Poole, UK) and diluted in physiological saline to make final concentrations of 0.5% DMSO.

Rats were injected with CNO i.p. 30 minutes prior to testing. All injections were given in a volume of 1ml/kg.

Rats were returned to their home cage following injection.

A.2.3 Statistics

The main measures of the animals' ability to learn the reversals and visual discrimination were: (i) the number of trials to criterion and (ii) the number of

incorrect responses to criterion (errors of commission). Secondary measures were not analysed in these experiments.

Data for each primary variable were analysed using a repeated measures mixed model ANOVA consisting of one between-subject factor (Region/virus type) and two within-subject factors (Dose and Phase). When significant interactions were found, further analysis was performed of Simple Main Effects or Simple Main Interactions, as appropriate. Analysis was followed by post-hoc Sidak's corrected pair-wise comparisons to vehicle.

For all comparisons, significant difference was assumed at $p < 0.05$.

A.3. Experiments

A.3.1 Experiment 1: Effects of post-synaptic stimulatory DREADD manipulations in the dorsolateral and dorsomedial striatum

A.3.1.1 Experimental rationale

As shown in Chapter 4, dopaminergic manipulations in the striatum through dopamine D_2 antagonism lead to significant changes in performance on a touchscreen serial reversal learning task. Dopamine D_2 receptors are inhibitory in their action, reducing cAMP signalling; therefore, antagonising the inhibitory action leads to a net activation and increase in cAMP signalling. To simulate this effect through DREADDs, a G_S coupled DREADD, such as M3Ds, can be utilised in the post-synaptic neuron (Figure 8.2). As dopamine D_2 receptors are also located pre-synaptically as auto-receptors, this could aid in the understanding of the location of action of raclopride in the DMS and DLS.

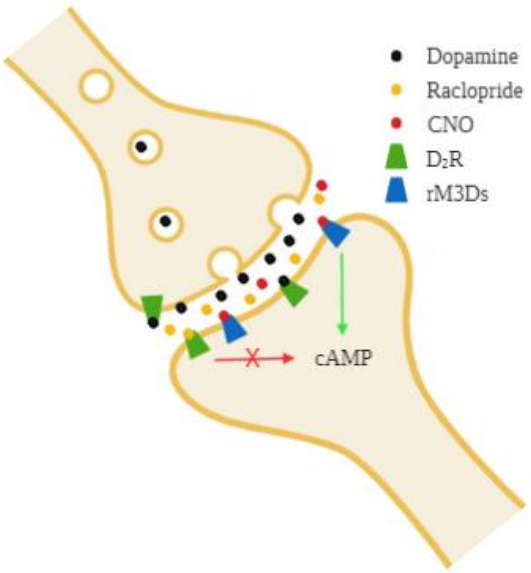


***Figure 8.2*: A schematic showing the hypothesis and rationale behind Experiment 1. Raclopride binds to the post-synaptic dopamine D_2 receptor, preventing the binding of endogenous dopamine; dopamine activation of D_2 receptors decreases cAMP signalling, therefore by antagonising this pathway there is a net increase in cAMP. Similarly, when CNO binds to rM3Ds DREADDs, there is a direct increase in cAMP signalling.**

A.3.1.2 Methods

A.3.1.2.1 Viral transfusion surgery

32 male Lister Hooded rats (Charles River, UK), were trained and surgically prepared as previously described (Chapter 2, Appendix A~ Methods). Rats were pseudo-randomly assigned into aDLS, pDMS or GFP to ensure equal performance across the groups, with numbers equalling 16 per group.

HSV-pENK-rM3Ds (or GFP control) were infused into the aDLS or pDMS (AP +1.2, ML ±3.5, DV -4.4 and AP -0.4, ML ±2.6, -4.4, respectively). A stainless steel bevelled needle was inserted to the required infusion location, lowered a further 0.1 mm and retracted back to the intended coordinates. The virus was infused at a rate of 200 nL/min for 10 minutes, 10 minutes after the needle was inserted. The needle was left in place for a further 10 minutes prior to lowering by 0.1 mm and slowly retracting fully.

This procedure was carried out in both hemispheres prior to suture closure. The virus was allowed three weeks to express prior to testing; during this time the rats were allowed one-week recovery and two more weeks of training. Following surgery rats were single housed overnight and then returned to their home cage for the remainder of the experiment.

A.3.1.2.2 Histology

Rats were anaesthetised with an intraperitoneal injection of pentobarbital and perfused transcardially with 0.01M PBS followed by 10% paraformaldehyde (PFA). The brains were removed, postfixed for 4 hours in PFA, and stored in 30% sucrose solution. The brain was then sliced 60 μm thick in the coronal plane. Slices were washed three times for 10 minutes in 0.1M PBS and then blocked at room temperature for 1 hour in blocking buffer (containing 3% Normal Goat Serum (Vector Laboratories Inc, Peterborough, UK), 0.3% Triton X-100 (Fisher Scientific, Loughborough, UK) in 0.1M PBS). Slices were then incubated overnight at 4°C in the primary antibody solution (1:1000 HA-Tag rabbit anti-mouse (Cell Signalling, Leiden, The Netherlands), in 1% Normal Goat Serum (Vector Laboratories Inc), 0.3% Triton X-100 (Fisher Scientific) in 0.1M PBS). Following incubation in the

primary antibody the slices were washed with PBS (3x10 mins) and incubated in goat anti-rabbit Alexafluor488 (1:250, Abcam, Life Technologies, Cambridge, UK) in blocking buffer (1% Normal Goat Serum (Vector Laboratories Inc), 0.3% Triton X-100 (Fisher Scientific) in 0.1M PBS) for 2 hours at room temperature.

Slices were then washed in 0.1M PBS (3x10 mins), mounted and coverslipped with FluorSave reagent (Calbiochem, Darmstadt, Germany).

Slices were then visualised using a Zeiss Axio Imager (Zeiss, Germany).

A.3.1.3 Results

A.3.1.3.1 Histology

Figure 8.3 shows infusion locations of aDLS (A) and pDMS (B) DREADDs following immunohistochemistry. Positions were correct as per the defined regions by Paxinos and Watson (2006).

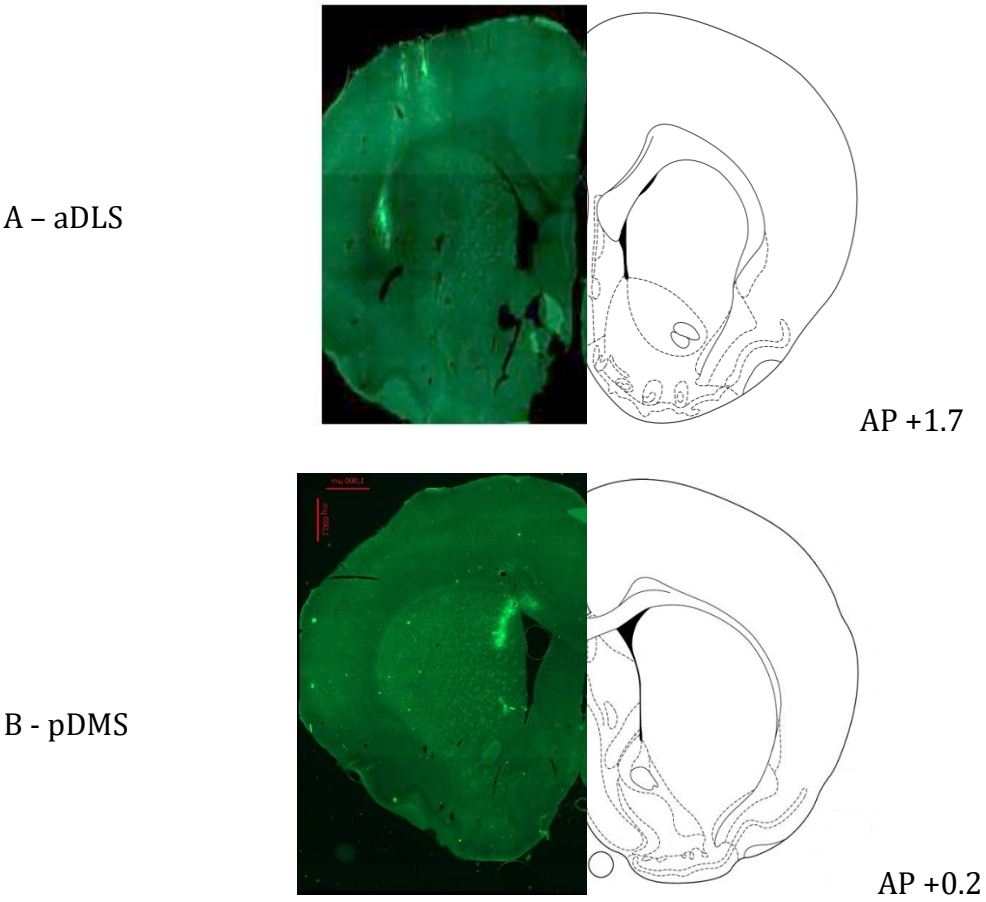


Figure 8.3: **Immunofluorescent images of DREADD infusion locations into the aDLS (A) and pDMS (B), alongside representative images from Paxinos and Watson (2006).**

A.3.1.3.2 Trials

A three-way repeated measures mixed design ANOVA revealed there were no significant main effects of Dose or Location/Virus type ($F_{2,4}$ = 0.544, p = 0.584 and $F_{2,25}$ = 2.159, p = 0.136, respectively). There was a main effect of Phase ($F_{2,50}$ = 19.620, $p \leq 0.001$).

There were also no significant two-way interactions (Dose x Phase $F_{4,100} = 0.513$, $p = 0.726$, Phase x Location $F_{4,50} = 0.212$, $p = 0.930$, and Dose x Location $F_{4,50} = 0.647$, $p = 0.632$), or three-way interaction ($F_{8,100} = 1.166$, $p = 0.327$). Results are displayed in Figure 8.4.

