THE NEUROPSYCHOPHARMACOLOGY OF REVERSAL LEARNING

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DECLARATION

This thesis, whether in the same or different form, has not been previously submitted to this or any other University for a degree

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THE NEUROPSYCHOPHARMACOLOGY OF REVERSAL LEARNING

Summary

Reversal learning deficits are a feature of many neuropsychiatric disorders, most notably schizophrenia. These deficits could be due, in part, to altered ability to dissipate either or both associations of previous positive (*perseverance*) and negative (*learned non-reward*) valence. Studies reported in this thesis developed an egocentric maze task and a visuospatial operant task for separate assessments of spatial reversal learning, perseverance and learned non-reward in mice. These tasks were subsequently used to assess the cognitive causes for altered performance after manipulations to brain systems recognised to be involved in reversal learning and relevant for human psychopathology, with a specific focus on schizophrenia.

NMDA receptor (NMDAr) antagonism through acute phencyclidine did not affect reversal learning in the operant task, but caused general impairments in the maze task. Orbitofrontal (OFC) lesioned mice showed perseverative impairments in the operant task. Mice treated with the 5-HT_{2C} receptor (5-HT_{2C}R) antagonist SB242084 and 5-HT_{2C}R KO mice showed facilitated reversal learning and decreased learned non-reward in the operant task. In the maze task, SB242084 decreased perseverance but increased learned non-reward, while 5-HT_{2C}R KO mice showed perseverance and discrimination learning deficits. The final experimental chapter investigated the effect of SB242084 on touch-screen visual reversal learning in the rat. SB242084 retarded learning in this task.

These studies demonstrate that previously non-reinforced associations can be of considerable importance in tasks of cognitive flexibility. The studies also show that the NMDAr, the 5-HT_{2C}R, and the OFC, are involved in reversal learning and can modulate mechanisms related to both perseverance and learned non-reward. Moreover, in reversal learning, few effects of manipulations affecting PFC-functioning, or activity at the NMDAr and 5-HT_{2C}R, generalise across the procedures in the visuospatial, egocentric spatial, and visual domains.

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LIST OF ABBREVIATIONS

5,7-DHT	5,7-Dihydroxytryptamine
5-CSRTT	5-choice serial reaction time task
5-HT	5-hydroxytryptamine
5-HT_NR	5-hydroxytryptamine receptor
5-HTT	5-HT transporter
6-OHDA	6-Hydroxydopamine
ACC	anterior cingulate cortex
ACh	acetylcholine
AMPAr	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANOVA	analysis of variance
Ca^{2+}	calcium
CANTAB	cambridge neuropsychological test automated battery
CS	conditioned stimulus
DA	dopamine
D_NR	dopamine receptor
DAG	diglyceride
DStr	dorsal striatum
GABA	γ-Aminobutyric acid
GPCR	G-protein coupled receptor
IL	infralimbic cortex
i.p.	intraperitoneal
IP	inositol triphosphate
ID/ED	intra-dimensional/extra-dimensional
ITI	inter-trial interval
KO	knock-out
LED	light-emitting diode
LO	lateral orbital
LSD	least square difference *or* lysergic acid diethylamide
LTP	long-term potentiation
mCPP	meta-Chlorophenylpiperazine

Mg^{2+}	magnesium
MO	medial orbital
mPFC	medial prefrontal cortex
mRNA	messenger Ribonucleic acid
NAc	nucleus accumbens
NMDA	N-Methyl-D-aspartic acid
NMDAr	N-Methyl-D-aspartic acid receptor
OCD	obsessive-compulsive disorder
OFC	orbitofrontal cortex
PCP	phencyclidine
PCPA	parachlorophenylalanine
PCR	polymerase chain reaction
PFC	prefrontal cortex
PL	prelimbic cortex
PLC	phospholipase C
s.c.	subcutaneous
SEM	standard error of the mean
SNc	substantia nigra pars compacta
SNP	single-nucleotide polymorphism
SSRI	selective serotonin re-uptake inhibitor
US	unconditioned stimulus
VO	ventral orbital
VTA	ventral tegmental area
WCST	wisconsin card sorting task
WT	wild-type

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Purposeful goal-directed behaviours require organisms to flexibly adapt to constantly changing motivational goals and situational demands by overcoming previously learned associations. The ability to perform goal-directed behaviours across different situations therefore requires flexible associations between stimuli and reward, or cognitive flexibility. Disruptions in cognitive flexibility are a common feature of neuropsychopathology, which closely correlate with long-term health outcomes (Green, 1996, 2006; Holthausen et al., 2007; Harvey et al., 1998; Keefe et al., 2006) as well as remaining largely unaddressed by currently available therapeutics (Weiss et al., 2002). Importantly, the inability to treat these deficits can often be the rate-limiter of treatment progression, trapping patients within life-long social and financial dependency despite existing medication (Meltzer, 2003).

This chapter discusses paradigms of cognitive flexibility with a particular focus on reversal learning in relation to schizophrenia. It defines the underlying components of cognitive flexibility, and stresses that reversal learning can be thought of as a schedule of concurrent *perseverance* and *learned non-reward*. It reviews previous experiments designed to separately assess these mechanisms, and discusses their consequence for interpreting reversal learning performances. Finally, the potential benefits of reducing reversal learning into it constituent components as well as the aims of the thesis are outlined.

1.2 COGNITIVE FLEXIBILITY

Cognitive flexibility is most commonly assessed through reversal learning and attentional set-shifting tasks. Both reversal learning and attentional set-shifting are now part of the CANTAB ID/ED-task, which can be used with non-human primates and human participants, as well as the rodent bowl-digging version. These tasks normally consist of seven tests of increasing levels of difficulty. Subjects initially learn a two-

choice discrimination. This is followed by a compound discrimination, where a second superimposed sensory or cognitive dimension is introduced but the correct and incorrect stimuli remain constant. Next, subjects are challenged by an intradimensional-shift, where learned stimuli are replaced by novel stimuli, with the relevant and irrelevant dimensions remaining constant. When acquired, subjects are challenged by an extradimentional or attentional-set shift, where the irrelevant dimension becomes relevant, and the relevant dimension becomes irrelevant. Each of these tests is typically followed by a reversal test where the contingencies reverse.

Hence, reversal learning involves a single sensory or cognitive domain typically containing two different stimuli. After learning an initial CS+ versus CS- discrimination, the contingencies reverse. In contrast to reversal learning, attentional set-shifting involves at least two different superimposed sensory or cognitive domains each containing at least two different stimuli. In an initial acquisition phase, two stimuli within one sensory or cognitive domain serves as CS+ and CS- while stimuli within other domains are irrelevant. In the following set-shifting phase, the previous CS+ and CS- become irrelevant while stimuli within the previous irrelevant domain become the relevant CS+ and CS-. Although broadly similar, attentional set-shifting but not reversal learning demands attentional relocation, posing demands upon different neuronal and cognitive modalities (Bissonette et al., 2008; Dias et al., 1996; Ghods-Sharifi et al., 2007). As a domain of cognitive flexibility and neuropsychopharmacological assessment, reversal learning has been somewhat overshadowed by attentional set-shifting. One reason is likely to be the relatively greater difficulty of attentional set-shifting – increasing the likelihood of observing deficits from pathology as well as alleviation through treatment.

However, there is some evidence suggesting that reversal learning may be more suited for cross-species translation. For example, attentional set-shifting but not reversal learning is related to intelligence and language. If accounting for schizophrenic patients' current IQ, patients still show reversal learning deficits but do not differ from healthy controls in attentional set-shifting (Laws, 1999; Leeson et al., 2009). Prompting schizophrenic patients to verbalise their decision-making remediates poor set-shifting performance (Choi and Kurtz, 2007; Perry et al., 2001; Rossel and David, 1997; Rossi et al., 2006; Stratta et al., 1994), and when figural stimuli are replaced by verbal stimuli, the performance of schizophrenics patients deteriorates while the performance of healthy controls improves (Rossel and David, 1997). Hence, attentional set-shifting is

compromised by general verbal and intelligence deficits while reversal learning can be relatively independent of verbal and general intelligence (Leeson et al., 2009) and a better predictor of social functioning (Shamay-Tsoory et al., 2007).

1.3 REVERSAL LEARNING

Reversal learning has been assessed in many neuropsychiatric disorders and their associated animal models. Most or many human psychopathologies been shown to display deficits within cognitive flexibility, with reversal learning deficits observed in Parkinson's disease (Cools et al., 2001; Freedman and Oscar-Berman, 1989), Alzheimer's disease (Freedman and Oscar-Berman, 1989), obsessive compulsive disorder (Remijnse et al., 2006), autism (Coldren and Halloran, 2003) unipolar (Reischies, 1999) and bipolar depression (McKirdy et al., 2009), Huntington's disease (Lange et al., 1995; Lawrence et al., 1999; Oscar-Berman and Zola-Morgan, 1980), schizophrenia (Ceaser et al., 2008; Crumpton, 1963; Jazbec et al., 2007; Leeson et al., 2009; Murray et al., 2008; Nolan, 1974; Pantelis et al., 1997, 1999, 2004, 2009; Shamay-Tsoory et al., 2007; Tyson et al., 2004), Korsakoff's syndrome (Oscar-Berman and Zola-Morgan, 1980), attention-deficit hyperactivity disorder (Reeve and Schandler, 2001) post-traumatic stress disorder (Koenen et al., 2001) and by cocaine abusers (Ersche et al., 2008).

As reversal learning tasks fail to distinguish between pathologies, with patients across widely disparate diagnoses and symptom profiles displaying 'similar' deficits, current reversal paradigms can as a consequence be thought of as rather crude measures of cognitive functioning.

1.4 DISSCOCIATING THE COMPONENTS OF COGNITIVE FLEXIBILITY

Yet pathology-related deficits in cognitive flexibility may be due to abnormalities in either or both of two separate processes. In attentional set-shifting, the initial discrimination can be reduced to conditioned attention towards the relevant dimension and conditioned inattention towards the irrelevant dimension. After the subsequent contingency shift, the relevant dimension becomes irrelevant, a process opposed by perseverance. Conversely, the irrelevant dimension becomes relevant, a process opposed by learned irrelevance. In reversal learning, the initial two-choice

discrimination could be reduced to an excitatory CS-US association, eliciting approach and contact, and an inhibitory CS – 'no US' association, eliciting withdrawal (Mackintosh, 1983). After the subsequent contingency shift, the CS predicting the US becomes associated with 'no US', a process opposed by perseverance. Conversely, the CS initially predicating 'no US' now predicts the US, a process opposed by learned non-reward. Reversal learning and attentional set-shifting can hence be thought of as schedules of concurrent perseverance and learned non-reward or learned irrelevance, with deficits being due to a failure to dissipate either or both associations of previous positive (*perseverance*) and negative (*learned non-reward* or *learned irrelevance*) valence.

1.4.1 *Perseverance*

Perseverance is a hypernym for a range of phenomena related to inappropriate repetition or maintenance of an activity, response, or abstract rule. This includes, for example, various forms of repetitions or maintenance of motor-outputs akin to catatonia (Albert and Sandson, 1986; Freeman and Gathercole, 1966; Helmick and Berg, 1976; Luria, 1965). However, in clinical and preclinical studies, perseverance is most often used to describe a breakdown of executive functioning whereby abstract information encoding relationships between stimuli and behavioural goals are excessively and inappropriately repeated or maintained (Garner, 2006). Perseverance is required to obtain difficult goals and is therefore an integral part of goal-directed behaviours (Albert and Sandson, 1986; Ramage et al., 1999). However, excessive perseveration has for long been recognised as a component of psychopathology (Hughlings-Jackson, 1879; Wilson, 1908).

The terminology used to refer to perseverative responding in tasks of cognitive flexibility can appear complex. One reason for this complexity is likely to be an aspiration to separate the perseverative responding observed in attentional set-shifting and reversal learning tasks. For example, deficits have been referred to as stuck-in-set perseverance (Sandson and Albert, 1984; Rolls et al., 1994), stimulus-driven perseverance (Kodituwakku et al., 2001), paradigmatic or affective perseveration (Hauser, 1999), recurrent perseveration (Nagahama et al., 2005) or intentional perseveration (Hudson, 1968).

Fundamentally, perseverative behaviour is never defined by the valence of the

association or behaviour that is inappropriately repeated or maintained. Thus, excessive avoidance of a previously negative pairing and excessive approach of a previously positive pairing are both perseverative responses. Yet, perseveration has become used specifically to specify an inability to overcome positively reinforced rather than irrelevant or non-reinforced associations (Boulougouris et al., 2008; Clarke et al., 2007). Importantly, the vast majority of preclinical manipulations of cognitive flexibility, as well as pathology-related deficits within cognitive flexibility, are interpreted as due to altered perseverance. This is often done without considering manipulations or alterations in the ability to overcome non-reinforced or irrelevant associations within reversal learning and attentional set-shifting tasks.

1.4.2 *Non-reinforcement in discrimination and reversal learning*

Learned non-reward is the consequence of a CS – 'no US' association formed in a two-choice discrimination paradigm. After a contingency shift, learned non-reward is the interference from learning a CS – US association from previously experiencing the CS in a pairing with 'no US'. It is closely related to the phenomenon of latent inhibition. However, learned non-reward is also different from latent inhibition, since it occurs in a context of a second stimulus presentation with opposite reward contingencies (Mackintosh, 1983). The inability to overcome a non-rewarded association in two-choice reversal learning has recently been referred to as either learned avoidance (Clarke et al., 2007) or learned irrelevance (Boulougouris et al., 2007). However, none of these terms accurately capture the phenomenon taking place in appetitive reversal learning (Table 1.1). Although relatively overlooked in modern clinical and preclinical studies, avoidance of non-rewarded responses has long been recognised as an important component in discriminatory paradigms. As theorists stressed that discrimination learning is a two-process phenomenon (Amsel, 1958, 1962; Hull, 1952; Spence, 1936; Skinner, 1938; Tolman, 1938), some experimental effort was made to determine the relative contribution of learned non-reward and perseverance in two-choice discrimination learning. These experiments typically showed that non-reinforcement exerts greater control upon choice behaviour in appetitive two-choice discrimination tasks than reinforcement.

For example, in the rhesus macaque monkey, if an object is presented alone, and later paired with a novel object in a two-choice discrimination, performance is best if

the object is non-rewarded rather than rewarded (Moss and Harlow, 1947). These results, referred to as an example of the 'Moss-Harlow effect', led Harlow and colleagues to favour a uni-process theory where discrimination learning is achieved solely through non-reinforcement without the guidance of reward (Harlow and Hicks, 1957).

The relative importance of the CS+ and the CS- in discrimination learning can also be assessed by replacing either the previous CS+ or the CS- with a new CS of the same contingency. When the CS+ is replaced, successful performance is dependent on avoidance of the CS-. When the CS- is replaced, successful performance is dependent on approach to the CS+. Within this paradigm, the rat, cat, and rhesus monkey make more errors with a novel CS- than with a novel CS+ (Mandler, 1968; Mandler, 1970; Stevens and Fechter, 1968; Riopelle, 1955; Warren and Kimball, 1958). This indicates that CS- variability is more detrimental for discrimination learning than CS+ variability, and that subjects primarily learn to avoid the CS- rather than to approach the CS+.

Table 1.1. Reinforcement correlation coefficients in two-stage discrimination paradigms.

	Reinforcement correlation coefficients			
	Conditioning phase		*Test phase*	
Phenomenon	*Stimulus A*	*Stimulus B*	*Stimulus A*	*Stimulus B*
Reversal learning	+1.0	-1.0	-1.0	+1.0
Learned non-reward		**-1.0**		**+1.0**
Attentional set-shifting	+1.0	+0.5	+0.5	+1.0
Learned irrelevance		**+0.5**		**+1.0**
Learned avoidance	1.0†	0	0	0
Latent inhibition	0	None	1.0	None

In learned irrelevance, a stimulus initially non-correlated with reinforcement becomes correlated with reinforcement. In a typical learned avoidance task, a stimulus initially correlated with reinforcement becomes neutral. In an appetitive two-stage latent inhibition task, an initially neutral stimulus becomes correlated with reinforcement. In learned non-reward, a stimulus initially negatively correlated with reinforcement becomes positively correlated with reinforcement. † = Aversive.

Moreover, Mason et al. (1980) trained rats on two separate visual two-choice discriminations, presented 54 and 6 times respectively. Subsequently, the CS+ and the CS- from each discrimination were paired into two new discriminations. Here, longer training with the CS- facilitated learning to a greater extent than longer training with the CS+. Similar results were also observed using an analogous visual bowl-digging task in the blackbird (Mason and Reidinger, 1982).

Furthermore, Mullins and Winefield (1979) used a circular apparatus surrounded by 12 response boxes blocked by painted doors serving as visual stimuli, and systematically varied the number of available CS- or CS+'s. They concluded that while learning is guided by constant stimuli and retarded by variable stimuli, variability in the number of CS-'s retard learning more than variability in the number of CS+'s.

There is also evidence suggesting that non-reinforcement is an important component of reversal learning. Sasaki (1969) trained animals on a visual and a spatial two-choice maze discrimination in two separate experiments. Animals subsequently received 20 forced-choice trials, either non-rewarded with the previous CS+, or rewarded with the previous CS-. A third group received no forced choices. In the reversal phase, previous experience of forced choices towards the previous CS-, but not the previous CS+, facilitated performance in both the visual and spatial tasks.

In a similar set-up, Cross and Brown (1965) trained squirrel monkeys in a two-choice object discrimination task. Prior to reversal, animals received non-rewarded forced-choice trials with the previous CS+, rewarded forced-choice trials with the previous CS-, or both forced-choice rewarded and non-rewarded trials. Animals with experience of forced trials involving the previous CS- showed better performance in the subsequent reversal than animals with experience of forced trials involving only the previous CS+. This suggests that experience of non-reward has a greater impact on reversal learning than experience of reward.

A further procedure has been to train animals on a simple two-choice CS+ and CS- discrimination, and subsequently assess performance in a perseverance or learned non-reward test. In a perseverance test, the previous CS+ becomes CS- while the previous CS- is replaced by a novel CS+. Here, only previous conditioning towards the previous CS+ can interfere with performance as the previous CS- has been removed. In a learned non-reward test, the previous CS+ is replaced by a novel CS- while the previous CS- becomes CS+. Thus, only previous conditioning towards the previous CS- can interfere with performance as the previous CS+ has been removed (Table 1.2). In

the capuchin, performance has been shown to be worse in the learned non-reward test than in the perseverance test, suggesting that learned non-reward contributes more than perseverance to the difficulty level of reversal learning (Beran et al., 2008; Goulart et al., 2005). This approach has also been used in neuropsychopharmacological studies of visual reversal learning in the marmoset (Clarke et al., 2007) and olfactory and somatosensory reversal learning in the rat (Tait and Brown, 2007).

Table 1.2. Assessing visual reversal learning, perseverance and learned non-reward.

Stage	Stimuli	Correct stimulus
Simple discrimination		
Full reversal test		
Perseverance test		
Learned non-reward test		

In a full reversal test, the contingencies from the initial simple discrimination test reverse. In a perseverance test, the initial CS+ becomes CS-, while a novel CS+ replaces the previous CS-. In a learned non-reward test, the initial CS- becomes CS+, while a novel CS- replaces the previous CS+ (adapted from Clarke et al., 2007).

Notably, non-reinforcement guided discrimination learning has also been used to explain secondary reversal effects. The overtraining reversal effect, the phenomenon whereby overtraining on a simple discrimination produces faster reversals, has been shown to be consequence of learned non-reward (D'Amato and Jagoda, 1961). During overtraining, there is little responding towards the CS-, which weakens its negative association, and forcing animals to respond towards the CS- during overtraining abolishes any overtraining reversal effect (D'Amato and Jagoda, 1962). Moreover, the

serial reversal effect, the phenomenon observed as a positive correlation between learning and the number of reversals completed, has been suggested to be related to a decreasing influence of the CS- upon choice behaviour as the animal learn over subsequent reversals that no stimuli remains consistently non-rewarded (Allen and Leri, 2011). In sum, although the vast majority of preclinical paradigms implicitly assume that positively reinforced associations guide choice-behaviour, the above experiments serve to highlight that non-reinforcement also is likely to be of considerable importance.

1.4.3 *Learned irrelevance*

Learned irrelevance is the analogue of learned non-reward in an attentional set-shifting task. In learned irrelevance, a stimulus initially non-correlated with reinforcement becomes correlated with reinforcement. That is, a stimulus rewarded 50% of the time become rewarded 100% of the time (Table 1.1). Learned irrelevance is more difficult to overcome than latent inhibition (Baker and Mackintosh, 1979; Bennett et al., 1995, 2000). Indeed, the difficulty discrepancy between reversal learning and attentional set-shifting has been speculated to be related to the difficulty discrepancy between learned non-reward and learned irrelevance (Buss, 1953). Relative to the learned non-reward and perseverance dissociation of reversal learning, the learned irrelevance and perseverance dissociation of attentional set-shifting is extensively investigated. The approach has been to modify the CANTAB ID/ED-task. In these tasks, either the previously relevant dimension is replaced by a novel irrelevant dimension to probe learned irrelevance, or the previously irrelevant dimension is replaced by a novel relevant dimension to probe perseverance (Table 1.3). Notably, these investigations have shown pathology-specific dissociations of cognitive causes for performance deficits. Prefrontal lesioned (Owen et al., 1993), schizophrenic (Elliot et al., 1995, 1998) and Huntington's patients (Lawrence et al., 1999) all exhibit perseverative set-shifting deficits, while unmedicated Parkinson's patients display deficits in both perseverance and learned irrelevance (Owen et al., 1993). However, L-Dopa medicated Parkinson patients show impaired learned irrelevance but no deficits in perseverance (Owen et al., 1993; Slabosz et al., 2006), suggesting that perseverance, but not learned irrelevance, is related to dopaminergic hypoactivity. Moreover, in healthy subjects, learned irrelevance appears to contribute more than perseverance to the difficulty of attentional acquisition (Maes et al., 2009) as well as attentional set-shifting

(Maes et al., 2004). Preclinically, this approach has been taken once, assessing perseverance in mice using the bowl-digging task (Garner et al., 2006).