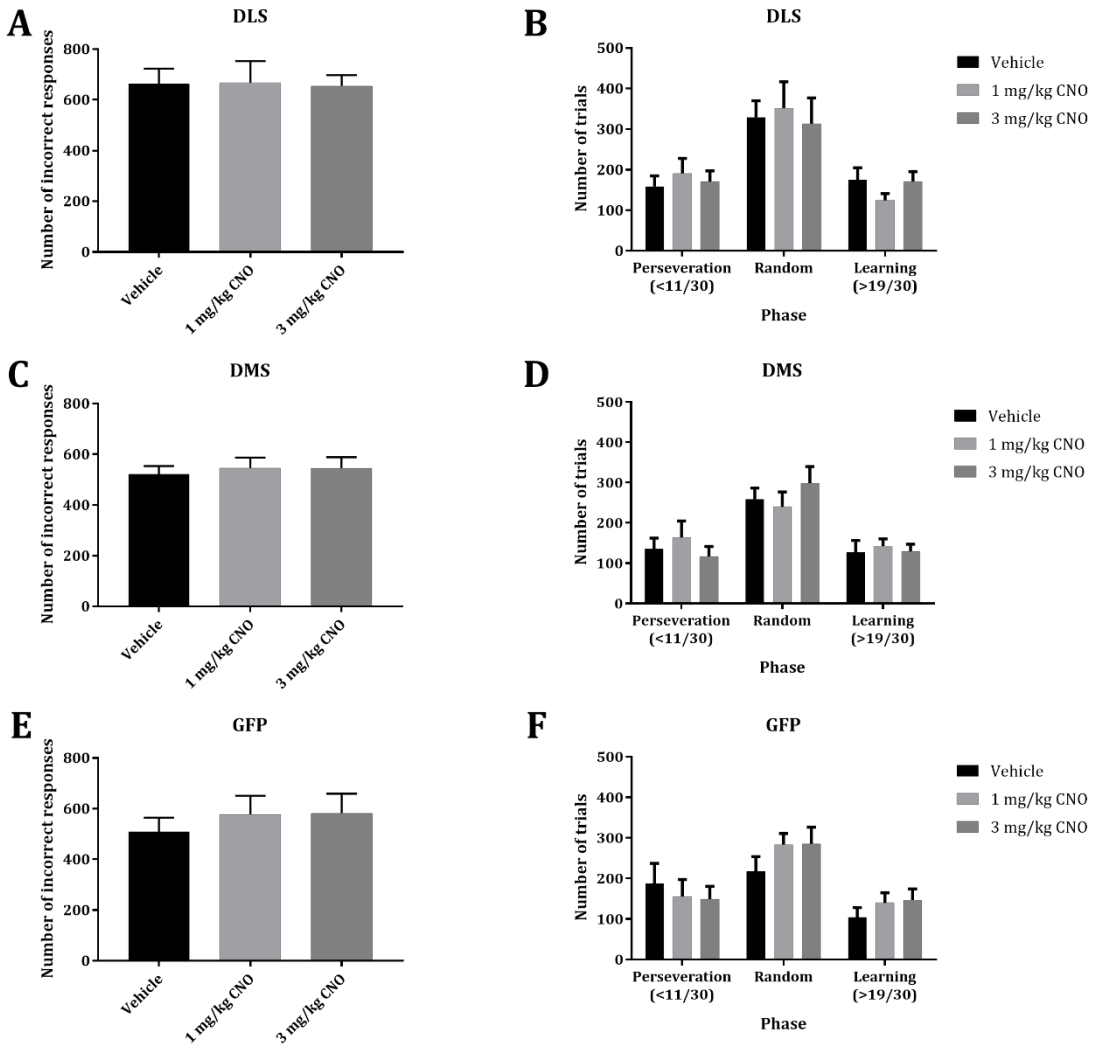


Figure 8.4: **Histograms showing the total number of trials to criterion following injection of CNO following DREADD infusion into the DLS (A), DMS (C), or GFP control (E), and the number of trials per phase (B, D, and F). No significant differences were found. Data are represented as mean values ± SEM.**

A.3.1.3.3 Incorrect responses

A three-way repeated measures mixed design ANOVA revealed there were no significant main effects of Dose or Location/Virus type ($F_{2,4} = 0.487$, $p = 0.617$ and

$F_{2,25}$ = 1.713, p = 0.201, respectively). There was a main effect of Phase ($F_{2,50}$ = 27.761, $p \leq 0.001$).

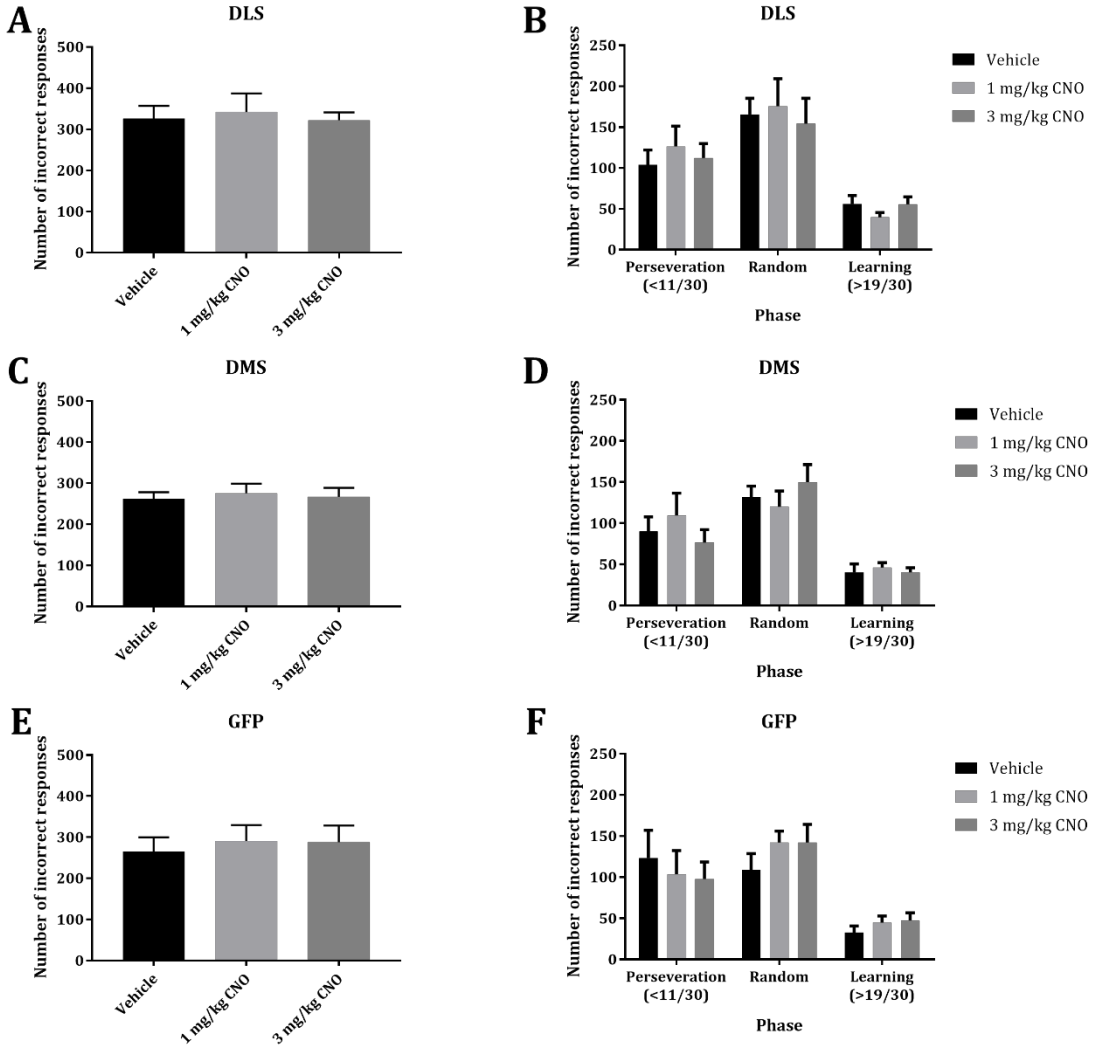


Figure 8.5: **Histograms showing the total number of incorrect responses to criterion following injection of CNO following DREADD infusion into the DLS (A), DMS (C), or GFP control (E), and the number of incorrect responses per phase (B, D, and F). No significant differences were found. Data are represented as mean values ± SEM.**

There were also no significant two-way interactions (Dose x Phase $F_{4,100}$ = 0.489, p = 0.744, Phase x Location $F_{4,50}$ = 0.217, p = 0.928, and Dose x Location $F_{4,50}$ = 0.503, p = 0.734), or three-way interaction ($F_{8,100}$ = 1.147, p = 0.339). Results are displayed in Figure 8.5.

A.3.2 Experiment 2: Effects of inhibitory DREADD manipulations in the medial and lateral OFC

A.3.2.1 Experimental rationale

Following the lack of results in Experiment 1, it was decided to attempt a different approach. As there were clear effects observed in Chapter 6 following inactivation of the orbitofrontal cortex, it seemed logical to attempt this using the DREADDs approach.

Chapter 6 reports dissociable effects following inactivation of the medial and lateral orbitofrontal cortex (mOFC and lOFC) following infusion of a baclofen/muscimol cocktail. Infusion of an inhibitory DREADD, such as hM4Di, into the same coordinates should produce similar dissociable results, with improved performance in the mOFC group and impaired performance in the lOFC group following DREADD activation with CNO.

A.3.2.2 Methods

A.3.2.2.1 Viral transfusion surgery

48 male Lister Hooded rats (Charles River, UK), were trained and surgically prepared as previously described (Chapter 2, Appendix A~ Methods). Rats were pseudo-randomly assigned into mOFC, lOFC or GFP to ensure equal performance across the groups, with numbers equalling 16 per group.

AAV5-CamKII-hM4Di (or YFP control) were infused into the mOFC or lOFC (AP +4.7, ML ±0.6, DV -2.5 or AP +3.7, ML ±2.5, DV -3.2, respectively). A stainless steel bevelled needle was inserted to the required infusion location, lowered a further 0.1 mm and retracted back to the intended coordinates. The virus was infused at a rate of 100 nL/min for seven and a half minutes, seven and a half minutes after the needle was inserted. The needle was left in place for a further seven and a half minutes prior to lowering by 0.1 mm and slowly retracting fully.

This procedure was carried out in both hemispheres prior to suture closure. The virus was allowed five weeks to express prior to testing; during this time the rats were allowed one-week recovery and two more weeks of training. Following surgery rats were single housed overnight and then returned to their home cage for the remainder of the experiment.

A.3.2.2.2 Histology

Rats were anaesthetised with an intraperitoneal injection of pentobarbital and perfused transcardially with 0.01M PBS followed by 10% PFA. The brains were removed, postfixed for 4 hours in PFA, and stored in 30% sucrose solution. The brain was then sliced 60 μm thick in the coronal plane. Slices were washed three times for 10 minutes in 0.01M PBS and then blocked at room temperature for 1 hour in blocking buffer (containing 3% Normal Goat Serum (Vector Laboratories Inc), 0.3% Triton X-100 (Fisher Scientific) in 0.01M PBS). Slices were then incubated overnight at 4°C in the primary antibody solution (1:5000 rabbit anti-GFP (ab290, Abcam), in 1% Normal Goat Serum (Vector Laboratories Inc), 0.3% Triton X-100 (Fisher Scientific) in 0.01M PBS). Following incubation in the primary antibody, the slices were washed with PBS (3x10 mins) and incubated in goat anti-rabbit Alexafluor488

(1:250, Abcam) in blocking buffer (1% Normal Goat Serum (Vector Laboratories Inc), 0.3% Triton X-100 (Fisher Scientific) in 0.01M PBS) for 2 hours at room temperature.

Slices were then washed in 0.01M PBS (3x10 mins), mounted and coverslipped with FluorSave reagent (Calbiochem).

Slices were then visualised using a Zeiss Axio Imager (Zeiss).

A.3.2.3 Results

A.3.2.3.1 Histology

Figure 8.6 shows infusion locations of mOFC (A) and lOFC (B) DREADDs following immunohistochemistry. Positions were correct as per the defined regions by Paxinos and Watson (2006).

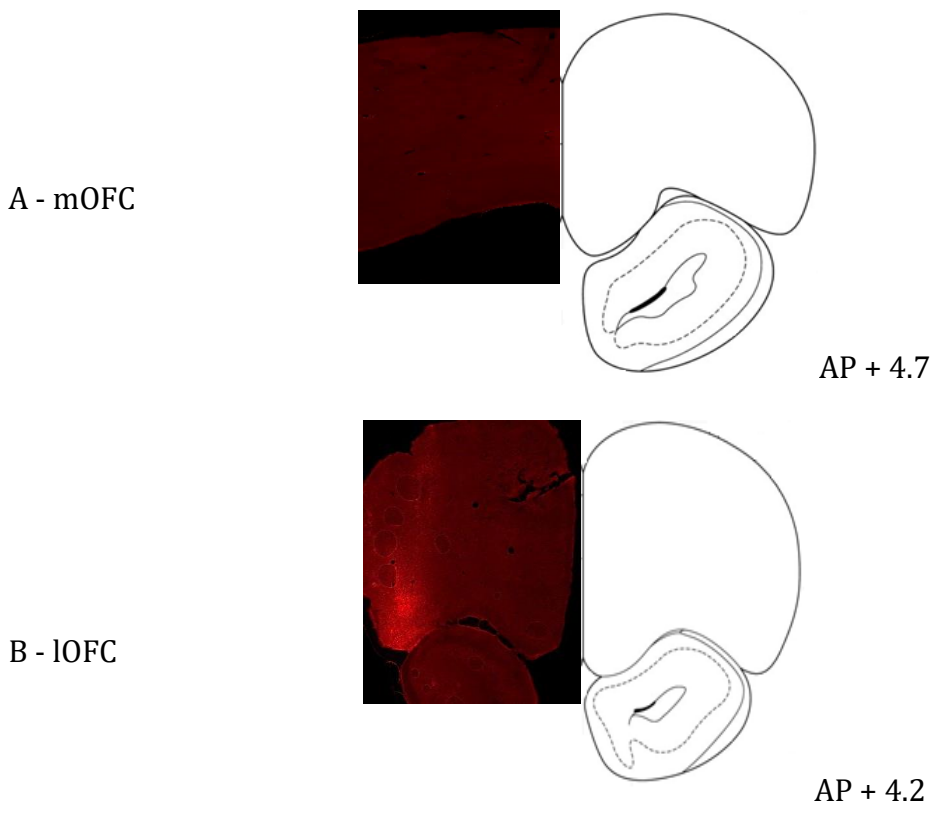


Figure 8.6: **Immunofluorescent images of DREADD infusion locations into the mOFC (A) and lOFC (B), alongside representative images from Paxinos and Watson (2006).**

A large number of the infusions were deemed to be in the incorrect location following imaging analysis, final numbers per group can found in Table 8.1. All animals with virus in the incorrect locations were combined together into a new "miss" group. Three animals died during testing and therefore were fully excluded from all analysis.

Location	Original number of rats	Final number of rats
mOFC	16	5
lOFC	16	12
GFP	16	15
Miss	-	13

Table 8.1: **Summary of the cohorts of animals used in Experiment 2.**

A.3.2.3.2 Trials

A three-way repeated measures mixed design ANOVA revealed there were no significant main effects of Dose or Location ($F_{3,123} = 0.215$, $p = 0.886$ and $F_{3,41} = 0.505$, $p = 0.681$, respectively). There was a main effect of Phase ($F_{2,82} = 43.096$, $p \leq 0.001$).

There were also no significant two-way interactions (Dose x Phase $F_{6,246} = 0.195$, $p = 0.978$, Phase x Location $F_{6,82} = 1.140$, $p = 0.347$, and Dose x Location $F_{9,123} = 0.907$, $p = 0.522$), or three-way interaction ($F_{18,246} = 1.479$, $p = 0.066$). Results are displayed in Figure 8.7.

A.3.2.3.3 Incorrect

A three-way repeated measures mixed design ANOVA revealed there were no significant main effects of Dose or Location ($F_{3,123} = 0.245$, $p = 0.865$ and $F_{3,41} = 0.513$, $p = 0.676$, respectively). There was a main effect of Phase ($F_{2,82} = 78.349$, $p \leq 0.001$).

There were also no significant two-way interactions (Dose x Phase $F_{6,246} = 0.195$, $p = 0.969$, Phase x Location $F_{6,82} = 1.11$, $p = 0.362$, and Dose x Location $F_{9,123} = 0.787$, $p = 0.629$), or three-way interaction ($F_{18,246} = 1.515$, $p = 0.085$). Results are displayed in Figure 8.8.

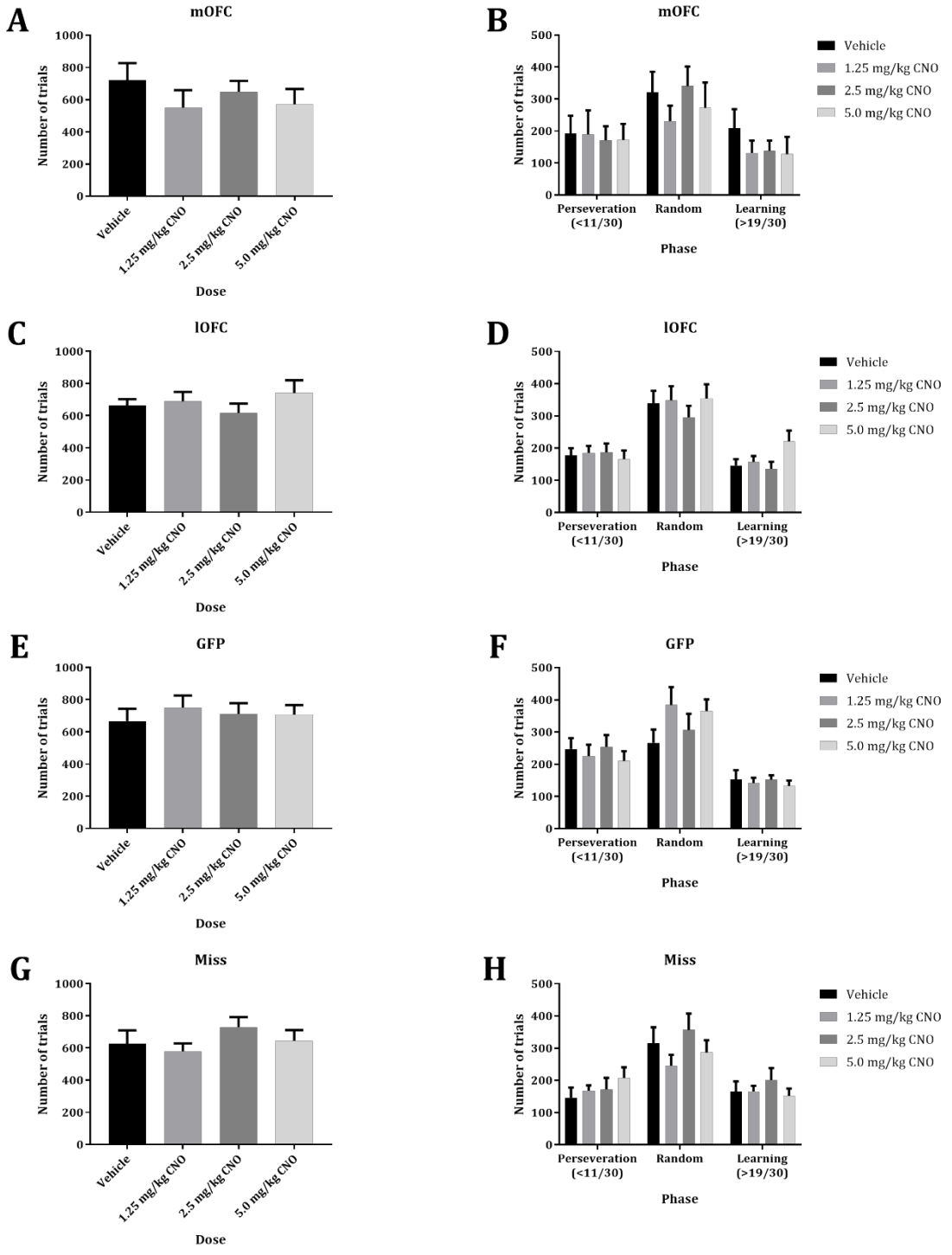


Figure 8.7: **Histograms showing the total number of trials to criterion following injection of CNO following DREADD infusion into the mOFC (A), lOFC (C), GFP control (E), or missing (G), and the number of trials per phase (B, D, F, and H). No significant differences were found. Data are represented as mean values ± SEM.**