Table 1.3A. Separate assessment of perseverance in attentional set-shifting

Stage	Stimuli	Relevant dimension	Irrelevant dimension	Correct stimulus
Stage 7 (IDR)		Shape	Line	
Stage 8 (EDS)		Solidity	Shape	

Table 1.3B. Separate assessment of learned irrelevance in attentional set-shifting

Stage	Stimuli	Relevant dimension	Irrelevant dimension	Correct stimulus
Stage 7 (IDR)		Shape	Line	
Stage 8 (EDS)		Solidity	Shape	

Separately assessing perseverance and learned irrelevance in attentional set-shifting using a modified version of the last two test phases of the CANTAB ID/ED-task (adapted from Owen et al., 1993). In the perseverance test (A), the previously relevant dimension becomes irrelevant, while the previously irrelevant dimension is replaced by a novel relevant dimension. In the learned irrelevance test (B), the previously irrelevant dimension becomes relevant, while the previously relevant dimension is replaced by a novel irrelevant dimension.

1.5 NOVELTY CONFOUND

Novelty is a feature in most of the studies cited so far attempting to separately probe perseverative versus learned non-reward or learned irrelevance. A manipulation of novelty attraction or avoidance could therefore confound any interpretation regarding

the manipulations effect on learning. In a perseverance test, a novel rewarded stimulus or dimension is paired with a previously rewarded but now non-rewarded stimulus or dimension. In this test condition, increased novelty attraction would be observed as facilitated learning while increased novelty avoidance would be observed as retarded learning. In a learned non-reward or learned irrelevance condition, a novel non-rewarded stimulus or dimension is paired with a previously non-rewarded but now rewarded stimulus or dimension. In this test condition, increased novelty attraction would be observed as retarded learning while increased novelty avoidance would be observed as facilitated learning. Hence, one intrinsic control for a manipulation of novelty-attraction or avoidance in these tasks is that it would cause opposing effects upon learning in the perseverance and learned non-reward or learned irrelevance conditions (Clarke et al., 2007). For example, a manipulation-induced or pathology-related increase of novelty-attraction would give rise to decreased perseverance, where the novel CS is correct, and increased learned non-reward, were the novel CS is incorrect.

Moreover, performance in a reversal learning test could also control for effects on novelty-attraction or novelty-recognition as no novel stimulus is presented in this test. If an effect of a manipulation is observed in perseverance and/or learned non-reward tests where novelty is a feature, as well as in a reversal learning test which lacks novelty, a fitting interpretation would be that the effect is related to shared features of the tests and unrelated to differences in the presentation of a novel stimulus.

A further approach to overcome novelty-related confounds has been to add control conditions where increases in perseverance and learned irrelevance facilitates learning (Table 1.4). In a perseverance control condition, this can be done by replacing the CS- with a novel CS-, while the CS+ remain constant. In a learned non-reward control condition, the CS+ is replaced by a novel CS+, while the CS- remain constant (Gauntlett-Gilbert et al., 1999). However, it has been noted that this set-up still allows for a novelty confound, as it involves a choice between a previously relevant or irrelevant dimension and a novel dimension (Slabosz et al., 2006). As yet, this form of novelty control has only been performed in the domain of attentional set-shifting, but could also be used in reversal learning.

Table 1.4. Controlling for novelty-attraction and novelty-avoidance in perseverance and learned irrelevance testing in attentional set-shifting.

		Relevant dimension	Irrelevant dimension
Learned irrelevance test	Pre-shift	A	B
	Post-shift	C	B
Perseverance test	Pre-shift	A	B
	Post-shift	A	C

In the learned irrelevance condition, the irrelevant dimension B stays irrelevant while the relevant dimension A is replaced by a novel relevant dimension C. In this condition, enhanced learned irrelevance should facilitate performance. In the perseverance condition, the relevant dimension A stays relevant while the irrelevant dimension B is replaced by a novel irrelevant dimension C. In this condition, enhanced perseverance should facilitate performance (adapted from Gauntlett-Gilbert et al., 1999)

1.6 DISCUSSION

This chapter has reviewed previous experimental designs used to separately assess the underlying cognitive components of two-choice discrimination learning and cognitive flexibility. This work suggests that reversal learning and attentional set-shifting consists of at least two independent cognitive mechanisms. Here I will note two following consequences for reversal learning task design and behavioural interpretations.

Firstly, 'normal' learning is expressed as a delay in learning with reversal learning, attentional set-shifting, perseveration, learned non-reward, and learned irrelevance all being observed as increases in trials and/or time taken to acquire a discrimination due to previous conditioning to avoid and approach CSs now associated

with reward and non-reward, respectively. Although it can be tempting to interpret experimentally induced increases and decreases in the speed of learning as representing cognitive enhancements and retardations, an equally valid interpretation is that a retardation of learning is represented through a increase in the speed of learning, while a cognitive enhancement is represented by a decrease in the speed of learning. Manipulations of cognitive flexibility in themselves can therefore not be interpreted as enhancing or retarding, but only evaluated against the cognitive profile of a given model or pathology.

Secondly, as the domains of perseverance, learned non-reward and learned irrelevance are independent, the effect of a manipulation upon the first cognitive domain may bear little or no relationship to the effect upon the second cognitive domain. This has complex implications for the validity of disease models.

For example, many animal models of psychiatric diseases, including schizophrenia, display reversal learning deficits believed to be due to increased perseverance. These animal models can also show predictive validity with established pharmaco-therapy blocking reversal deficits, believed to be due to blocking the model's perseverative deficits. However, an alternative interpretation is that the perseverative reversal learning deficits are blocked by a co-manipulation disrupting learned non-reward. Or conversely, a non-reward related reversal learning deficit could be blocked by reducing perseverance. The observed deficit within reversal learning could thereby be blocked by causing a second deficit. A further possibility is that a strong increase in perseverance can be masked through a decrease or no effects within learned non-reward, giving no observable effects on reversal learning.

It has been suggested that if a perseverative response strategy is to be modelled, a test of perseveration rather than a reversal or set-shifting test, where perseveration and learned irrelevance or learned non-reward is combined, may be more suitable (Garner et al., 2006). Thus, tests of perseveration and learned non-reward should therefore also be viewed as tests in their own right, similar to the tests preceding the attentional set-shifting test in the ID/ED task.

1.7 CONCLUSIONS

Although manipulations of reversal learning traditionally have been interpreted as manipulations of perseverance, the element of learned non-reward within typical reversal learning tasks should also be considered. This approach has several important consequences:

(1) Highlighting pathology-specific deficits and thereby give indications of relevant drug targets.

(2) Aiding translational approaches by clarifying species and task related differences in problem solving.

(3) Inferring greater construct and predictive validity by observing that the model and potential therapeutic act upon the same or similar cognitive constructs.

1.8 THESIS AIMS

The mouse carries the values of being more cost-effective for high-throughput tasks, as well as the availability of a great number of available genotypic models relevant for psychopathology. As such, the main aims of this thesis were to develop tasks of reversal learning, perseverance and learned non-reward in the mouse, and to use these tasks to investigate the effects of manipulations to brain systems recognised to be involved in reversal learning and relevant to schizophrenia.

Firstly, Chapter 2 describes a group of experiments designed to explore suitable protocols for assessing reversal learning, perseverance, and learned non-reward using the radial-arm maze and operant chamber in the mouse. Chapter 3 then outlines the method used in the majority of the following neuropharmacological experiments. Chapter 4-8 assesses a range of manipulations recognised to be involved in reversal learning and relevant for human psychopathology in general, and schizophrenia in particular. This includes acute phencyclidine (Chapter 4), medial prefrontal cortical and orbitoprefrontal cortical excitotoxic lesioning (Chapter 5), 5-HT_{2C} receptor antagonism

(Chapter 6), and 5-HT_{2C} receptor knock-out mice (Chapter 7). The final experimental chapter investigates the effects of 5-HT_{2C} receptor antagonism using a visual touch reversal learning task in the rat (Chapter 8).

To summarise, there were two main aims of the thesis;

(1) Develop assays for investigating reversal learning, perseverance and learned non-reward in the mouse.

(2) Use these tasks to assess the cognitive mechanisms for altered performance after manipulations to brain systems recognised to be involved in reversal learning and relevant for human psychopathology, with a specific focus on schizophrenia.

CHAPTER 2

VISOUSPATIAL AND EGOCENTRIC REVERSAL LEARNING

2.1 INTRODUCTION

As described in Chapter 1, reversal learning, perseverance and learned non-reward has been explored by replacing either the previously correct or incorrect response alternative across reversal trials with a novel response option. The following sets of experiments aimed to explore protocols for using this method to investigate reversal learning and its cognitive components in the mouse using a spatial dimension. These methods are then used in the following empirical studies of the mouse presented in this thesis. Experiment 1-3 used an eight-arm radial maze in an egocentric design, while experiment 4-6 used the operant chamber in a visuospatial design.

2.2 EXPERIMENT 1: MAZE REVERSAL LEARNING

2.2.1 *Method*

2.2.1.1 Animals

The animals were 8 C57BL/6J male mice (Charles River, UK) weighing a mean 24.4g at the start of the experiment.

2.2.1.2 Apparatus and procedure

The experiment used an eight-arm radial maze made of Plexiglas elevated 55 cm above the floor. Each arm (33.5 × 5 × 8.3 cm) extended from a circular central platform (15.5 cm diameter). Black-painted vial bottle tops (80 mm diameter, 40 mm deep) served as food-wells. The maze was surrounded by featureless circular blackout material. An around 2m high tripod holding a camcorder was placed behind the curtain and the W-arm of the maze. The room was lit by a white-light located in the ceiling.

Habituation. Before being placed in the maze, the maze was always wiped with a sponge moistened with disinfectant to minimise intra-maze olfactory cues. On the first

day, each mouse was allowed to explore a cross-maze over 30 min with five pellets placed in each of the four arms (three along their lengths and two in the food-wells). Over the next four days, each mouse was placed in the maze ≈ 2×10-15 min/day. Again, five pellets were initially placed in each of the four arms (three along their lengths and two in the food-wells). This was gradually decreased over the week until only one pellet was located in each of the four food-wells. If all pellets were consumed within a 10-15 min interval, the mouse was removed from the maze, the maze was re-baited, and the next 10-15 min interval began. The intervals served to habituate the animals to repeated handling after consumption and exploration.

Turn bias. The mouse turn bias was determined in a T-maze prior to discrimination learning (Floresco et al., 2006; Ragozzino et al., 1999). The start-arm for each of the seven trials was predetermined in a pseudorandom order identical for each mouse. The start-arm was S (south), E (east), or W (west) across trials but never N (north). Each animal was given seven trials. The mouse was placed in the start-arm and always had the choice of turning 90° left or 90° right, with both arms baited in order to delay association between response and reinforcement. One trial comprised one left and one right response. For example, if the mouse turned left, it was allowed to consume the pellet and thereafter immediately returned to the start-arm to make a new choice. If the animal turned left again, it was again returned to the start-arm. This continued until the mouse had turned right. After the animal had made both a left and a right turn, it was returned to the cage while the maze was prepared for the next trial. This was repeated seven times. To calculate the mouse turn-bias, the directions of the first turn of each trial were summed, with the majority of responses being the mouse turn-bias.

Spatial discrimination (Fig. 2.1A). Again, the mouse always had the choice of turning 90° to the left or 90° to the right. The start arm for each trial was predetermined in a pseudorandom order identical for each mouse, and was S, E, or W across trials but never N. However, the start-arm never remained the same for more than two consecutive trials. Only the arm opposite the mouse turn bias was baited. Each animal was given 25 trials/day. After every ≈ 7th trial, the maze was turned 90° to minimise the use of extra-maze cues. After making a response, the mouse was removed from the maze and returned to its home-cage while the maze was set up for the next trial. The ITI was approximately 30s. If the mouse failed to leave the start-arm within ≈ 10s or choose to reverse into the start-arm from the centre of the maze it was given a gentle push. The criterion in this and all subsequent tests were nine consecutive correct responses. If a

mouse made nine consecutive correct responses it was given a probe-trial. In the probe-trial, N figured as start-arm. If successful, the spatial discrimination was completed. If unsuccessful, a further five correct responses lead to a new probe-trial. Each mouse was given a maximum of 200 trials. When completed, all animals were assessed in a full reversal condition. The data collected was trials, correct responses and incorrect responses to criterion.

Full reversal test (Fig. 2.1B). Now the bait was moved to the opposite arm. Thus, an animal trained to turn 90° right now had to turn 90° left without any additional changes to the maze-configuration. When completed, half of the animals were tested in a learned non-reward test while the other half were tested in a perseverance test.

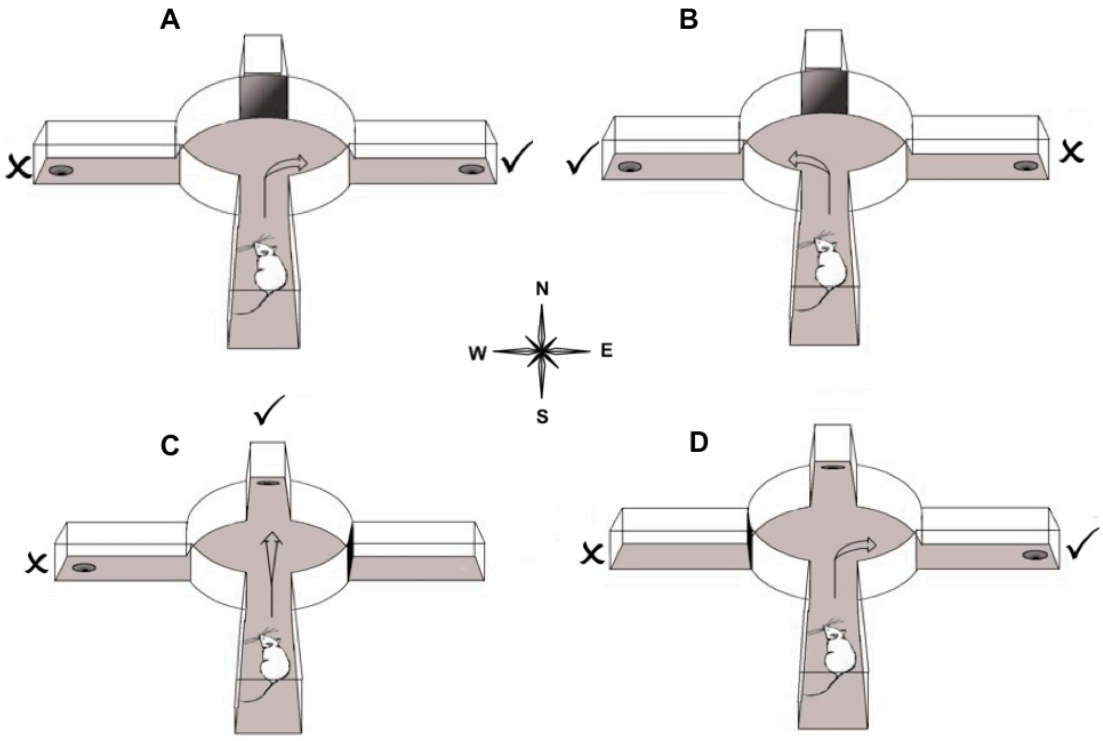

Figure 2.1. Example of the experimental procedure used in Experiment 1. All animals completed an initial spatial discrimination (A) followed by a full reversal test (B). After reaching criterion, animals were assigned to either a perseverance test (C) or a learned non-reward test (D).

Perseverance test (Fig. 2.1C). Here the previously correct arm remained opened while a novel arm replaced the previously incorrect arm. For example, a previously incorrect arm 90° to the left was replaced by a novel correct arm straight on. Only the novel arm was baited. Hence, altered performance in this test must be due to a manipulation of the association of reward, as the previously incorrect alternative no longer is present. That is, the only error the mouse could make was to enter the previously correct arm.

Learned non-reward test (Fig. 2.1D). Here the previously incorrect arm remained opened while a novel arm replaced the previously correct arm. For example, a previously correct arm 90° to the right was replaced by a novel incorrect arm straight on. Only the previously incorrect arm was baited. Hence, altered performance in this test must be due to a manipulation of the association of non-reward, as the previously correct alternative no longer is present. That is, the only error the mouse could make was to avoid the previously incorrect arm.

2.2.2 *Results and discussion*

Seven of the eight animals reached criterion on the spatial discrimination within 200 trials. Six of the seven remaining animals reached criterion in the full reversal test. Animals required significantly more trials ($t_6 = 2.6$, $p < .05$) and made more incorrect responses to criterion ($t_6 = 2.6$, $p < .05$) in the full reversal test compared to the spatial discrimination (Fig. 2.2). They also made more correct responses in the full reversal test, although the difference failed to reach significance ($t_6 = 2.1$, $p < .09$).

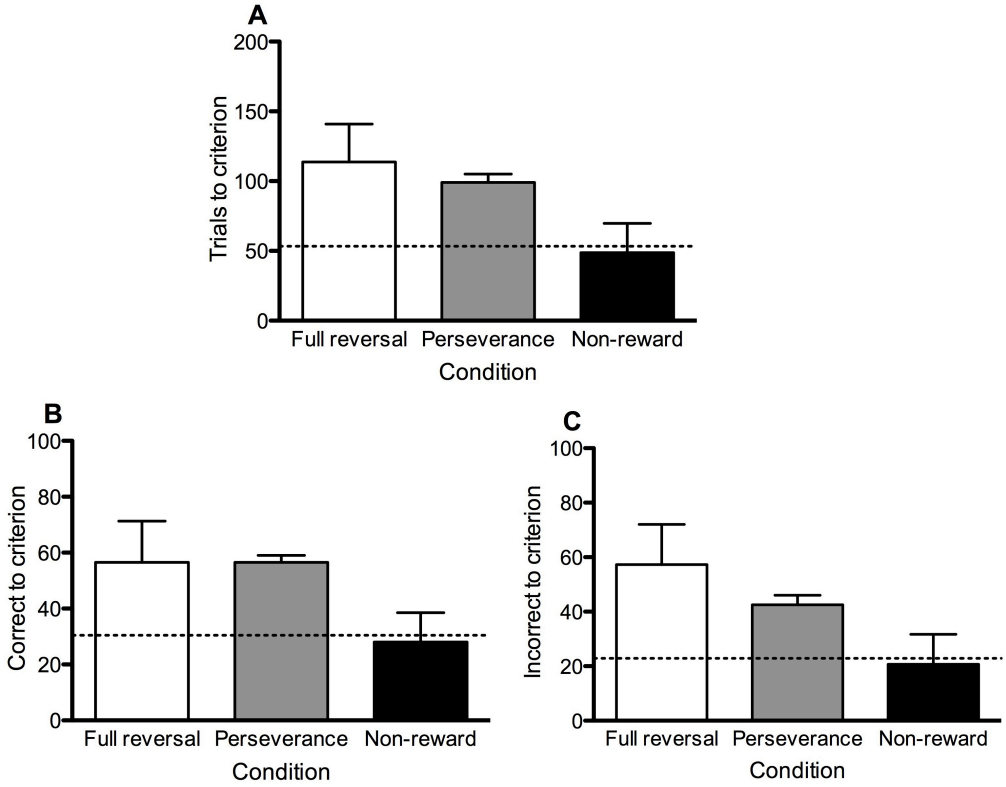

Figure 2.2 Mean trials (A) correct (B) and incorrect (C) responses to criterion in the three test conditions of Experiment 1. Broken line represents mean spatial discrimination performance.

Two of the three animals assessed in the perseverance test reached criterion within 200 trials. In the learned non-reward test, all three animals reached criterion. There were no significant effects of test condition (perseverance and learned non-reward) on trials, correct responses or incorrect responses to criterion.

These results suggest that mice can be taught egocentric spatial discrimination and reversal learning in the maze. Animals required more trials to criterion in the full reversal test than in the initial spatial discrimination, showing that choice-behaviour during reversal is guided by the reward contingencies established during the spatial discrimination. Rats typically learn egocentric maze discriminations and reverse in about 60 trials (Floresco et al., 2006; Ragozzino et al., 1999). Here, mice seem to require almost twice as many trials to reverse.

There were signs that animals used non-egocentric spatial cues to navigate the maze. Two animals had a deficit in locating the reward when a specific start-arm was

used. For example, these animals persisted in taking left only when starting from the W-arm. It is possible that the location of the maze in relation to the room lighting gave each of the four arms particular shadowing and visual cues. Keeping the camera behind the W-arm throughout the experiment also provided a prominent visual cue. It is also possible that animals were guided by intra-maze cues. During the ITI, the animals were held in their home-cages containing sawdust that often transferred to the maze and were not completely removed between trials.

The perseverance test appeared to be more difficult than the learned non-reward test. Under the current protocol, there are two critical differences between the perseverance and learned non-reward tests. In the initial spatial discrimination and full reversal tests, animals had been trained to turn 90°. This is still true in the learned non-reward test, but not in the perseverance test, where animals now have to go straight on. Also, the response required in the spatial discrimination and non-reward test are identical while they differ between the spatial discrimination and the perseverance test (Fig 2.1). This could account for the relative difficulty of the perseverance test and ease of the non-reward test.