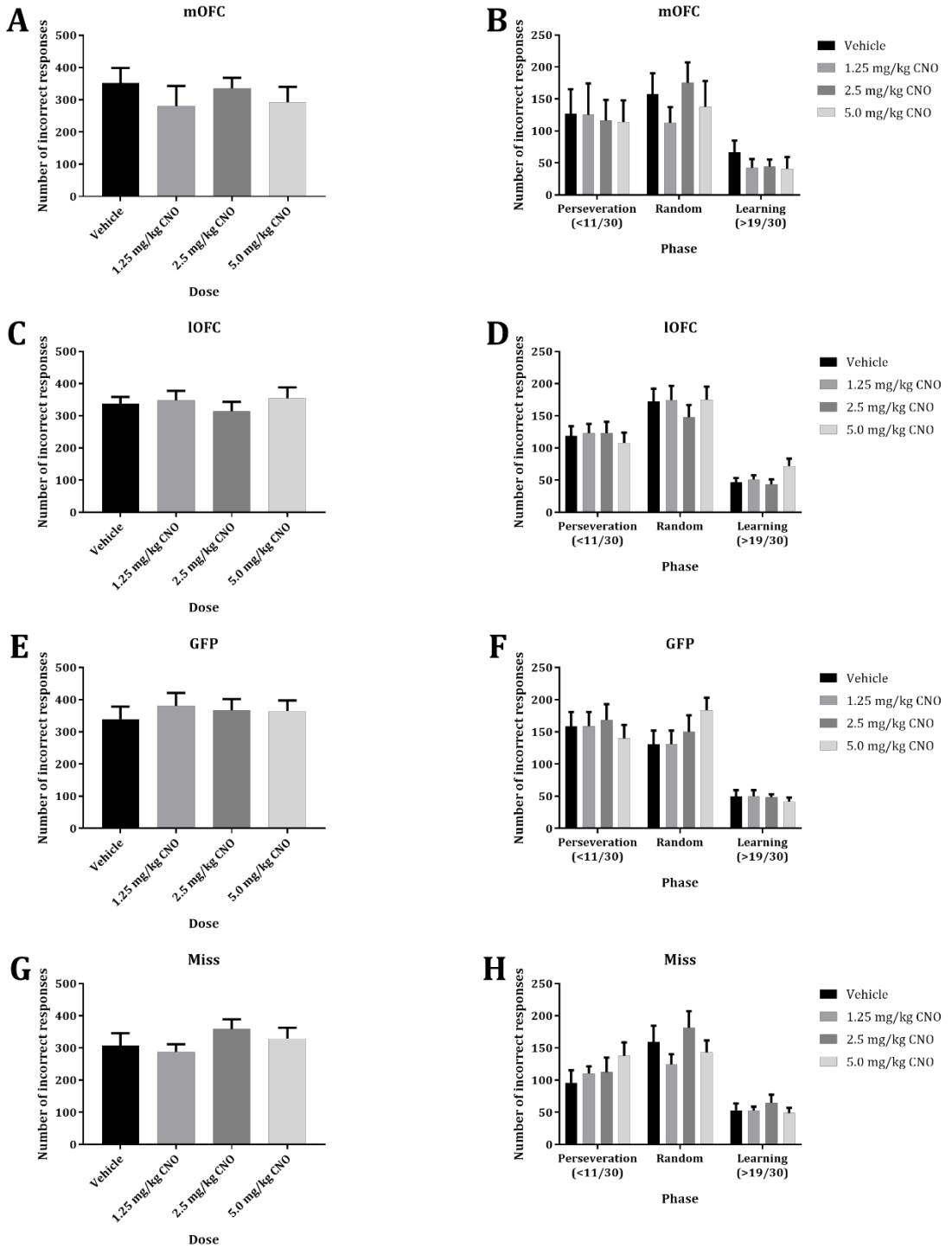


Figure 8.8: **Histograms showing the total number of incorrect responses to criterion following injection of CNO following DREADD infusion into the mOFC (A), lOFC (C), GFP control (E), or missing (G), and the number of incorrect responses per phase (B, D, F, and H). No significant differences were found. Data are represented as mean values ± SEM.**

A.3.3 Experiment 3: Investigating the effects of different inhibitory DREADD promotors and viral titres in the medial OFC

A.3.3.1 Experimental rationale

Following the lack of results in both Experiments 1 and 2, it was decided to pilot the DREADDs using different promotor dependent DREADDs and different viral loads in the medial OFC.

Human synapsin 1 (hSYN) is a promotor found in neurons which has been used previously to target neuronal expression of transgenes in rats (Kügler, Kilic and Bähr, 2003). Calcium/calmodulin-dependent protein kinase (CamKII) is also found in the rat cortex (Liu and Jones, 1996), as well as in monkeys (Jones, Huntley and Benson, 1994). Previous work by Lopez *et al.* (2016) has shown that there are differences between hSYN and CamKII in the modulation of hippocampal plasticity and memory. The use of hSYN and CamKII as DREADDs promotors should allow for viral expression in the mOFC and therefore an improvement in reversal performance following CNO activation.

In addition to using two different promotors, the effect of viral titre was also investigated. Using a high viral load should lead to high expression of DREADDs in the target neurons; however, a high expression could lead to basal activity of the DREADDs and therefore an effect would not be able to be measured in this instance (Roth, 2016). In order to ensure that there was no basal activity, and to ensure the neuron does not become overwhelmed and unstable, two viral titres were also compared.

A.3.3.2 Methods

A.3.3.2.1 Viral transfusion surgery

48 male Lister Hooded rats (Charles River, UK), were trained and surgically prepared as previously described (Chapter 2, Appendix A~ Methods).

AAV5-CamKII-hM4Di or AAV5-hSYN-hM4Di were infused into the mOFC (AP +4.7, ML ±0.6, DV -2.5). A stainless steel bevelled needle was inserted to the required infusion location, lowered a further 0.1 mm and retracted back to the intended coordinates. The virus was infused at a rate of 100 nL/min for seven and a half minutes, five minutes after the needle was inserted. The needle was left in place for a further five minutes prior to lowering by 0.1 mm and slowly retracting fully.

This procedure was carried out in both hemispheres prior to suture closure. The virus was allowed five weeks to express prior to testing; during this time the rats were allowed one-week recovery and two more weeks of training. Following surgery rats were single housed overnight and then returned to their home cage for the remainder of the experiment.

A.3.3.2.2 Histology

Rats were anaesthetised with an intraperitoneal injection of pentobarbital and perfused transcardially with 0.01M PBS followed by 10% PFA. The brains were removed, postfixed for 4 hours in PFA, and stored in 30% sucrose solution. The brain was then sliced 60 μm thick in the coronal plane. Slices were washed three times for 10 minutes in 0.01M PBS and then blocked at room temperature for 1 hour in blocking buffer (containing 3% Normal Goat Serum (Vector Laboratories Inc), 0.3% Triton X-100 (Fisher Scientific) in 0.01M PBS). Slices were then incubated overnight at 4°C in the primary antibody solution (1:5000 rabbit anti-GFP (ab290, Abcam), in 1% Normal Goat Serum (Vector Laboratories Inc), 0.3% Triton X-100 (Fisher Scientific) in 0.01M PBS). Following incubation in the primary antibody, the slices were washed with PBS (3x10 mins) and incubated in goat anti-rabbit Alexafluor488 (1:250, Abcam) in blocking buffer (1% Normal Goat Serum (Vector Laboratories Inc), 0.3% Triton X-100 (Fisher Scientific) in 0.01M PBS) for 2 hours at room temperature.

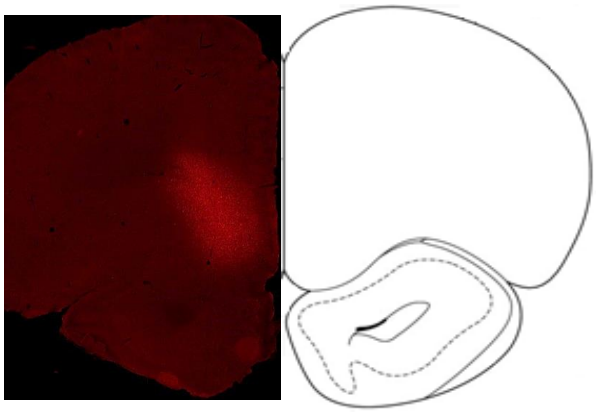
Slices were then washed in 0.01M PBS (3x10 mins), mounted and coverslipped with FluorSave reagent (Calbiochem).

Slices were then visualised using a Zeiss Axio Imager (Zeiss).

A.3.3.3 Results

A.3.3.3.1 Histology

Figure 8.9 shows infusion of DREADDs into the mOFC following immunohistochemistry. Positions were correct as per the defined regions by Paxinos and Watson (2006).



AP +4.2

Figure 8.9: **Representative Immunofluorescent image of DREADD infusion into the mOFC, alongside representative images from Paxinos and Watson (2006).**

A.3.3.3.2 Trials and incorrect responses independent of virus type or titre

A two-way repeated measures ANOVA revealed that there was no significant main effect of Dose on the number of trials ($F_{2,28}$ = 1.039, p = 0.367); however, there was a significant main effect of Phase ($F_{2,28}$ = 8.768, p = 0.001, Figure 8.10A).

There was also no significant Dose x Phase interaction ($F_{4,56}$ = 0.640, p = 0.636, Figure 8.10B).

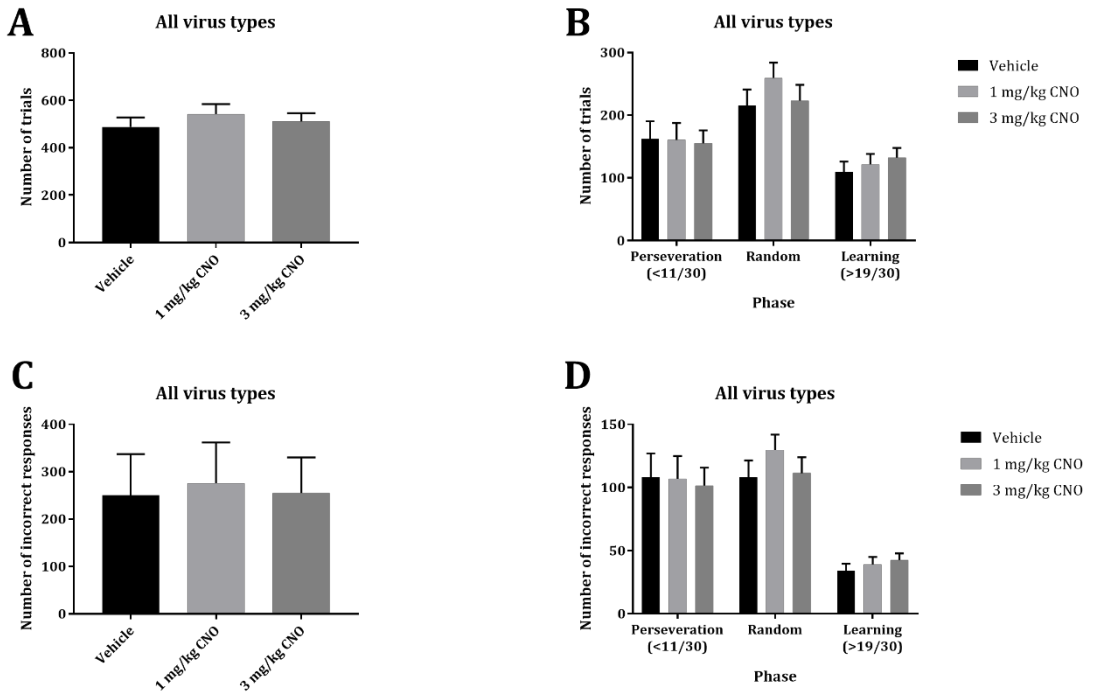


Figure 8.10: **Histograms showing the total number of trials or incorrect responses to criterion following injection of CNO following DREADD infusion into the mOFC (A and C), and the number of trials and incorrect responses per phase (B and D). No significant differences were found. Data are represented as mean values ± SEM.**

There was a significant main effect of Phase on the number of incorrect responses ($F_{2,28}$ = 17.141, $p \leq 0.001$,); however, there was no significant effect of Dose or Dose x Phase interaction observed ($F_{2,28}$ = 0.996, p = 0.382, and $F_{4,56}$ = 0.607, p = 0.659, respectively, Figure 8.10C and D).

A.3.3.3.3 Trials

When data were analysed with virus type/titre included in the analysis, a three-way mixed design repeated measures ANOVA revealed there was a significant main effect of Phase ($F_{2,22}$ = 9.085, $p \leq 0.001$); however, there were no significant main effects of Dose or Virus ($F_{2,22}$ = 0.966, p = 0.396, and $F_{3,11}$ = 0.522, p = 0.676, respectively, Figure 8.11 A, C, E, and G).

No significant two-way interactions were found (Dose x Phase $F_{4,44}$ = 0.615, p = 0.654, Dose x Virus $F_{2,22}$ = 0.635, p = 0.701, Virus x Phase $F_{6,22}$ = 0.892, p = 0.518),

there was also no significant three-way interaction ($F_{12,44}$ = 0.828, p = 0.621, Figure 8.11B, D, F and H).

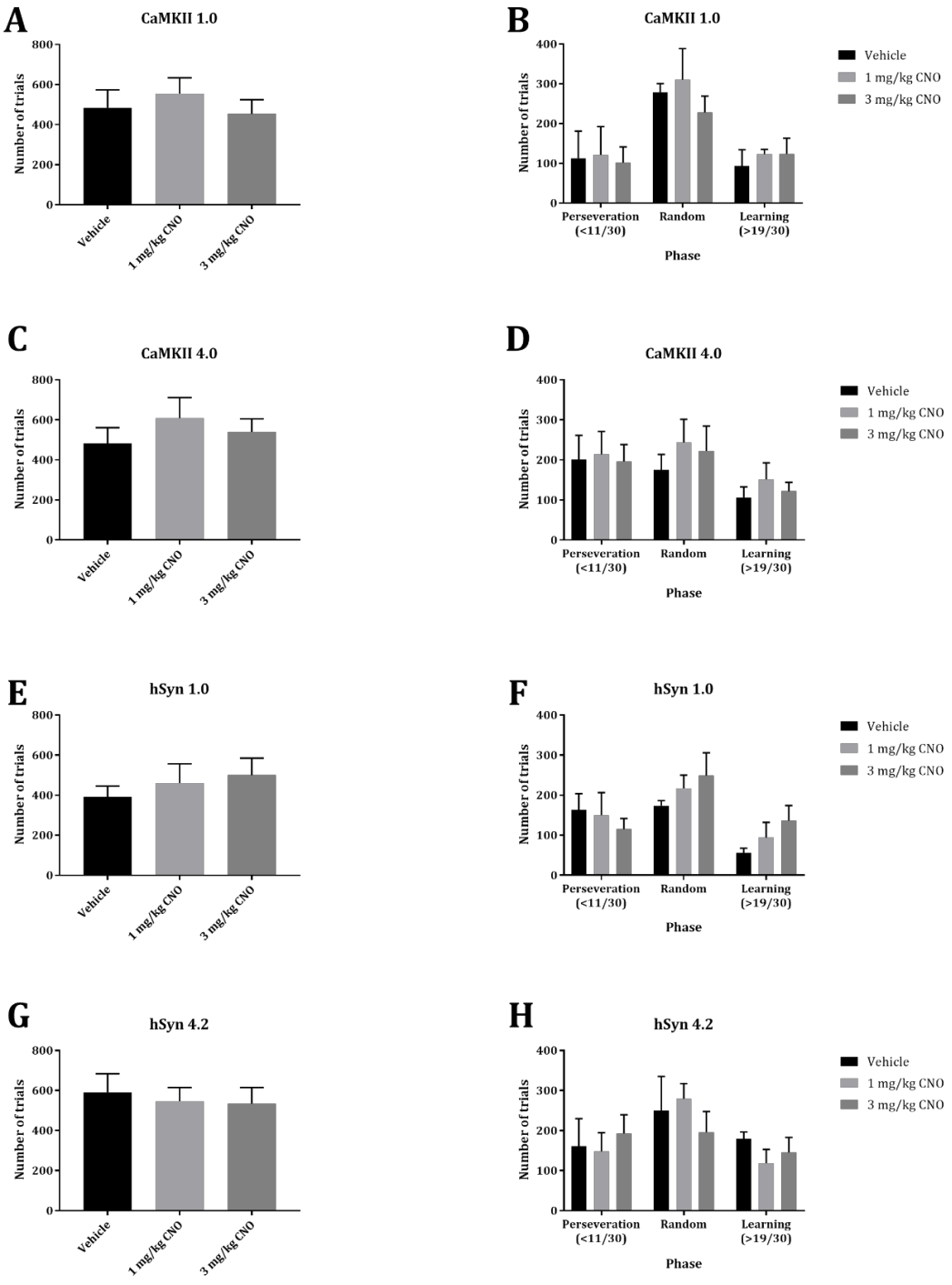


Figure 8.11: **Histograms showing the total number of trials to criterion following injection of CNO following infusion into the mOFC for CaMKII 1.0 (A), 4.0 (C) and hSyn 1.0 (E) and 4.2 (G), dependent DREADDs, and the number of trials per phase (B, D, F, and H). No significant differences were found. Data are represented as mean values ± SEM.**