2.3. EXPERIMENT 2: MAZE REVERSAL LEARNING

2.3.1 *Introduction and method*

The experiment used 18 C57BL/6J male mice (Charles River, UK) weighing a mean 23.5g at the start of the experiment.

Steps were taken to limit the availability of visual cues present in Experiment 1:

- The maze was completely enclosed in a 'tent'-like structure of blackout material, with a red light and bullet-camera hanging directly above the central platform of the maze. The ceiling light was also changed from white to red.
- During the inter-trial interval, the mouse was placed in a sawdust free holding-cage containing heavy-absorbent paper to avoid contamination of the test apparatus and intra-maze cues.

Further changes were made to the protocol to avoid confounds between the perseverance and learned non-reward tests:

- The novel path in the perseverance and learned non-reward tests was introduced as a 45° turn rather than a non-turn. This meant that both the perseverance and learned non-reward tests consist of a two-choice simultaneous discrimination between two turning directions rather than a discrimination between a turn and a non-turn.
- The three test conditions were run between-subjects to avoid the spatial discrimination and learned non-reward test requiring identical responses.
- The maximum trials allowed within each condition was changed from 200 to 250.

2.3.2 *Results and discussion*

Sixteen of the 18 animals reached criterion on the spatial discrimination phase within 250 trials, while all remaining animals reached criterion in the following test conditions (Fig. 2.3). There were significant effects of test condition on trials to criterion ($F_{2,18} = 5.5$, $p < .05$), as well as correct ($F_{2,13} = 5$, $p < .05$) and incorrect responses to criterion ($F_{2,13} = 4.9$, $p < .05$). Post-hoc comparisons using Fisher's LSD test showed that animals required significantly fewer trials to complete the perseverance ($p = .03$) and learned non-reward tests ($p = .02$) than the full reversal test. Animals also

made fewer incorrect and correct responses in the perseverance ($p = .03$; $p = .04$) and the learned non-reward tests ($p = .02$; $p = .02$) than in the full reversal test.

An issue in Experiment 1 was the use of non-spatial cues to navigate in the maze as, for some animals, start-arm predicted choice of response-arm. To minimise the use of non-spatial cues, the maze was enclosed in black material, the room and 'tent' lighting was changed from white to red, and the animals were held in a sawdust-free cage between trials. In this experiment there were no signs indicating the use of non-spatial cues.

However, a possible further issue is the order the animals encounter the novel and now blocked arms. In the learned non-reward test, after a turn towards the previously correct arm the animal encounters the unexpectedly blocked arm first followed by the novel non-rewarded arm. In the perseverance test condition, after a turn towards the previously incorrect arm the animal encounters the unexpectedly blocked arm first followed by the novel rewarded arm. To account for the order at which the correct and incorrect arms could be encountered in the perseverance and learned non-reward tests, it would be appropriate to counterbalance the task as such the animal also can encounter the novel arm first followed by the now blocked arm after making incorrect and correct turns in both the learned non-reward and perseverance tests.

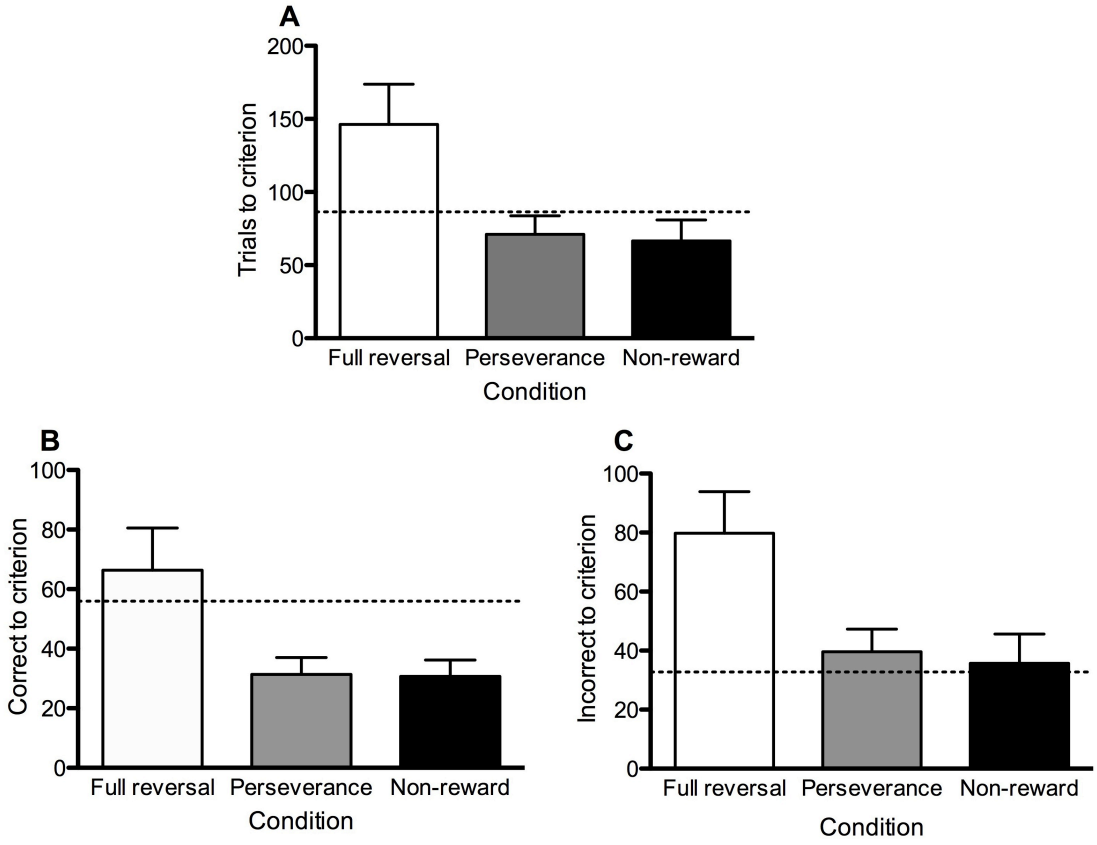

Figure 2.3. Mean trials (A) correct (B) and incorrect (C) responses to criterion in the three test conditions of Experiment 2. Broken line represents mean spatial discrimination performance.

2.4 EXPERIMENT 3: MAZE REVERSAL LEARNING

2.4.1 *Introduction and method*

The experiment used 16 C57BL/6J male mice (Charles River, UK) weighing a mean 25g at the start of the experiment. Now the egocentric turning directions were counterbalanced. For one half of the animals, the spatial discrimination phase constituted a T-maze with the novel turning option being a 45° turn. For the other half, the spatial discrimination constituted a Y-maze with the novel turning option being a 90° turn. Now, animals could encounter the novel arm first after making a turn in the incorrect or correct direction in the learned non-reward or perseverance tests. The maze habituation was also changed, taking place over 4 days at 3×12 min/day with the initial day of 30 min free exploration omitted.

2.4.2 *Results and discussion*

Fifteen of the 16 animals reached criterion on the spatial discrimination phase within 250 trials, while all animals reached criterion on the following three tests (Fig. 2.4). There were no significant differences in trials, correct or incorrect responses to criterion in the three test conditions or two maze-configurations.

Compared to animals in Experiment 2, animals in Experiment 3 learned the spatial discrimination and full reversal faster while requiring more trials to reach criterion in the learned non-reward test. Yet, none of these differences reached significance (all $p > .05$). Here, an issue is the degree of independence of the full reversal, learned non-reward, and perseverance tests. Generalisations between a 45° and a 90° turn in the same direction would cause the perseverance and learned non-reward tests to measure reversal learning rather than the components of reversal learning. However, as there were no differences in learning between animals exposed to the Y-maze or T-Maze, this cannot be attributed to the different maze-configurations.

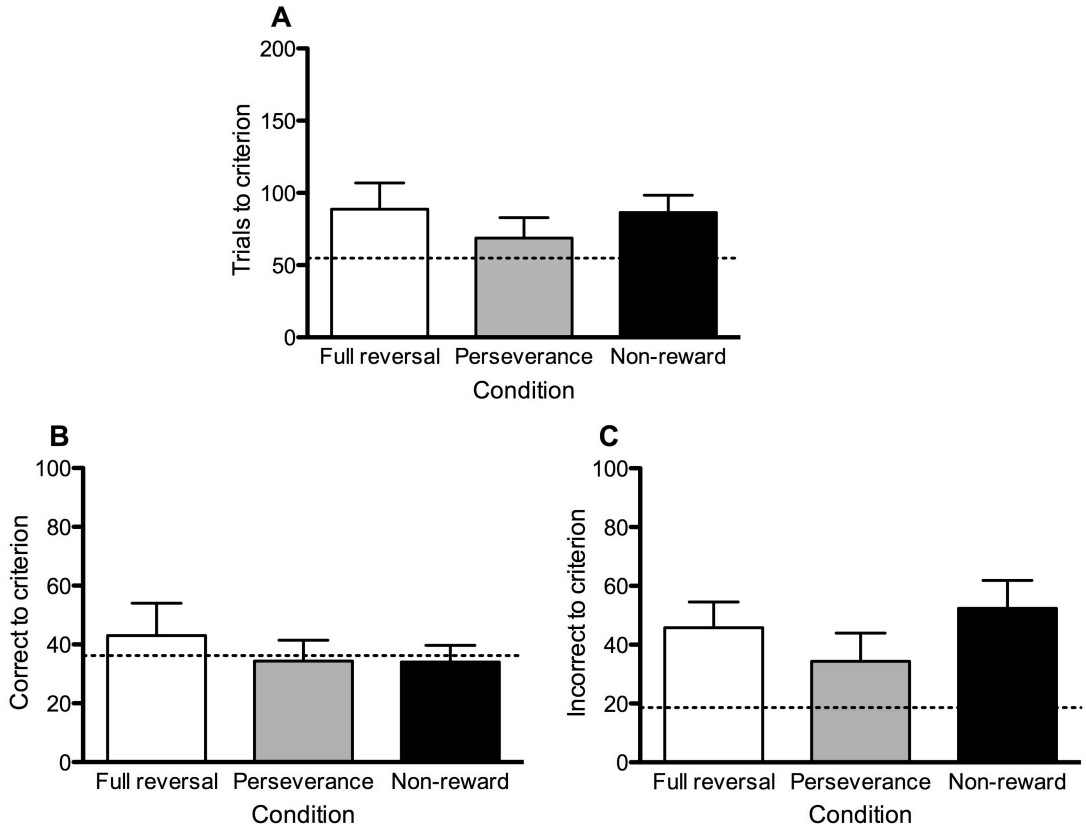

Figure 2.4. Mean trials (A) correct (B) and incorrect (C) responses to criterion in the three test conditions of of Experiment 3. Broken line represents mean spatial discrimination performance.

2.5 EXPERIMENT 4: OPERANT REVERSAL LEARNING

2.5.1 *Introduction*

The following set of experiments used a similar rationale to the experiments in the radial-arm maze to explore protocols for assessing reversal learning, perseverance and learned non-reward in the operant chamber, with the location of the previously correct or incorrect nosepoke-hole location being changed across trials.

2.5.2 *Method*

2.5.2.1 Animals

The experiment used 24 C57BL/6J male mice (Charles River, UK) weighing a mean 25g at the start of the experiment.

2.5.2.2 Apparatus and procedure

The experiments used eight operant chambers (22.5 × 18 × 13 cm; Med Associates, Georgia, VT, USA) placed in sound-attenuating wooden chambers with fans for purpose of ventilation and attenuating external noise. Each chamber was fitted with a central magazine (W = 2.5 cm, H = 2 cm) connected to an external pellet dispenser delivering 20 mg sucrose pellets (Sandown Scientific, Middlesex, UK). The chambers contained two nosepoke-holes (3.2 cm diameter), located 16.2 cm apart and 5.5 cm above a grid-floor, initially placed on the distal side of the chamber relative to the food dispenser. A houselight was located centrally above the nosepoke-holes 9 cm above the floor.

Training stage 1. Each trial began with the illumination of a single nosepoke-hole and the houselight. The illuminated nosepoke-hole was counterbalanced across the left and right sides. The lit-up nosepoke-hole remained the same across all trials. A nosepoke in the lit-up nosepoke-hole led to pellet delivery, the houselight turning off, and the beginning of a 10s ITI when the chamber was kept dark. The criterion was ≥ 20 correct responses over a single 40 min session. On the fifth day of this schedule, only two animals had passed criterion, with animals making a mean three correct responses

over 40 min. The program was therefore changed in order to find a more effective way of getting the mice to nosepoke for reward.

Training stage 2. The houselight-cycle was now reversed. Each trial began with the offset of the houselight and illumination of a single nosepoke-LED. A correct response now led to pellet delivery, the houselight turning on, and the beginning of a 10s ITI when the houselight was kept on. All animals reached the criterion of ≥ 20 correct responses in a 40 min session over a mean 3 days. After criterion was achieved, this stage was repeated the next day with the opposite nosepoke-hole activated. All animals now reached criterion on the first day.

Training stage 3. The animals were now required to nosepoke in the magazine within 20s, which activated a single nosepoke-LED and the houselight. A response in the correct nosepoke-hole within 10s led to pellet delivery, the nosepoke-LED turning off, and the beginning of a 10s ITI when the chamber was kept dark. However, the houselight stayed on for an additional 4s after a correct response. Failure to respond in the magazine within 20s or failure to respond in the nosepoke-hole within 10s counted as an omission and led to the immediate onset of the 10s ITI. The criterion was ≥ 75 correct responses over 40 min.

After eight days on this schedule, only seven animals had reached criterion. Moreover, the number of correct responses seemed to reach a plateau around day 4 or 5 and subsequently dwindle. This again prompted changes to the protocol.

Training stage 4. Each mouse was now exposed to 50 trials/day. A trial began with the onset of the houselight and a single nosepoke-LED. Thus, an initial nosepoke in the magazine was no longer required. A nosepoke in the lit-up nosepoke-hole within 10s led to pellet delivery, the nosepoke-LED turning off and the beginning of a 30s ITI. This longer ITI was used to increase the motivation for animals to nosepoke. The criterion was ≥ 35 correct responses for two consecutive sessions of 50 trials or ≥ 40 correct responses in a single session of 50 trials. All animals reached criterion in a mean 4 days. When criterion was achieved, this stage was repeated with the opposite nosepoke-hole activated. All animals now reached criterion in a mean 3 days.

Training stage 5. The ITI was now adapted to the test condition of 15s. The criterion was 40 correct responses over 50 trials for two consecutive sessions. Everything else was identical to the previous stage. All animals reached criterion in a mean 3 days. When criterion was achieved, the stage was repeated with the opposite nosepoke-hole activated. All animals now reached criterion in a mean 2 days.

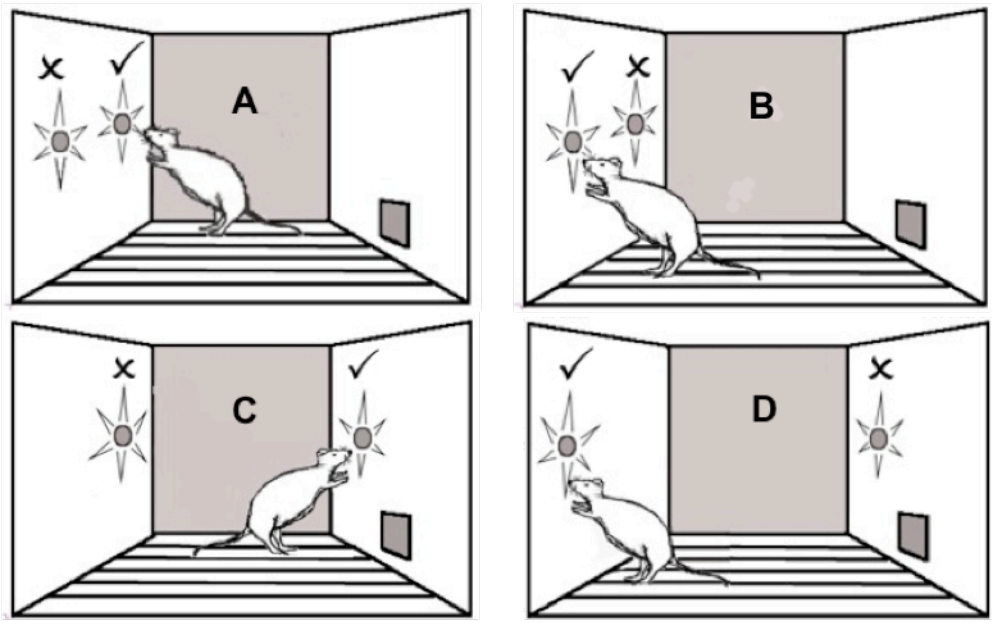

Figure 2.5. Example of the experimental procedure used in Experiment 4. All animals initially completed a spatial discrimination (A), followed either by a full reversal (B), a perseverance (C) or a learned non-reward test (D).

Spatial discrimination (Fig. 2.5A). A trial began with the onset of the houselight and both nosepoke-LEDs. A nosepoke in the correct nosepoke-hole within 10s led to pellet delivery, the nosepoke-LED turning off, and the beginning of a 15s ITI when the chamber was dark. Failure to respond within 10s counted as an omission. The correct and incorrect nosepoke-holes were counterbalanced. A nosepoke in the incorrect nosepoke-hole counted as an incorrect response and leads to immediate onset of the 15s ITI. Each animal was exposed to five 10-trial blocks. The criterion in the spatial discrimination and all subsequent conditions was at least 9 correct responses in a 10-trial block. When achieved, the session ended and the animals were removed from the chambers. If the animal failed to reach criterion this was repeated the following day until criterion was reached. When achieved, a retention test followed when animals again required to reach criterion on the same contingencies. All animals successfully reached criterion on day 1 of retention. Subsequently, the animals were allocated to one out of three test conditions.

Full reversal test (Fig. 2.5B). Here the previously incorrect nosepoke-hole become correct while the previously correct nosepoke-hole became incorrect. Everything else was identical to the spatial discrimination phase.

Perseverance test (Fig. 2.5C). Here the previously correct nosepoke-hole became incorrect, while the previously incorrect nosepoke-hole became correct and moved to the opposite side of the chamber. Hence, the only error the mouse could make was to nosepoke in the previously correct nosepoke-hole. The established non-rewarded association should not influence the performance as the previous CS- had been removed.

Learned non-reward test (Fig 2.5D). Here the previously incorrect nosepoke-hole became correct, while the previously correct nosepoke became incorrect and moved to the opposite side of the chamber. Hence, the only error the mouse could make was to avoid the previously incorrect nosepoke-hole. The established rewarded association should not influence the performance as the previous CS+ had been removed.

2.5.3 *Results and discussion*

All animals reached criterion on the initial spatial discrimination phase and the following test conditions (Fig. 2.6). There were significant main effects of test condition on trials ($F_{2,21} = 7.1$, $p < .01$) and incorrect responses to criterion ($F_{2,21} = 6.6$, $p < .01$). Animals required significantly fewer trials and made less incorrect responses in the learned non-reward test than in the perseverance and full reversal tests (all $p \geq .04$). There were no significant differences in correct responses or omissions across the three test conditions.

This study suggests that reversal learning, perseverance and learned non-reward can be assessed in the mouse using the operant chamber. Although a lack of difference in any performance index between the spatial discrimination and full reversal tests, animals made more incorrect responses and omissions in the full reversal test. This study also highlights important protocol parameters relevant to training and testing. Mice more readily learn to nosepoke for reward when stimuli saliency is increased by turning the houselight off.

However, the current design also allows for critical confounds between the perseverance and learned non-reward tests. As animals are required to nosepoke on the

left wall of the chamber throughout training, there were signs of mediating behaviour to the left wall of the chamber during the test conditions.

Moreover, in the learned non-reward test, the animals are required to nosepoke in the only nosepoke-hole present on the left wall of the chamber, a hole that has been repeatedly associated with reward during training, whereas the perseverance test requires a shift to responding next to the magazine. This could partly be overcome through trial self-initiation, where animals have to start each trial through a nosepoke in the magazine.