A.3.3.3.4 Incorrect responses

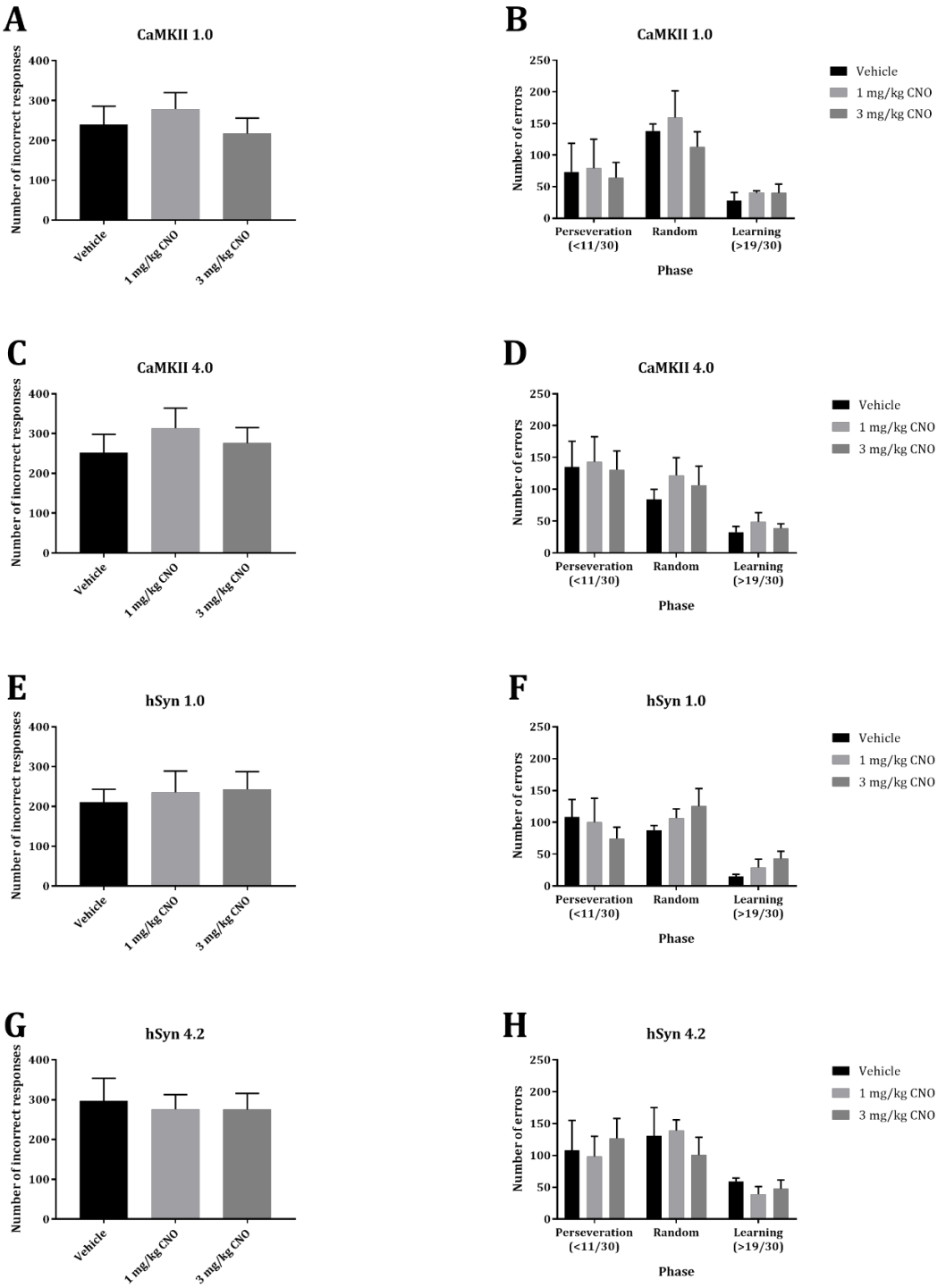


Figure 8.12: **Histograms showing the total number of incorrect responses to criterion following injection of CNO following infusion into the mOFC for CaMKII 1.0 (A), 4.0 (C) and hSyn 1.0 (E) and 4.2 (G) dependent DREADDS, and the number of incorrect responses per phase (B, D, F, and H). No significant differences were found. Data are represented as mean values ± SEM.**

A three-way ANOVA revealed there was a significant main effect of Phase on the number of incorrect responses ($F_{2,22}$ = 16.746, $p \leq 0.001$); however, there was no effect of Dose or Virus ($F_{2,22}$ = 0.950, p = 0.402, and $F_{3,11}$ = 0.523, p = 0.675, respectively, Figure 8.12 A, C, E and G).

There were also no significant two-way interactions (Dose x Phase $F_{4,44}$ = 0.586, p = 0.675, Dose x Virus $F_{2,22}$ = 0.613, p = 0.717, and Virus x Phase $F_{6,22}$ = 0.951, p = 0.480).

There was no significant three-way interaction ($F_{12,44}$ = 0.773, p = 0.674, Figure 8.12 B, D, F and H).

A.4. Discussion

Unfortunately, the DREADDs experiments did not produce validated results to further our understanding of pathway specific modulation.

Validation of DREADDs function and expression could not be obtained due to the lack of access to patch-clamp technology; also the DREADDs could either be confirmed functionally or their location, though not both, and therefore their correct location was deemed a priority at the time. Immunohistochemistry analysis were performed to confirm the expression and location of the DREADDs virus, however this relied on the expression of a secondary fluorescent protein that was not bound to the DREADD, therefore expression and trafficking of the DREADD protein was a secondary assumption. Also, immunohistochemistry of a bound fluorescent marker was not able to produce validation due to lack of fluorescence. Fluorescence of the secondary marker was unable to be quantified due to a lack of stereology equipment; and electrophysiology confirmation of DREADDs effects was not able to be performed to due tissue collection methods and visualisation protocols.

Recent findings by Gomez et al. (2017) report a lack of mechanism of action for clozapine-N-oxide (CNO) at DREADDs in vivo, with CNO not entering the brain following systemic injection, and a low affinity for DREADDs. Taken together, the lack of confirmation of expression, functional quantification, and systemic CNO action compound the lack of behavioural effects.

Appendix B. FURTHER STATISTICAL CONSIDERATIONS

To further interrogate the data obtained in this thesis, the combined vehicle data from the DREADDs animals (n=88) were analysed.

Using the DREADDs data I was able to obtain representative numbers for the average number of trials, incorrect response, and omissions to criterion per phase of the reversal.

Using this bank of data, I also analysed the amount of incorrect responses and omissions as a proportion of the number of trials per phase of the reversal task, this allows for the understanding of the distribution of these types of responses throughout the task.

Finally, using the representative variable size, I calculated *a priori* sample sizes in order to obtain adequate power for the studies. This is followed by tables displaying the observed effect sizes and achieved power for each study.

B.1. Variable size

B.1.1 Number of Trials

Figure 9.1 shows the number of incorrect responses per phase of reversal, these values are represented in Table 9.1.

The number of trials to criterion per phase of reversal are not normally distributed when analysed in their original number for format ($p = 0.007$, $p = 0.0003$, and $p < 0.0001$ for the perseveration, random and learning phases, respectively); therefore, all further analysis of the number of trials will be normalised through a square root transformation ($p > 0.05$ for all phases). Further interrogation of the number of trials through a repeated measures one-way ANOVA revealed a significant effect of phase ($F_{2,174} = 35.59$, $p < 0.001$); therefore, further analysis will exclude a main effect of phase.

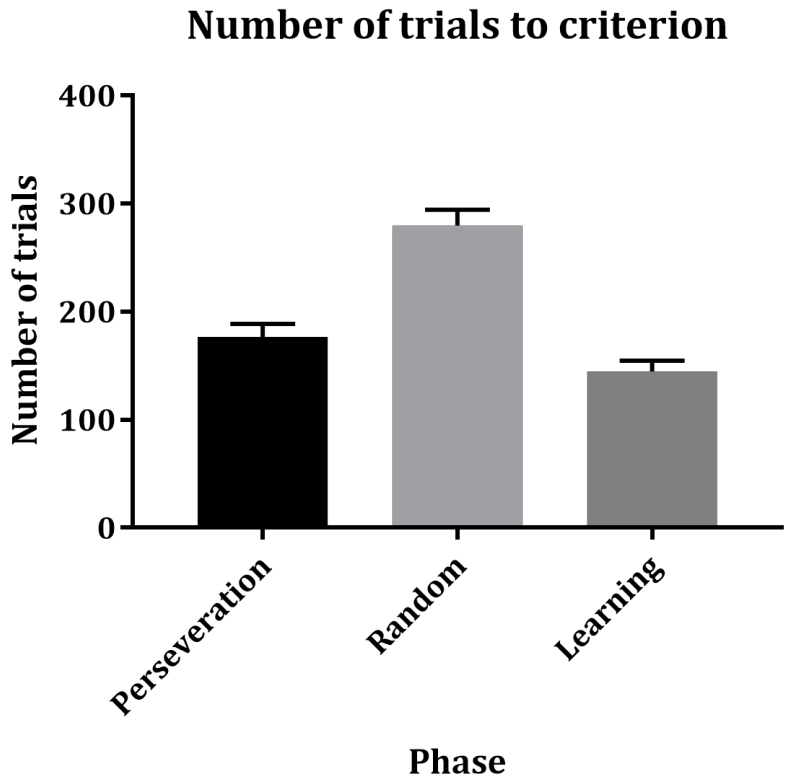


Figure 9.1: **A histogram representing the number of trials to criterion per phase of the reversal paradigm (n=88). The average number of responses is represented in Table 9.1. All data are represented as mean ± SEM.**

Perseveration	Random	Learning
176.9 ± 11.80	279.9 ± 14.66	144.9 ± 9.94

Table 9.1: **A table showing the mean number of trials to criterion per phase of reversal. Data are represented as number of trials ± SEM.**

A significant effect of phase shows that the number of trials completed in each part of the reversal task are not equal, and it is clear that animals will spend more time in the Random phase of the task. An increase in the number of trials in the Random (new learning) phase of the task would suggest that this period of learning requires more choice input; an animal can successfully overcome previously learned reward

to complete the perseveration phase, however more trials and more information are required to successfully learn the new parameters of the task.

B.1.2 Number of Incorrect responses

Figure 9.2 shows the number of incorrect responses per phase of reversal, these values are represented in Table 9.2.

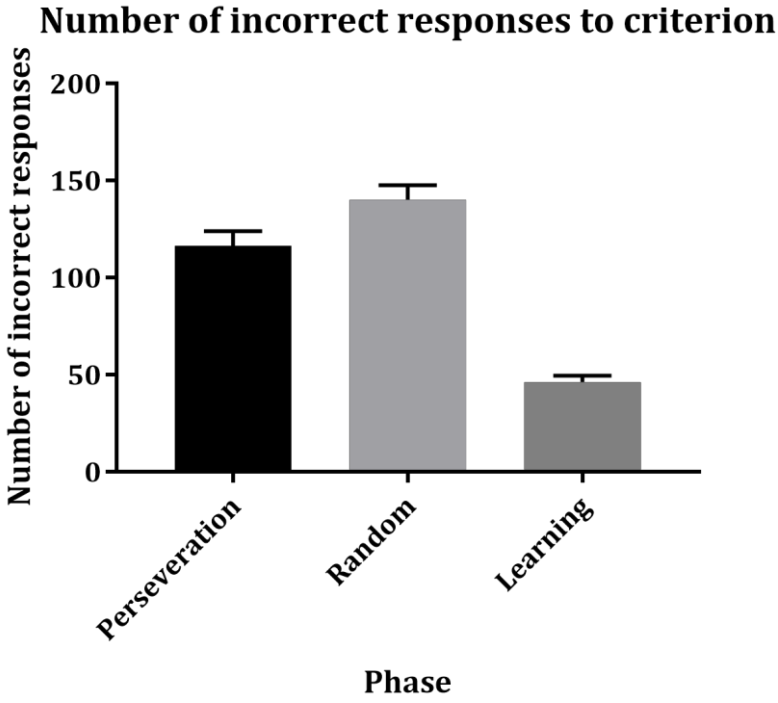


Figure 9.2: **A histogram representing the number of incorrect responses to criterion per phase of the reversal paradigm (n=88). The average number of responses is represented in Table 9.2. All data are represented as mean ± SEM.**

Perseveration	Random	Learning
116.4 ± 7.73	140.3 ± 7.50	46.3 ± 3.35

Table 9.2: **A table showing the mean number of incorrect responses to criterion per phase of reversal. Data are represented as number of trials ± SEM.**

The number of trials to criterion per phase of reversal are not normally distributed when analysed in their original number for format (p = 0.01, p = 0.0007, and p < 0.0001 for the perseveration, random and learning phases, respectively); therefore, all further analysis of the number of trials will be normalised through a square root transformation (p > 0.05 for all phases). Further interrogation of the number of trials through a repeated measures one-way ANOVA revealed a significant effect of phase ($F_{2,174}$ = 68.48, p < 0.001); therefore, further analysis will exclude a main effect of phase.