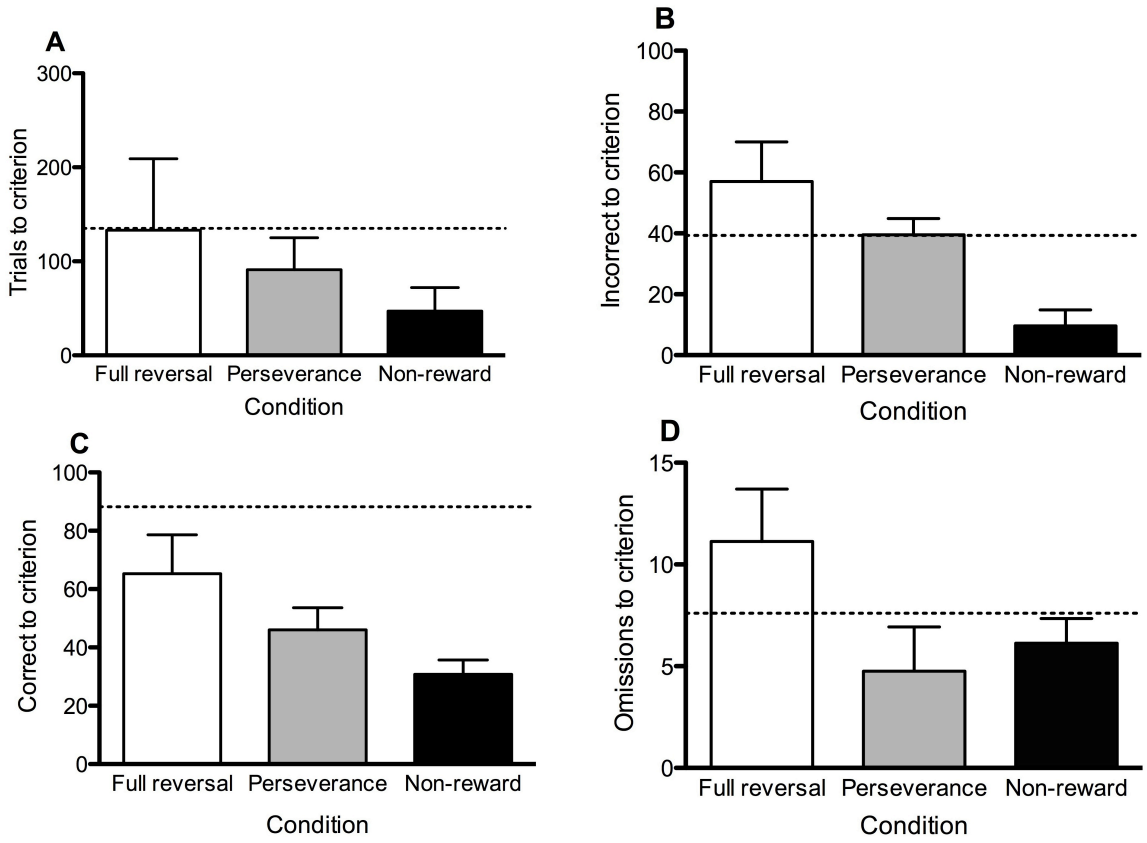

Figure 2.6. Mean trials (A) incorrect (B) correct (C) and omissions (D) to criterion in the three test conditions of Experiment 4. Broken line represents mean spatial discrimination performance.

2.6 EXPERIMENT 5: OPERANT REVERSAL LEARNING

2.6.1 *Introduction*

This experiment attempted to counterbalance the location of the correct and incorrect nosepoke-hole across the two walls of the chamber. Initially, two nosepoke-holes were placed in two out of four possible location, on opposite sides on the operant chamber, creating four different possible nosepoke-hole combinations (Fig. 2.7). In the subsequent perseverance and learned non-reward tests, the previously incorrect or correct nosepoke-hole was moved to the adjacent side of the chamber.

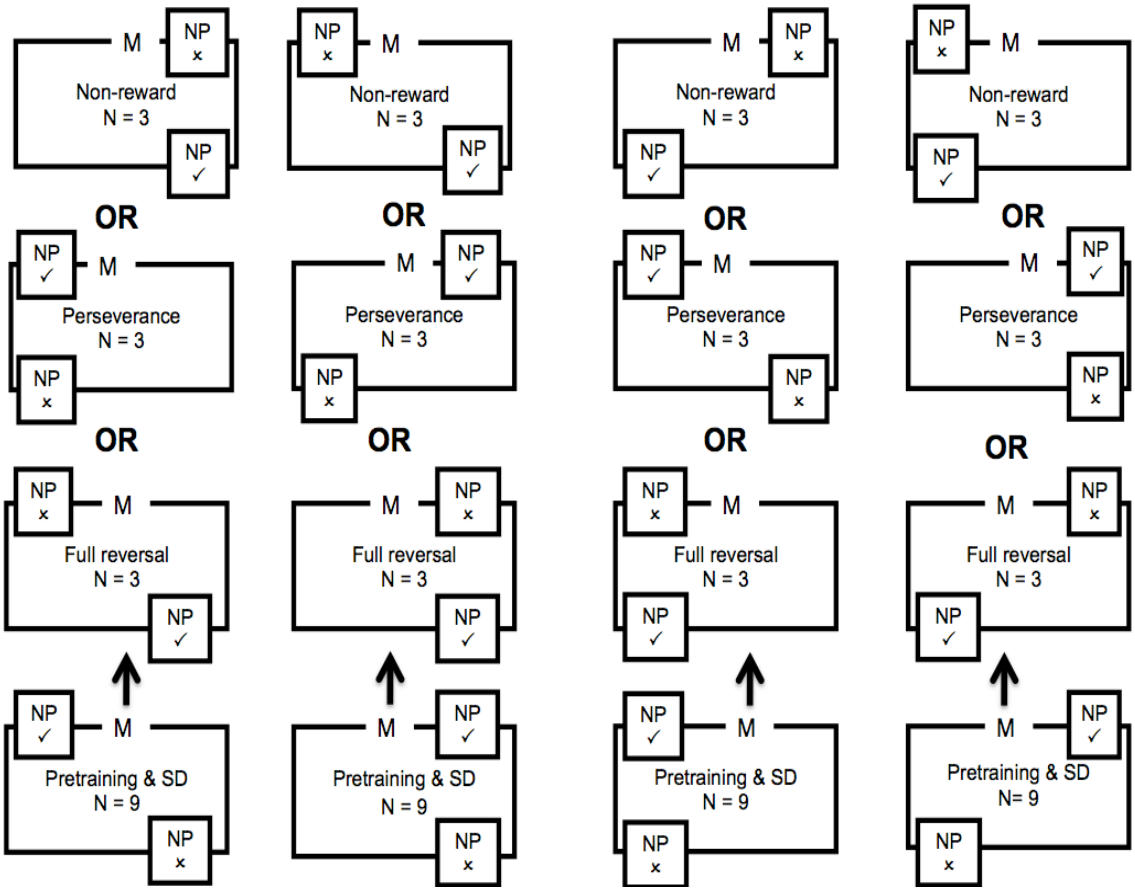

Figure 2.7. Example of counterbalance of nosepoke-locations in Experiment 5. One nosepoke-hole was always located in one out of two possible position on each wall of the chamber, giving four possible arrangements. The correct and incorrect nosepoke-hole was determined by the animals side-bias (see text). In the perseverance and learned non-reward tests, the previously incorrect or correct nosepoke-hole, respectively, is moved to the adjacent location in the chamber. In this example, the animals side-bias is to the left throughout. SD = spatial discrimination, NP = nosepoke-hole.

2.6.2 *Method*

The experiment used 18 C57BL/6J male mice (Charles River, UK) weighing a mean 25.6g at the start of the experiment.

Training stage 1. Trial onset was signalled by the houselight turning off and a single nosepoke-LED turning on. This houselight-phase was initially used in order to

increase the saliency of the nosepoke-LED and more effectively get the animals to begin to nosepoke for pellet delivery. A nosepoke in the lit-up nosepoke-hole led to a pellet delivery, the houselight turning on, and the beginning of a 30s ITI when the houselight was kept on. However, after a correct response, the houselight stayed on for a further 4s, before it turned off and stayed off until the end of the ITI. All animals reached the criterion of ≥ 20 correct responses over 40 min over a mean 3 days. After criterion was achieved, this stage was repeated the following day with the opposite nosepoke-hole activated. All animals now reached criterion on day one.

Training stage 2. The animals were required to nosepoke in the magazine within 20s, which activated the houselight and a single nosepoke-LED. A response in the lit-up nosepoke-hole within 10s lead to pellet delivery, the nosepoke-LED turning off, and the beginning of a 30s ITI when the chamber was kept dark. After a correct response, the houselight stayed on for an additional 4s. Failure to either respond in the magazine within 20s or to respond in the nosepoke-hole within 10s counted as an omission and caused immediate onset of the 30s ITI. The criterion was ≥ 35 correct responses over two consecutive sessions or ≥ 40 correct responses in a single session of 50 trials. All animals reached criterion over a mean 7 days. After criterion was achieved, this stage was repeated the following day with the opposite nosepoke-hole activated. All animals now reached criterion over a mean 6 days.

Training stage 3. This was as stage 2, except that the ITI was now adapted to the test condition of 15s. The criterion was ≥ 40 correct responses over 50 trials for two consecutive sessions. Everything else was identical to the previous stage. All animals reached criterion over a mean 3 days. When criterion was achieved, this stage was repeated the following sessions with the opposite nosepoke-hole activated. All animals again reached criterion over a mean 3 days.

Side-bias. The mouse side-bias was determined prior to the spatial discrimination (Floresco et al., 2006). A nosepoke in the magazine activated both nosepoke-LEDs. On the first attempt, a nosepoke in either nosepoke-hole resulted in pellet delivery. On the second attempt, only a nosepoke in the nosepoke-hole opposite the one responded to in the first attempt resulted in pellet delivery. For example, if the mouse initially responded in the left nosepoke-hole, it was rewarded with a pellet and a new trial commenced. If choosing left once more, no pellet was delivered and the houselight was extinguished. This continued until the mouse responded in the right nosepoke-hole. The second trial began once the animal had responded in both the left and the right nosepoke-hole. Thus,

one trial comprised at least one left and one right response. Each animal was given seven trials. To calculate the mouse side-bias, the first response of each trial was added together, with the majority of responses being the mouse side-bias.

Spatial discrimination. A trial began with the offset of the houselight. A nosepoke in the magazine lead to the onset of the houselight and both nosepoke-LEDs. A nosepoke in the correct nosepoke-hole within 10s lead to pellet delivery, nosepoke-light turning off, and beginning of a 15s ITI when the chamber was dark. Failure to respond within 10s counted as an omission. Nosepoke in the inactive nosepoke-hole counted as an incorrect response and lead to immediate onset of the 15s ITI. Each animal was exposed to five 10-trial blocks. After reaching criterion, animals where assigned to one of three test conditions.

Full reversal test. The previously incorrect nosepoke-hole became correct while the previously correct nosepoke-hole became incorrect. Everything else was identical to the spatial discrimination phase.

Perseverance test. The previously correct nosepoke-hole became incorrect, while the previously incorrect nosepoke-hole became correct and moved to the adjacent location of the operant chamber.

Learned non-reward test. The previously incorrect nosepoke-hole became correct, while the previously correct nosepoke-hole became incorrect and moved to the opposite side of the operant chamber.

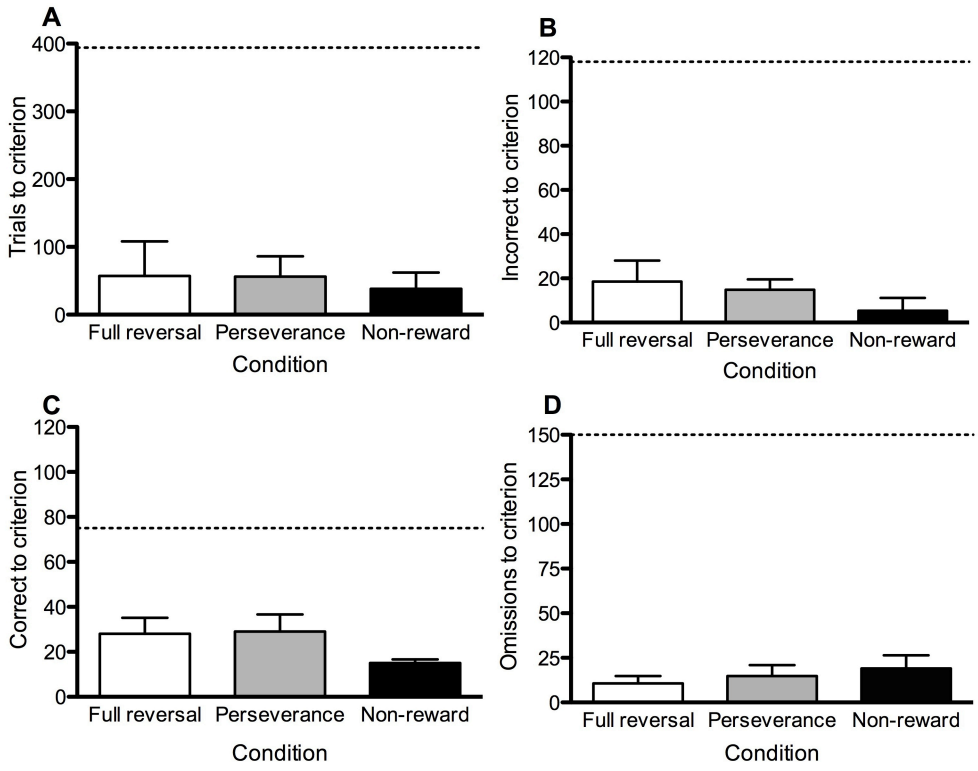

Figure 2.8. Mean trials (A) incorrect (B) correct (C) and omissions (D) to criterion in the three test conditions of Experiment 5. Broken line represents mean spatial discrimination performance.

2.6.3 *Results and discussion*

All animals side-bias were to the right side of the chamber, towards the nosepoke-hole next to the food magazine. Sixteen of the 18 animals reached criterion on the spatial discrimination phase, and the remaining 16 animals also reached criterion in the three following test conditions (Fig. 2.8). Animals required significantly more trials on the spatial discrimination phase compared to any of the following test conditions. There were no significant differences in performance across the three test conditions (all $p >. 05$). In sum, there was a strong bias for animals to respond in the nosepoke-hole located next to the food magazine, making this protocol unsuitable for assessing cognitive flexibility.

2.7 EXPERIMENT 6: OPERANT REVERSAL LEARNING

2.7.1 *Introduction and method*

In order to avoid wall related confounds, three nosepoke-holes were now placed on a single wall of the operant chamber. The houselight-phase was also reversed throughout training and testing in order to increase the saliency of the nosepoke-LEDs.

Animals. The experiment used 24 C57BL/6J male mice (Charles River, UK) weighing a mean 24.9g at the start of the experiment.

Counterbalancing. Three nosepoke-holes where placed on a single wall of the chamber, located opposite to the food magazine (Fig. 2.9). For each animal, the initial training and spatial discrimination took place using two of these three nosepoke-holes, with the location of the two nosepoke-holes fully counterbalanced. Thus, animals completed training and spatial discrimination using either the left and right, central and right, or the central and left nosepoke-holes. The third nosepoke-hole remained idle, never lighting up. A response in this idle nosepoke-hole was always without consequence.

Training stage 1. Trial onset was signalled by the houselight turning off and a single nosepoke-LED turning on. A nosepoke in the lit-up nosepoke-hole led to pellet delivery, houselight turning on, and beginning of a 30s ITI when the houselight was kept on. All animals reached criterion of ≥ 20 correct responses over 40 min in a mean 3 days. After the criterion was achieved, this stage was repeated the following day with the second nosepoke-hole activated. All animals now reached criterion on day one.

Training stage 2. As stage 1, however, animals were now required to nosepoke in the magazine within 20s, which activated a single nosepoke-LED. A response in the lit-up nosepoke-hole within 10s lead to pellet delivery, nosepoke-light turning off, and the beginning of a 30s ITI when the chamber was kept lit-up. However, after a correct response, the houselight stayed off for a further 4s before it turned on and stayed on until the end of the ITI. Failure to either respond in the magazine within 20s or to respond in the nosepoke-hole within 10s counted as an omission and caused immediate onset of the 30s ITI. The criterion was ≥ 35 correct responses over two consecutive sessions or ≥ 40 correct responses in a single session of 50 trials. All animals reached criterion in a mean 7 days. After the criterion was achieved, this stage was repeated the

following day with the opposite nosepoke-hole activated. All animals now reached criterion over a mean 6 days.

Training stage 3. As stage 2, however, the ITI was now adapted to the test condition of 15s. Criterion was ≥ 40 correct responses over 50 trials for two consecutive sessions. Everything else was identical to the previous stage. All animals reached criterion over a mean 3 days. When criterion was achieved, this stage was repeated with the second nosepoke-hole activated. All animals again reached criterion in a mean 3 days. Animals required a mean 20 days in total to complete training.

Side-bias and spatial discrimination. As in experiment 4 and 5, each animals side-bias was assessed prior to the spatial discrimination, and this was followed by the acquisition of spatial discrimination (Fig. 2.9A).

Full reversal test (Fig. 2.9B). Here the previously incorrect nosepoke-hole became correct while the previously correct nosepoke-hole became incorrect.

Perseverance test (Fig. 2.9C). Here the previously correct nosepoke-hole became incorrect, while the previously idle nosepoke-hole became correct and the previously correct nosepoke-hole became idle.

Learned non-reward test (Fig. 2.9D). Here the previously incorrect nosepoke-hole became correct, while the previously correct nosepoke-hole became idle and the previously idle nosepoke-hole became incorrect.

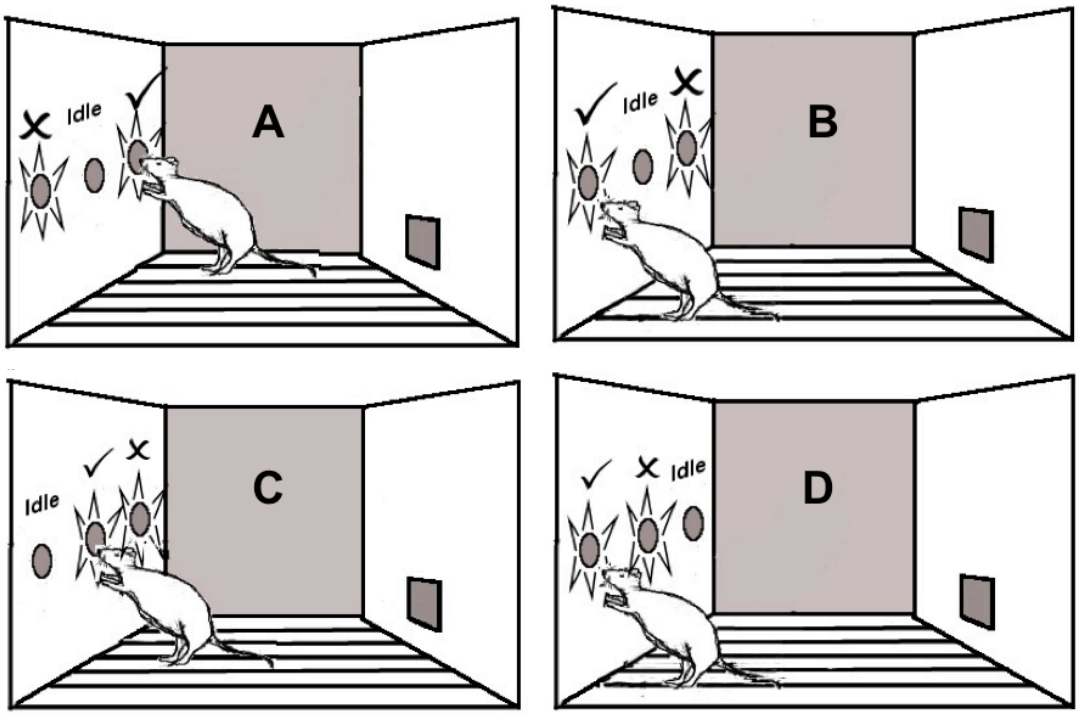

Figure 2.9. Example of the experimental procedure used in Experiment 6. Animals initially completed a spatial discrimination (A). This was followed by either a full reversal (B) perseverance (C) or learned non-reward test (D).

2.7.2 *Results and discussion*

Two animals were omitted after failing to reach criterion in pretraining stage 3, while one animal was omitted after failing to reach criterion in the full reversal test after 23 days of testing. There were no significant differences in performance between animals exposed to the three different nosepoke-hole combinations.

There was a significant effect of test condition on trials ($F_{2,18} = 4.49$, $p < .05$), correct ($F_{2,18} = 6.19$, $p < .01$), and incorrect ($F_{2,18} = 12.9$, $p < .0001$) responses to criterion (Fig. 2.10). Post-hoc comparisons using Fisher's LSD test showed that animals in the learned non-reward test reached criterion faster than animals in the full reversal ($p < .05$) and perseverance tests ($p < .05$). Animals required significantly more correct responses to criterion in the full reversal test compared to the learned non-reward ($p < .01$) and perseverance tests ($p < .01$). Animals also committed significantly more errors in the perseverance test compared to the learned non-reward ($p < .0001$) and full reversal tests ($p < .05$). There were no significant differences between test conditions on latencies to response in the magazine, the nosepoke-hole, or for pellet retrieval latencies.

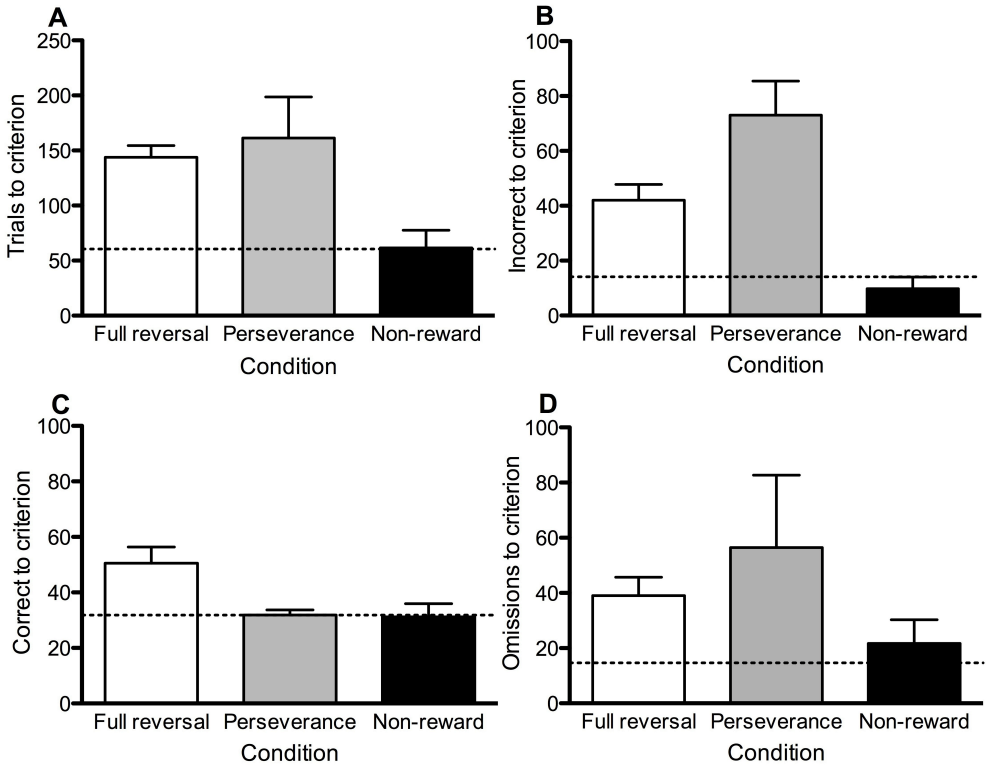

Figure 2.10. Mean trials (A) incorrect (B) correct (C) and omissions (D) to criterion in the three test conditions of Experiment 6. Broken line represents mean spatial discrimination performance.

Animals required significantly more trials to reach criterion in the perseverance test compared to the learned non-reward test. This was mainly due to a large difference in incorrect responses between the two test conditions. Indeed, animals also made more incorrect responses in the perseverance than the full reversal test.

In studies of reversal learning, it is implicitly assumed that only learning in the spatial discrimination proactively interferes with learning in the test phase. However, if one treats two-choice simultaneous discrimination learning as excitatory and inhibitory conditioning to the CS+ and the CS-, the high and low numbers of incorrect responses in the perseverance and learned non-reward tests could be explained by proactive interference from the training stages (Table 2.1).

In the perseverance test, *only* A+ proactively interferes with new learning A-N+, while previous learning of B+ and B- interfere minimally as B is absent in the test condition. In the learned non-reward test, *both* B- and B+ proactively interfere with new learning B+N-, while previous learning of A- and A+ interfere minimally as A is absent in the test condition. Thus, there is previous excitatory conditioning to the incorrect stimulus in the perseverance test, and previous excitatory and inhibitory conditioning to the correct stimulus in the learned non-reward test.

Further, animals may treat the nosepoke-holes rather than the nosepoke-LEDs as stimuli. This would cause the development of latent inhibition towards N throughout training and in the spatial discrimination, and the development of latent inhibition towards B and A in half of the training phases (Table 2.1). Importantly, latent inhibition towards N would further facilitate learning in the learned non-reward test, where N is non-rewarded, and further impair learning in the perseverance test, where N is rewarded. This could explain the relative ease and difficulty of the perseverance and learned non-reward tests. In order to avoid this, idle nosepoke-holes could be covered to avoid responses to them.

The animals should also be trained to nosepoke over a shorter time. This can be done by reducing the criterion in each training phase, training animals to nosepoke in one rather than two nosepoke-holes, and omitting assessment of animal side-biases. This would shorten the training and minimise its effects upon learning in the test conditions.

Table 2.1. Stimuli reward contingencies across the different training and test stages.

Condition/Stage	*Training 1, 3, & 5*	*Training 2, 4, & 6*	*Discrimination*	*Test*
Full reversal	A+(N^{LI}/B^{LI})	B+(N^{LI}/A^{LI})	A+B-(N^{LI})	A-B+(N^{LI})
Perseverance	**A+(**N^{LI}/B^{LI})	B+(N^{LI}/A^{LI})	**A**+B-(N^{LI})	A-N+(B^{LI})
Non-reward	A+(N^{LI}/B^{LI})	**B+(**N^{LI}/A^{LI})	A+**B-(**N^{LI})	**B**+N-(A^{LI})

Stimuli in bold signify interfering associations relevant to the specific test condition. LI signify latent inhibition effects formed at each training phase.

2.8 GENERAL DISCUSSION

The experiments in this chapter have evaluated different protocols for spatial reversal learning, perseverance and learned non-reward testing in the mouse using the operant chamber (Experiments 4-6) and the eight-arm radial maze (Experiments 1-3). Importantly, across these experiments, it is clear that procedural differences can have a large effect on the relative strength of learned non-reward versus perseverance on reversal learning. Moreover, although the operant chamber and eight-arm radial maze tasks appear analogous, they differ in important parameters that are likely to affect the outcome of any manipulation used.

In the maze, the animal is required to make a turn relative to its body. Thus, reliable information for successful performance can only be provided internally through proprioceptive systems. The use of egocentric cues is determined by limiting the number of visouspatial cues and a final 'probe-trial', where egocentric guidance is pitted directly against the use of exteroceptive cues.

The operant task, however, is a visuospatial reversal learning task. Visuospatial cues are available, most clearly through the locations of the two lit-up nosepoke-holes. As opposed to the maze task, this task can be solved with a variety of place, allocentric or egocentric spatial strategies. This is important, since both the neuroanatomy of reversal learning and pathology-related reversal learning deficits can depend upon modality of input (e.g., Brigman et al., 2008; Hölscher and Schmidt, 1994; Oscar-Berman and Zola-Morgan, 1980; Wirtshafter and Asin, 1986).

The two tasks also differ in constrains upon response-time and length of the inter-trial interval. In the operant chamber, animals are forced to respond faster and more often and animals can omit responses in the operant chamber but not in the maze.

Thus, the operant task may carry a greater sensitivity to manipulations of impulsivity than the maze task.

Moreover, in the maze, animals only perceive the CS- when responding to it. In the operant chamber however, the animal is visually exposed to the CS- during each trial regardless of outcome. Thus, as the CS- also is present in positively reinforced trials, the association formed may be closer in form to learned irrelevance, an association of greater magnitude than other non-reinforced associations (Baker et al., 1979; Bennett et al., 1995, 2000).

The two tasks also differ in a range of parameters known to be important in latent inhibition and extinction. This includes the number of positive and negative reinforcements prior to the contingency shift (Lubow et al., 1973), stimuli intensity (Crowell and Anderson, 1972; Schnur and Lubow, 1976), length of context experience (Escobar et al., 2002; Hall and Channell, 1985; Hall and Minor, 1984), handling (Weiner et al., 1985), and the length of the ITI (Schnur and Lubow, 1976; Crowell and Anderson, 1972; Lantz, 1973).

In conclusion, this chapter has shown that mice can be taught egocentric and visuospatial reversal learning in the maze and operant chamber. It has further been shown that variations in training and testing protocols can have a large affect the relative loading upon perseverance and learned non-reward. Although the tasks have considerable theoretical similarity, they differ in many parameters likely to affect the results of any manipulation used. The fully developed versions of each paradigm, (derived from Experiments 1 to 3 for the maze and 4 – 6 for the operant chamber) are outlined in the next chapter and will be used to explore the neurochemical and anatomical substrates of reversal learning in the remaining chapters of this thesis.

CHAPTER 3

EXPERIMENTAL METHODS

3.1 INTRODUCTION

This chapter outlines the protocols chosen for the majority of experiments investigating the neuropharmacology of reversal learning, perseverance and learned non-reward in the maze and operant chamber. These protocols derive both from previously published studies and the preliminary experiments of Chapter 2. This chapter also includes detailed explanations of additional techniques and methods used in the experiments reported in Chapters 4-8 of this thesis. Methods only relevant to individual chapters, including drug administration methods, lesioning procedures and genotyping techniques, are included in the relevant chapter.

3.2 ANIMALS

Animals were C57BL6/J male mice (Charles River, UK) housed in solid-bottomed cages (North Kent Plastics, type M2) with sawdust-lined flooring and paper bedding. They were housed in a controlled environment held at 21 ± 2°C and 50 ± 15% relative humidity with a 12:12h light-dark period (lights on: 07:00h) and 15-20 air-changes/min. Animals had ad libitum access to standard laboratory chow (Special Diet Service Ltd, Witham, UK) and tap water, with home cages being cleaned weekly. On arrival, animals were allowed an acclimatisation period of at least 1 week before the beginning of food deprivation. Food deprivation started 7 days before behavioural training, with animals being kept at around 85-90% of their ad libitum weight throughout testing. During this first week, animals were given 2-3 sucrose pellets daily for habituation to the test diet. Behavioural training and testing took place six days a week (Mon-Sat) between 7am and 7pm with animals being fed 1h after testing. In pharmacological experiments using mice, subcutaneous sham saline injections (4 ml/kg) for habituation to the injection procedure were given in the nape of the neck on the day before commencing behavioural training. All procedures in this thesis were conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986 (Project License 70/6654) following internal review by the University of Sussex

Local Ethical Review Committee.

3.3 OPERANT TASK

3.3.1 *Apparatus*

The experiments were conducted in eight operant chambers (22.5 × 18 × 13 cm; Med Associates, Georgia, VT, USA) placed in sound-attenuating wooden boxes with fans for the purpose of ventilation and concealing external noise (Fig. 3.1). Each box was fitted with a central magazine (W = 2.5 cm, H = 2 cm) connected to an external pellet dispenser delivering 20 mg sucrose pellets (Sandown Scientific, Middlesex, UK). The opposite side of the chamber contained three nosepoke-holes (3.2 cm diameter), located 6.5 cm apart and 5.5 cm above a grid-floor. A houselight was located centrally above the nosepoke-holes 9 cm above the floor. Nosepoke-holes and magazine entries were detected through the breaking of infrared photocell beams located horizontally across the entrances. The chambers where controlled by Med-PC (version 5) and the tasks programmed in Medstate notation.

Figure 3.1. Photo of the operant chamber set-up. Three nosepoke-holes (all open) can be seen on the left side and a central magazine-tray on the right side of the photo. A houselight was located centrally above the nosepoke-holes. A board-camera was mounted centrally behind the operant chamber. An infrared-light was placed on top of the operant box to make video-recordings in the dark possible.

3.3.2 *Procedure*

Training stage 1. On the first day, animals were exposed to the apparatus for 1h in the dark with the fan on and the magazine loaded with pellets. Training to nosepoke for food-reward began on day 2. A trial began with the offset of the houselight and the onset of a single nosepoke-hole LED (fully counterbalanced across the left or right nosepoke-holes). The other two nosepoke-holes remained covered with metallic plates. Training and initial testing was restricted to these two nosepoke-holes, as earlier work (Chapters 2 and 4) had shown this to be sufficient. Responding in the nosepoke-hole caused the nosepoke-LED to turn off, delivery of a single 20 mg sucrose pellet, and the beginning of a 15s ITI. After a correct response, the houselight remained on for an additional 4s before turning off and remaining off for the rest of the ITI. Animals reached criterion when completing ≥ 20 correct responses over 60 min.

Training stage 2. As stage 1, except animals were now required to self-initiate each trial with a nosepoke in the magazine. The available nosepoke-hole stayed the same as in the previous training phase. A nosepoke in the magazine triggered the onset of a single nosepoke-hole LED. Failure to self-initiate a trial within 20s counted as an omission and caused the immediate onset of the 15s ITI. Responding in the nosepoke-hole lead to the nosepoke-LED turning off, pellet delivery, and the beginning of the ITI. Failure to respond in the lit-up nosepoke-hole within 12s counted as an omission and caused the offset of the nosepoke-LED and the immediate onset of the ITI. Animals were required to complete 49 correct responses over 70 trials to meet criterion (≥ 70%). After reaching criterion, each animal received a single session with the opposite nosepoke-hole available (left or right). Animals had no problems transferring responding to a different location of a single lit-up nosepoke hole. Animals were subsequently trained on a two-choice discrimination

Spatial discrimination (Fig. 3.2A). Now both the left and right nosepoke-holes were presented while the central nosepoke-hole remained covered (see Figure 3.2 for flow-chart of the discrimination and reversal procedures).

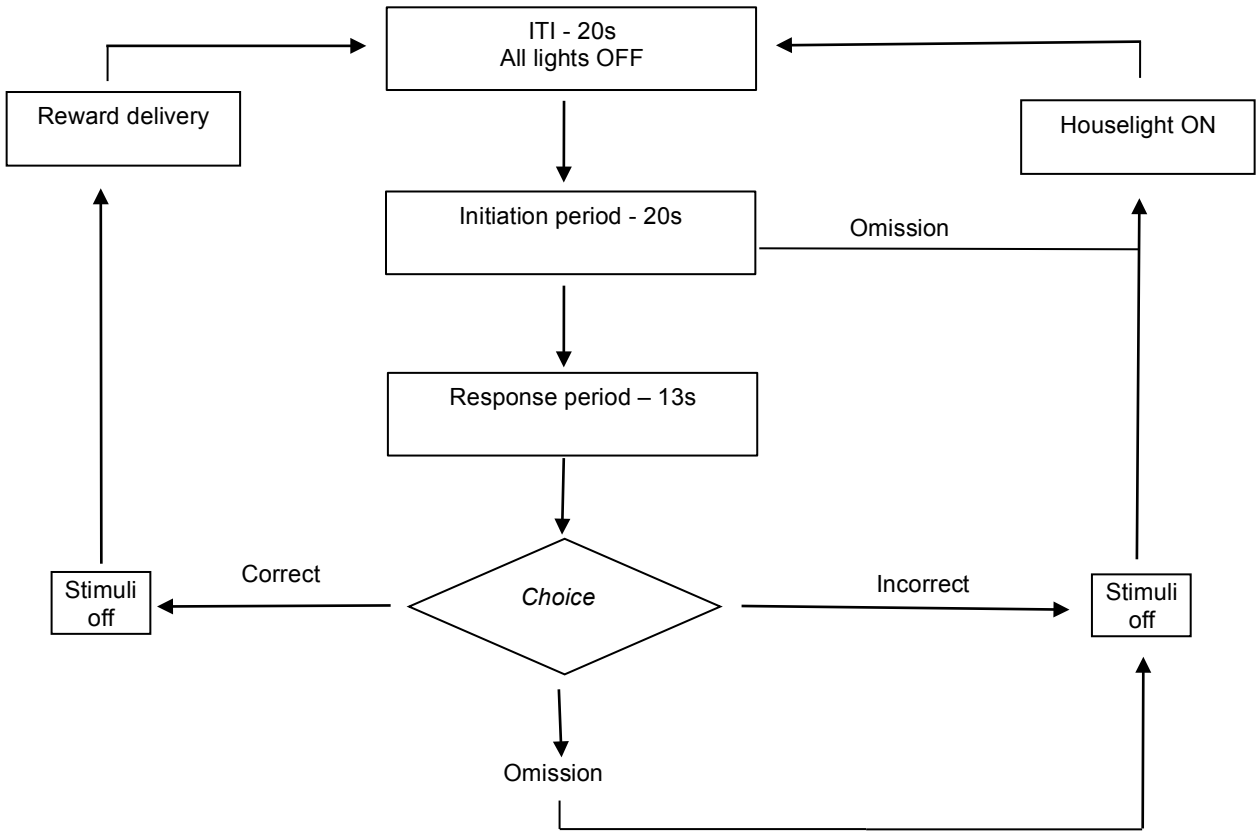

Figure 3.2. Trial sequence in the operant visual discrimination and reversal learning task. A trial is initiated when the mouse enters its head into the food magazine. Trial initiation leads the onset of two nosepoke-hole LEDs (CS+, CS-). Failure to initiate a trial within 20s is scored as an omission and causes the immediate onset of the houselight. A response towards the CS+ is scored as a correct response, and leads to the immediate offset of the two nosepoke-hole LED's, reward delivery, and beginning of a 20s ITI. A response towards the CS- is scored as an incorrect response, which triggers the immediate onset of the houselight. Failure to respond in a nosepoke-hole within 13s is scored as an omission and leads to the offset of the nosepoke-hole LED's and the onset of the houselight.

The correct nosepoke-hole was the opposite nosepoke-hole to which the animal had been trained to respond in stages 1 and 2 of training. A nosepoke in the correct nosepoke-hole within 12s lead to pellet delivery, nosepoke-lights turning off, and the beginning of the 15s ITI. A nosepoke in the incorrect nosepoke-hole counted as an incorrect response and led to the immediate onset of the 15s ITI. Failure to either self-initiate a trial within 20s or

respond in the lit-up nosepoke-hole within 12s counted as omissions and caused the immediate onset of the ITI. A trial was scored as either correct, incorrect or an omission, and the criterion for this and all subsequent conditions were at least nine correct responses within a single block of 10 trials (Boulougouris et al., 2008; Boulougouris and Robbins, 2010). Each session consisted of seven 10-trial blocks and each animal received one session per day. If the animal failed to reach criterion the schedule was repeated on the following day until criterion was reached.

Full reversal test (Fig. 3.3B). Here the contingencies from the previous phase were reversed. A response in the previously incorrect nosepoke-hole was now correct while a response in the previously correct nosepoke hole now was incorrect.

Perseverance test (Fig. 3.3C). Here the previously correct nosepoke-hole became incorrect, while the previously incorrect nosepoke-hole was covered and replaced by a new correct nosepoke-hole. Thus, avoidance of the previously incorrect nosepoke-hole could no longer interfere with performance as the previously incorrect nosepoke-hole had been removed.

Learned non-reward test (Fig. 3.3D). Here the previously incorrect nosepoke-hole became correct, while the previously correct nosepoke-hole was covered and replaced by a new incorrect nosepoke-hole. Thus, approach towards the previously correct nosepoke-hole could no longer interfere with performance as the previously

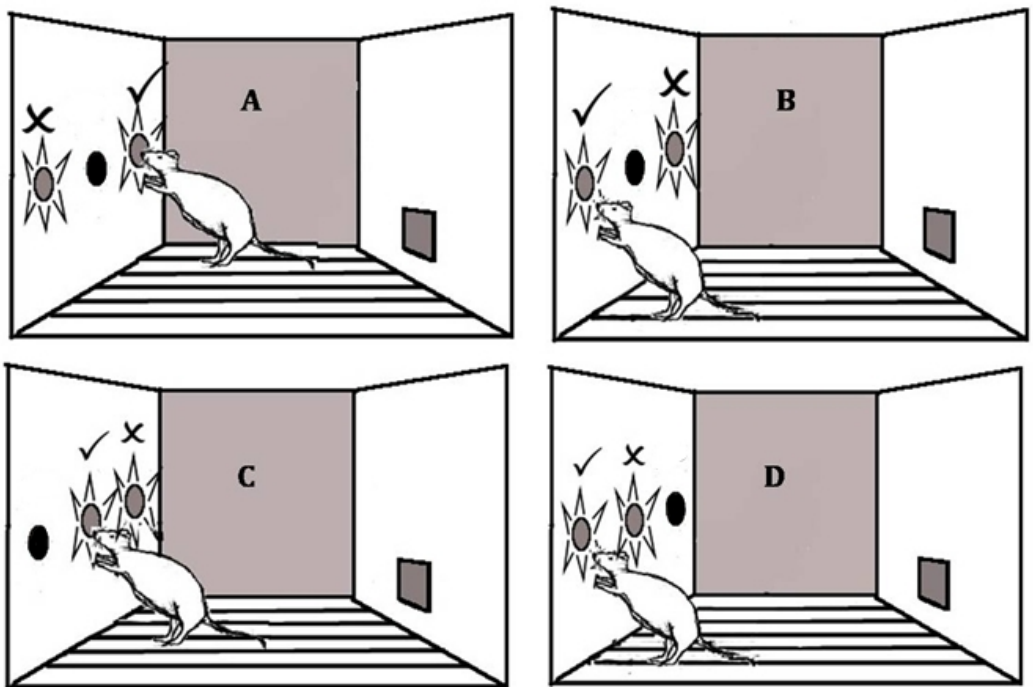

Figure 3.3. Diagram of the four types of discrimination in the operant task. All animals initially completed a spatial discrimination test (A). Example of a subsequent full reversal test (B), perseverance test (C) or learned non-reward test (D).

correct nosepoke-hole had been removed.

Experimental designs. Pharmacological experiments used a three-stage between-subjects serial design (Fig. 3.4A). After completing the spatial discrimination drug-free, animals were matched in pairs for trials to criterion and randomly assigned to a drug and test condition. Animals subsequently completed three tests, each proceeded by a drug-free retention test. Lesion and transgenic experiments used a within-subjects serial design (Fig. 3.4B). After reaching criterion in the spatial discrimination test, all animals completed a full reversal test followed either by a learned non-reward test and a perseverance test. The relative order of the perseverance and the learned non-reward tests where counterbalanced across the experimental groups. Again, each test was preceded by a retention test of the previously learned response.

Data analyses and statistics. Measures collected from each test phase were trials to criterion, omissions, correct and incorrect responses as well as latencies to respond in the nosepoke-hole, trial self-initiation through a magazine entry and latency to retrieve pellet reward. Moreover, incorrect responses to criterion was further analysed as 'early-errors' and 'late-errors' (Boulougoris et al., 2008; Brigman et al., 2008; Gastambide et al., 2012; Jones and Mishkin, 1972; Ragozzino et al., 2002). Early-errors were defined as errors made while the animals responding still was biased towards the previously correct stimulus. That is, when animals still made more than 50 % incorrect responses in a 10-trial bin. This corresponds to what other studies refer to as 'perseverative errors'. Late-errors were defined as errors made after making 50% or more correct responses in a 10-trial bin. This corresponds to what other studies refer to as 'learning-errors', 'regressive-errors', or 'maintenance-errors'. These labels 'early' and 'late' were adopted to avoid potential confusion with the different conditions.