A significant effect of phase shows that the number of incorrect responses completed in each part of the reversal task are not equal, and it is clear that animals will spend more time in the Random phase of the task. The reasoning for this is similar to that for the number of trials; also, if an animal is spending more time in the Random phase of the task and performing more trials, then there are more opportunities to commit an incorrect response.

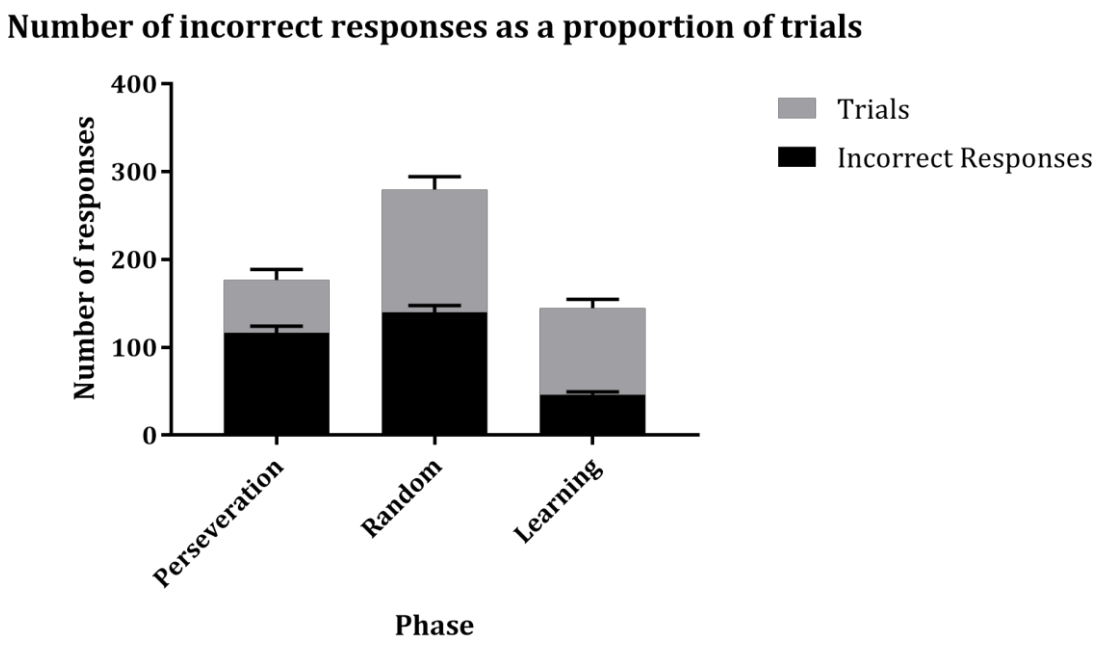


Figure 9.3: **A histogram representing the number of incorrect responses as a proportion of the number of trials taken to criterion per phase of the reversal task. Data are represented as mean ± SEM.**

Figure 9.3 shows the number of incorrect responses as a proportion of the number of trials per phase of the reversal task. There is a 65.8% chance that a trial during the Perseveration phase will be incorrect, whereas this drops to 50.1% during the Random phase and 32.0% during the Learning phase. This reduction in incorrect responses is to be expected as the animal learns throughout the course of the reversal; this is also generated by the type of analysis performed on the reversal data, by separating the data into phases dependent on the proportion of incorrect responses.

B.1.3 Number of Omissions

Figure 9.4 shows the number of omissions observed during each phase of the reversal. This data is represented as mean ± SEM (Figure 9.4A), box and whisker plot (Figure 9.4B) and a scatter plot of each individual data point (Figure 9.4C). Omission data is not normally distributed ($p < 0.0001$ for each phase); following square-root transformation the number of omissions per phase are still not normally distributed ($p < 0.0001$), therefore data will be analysed in a non-parametric approach.

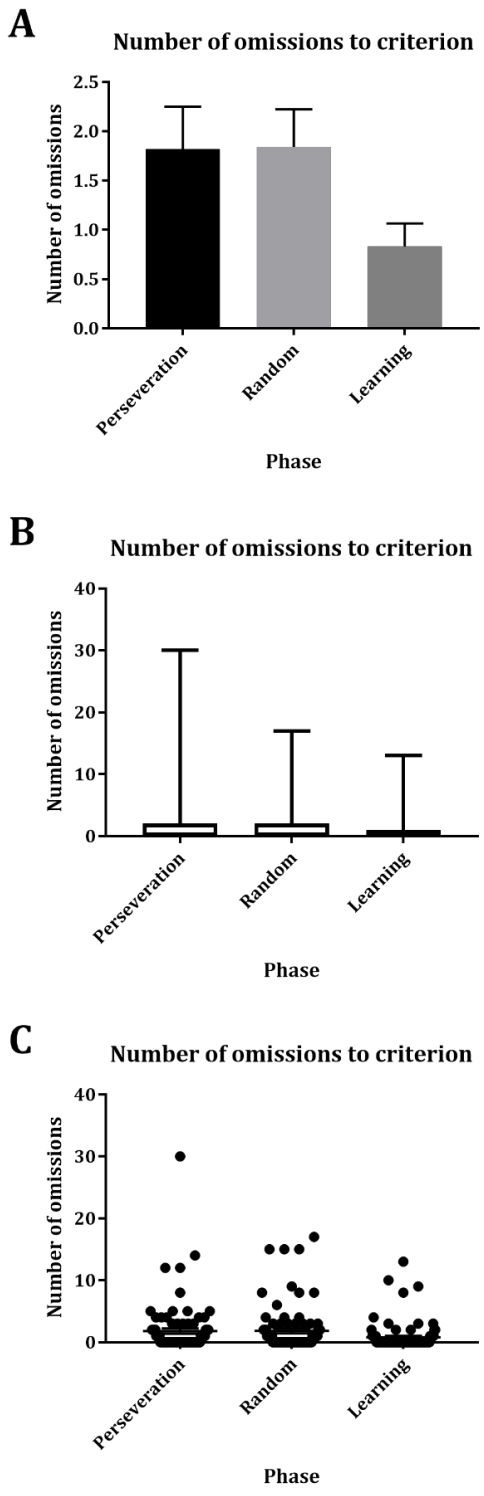


***Figure 9.4*: Graphical representations of the number of omissions to criterion per phase of the reversal paradigm (n=88). The average number of responses is represented in Table 9.3. Data are represented as mean ± SEM (A), box and whisker plot (B), and scatter plot (C).**

Perseveration	Random	Learning
1.82 ± 0.43	1.84 ± 0.38	0.83 ± 0.24

Table 9.3: **A table showing the mean number of trials to criterion per phase of reversal. Data are represented as mean ± SEM.**

B.2. Omission frequency distribution

To understand the distribution of omissions throughout the phases of the task, the number of omissions as a proportion of the number of trials for each phase was investigated (Figure 9.5).

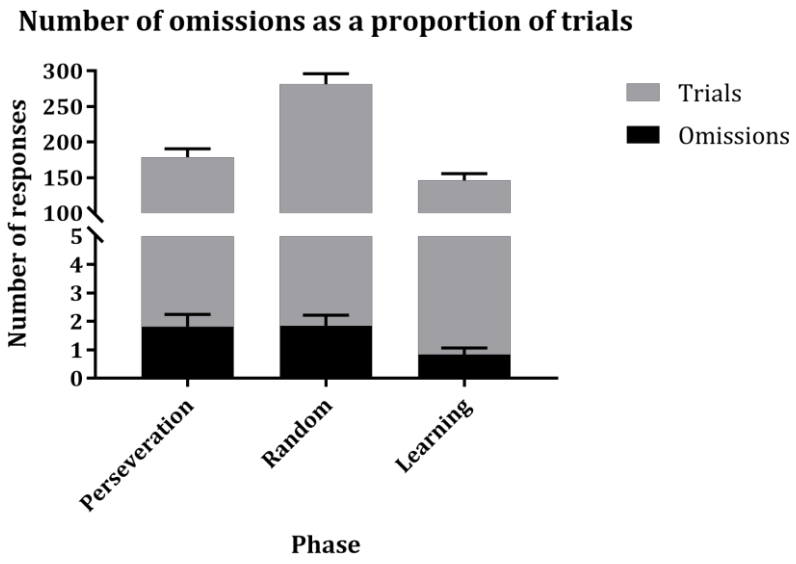


Figure 9.5: **A histogram representing the number of omissions as a proportion of the number of trials taken to criterion per phase of the reversal task. Data are represented as mean ± SEM.**

Figure 9.5 shows that while the number of omissions observed in the perseveration and random (new learning) phases are approximately equal, 1.82 and 1.84 respectively, it is clear that the proportion of omissions per phase compared to the number trials is higher in the perseveration phase of the reversal. There is a 1.03%

chance that a trial during the perseveration phase will be an omission, however there is a 0.66% chance that a trial will be omitted during the middle phase of the reversal task.

This increase in probability of an omission during the perseveration phase could be due to the animal starting to inhibit the prepotent response to the previous CS+, however they have not overcome the learned non-reward to attend to the new CS+, and therefore it appears that a decision cannot be made, resulting in an omission.

B.3. Estimates of sample sizes required – *a priori*

For Raclopride and SB-242,084 studies I intended to use three or four doses of compound (including Vehicle), therefore to calculate the required sample size for each study I assumed three groups.