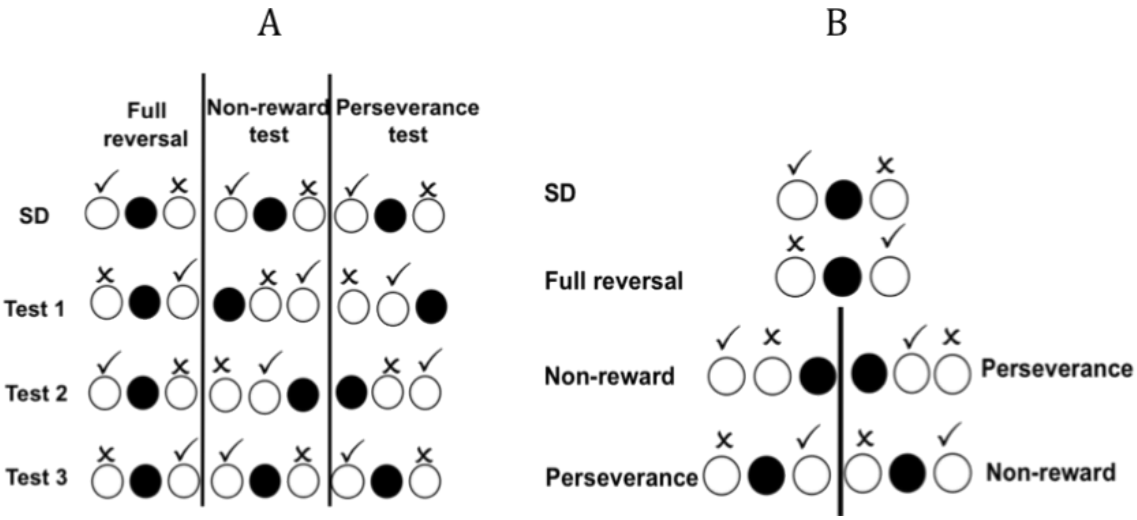

Figure 3.4. Example of the between-subjects and within-subjects experimental designs used in the operant procedure. (A) Between-subjects. Across each test in the full reversal condition, the contingencies reversed between the left and right nosepoke-hole. The central nosepoke-hole remained blocked. Across each test in the learned non-reward condition, the previously incorrect nosepoke-hole became correct, the previously correct nosepoke-hole was blocked, and a previously blocked nosepoke-hole was introduced as a incorrect alternative. Across each test in the perseverance condition, the previously correct nosepoke-hole became incorrect, the previously incorrect nosepoke-hole was blocked, and a previously blocked nosepoke-hole was introduced as a correct alternative. (B) Within-subjects. Each animal was initially exposed to a full reversal test. This was followed by a learned non-reward test and a perseverance test with order of tests counterbalanced.

3.4 MAZE TASK

3.4.1 *Apparatus*

The experiments used an eight-arm radial maze made of clear Plexiglas elevated 55 cm above the floor. Each arm (33.5 × 5 × 8.3 cm) extended from a circular central platform (15.5 cm diameter). Access to each arm was controlled by inserting or removing a clear Plexiglas insert at its entrance. Black-painted vial bottle tops (80 mm diameter, 40 mm deep) figured as food-wells. The maze was enclosed by a featureless circular 'tent' of blackout material. A red light bulb and bullet-camera was located 63 cm above the central platform. The camera connected to a monitor and DVD recorder located in the corner of the room. Animal choice-behaviour were observed through the monitor, which was kept at minimal luminance to minimise visual-cues.

3.4.2 *Procedure*

Maze habituation. Each animal received four days of habituation to the cross-maze, for a maximum of 3×12 min/day. Before being placed in the maze, the maze was always wiped with a sponge moistened disinfectant to minimise intra-maze olfactory cues. Initially five pellets were placed in each of the four arms (three along their lengths and two in the food-wells located at the end of each arm). This was gradually decreased over the week until only one pellet was located in each of the four food-wells. If all pellets were consumed within a 12 min interval, the mouse was removed from the maze, the maze was re-baited, and the next interval began. During the interval, the mouse was

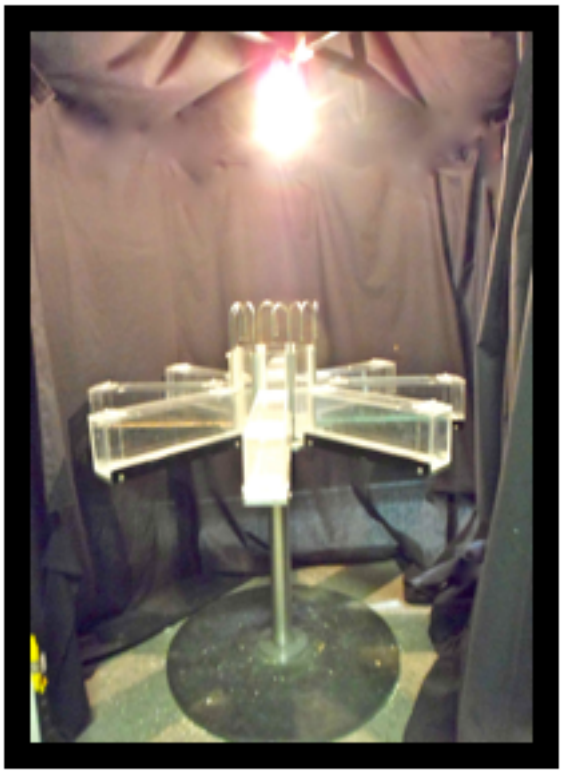

Figure 3.5. Photo of the radial-arm maze set-up used for the reversal learning and novelty experiments. For clarity, the red light used for the experiments has been replaced by a white light.

placed in a holding cage with heavy-absorbent paper to avoid intra-maze cues contaminating the test apparatus. The intervals served to habituate the animals to repeated handling after consumption and exploration.

Turn bias. The mouse turn-bias was determined prior to spatial discrimination (Floresco et al., 2006; Ragozzino et al., 1999). The maze was given a T- or Y-configuration with the start-arm being South (S), West (W) or East (E) across trials but never North (N). The maze-configuration (Y-maze vs. T-maze) was counterbalanced across the different test conditions and experimental groups. The mouse was placed in the start-arm and always had the choice of turning left or right, with both arms baited in order to delay any association between response and reinforcement. The start-arm for each trial was predetermined in a pseudorandom order identical for each mouse. Each animal was given seven trials. One trial comprised one left and one right response. For example, if the mouse turned left, it was allowed to consume the pellet and thereafter returned to the start-arm to make a new choice. If choosing left once more, the mouse was immediately returned to the start-arm. This continued until the mouse turned right. To calculate the mouse turn-bias, the first turn of each trial were added together, with the majority of responses being the mouse turn-bias.

Spatial discrimination (Fig. 3.6A). Again, the start-arm was S, W or E across trials but never N. The start-arm for each trial was predetermined in a pseudorandom order identical for each mouse. Over every nine trials, each arm figured as start-arm equal number of times but the same arm never figured as start-arm for more than two consecutive trials. The mouse always had the choice of turning 90° (T-maze) or 45° (Y-maze) to the left and right. Only the arm opposite the mouse turn-bias was baited. After every ≈7th trial, the maze was turned 90° to minimise intra-maze cues. After making a response, the mouse was removed from the maze and returned to the holding cage while the maze was set up for the next trial. The inter-trial interval was approximately 40s. If a mouse made nine consecutive correct responses it was given a probe-trial (Ragozzino et al. 1999; Floresco et al. 2006). In the probe-trial, the use of an egocentric response strategy was pitted against the use of exteroceptive cues by using N as the start-arm. If successful, spatial discrimination was completed and the animal was returned to its home-cage. If unsuccessful, a further five correct responses lead to a new probe-trial. Each animal was given 25 trials/day. However, if the animal had completed ≥ 6 consecutive correct responses after 25 trials, it was given the chance to reach criterion.

Full reversal (Fig 3.6B). An animal trained to turn right now had to turn left. Thus, the bait was moved to the opposite arm. Everything else was identical to the preceding spatial discrimination, with no additional changes to the maze-configuration.

Perseverance test (Fig. 3.6C). Here the previously correct arm remained open while the previously incorrect arm was replaced by a novel arm. For example, a previously incorrect arm 90° to the left was replaced by a novel correct arm 45° to the left. Only the novel arm was baited. A learning deficit in this test must be due to a failure in suppressing the association of reward, as the previously incorrect alternative no longer is present. That is, the only mistake the mouse can make is to enter the previously correct arm.

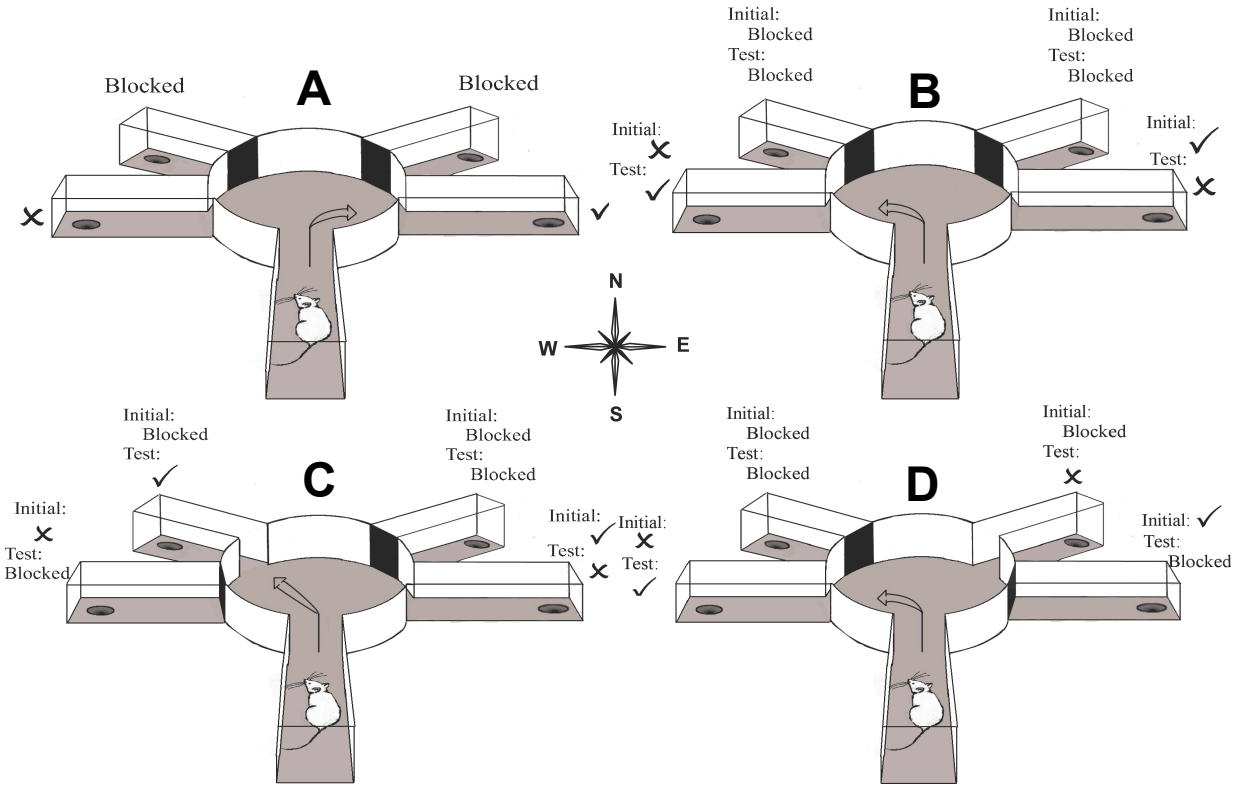

Figure 3.6. Diagram of the four types of discrimination in the maze task. All animals initially completed a spatial discrimination test (A). Example of a subsequent full reversal test (B), perseverance test (C) or learned non-reward test (C).

Learned non-reward test (Fig. 3.6D). Here the previously incorrect arm remained open while the previously correct arm was replaced by a novel arm. For example, a previously correct arm 90° to the right was replaced by a novel incorrect arm 45° to the right. Only the previously incorrect arm was baited. Hence, a learning deficit in this test must be due to a failure in suppressing the association of non-reward, as the previously correct alternative no longer is present. That is, the only mistake the mouse can make is to avoid the previously incorrect arm.

Experimental designs. Pharmacological experiments assessed reversal learning, perseverance and learned non-reward using a three-stage between-subjects serial design. After completing the spatial discrimination drug free, animals were pair-matched for trials to criterion and randomly assigned to a drug and test condition. Animals subsequently completed one of the three test conditions. Lesion and transgenic experiments assessed reversal learning, perseverance and learned non-reward using a within-subjects serial design. After reaching criterion in the spatial discrimination phase, all animals completed a full reversal test followed by a learned non-reward test and a perseverance test. The relative order of the perseverance and the learned non-reward tests were counterbalanced across the experimental groups.

Data analyses and statistics. Total trials and probe trials to criterion and total incorrect and correct responses to criterion at each phase of the experiment were collected from each animal. As in the operant task, errors were further analysed as early-errors and late-errors. Before making five correct responses within a block of ten trials, errors were coded as early-errors. After making five or more correct responses within a block of ten trials, errors were coded as late-errors. Animals failing to reach criterion in a test within 250 trials were counted as 250 and removed from further testing. Video analyses of mean trial times were done using JWatcher (version 1.0)

3.5 MAZE NOVELTY RECOGNITION

Novelty recognition and attraction was assessed using the eight-arm radial maze described earlier in this chapter (Fig 3.5). Animals were initially habituated to a T-maze or a Y-maze for 3×12 min/day for three days. After each 12 min interval, the maze was wiped with a disinfectant to eliminate intra-maze cues. On the last two days of maze habituation, animals received sham saline injection for habituation to the injection procedure (4 ml/kg).

Testing took place on the fourth day over 2×15 min intervals. In the first 15 min interval, the maze was maintained in the same configuration as during maze habituation. In the second 15 min interval, one of the previously open arms was closed while an arm 45° to the north or south was opened. The maze-configuration (T-maze vs. Y-maze) and location of the novel arm (N vs. S) was counterbalanced across the experimental groups.

The 2×15 min test-phase was recorded and analysed using JWatcher (version 1.0). Total and proportion of time and arm entries in each arm was scored before and after the 45° change in arm location. An arm-entry was scored when an animal placed its back-paws behind the small regress separating the central platform from the extending arm. With high degree of similarity, only proportions and change in proportions of arm-entries and time is reported.

3.6 LOCOMOTOR ACTIVITY

Locomotor activity was monitored in nine polypropylene cylinders (H = 25.5 cm, D = 24.5 cm) with a central hollow tube and a circular runway (D = 7.5 cm; Fig. 3.6). The cylinders were placed on a transparent table above a video camera. Between each test, the cylinders and table were wiped with a disinfectant followed by hot water. Recordings from each session were analysed using software (written by John Anderson, School of Life Sciences Workshop, University of Sussex) running on Matlab (version 15). Total number of full 360° and total number of 45° turns where collected from each animal and summed into 15 min time-bins. However, as full 360° turns and 45° turns consistently showed high similarity, only 45° turns are reported. Studies using this system have previously been reported by Dalton et al. (2004) and Clifton et al. (2003).

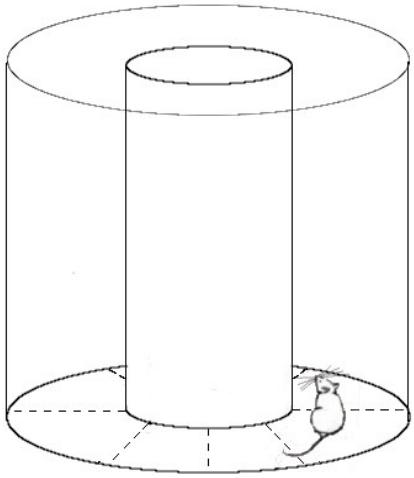

Figure 3.7. Example of the locomotor-box. The dotted lines represents ¼ turns.

CHAPTER 4

PHENCYCLIDINE AND REVERSAL LEARNING

4.1 INTRODUCTION

Schizophrenia is characterised by independent disturbances within three domains; perception, emotion and thinking. These reflect the positive, negative and cognitive symptoms, respectively, described in diagnostic schema such as the DSM IV. Of these, cognitive symptoms precede and outlast all other symptoms and are the best predictors of long-term health (Barch, 2005). Although available neuroleptics can show good efficacy against the positive symptoms as well as moderate efficacy against the negative symptoms, they have frequently been found to have no effects or even detrimental effects on cognition (Weiss et al., 2002). The inability of available neuroleptics to ameliorate these deficits severely limits treatment progression and is believed to be the cause of the often poor long-term health outcomes associated with diagnosis despite existing medication (Green, 1996, 2006; Harvey et al., 1998; Holthausen et al., 2007; Keefe et al., 2006).

One of these cognitive deficits is what Bleuler (1905) referred to as ‘adhesive thoughts’ producing behaviour resistant to change across situations. This form of cognition can be assessed in reversal learning tasks, in which schizophrenic patients repeatedly have been shown to express deficits (Ceaser et al., 2008; Crumpton, 1963; Jazbec et al., 2007; Leeson et al., 2009; Murray et al., 2008; Nolan 1974; Pantelis et al., 1999, 1997, 2004, 2009; Shamay-Tsoory et al., 2007; Tyson et al., 2004). These deficits appear to be due to increased perseverance. Within attentional set-shifting, schizophrenic patients have been challenged with separate tests of perseverance and learned irrelevance. In a perseverance test, the previously correct dimension becomes incorrect while a novel dimension becomes correct. In a learned irrelevance test, a novel correct dimension is introduced while the previously incorrect dimension becomes correct. Here schizophrenic patients display deficits attributable to perseveration towards the previous correct dimension rather than a potentiation of irrelevant associations (Elliott et al., 1995, 1998).

4.1.1 *Schizophrenia NMDA-receptor functioning*

Schizophrenia is associated with compromised glutamatergic and N-methyl-D-aspartate-receptor (NMDAr) functioning. NMDArs' are heteromeric assemblies consisting of multiple NR1 subunits coupled with at least one NR2 and/or NR3 subunit. Four NR2, eight NR1 and two NR3 splice variants has so far been identified. NMDAr subunit composition is tightly controlled throughout development, shows subregion-specific distributions, and determines ligand affinity. Conductance is regulated through a central Ca^{+}-channel which at rest is gated by a voltage-dependent Mg^{2+} blockade. Glycine and d-serine also function as endogenous agonists at the NMDAr, with binding being a prerequisite for glutamate-induced hyperpolarisation (Cull-Candy et al., 2001).

There is a wealth of evidence for decreased glutamatergic and NMDAr activity in schizophrenia. Prefrontally, there are altered levels of NR2C and NR2D (Akbarian et al., 1996) and decreased NR1 mRNA-expression correlating with cognitive impairments (Humphries et al., 1996; Sokolov, 1998). Specific gene variants of the neuregulin-1 and dysbindin-1 genes, controlling for NMDAr subunit expression, have also been associated with schizophrenia (Stefansson et al., 2002; Straub et al., 2002).

Furthermore, schizophrenic patients have lower levels of the NMDAr co-agonists glycine (Neeman et al., 2005; Sumiyoshi et al., 2004) and d-serine (Bendikov et al., 2007; Hashimoto et al., 2003; Yamada et al., 2005) as well as increased levels of the d-serine catabolic enzyme DAAO in the parietal cortex and cerebellum (Madeira et al., 2008; Verrall et al., 2007). G72, a gene controlling DAAO transcription, has also been linked to susceptibility to schizophrenia (Chumakov et al., 2002). Kyneurenic acid and homocystein, two endogenous NMDAr antagonists, are elevated in the brains of schizophrenic patients (Erhardt et al., 2001; Neeman et al., 2005; Nilsson et al., 2005; Schwarcz et al., 2001). Reduced levels of glutamate in the cerebrospinal fluid (Kim et al., 1980) and prefrontal cortex (Tsai et al., 1995), and reduced levels of the NMDAr and glutamate in the hippocampus (Pilowsky et al., 2005; Tsai et al., 1995) have also been observed. Most of these alterations correspond to a NMDAr hypoactivation, which has been suggested as sufficient for the expression of schizophrenic symptoms (Moghaddam, 2003). Yet, there is evidence using proton magnetic resonance spectroscopy of region and disease-stage dependent variations of glutamate-levels in the brains of schizophrenic patients. Levels of glutamine, a glutamate precursor, are elevated in the medial prefrontal cortex (mPFC) and thalamus of never-treated first

episode schizophrenics (Bartha et al., 1997; Théberge et al., 2002) and in patients treated with antipsychotic medication for 9 months (Williamson et al., 2001). In chronic patients, however, glutamate and glutamine levels are decreased in the anterior cingulate while glutamine levels remain elevated in the thalamus (Théberge et al., 2003).

The mechanism underlying the symptom expression is believed to be altered activity at NMDArs' located on prefrontal GABAergic interneurons (chandelier and basket cells), causing aberrant glutamatergic pyramidal cell firing, related to the cognitive deficits, which subsequently produce a downstream striatal hyperdopaminergic state, related to the positive symptoms (Moghaddam and Pehrson, 2010; Morris et al., 2005).

4.1.2 *Modelling schizophrenia through NMDA receptor antagonism*

The anesthetic Sernyl (phencyclidine hydrochloride, or PCP) was early noted to cause acute post-operative psychosis (Johnstone et al., 1959) and exacerbate symptoms in schizophrenic patients (Luby et al., 1959). The psychotomimetic effects of PCP are now considered to be due to its non-competitive antagonist effects at the NMDAr (Javitt and Zukin, 1991; Olney and Farber, 1995), and PCP is now widely used to preclinically model the neurochemical disturbances and symptoms observed in schizophrenic patients.

In healthy subjects, NMDAr antagonism induces a spectrum of symptoms analogous to schizophrenia, including the cognitive deficits (Adler et al., 1998; Lahti et al., 1995; Krystal et al., 1994). However, dosing regimens differ substantially across labs and studies, making interpretations regarding its effect on cognition difficult. Subchronic PCP-treatments are commonly viewed to have greater construct validity than acute treatments to the pathophysiology of schizophrenia (Jentsch et al., 1997a, 1997b). These dosing protocols also retard cognitive flexibility. Rats challenged with 5 mg/kg twice daily for 7 days followed by a 7-day washout period show deficits in maze visual reversal learning (Jentsch and Taylor, 2001). In the bowl-digging task, rats treated with 0.63 or 1.3 mg/kg once daily across 5 days prior to testing and throughout testing show discrimination and reversal deficits (Laurent and Podhorna, 2004). Subchronic PCP-treatment at 2 mg/kg twice daily for 7 days followed by a 7-day washout period also retards learning in a visuospatial lever reversal task (Abdul-Monim

et al., 2006, 2007; Idris et al., 2010; McLean et al., 2009, 2010).

That said, an acute dose of PCP or alternative NMDAr antagonists also produces cognitive impairments in a range of tasks believed to be relevant to the deficits observed in schizophrenia. In humans, acute ketamine impairs working memory (Adler et al., 1998; Malhotra et al., 1996) and attentional set-shifting (Krystal et al., 1994). Acute PCP similarly impairs working memory (Bakker and Amini 1961), associative memory and recall (Davies and Beech, 1960). Acute PCP also retards delayed matching-to-sample in the primate (Hudzik and Wenger, 1993), while acute PCP, ketamine, or MK-801 impairs spatial delayed alternation in the rat (Verma and Moghaddam 1996) and water-maze spatial learning and memory in the mouse (Wesierska et al., 1990). In the 5-choice serial reaction time task (5-CSRTT), performance is also disrupted by an acute dose of PCP or MK-801 (Amitai et al., 2007, 2010; Auclair et al., 2009; Greco et al., 2005; Higgins et al., 2003; Le Pen et al., 2003; Paine et al., 2007, 2009).

Within tests of cognitive flexibility, acute PCP or MK-801 disrupts performance in the response sequence reversal task (Shannon and Love, 2004) and acute ketamine retards attentional set-shifting in the bowl-digging task (Nikiforuk et al., 2010). MK-801 also impairs operant attentional set-shifting when administered systemically (Darrah et al., 2008) and maze attentional set-shifting when infused into the mPFC (Stefani et al., 2003). In the rat, acute doses of PCP have been shown to retard both bowl-digging reversal learning (Gastambide et al., 2010) and water maze reversal learning (Wass et al., 2008). Acute PCP also retards performance in a visuospatial reversal learning task in the rat, a deficit blocked by a range of compounds, including atypical antipsychotics (Abdul-Monim et al., 2003; Idris et al., 2005, 2009).

However, acute PCP also produces dose-dependent increases in activity in the mouse, a response sometimes viewed as analogous to the positive symptoms of schizophrenia (Arguello and Gogos, 2006; Powell and Miyakawa, 2006). This hyperactivity response can interfere with performance in learning tasks and confound interpretations regarding the compounds effect on cognition (Gilmour et al., 2011).

Acute systemic or PFC-specific NMDAr antagonism leads to increased prefrontal pyramidal neuronal burst firing and increased prefrontal glutamate release, assumed to be relevant to cognitive impairments, which causes a downstream increase of striatal dopamine levels, thought to be related to the positive symptoms (Adams and Moghaddam, 1998; Ceglia et al., 2004; Jackson et al., 2004).

There is evidence suggesting that the hyperactivity effects are independent from the effect of acute PCP on prefrontal and striatal glutamate and dopamine levels, as well as its effect on cognition. That is, as activity levels return to baseline, the prefrontally elevated levels of glutamate and dopamine and the accumbal elevation of dopamine persist (Adams and Moghaddam, 1998). A systemic dose of PCP at 5 mg/kg significantly increases prefrontal dopamine levels in the rat, causing a 600% increase 40 min after treatment. This subsequently declines towards baseline but remains roughly doubled compared to vehicle treated controls 140 min post-treatment. Similar effects are also seen in the NAc. Its effect on prefrontal glutamate, however, reaches its peak and plateaus just under 300% over baseline and remains at this levels 140 min post-treatment. Just as with elevated prefrontal glutamate and dopamine levels, deficits in cognitive flexibility have been shown to be independent of increases in activity. In the rat using the bowl-digging task, an acute dose of 2.58 mg/kg PCP retards attentional set-shifting 24h after treatment (Egerton et al., 2005) and 2.5 mg/kg retards bowl-digging reversal learning 2h after treatment (Gastambide et al., 2010).

Notably, although the cognitive symptoms of schizophrenia and the analogous PCP-induced impairments are believed to be related to aberrant glutamate signaling in the PFC, the elevated glutamate levels produced by acute PCP is the opposite to the reduced levels most often observed in schizophrenic patients (Kim et al., 1980; Pilowsky et al., 2005; Tsai et al., 1995). In relation to its effect on glutamate levels in the PFC, it may be that acute PCP only appropriately models non-treated first-episode schizophrenia (Théberge et al., 2003).

The cognitive inflexibility deficits seen after both acute and subchronic NMDAr antagonism are generally assumed to be due to increased perseverance, and analogous to the perseverative deficits seen in schizophrenic patients assessed in attentional set-shifting (Elliott et al., 1995, 1998). However, NMDAr antagonism also affects latent inhibition, and it has been suggested that an inability to overcome non-reinforced associations contribute to the deficits induced by NMDAr antagonists in tasks of cognitive flexibility. For example, acute PCP potentiates latent inhibition in the conditioned taste aversion paradigm (Pålsson et al., 2005; Klamer et al., 2005), while MK-801 potentiates latent inhibition in a thirst-motivated conditional emotional response task (Gaisler-Solomon et al., 2003, 2008; Lipina et al., 2005). However, as yet, the effect of PCP on separate cognitive components in reversal learning has not been

assessed. Hence, the experiments in this chapter looked at the effect of acute PCP in the mouse on reversal learning, perseverance and learned non-reward.

Experiment 1 looked at the time and dose-dependent effects of PCP on activity levels in order to determine an appropriate dose and pre-treatment time at which PCP has no effects on activity levels. Experiment 2 and 3 examined the effect of PCP on operant serial reversal learning and maze reversal learning. Furthermore, as PCP has been found to retard novelty recognition in the rat (Grayson et al., 2007; McLean et al., 2010), and novelty is an intrinsic feature of these tasks, Experiment 4 investigated the effect of PCP on novelty place recognition in the maze.

4.2 METHOD

4.2.1 *Drug*

Phencyclidine hydrochloride (0, 1, 2.5, 5mg/kg; Sigma-Aldrich, UK) was diluted in 0.9% v/w saline and sonicated before being aliquoted and frozen at -80°C in quantities needed for each test day. The salt base was corrected for. Saline alone served as vehicle control and was prepared and stored in the same way. Solutions were administered subcutaneously in the nape of the neck at 4 ml/kg.

4.2.2 *Locomotor activity*

The experiment used 32 C57BL/6J male mice (Charles River, UK) weighing a mean 26.5g at the start of the experiment. The apparatus were as described in Chapter 3. A session began with a drug-free 60 min habituation-phase to the test environment. Animals were subsequently removed from the boxes and administered a dose of 0, 1, 2.5 or 5 mg/kg of PCP (N = 8 within each drug condition) and immediately replaced in the boxes with their activity monitored for a further 240 min. The number of 45° turns was analysed by a 4 (between-subjects: dose) × 16 (within-subjects: time-bin) mixed ANOVA.

4.2.3 *Operant task*

4.2.3.1 Animals and Procedure

The experiment used 24 wild-type male C57BL/6J mice (Charles River, UK) weighing a mean 24.2g at the start of the experiment. The training and testing procedure differed from the protocol outlined in Chapter 3 and is therefore described below. The operant chambers were set-up as depicted in Chapter 3, Figure 3.1. Nosepoke-hole blocks were not used.

Training stage 1. Trial onset was signalled by houselight turning off and one of the three nosepoke-lights turning on. The location of the lit-up nosepoke-hole was pseudorandom. However, the same nosepoke-hole did not light-up for more than three consecutive trials. A nosepoke in the lit-up nosepoke-hole led to pellet delivery, the

nosepoke-light turning off and beginning of a 30s ITI when the houselight remained lit. However, after a correct response throughout the experiment, the houselight remained off for the first 4s of the ITI before switching on and staying on for the remainder of the ITI. Responses in un-lit nosepoke-holes were without consequence. The criterion was ≥ 20 correct responses in a 40 min session.

Training stage 2. Identical to stage 1, however, animals were now required to nosepoke in the magazine within 20s, which activated the single nosepoke-light. A response in the lit-up nosepoke-hole within 12s lead to pellet delivery, the nosepoke-light turning off, and beginning of the 30s ITI when the chamber remained dark. Failure to either respond in the magazine within 20s or respond in the nosepoke-hole within 12s counted as omissions and caused immediate onset of the 30s ITI. The criterion was ≥ 35 correct responses over two consecutive days or ≥ 40 correct responses in a single day of 50 trials.

Side-bias. As described in Chapter 3, each animals side-bias was assessed prior to the spatial discrimination. The location of the two initial nosepoke-holes (right vs left, centre vs. left, centre vs. right) was fully counterbalanced across the different drug and test conditions. The test conditions constituted full reversal, learned non-reward, and perseverance, and were as described in Chapter 3 with the exception of no nosepoke-hole blocks being used.

4.2.3.2 Experimental design and statistical analysis

After reaching criterion in the initial drug-free spatial discrimination phase, animals were pair-matched for trials to criterion and assigned to a drug and test condition. The experiment used a between-subjects serial design, where each animal completed three full reversal, perseverance or learned non-reward tests (Fig. 3.2A). Each test was preceded by a drug-free retention test of the previously learned contingencies. The dependent variables were trials, omissions, correct and incorrect responses to criterion. Incorrect responses were further analysed as early-errors and late-errors. The data was analysed using 3 (within subjects: test phase) × 2 (between subjects: drug) × 2 (between subjects: test condition) mixed ANOVAs.

4.2.4 *Maze task*

4.2.4.1 Animals and procedure

The experiment used 72 wild-type C57BL/6J male mice (Charles River, UK) weighing a mean 24.9g at the start of the experiment. The experiment was run in two batches of 36, with each animal being tested every other day. The training and testing procedure was as exactly as described in Chapter 3.

4.2.4.2 Experimental design and statistical analysis

After reaching criterion in the initial drug-free spatial discrimination phase, animals where pair-matched for trials to criterion and assigned to a drug and test condition. The maze experiment used a between-subjects single test design where each animal completed a single full reversal, perseverance or learned non-reward test. The dependent variables were trials, correct and incorrect responses to criterion, and early- and late-errors. Mean trial-time when drug-challenged in the test phase was collected through video analysis. The data was analysed using a 2 (drug) × 3 (test condition) between-subjects ANOVA.

4.2.5 *Maze novelty recognition*

The experiment used 16 C57BL6/J male mice (Charles River, UK) weighing a mean 27.0g at the start of the experiment. The procedure was as as described in Chapter 3. On the test-day, animals received an acute dose of PCP at 5 mg/kg 2h and 45min before being placed in the maze. Thus, the novel response arm location was introduced 3h after drug administration.

4.3 RESULTS

4.3.1 Experiment 1: PCP and locomotor activity

PCP significantly increased activity over 180 min (Fig. 4.1). There was a significant main effect of dose ($F_{3, 28} = 33.1$, $p < .0001$) and a significant dose × time interaction on activity levels over the 240 min test-phase ($F_{45, 420} = 14.7$, $p < .0001$), with PCP increasing activity levels in a dose-dependent manner (Fig. 4.1B). Separate ANOVAs for each time-bin revealed non-significant effects of dose in the last five time-bins. Accordingly, PCP at a dose of 5 mg/kg and a 180 min pre-treatment time was chosen for assessing the effects of PCP in the maze, operant and novelty tasks.

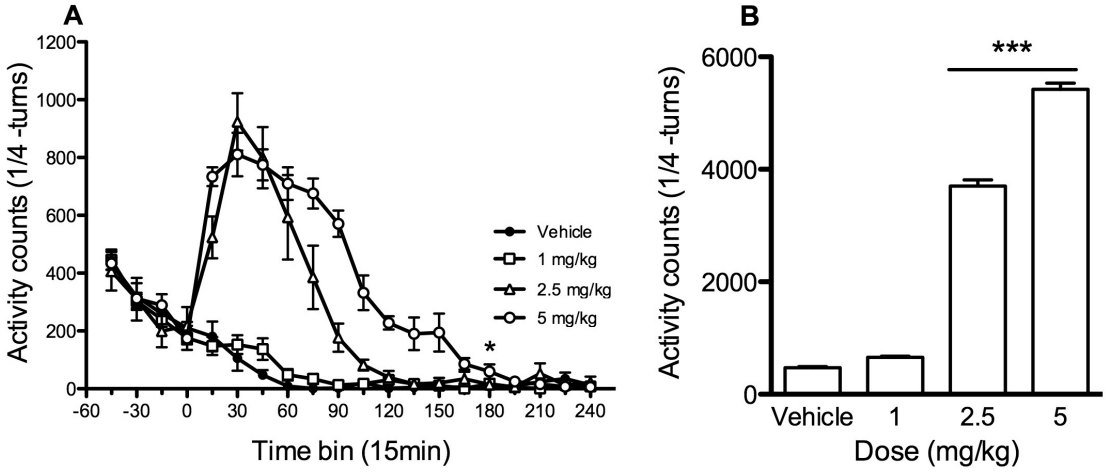

Figure 4.1. Effect of PCP on locomotor activity. (A) Activity expressed in 15 min time-bins. Animals were dosed at time 0, following 60 min of habituation to the test environment. Asterisk denote time-point at which drug effect upon activity equals $p \geq 0.05$. (B) Total activity counts over the 240 min test phase. PCP significantly increased activity at 2.5 and 5 mg/kg. (*** = $p < .0001$).

4.3.2 *Experiment 2: PCP and operant reversal learning*

There were no significant differences in performance between animals exposed to the different nosepoke-hole configurations (left vs right, right vs centre, left vs centre). PCP had no effect on learning in the three conditions, but decreased the number of omissions as well as trial-initiation and nosepoke-response latencies.

There were no significant main effects of drug or drug × condition interactions on trials (Fig 4.2; Drug: $F_{1,17} = 2.7$, p = ns, Drug × condition: $F_{2,17} = 2.1$, p = ns) incorrect responses (Fig 4.3; Drug: $F_{1,17} = 1.3$, p = ns, Drug × condition: $F_{2,17} = 1.2$, p = ns) or correct responses to criterion (Fig. 4.4; Drug: $F_{1,17} = 0.4$, p = ns, Drug × condition: $F_{2,17} = 0.6$, p = ns).

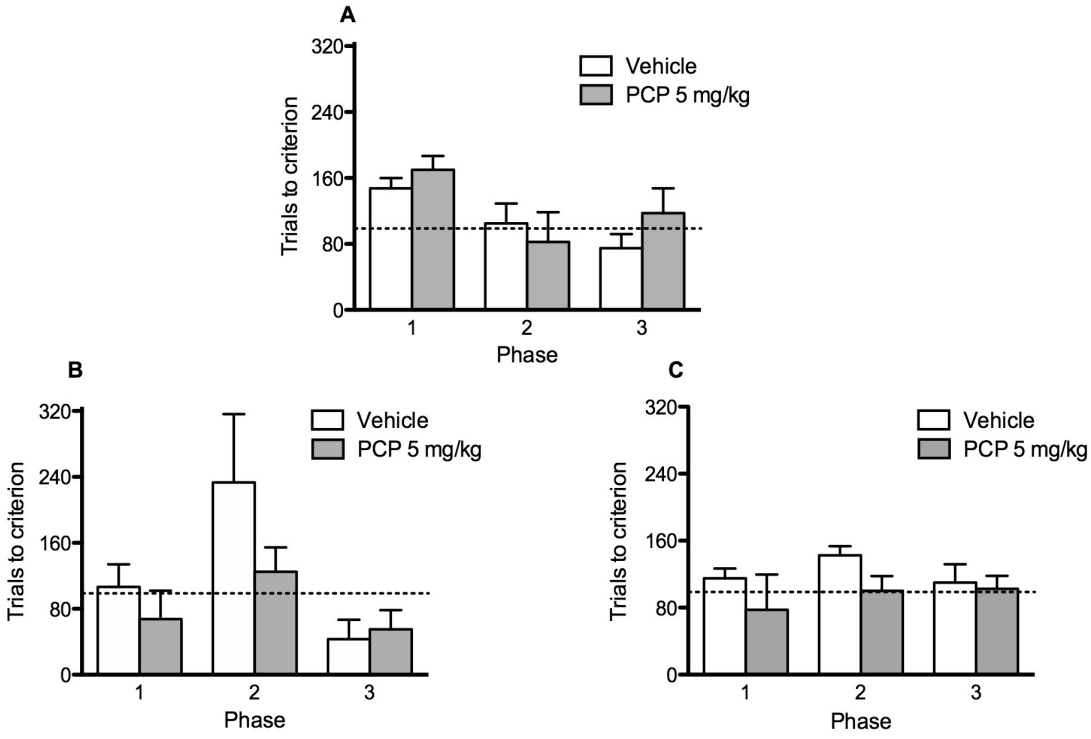

Figure 4.2. Effect of PCP on trials to criterion in the three test phases of the full reversal (A) learned non-reward (B) and perseverance conditions (C) in the operant procedure. Broken line represents mean performance in the initial spatial discrimination phase. Significant main effect of phase ($F_{2,34} = 3.8$, p = .03) and phase × condition interaction ($F_{4,34} = 4.5$, p = .005). In the first test phase, animals required more trials in the full reversal condition than in the perseverance (p = .02) and the learned non-reward conditions (p = .01). No main effect of PCP or interactions with test condition or test phase.

However, there was a significant effect of drug ($F_{1,17} = 11.9$, $p < .01$) as well as significant drug × condition ($F_{2,17} = 5.6$, $p < .05$) and phase × drug × condition ($F_{4,34} = 2.9$, $p < .05$) interactions on omissions to criterion (Fig. 4.5). Separate one-way ANOVAs within each drug and test condition showed that PCP significantly decreased omissions only in phase 2 of the in the perseverance condition ($F_{1,6} = 18.0$, $p < .01$). PCP also decreased trial initiation ($F_{1,17} = 13.5$, $p < .01$) and nosepoke-hole response times (Table 4.1; $F_{1,17} = 12.8$, $p < .01$). There were no effects of PCP or interactions with condition or test phase on early- or late-errors to criterion.

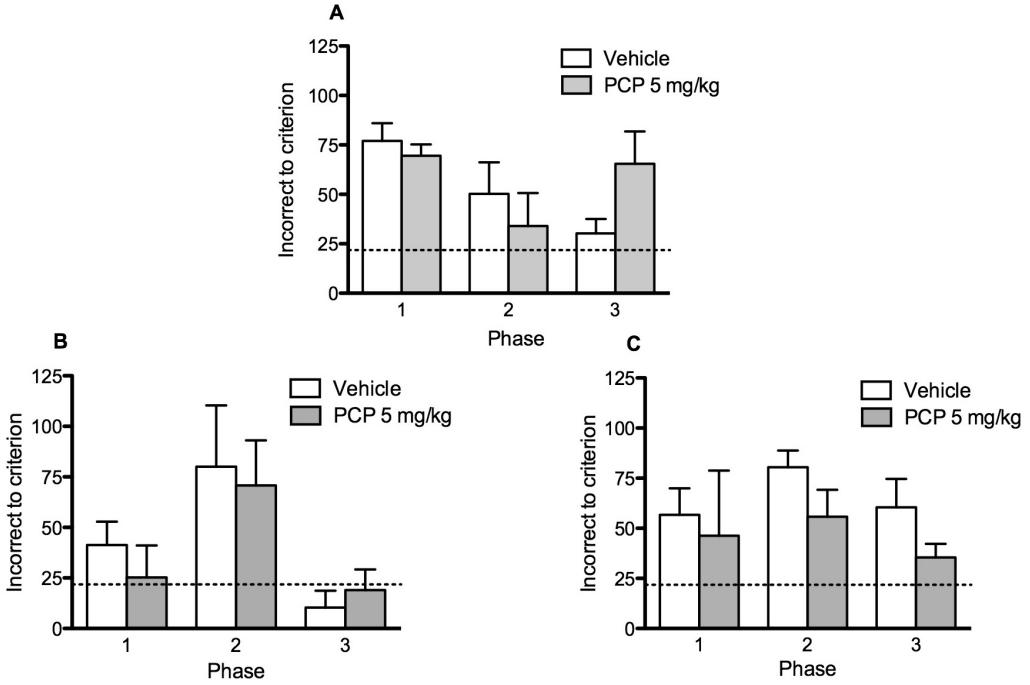

Figure 4.3. Effect of PCP on incorrect responses to criterion in the three test phases of the full reversal (A) learned non-reward (B) and perseverance conditions (C) in the operant procedure. Broken line represents mean performance in the initial spatial discrimination phase. Significant effect of phase ($F_{2,34} = 3.3$, $p = .048$) and phase × condition interaction ($F_{4,34} = 3.0$, $p = .032$). In the last test phase, animals made fewer incorrect responses in the learned non-reward condition than in the full reversal ($p = .02$) and perseverance conditions ($p = .02$). No main effect of PCP or interactions with test condition or test phase.