B.3.1 Three group studies

Analysis of the DREADDs data for the number of trials showed a significant matching effect during the repeated measures analysis, therefore this was used this to predict a high correlation among repeated measures (0.7). As the effect size of Raclopride is not known in this task, either systemically or locally, I calculated the required sample size for numerous estimates of effect size based on Cohen's *d* (Cohen, 1988). In order to estimate the required sample size $\alpha = 0.05$ and Power $(1-\beta) = 0.8$

Effect size (*d*)	**Total required sample size**	**Required size of each group**
0.2	33	11
0.5	9	3
0.8	6	2

Table 9.4: **A table showing the estimates sample size required to study the number of trials for the Raclopride studies for small (*d* = 0.2) , medium (*d* = 0.5) and large (*d* = 0.8) estimates of effect size.**

Analysis of the DREADDs data for the number of incorrect responses did not show a significant matching effect during the repeated measures analysis, therefore it was assumed that there was no this was correlation among repeated measures (0.5).

Effect size (*d*)	**Total required sample size**	**Required size of each group**
0.2	54	18
0.5	12	4
0.8	9	3

Table 9.5: **A table showing the estimates sample size required to study the number of incorrect responses for the Raclopride studies for small (*d* = 0.2) , medium (*d* = 0.5) and large (*d* = 0.8) estimates of effect size.**

B.3.2 Two group studies

In order to estimate the sample size required for the frontal lobe and SCH-23390 studies, the same analysis was performed assuming two groups, drug and vehicle, for the number of trials (Table 9.6) and the number of incorrect responses (Table 9.7).

Effect size (*d*)	**Total required sample size**	**Required size of each group**
0.2	26	13
0.5	6	3
0.8	4	2

Table 9.6: **A table showing the estimates sample size required to study the number of trials for the inactivation studies for small (*d* = 0.2) , medium (*d* = 0.5) and large (*d* = 0.8) estimates of effect size.**

Effect size (*d*)	Total required sample size	Required size of each group
0.2	42	21
0.5	10	5
0.8	6	3

Table 9.7: **A table showing the estimates sample size required to study the number of incorrect responses for the inactivation studies for small (*d* = 0.2) , medium (*d* = 0.5) and large (*d* = 0.8) estimates of effect size.**

For all experiment types, dopamine and 5-HT manipulation and frontal lobe inactivation, fewer animals are required to achieve the same level of power to analyse the number of trials compared to the number of incorrect responses, this is due to the lack of correlation between repeated measures in the analysis of errors.

Due to the nature of the task it must be assumed that we allow for analysis in the weakest parameter, number of incorrect responses, therefore the required number of animals in order to see an effect must be weighted towards the higher end of the sample size.

B.4. Achieved statistical power – post hoc

Below are a series of tables summarising the statistical findings of this these, their estimated effect size as Partial Eta Squared, and the Observed Power. Effects that were investigated are highlighted in bold.

B.4.1 Chapter 3 – Systemic dopamine serial reversal

	Measure	**Significance**	**Partial Eta squared**	**Observed power**
Dose	**Trials**	$\boldsymbol{F_{2,24} = 5.85, p = 0.009}$	**0.33**	**0.837**
	Incorrect	$\boldsymbol{F_{2,24} = 5.86, p = 0.008}$	**0.33**	**0.838**
Phase	**Trials**	$\boldsymbol{F_{2,24} = 14.62, p < 0.001}$	**0.55**	**0.997**
	Incorrect	$\boldsymbol{F_{2,24} = 15.88, p < 0.001}$	**0.57**	**0.998**
Dose x Phase	Trials	$F_{4,48} = 0.45$, $p = 0.77$	0.04	0.147
	Incorrect	$F_{4,48} = 0.67$, $p = 0.61$	0.05	0.203

B.4.2 Chapter 4 – Local D_2 manipulations

	Measure	**Significance**	**Partial Eta squared**	**Observed power**
Dose	Trials	$F_{2,70} = 0.193$, $p = 0.825$	0.005	0.079
	Incorrect	$F_{2,70} = 0.165$, $p = 0.848$	0.005	0.075
Phase	**Trials**	$\boldsymbol{F_{2,70} = 46.748, p < 0.001}$	**0.572**	**1.00**
	Incorrect	$\boldsymbol{F_{2,70} = 64.807, p < 0.001}$	**0.649**	**1.00**
Location	Trials	$F_{3,35} = 2.562$, $p = 0.070$	0.180	0.581

	Measure	**Significance**	**Partial Eta squared**	**Observed power**
	Incorrect	$F_{3,35}$ =2.297, p = 0.095	0.164	0.530
Dose x Phase	Trials	$F_{4,120}$ = 1.190, p = 0.318	0.033	0.366
	Incorrect	$F_{4,120}$ = 1.068, p = 0.375	0.030	0.330
Dose x Location	**Trials**	$\boldsymbol{F_{6,70} = 2.25, p = 0.048}$	**0.162**	**0.753**
	Incorrect	$\boldsymbol{F_{6,70} = 2.46, p = 0.032}$	**0.174**	**0.796**
Phase x Location	Trials	$F_{6,70}$ = 1.068, p = 0.390	0.084	0.394
	Incorrect	$F_{6,70}$ = 0.870, p = 0.521	0.069	0.322
Dose x Phase x Location	**Trials**	$\boldsymbol{F_{4,70} = 1.704, p = 0.072}$	**0.127**	**0.844**
	Incorrect	$\boldsymbol{F_{4,70} = 1.828, p = 0.049}$	**0.136**	**0.874**

B.4.3 Chapter 5 – Local D_1 and 5-HT_{2C} manipulations

B.4.3.1 Local D_1 manipulations

	Measure	**Significance**	**Partial Eta squared**	**Observed power**
Dose	Trials	$F_{1,9}$ = 0.130, p = 0.727	0.014	0.062
	Incorrect	$F_{1,9}$ = 0.336, p = 0.576	0.036	0.082
Phase	Trials	$F_{2,18}$ = 5.105, p = 0.018	0.362	0.751
	Incorrect	$F_{2,18}$ = 9.114, p = 0.002	0.503	0.948
Location	Trials	$F_{1,9}$ = 1.300, p = 0.294	0.126	0.175
	Incorrect	$F_{1,9}$ = 1.293, p = 0.295	0.126	0.175
Dose x Phase	Trials	$F_{2,18}$ = 1.351, p = 0.284	0.131	0.253
	Incorrect	$F_{2,18}$ = 1.963, p = 0.169	0.179	0.352
Dose x Location	Trials	$F_{1,9}$ = 0.08, p = 0.784	0.009	0.057
	Incorrect	$F_{1,9}$ = 0.08, p = 0.784	0.009	0.057
Phase x Location	Trials	$F_{2,18}$ = 0.082, p = 0.922	0.009	0.061
	Incorrect	$F_{2,18}$ = 0.064, p = 0.938	0.007	0.058

	Measure	Significance	Partial Eta squared	Observed power
Dose x Phase x Location	Trials	$F_{2,18}$ = 0.131, p = 0.878	0.014	0.067
	Incorrect	$F_{2,18}$ = 0.158, p = 0.855	0.017	0.071

B.4.3.2 Local 5-HT$_{2C}$ manipulations

	Measure	Significance	Partial Eta squared	Observed power
Dose	Trials	$F_{2,\ 24}$ = 1.523, p = 0.238	0.113	0.292
	Incorrect	$F_{2,24}$ = 1.881, p = 0.174	0.135	0.352
Phase	Trials	$F_{2,24}$ = 10.393, p = 0.001	0.464	0.976
	Incorrect	$F_{2,24}$ = 14.939, p < 0.001	0.555	0.998
Location	Trials	$F_{1,12}$ = 0.075, p = 0.789	0.006	0.057
	Incorrect	$F_{1,12}$= 0.125, p = 0.730	0.010	0.062
Dose x Phase	Trials	$F_{4,48}$= 1.284, p = 0.290	0.097	0.370
	Incorrect	$F_{4,48}$= 1.691, p = 0.168	0.123	0.480

	Measure	Significance	Partial Eta squared	Observed power
Dose x Location	Trials	$F_{2,24}$ = 0.827, p = 0.449	0.064	0.175
	Incorrect	$F_{2,24}$ = 1.023, p = 0.375	0.079	0.207
Phase x Location	Trials	$F_{2,24}$ = 0.751, p = 0.483	0.059	0.162
	Incorrect	$F_{2,24}$ = 0.779, p = 0.470	0.061	0.167
Dose x Phase x Location	Trials	$F_{4,48}$ = 0.596, p = 0.667	0.047	0.183
	Incorrect	$F_{4,48}$ = 0.795, p = 0.534	0.062	0.236

B.4.4 Chapter 6 – Frontal lobe inactivation

	Measure	Significance	Partial Eta squared	Observed power
Inactivation	**Trials**	$\boldsymbol{F_{1,41} = 6.005, p = 0.019}$	**0.128**	**0.667**
	Incorrect	$\boldsymbol{F_{1,41} = 5.787, p = 0.021}$	**0.124**	**0.651**
Phase	**Trials**	$\boldsymbol{F_{2,82} = 25.77, p < 0.001}$	**0.386**	**1.00**
	Incorrect	$\boldsymbol{F_{2,82} = 33.979, p < 0.001}$	**0.453**	**1.00**

	Measure	**Significance**	**Partial Eta squared**	**Observed power**
Location	Trials	$F_{3,41}$ = 1.416, p = 0.252	0.094	0.347
	Incorrect	$F_{3,41}$ = 1.624, p = 0.198	0.106	0.395
Inactivation x Phase	Trials	$F_{2,82}$ = 0.047, p = 0.954	0.001	0.057
	Incorrect	$F_{2,82}$ = 0.063, p = 0.939	0.002	0.059
Inactivation x Location	**Trials**	$\boldsymbol{F_{3,41} = 4.134, p = 0.012}$	**0.232**	**0.814**
	Incorrect	$\boldsymbol{F_{3,41} = 4.546, p = 0.008}$	**0.250**	**0.853**
Phase x Location	Trials	$F_{6,82}$ = 1.665, p = 0.140	0.109	0.604
	Incorrect	$F_{6,82}$ = 1.826, p = 0.104	0.118	0.652
Inactivation x Phase x Location	Trials	$F_{6,82}$ = 2.165, p = 0.055	0.137	0.740
	Incorrect	$\boldsymbol{F_{6,82} = 2.676, p = 0.02}$	**0.164**	**0.840**