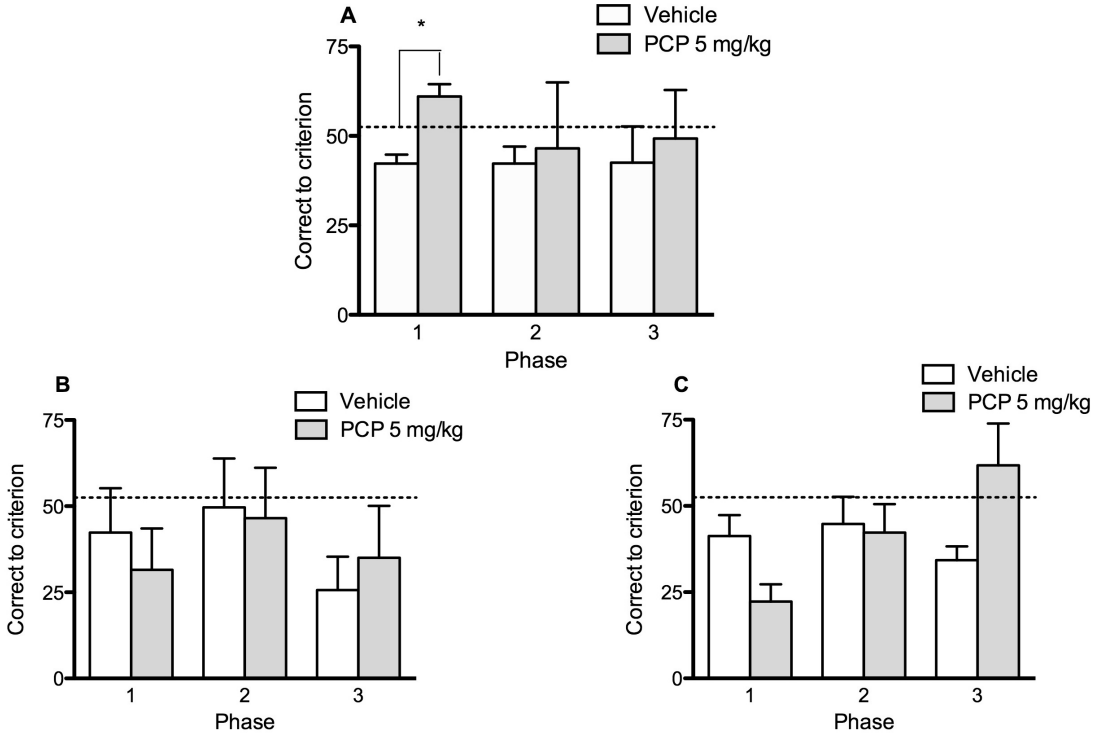

Figure 4.4. Effect of PCP on correct responses to criterion in the three test phases of the full reversal (A) learned non-reward (B) and perseverance conditions (C) in the operant procedure. Broken line represents mean performance in the initial spatial discrimination phase. No significant main effect of PCP or interactions with test condition or test phase.

Table 4.1. Mean latencies (± SEM) in the operant task collapsed over the three test phases and three test conditions.

Drug	Trial initiation	Nosepoke response	Pellet retrieval
Vehicle	5.17 ± .18	3.94 ± .13	2.06 ± .05
PCP 5 mg/kg	4.24 ± .17**	3.29 ± .13**	1.99 ± .05

Trial initiation: Significant effect of drug ($F_{1,17}$ = 13.5, p = .002) and drug × test phase interaction ($F_{2,34}$ = 4.5, p = .018). PCP decreased trial initiation-time in the second and third test phases. *Nosepoke response:* Significant main effects of drug ($F_{1,17}$ = 12.8, p = .002) and condition ($F_{1,17}$ = 5.7, p = .013). PCP decreased nosepoke-response times while animals in the non-reward condition were slower to respond in the nosepoke-hole than animals in the full reversal (p = .009) and the perseverance conditions (p = .008). *Pellet retrieval*: no significant effects.

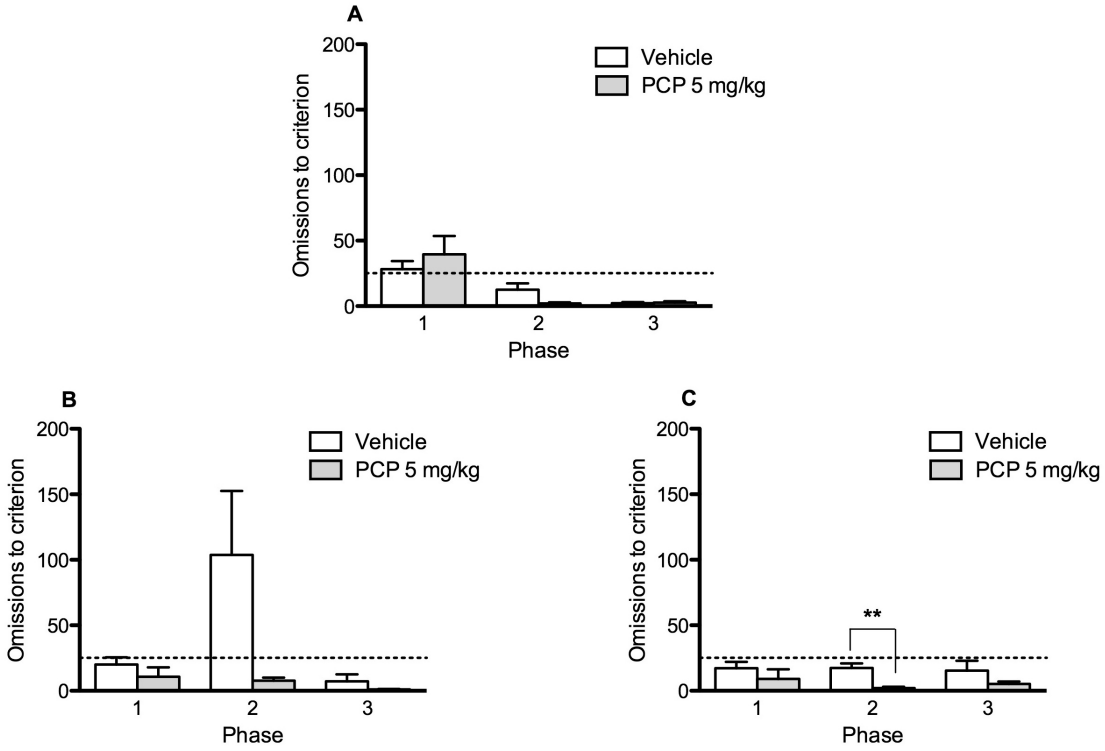

Figure 4.5. Effect of PCP on omissions to criterion in the three test phases of the full reversal (A) learned non-reward (B) and perseverance conditions (C) in the operant procedure. Broken line represents mean performance in the initial spatial discrimination phase. Significant main effects of drug ($F_{1,17} = 11.92$, $p = .003$) and phase ($F_{1,34} = 5.3$, $p = .01$) and drug × condition ($F_{2,17} = 5.6$, $p = .014$) and phase × drug × condition interactions ($F_{4,34} = 2.9$, $p = .035$). Animals made fewer omissions in phase three than phase 1 ($p = .002$) and phase 2 ($p = .017$) . PCP significantly decreased omissions only in phase 2 of the perseverance test ($F_{1,6} = 18.0$, $p = .005$).

4.3.3 *Experiment 3: PCP and maze reversal learning*

Acute PCP caused a non-condition dependent increase in trials and incorrect responses to criterion (Fig. 4.6). PCP also decreased mean trial times across all test conditions. PCP had no effect on late-errors, but significantly increased the number of early-errors.

There was a significant main effect of drug on trials ($F_{1,44} = 8.0$, $p < .01$) and incorrect responses to criterion ($F_{1,44} = 10.2$, $p < .01$) but no effects on correct trials to criterion. To further investigate how PCP affected learning in the three test conditions, incorrect responses was broken down and analysed in terms of pre- and post > 50% correct responses in a nine-trial `bin. PCP significantly increased the number of early-errors ($F_{2,44} = 7.6$, $p < .01$) but not late-errors across all conditions, with no significant effect of test condition or drug × test condition interaction.

For mean trial times, there was a significant effect of drug (Vehicle, 4.9s ± 0.3; PCP, 3.8 ± 0.1; $F_{1,44} = 10.1$, $p < .01$) but no effect of test condition or drug × test condition interaction.

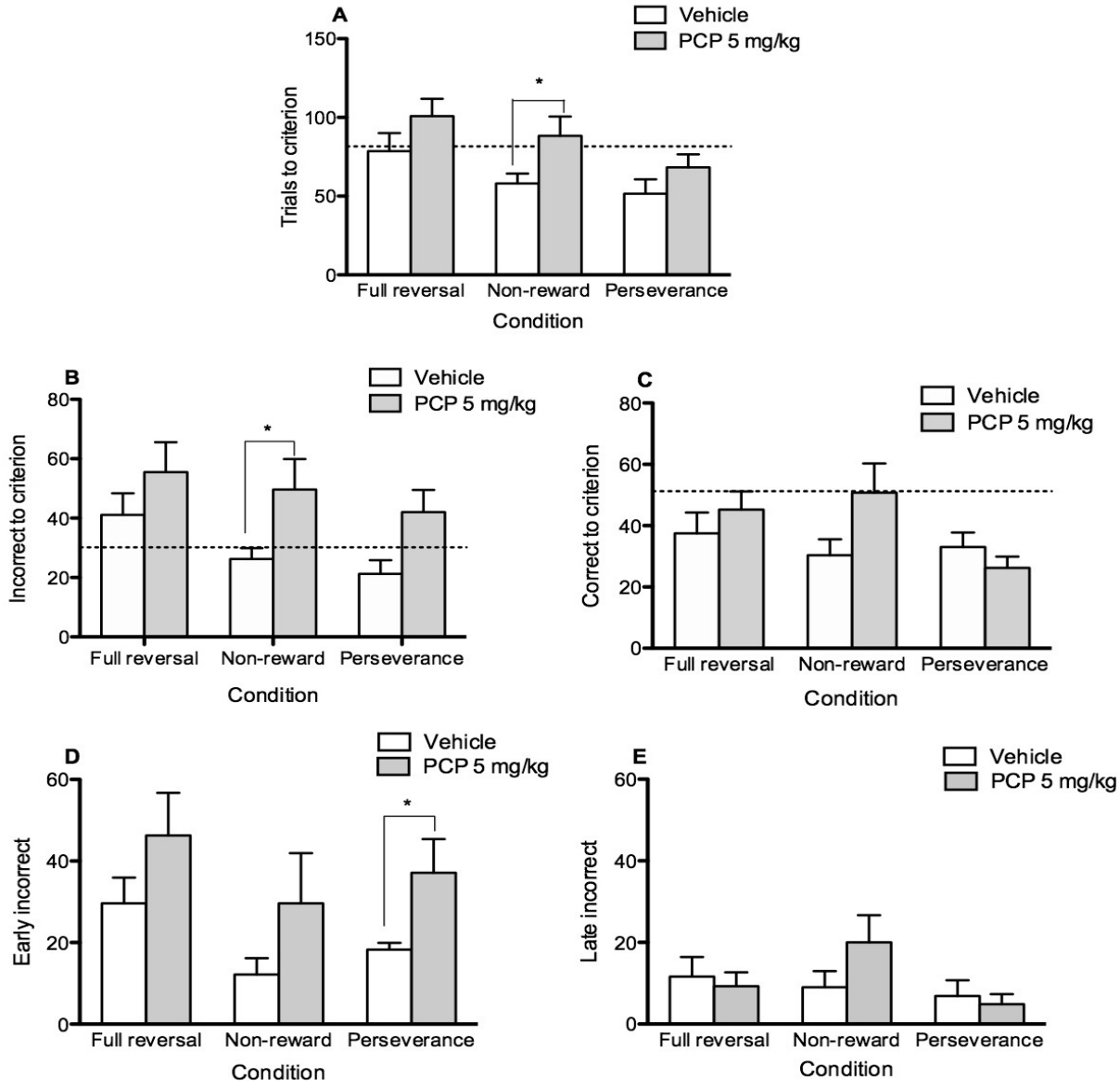

Figure 4.6. Effect of PCP in the three test conditions of the maze procedure. Asterisk denote differences at which $p < .05$. Broken line represents mean performance in the initial spatial discrimination phase. (A) *Trials to criterion.* Significant main effects of drug ($F_{1,44} = 8.1$, $p = .007$) and test condition ($F_{2,44} = 3.7$, $p = .032$). Animals in the full reversal test required more trials to criterion than animals in the perseverance ($p = .012$) and learned non-reward tests ($p = .052$). (B) *Incorrect responses to criterion.* Significant main effect of drug ($F_{1,44} = 10.2$, $p = .003$). (C) *Correct responses to criterion*. No significant effect of drug, test condition, or drug × test condition interaction. (D) *Early-errors.* Significant effect of drug ($F_{2,44} = 7.6$, $p = .008$) but no effect of test condition or drug × test condition interaction. (E) *Late-errors*. No effect of drug, test condition, or drug × test condition interaction.

4.3.4 *Experiment 4: PCP and maze novelty recognition*

Animals recognised the 45° shift in response-arm location, seen as significant increases in the proportion of arm entries and proportion of time spent in the novel arm. PCP had no effect on either measure.

Before the presentation of a novel arm, animals spent equal proportions of time and made equal proportions of arm entries in to each of the three arms (Table 4.2). Shifting the arm 45°, significantly increased the proportion of time spent in the arm ($F_{1,20} = 23.8$, $p < .0001$) and the proportion of arm entries into the arm ($F_{1,20} = 42.6$, $p < .0001$). There were no significant effects of drug on proportion of time spent in the novel arm or proportion of arm entries made into the novel arm.

Table 4.2. Effect of PCP on novelty recognition. Proportion of time and proportion of entries ± SEM (in brackets) before a 45° shift (pre-shift) and after a 45° shift (post-shift) in vehicle and PCP treated animals.

	Proportion of time (%)			**Proportion of entry counts (%)**		
	Pre-shift	*Post-shift*	*Change*	*Pre-shift*	*Post-shift*	*Change*
Vehicle	34.0 ± 2.2	51.8 ± 3.3	+17.8	36.7 ± 1.7	46.4 ± 3.3	+9.74
5 mg/kg PCP	31.0 ± 3.1	52.2 ± 5.0	+21.2	32.9 ± 0.8	46.0 ± 2.2	+13.14
Total	32.5 ± 1.9	52.0 ± 2.9	+19.5**	34.8 ± 1.0	46.2 ± 2.0	+11.44**

** $p < .001$

4.4 DISCUSSION

An acute dose of 5 mg/kg of PCP under a 3h pretreatment time had different effects on performance in the maze and operant tasks, leaving learning intact in the operant procedure while causing general disruptions in the maze. PCP affected latency-measures in both tasks, with PCP causing reductions in response-latencies and mean trial-times.

4.4.1 *Locomotor activity*

PCP have marked effect on motor function that may influence performance in procedures involving complex motor sequences, such as reversal learning tasks (Gilmour et al. 2011). In Experiment 1, PCP increases activity levels for 120 min at 2.5 mg/kg and for 180 min at 5 mg/kg. Most work investigating the effects on acute PCP on cognitive flexibility do not assess the dose and pretreatment times affect on activity, and it may be that motor disruptions could account for some of the previous reversal learning deficits observed.

4.4.2 *Operant task*

In the operant task, PCP decreased the number of omissions and trial self-initiation and nosepoke-hole response latencies yet failed to effect learning, with no significant effects on trials to criterion, correct or incorrect responses to criterion.

Previously, O'Neil and collegues have shown that acute treatment with PCP at 1-1.5 mg/kg 30 min prior to testing potently retards operant visuospatial reversal learning performance in the rat (Abdul-Monim et al., 2003; Idris et al., 2005, 2009). However, their procedure differs substantially from most other reversal learning procedures. In their task, animals do a within-session reversal between levers, with contingency shifts being cued by a 1 min dark-out period. Animals undergo repeated reversals during training, prior to being drug-challenged. When drug-challenged, the protocol remains identical to the protocol of the training phase. Thus, it can be argued that successful performance in this task, contrary to standard reversal learning assays, require the animal to preserve the response strategy adopted during training rather than to acquire an alternative response strategy. The PCP-induced reversal learning deficit in

their task is also blocked by a range of compounds, including clozapine, olanzapine, ziprasidone, lamotrigine, sertindole, risperidone, asenapine, the 5-HT_{2A} receptor (5-HT_{2A}R) antagonist M100907, the 5-HT_6R antagonist SB742457, the 5-HT_{2C}R antagonist SB243213A, the 5-HT_7R antagonist SB269970A, the α_7 nAChR agonist PNU-282987, the Na^+-channel inhibitor phenytoin, and the D_1R agonist SKF-38393. The validity of the task in relation to the cognitive deficits of schizophrenia is unclear, as these atypical antipsychotics lack in reliable therapeutic efficacy against the cognitive symptoms of schizophrenia.

Notably, PCP has also repeatedly been shown to be without effect on reversal learning. Subchronic PCP has recently been found to be without effect in a touch-screen visual reversal learning task in the mouse when administered twice daily at 5 mg/kg for 7 days followed by a 7 day washout period (Brigman et al., 2009). In the bowl-digging task, a range of subchronic dosing regiments have failed to affect performance in the reversal phases (Dawson et al., 2012; Deschenes et al., 2006; Goetghebeur et al., 2008, 2010; McLean et al., 2008; Rodefer et al., 2005, 2008) Also, an acute dose of 2.58 mg/kg 24h prior to testing has no effect on bowl-digging reversal (Egerton et al., 2005). In maze spatial reversal learning, acute PCP at 1 mg/kg dose 10 min prior to testing has no effect on acquisition of reversal learning, but affects retention 24h later (Handelmann et al., 1987). Here acute PCP was similarly found to be without effect on operant reversal learning.

More difficult to reconcile with the current negative data are reports of impaired reversal learning from a similar dosing protocol. In the rat, an acute dose of PCP at 2.5 mg/kg retards reversal learning 120 min post treatment in the bowl-digging task (Gastambide et al., 2010). These discrepancies could be explained by differences in methodology including task, species and dosing protocol. Notably, 2.5 mg/kg may still significantly increase activity levels 2h post-treatment.

PCP did however significantly reduce the number of omissions, most potently in the perseverance condition. With no effects of PCP on measures of trials, incorrect or correct responses to criterion in any of the test conditions, the effect is likely to be unrelated to learning. Omissions are most often are interpreted as a measure of motivation (Robbins, 2002). It is however unlikely that the PCP-induced decrease in omissions could be explained by a direct elevation in the motivation for sucrose. PCP has been found to decrease or have no hedonic effects when evaluated in licking microstructure (Lydall et al., 2010) and sucrose consumption tests (Turgeon and Hodge,

2003). Also, PCP failed to affect pellet-retrieval latencies, considered the more potent measure of reinforcer evaluation (Robbins, 2002).

A further possibility is that the decreased omissions is the product of exaggerated motivational salience of the reward related stimuli, or impulsive decision-making. Subchronic PCP has been shown to increase impulsive responding in the vervet monkey when having to inhibit reaching towards a barrier (Jentsch et al., 1997, 2000). However, the increased impulsivity following subchronic PCP has been explained by reduced corticostraital dopamine, with similar effects are seen after MPTP-induced dopamine depletions (Schneider and Kovelowski, 1990; Taylor et al., 1990a, 1990b). If anything, the opposite effect is produced after an acute treatment of PCP using the current dosing protocol, which increases prefrontal and striatal levels of dopamine (Adams and Moghaddam, 1999). Moreover, normal acquisition was observed in each of the three test conditions following acute PCP. It could be argued that a PCP-induced increase in behavioural control of reward related stimuli should cause profound perseverative deficits in this task.

The reduced omissions could also be an effect of increased activity. In the rat, the cocaine-induced elevation in responding for sucrose rats under a progressive-ratio schedule has been attributed to elevated activity, as cocaine also causes a parallel decrease in sucrose consumption and preference (Brown and Stephens 1999). However, a direct effect of locomotor activity on omissions is unlikely. Experiment 1 showed that 5 mg/kg of PCP failed to increase activity 3h post-treatment. Also, PCP had no effect on the number of nosepoke-hole responses made. It could be predicted that such effects also should increase the number of correct and incorrect responses by PCP-treated animals.

Alternatively, the effect on omissions could be related to motor-impulsivity. Motor impulsivity is most often assessed in the 5-CSRTT, where animals have to respond in one of five nosepoke-holes signaled by a brief illumination. Prior to the illumination, there is an ITI during which the animal must withhold from responding. Responses made during the ITI are described as premature, and believed to index motor impulsivity. Other performance measures include accuracy, measuring the correct response ratio, and 'perseverative-responses', measuring excessive non-reinforced nosepoke-hole responses.

In the rat, acute NMDAr antagonism through PCP, MK-801, Ro63-1908 or CPP increases premature responses in the 5-CSRTT (Higgins et al., 2003; Le Pen et al.,

2003; Paine et al., 2007). In the mouse, an acute dose of 3 mg/kg PCP 10 min before testing has been found to increase impulsivity in the 5-CSRTT in the mouse without significantly affecting other performance measures (Greco et al., 2005) although there is likely significant hyperactivity at this point. The decreased accuracy in the 5-CSRTT following an 1-2 mg/kg acute dose of PCP 30 min prior to testing has also been explained in terms of increased motor impulsivity, as decreased accuracy is independent of delay and associated with decreased correct response latencies (Smith et al., 2011).

The impulsivity effects of NMDAr antagonism are believed to be caused by a prefrontal hyperglutamatergic state (Ceglia et al., 2004). The competitive NMDAr antagonists CPP and MK-801 increases premature responses in the 5-CSRTT if infused into the mPFC (Agnoli and Carli, 2012; Baviera et al., 2008; Lehmann et al., 1987) or infralimbic cortex (Murphy et al., 2005). Increased glutamate within the mPFC also appears to be selectively involved in motor impulsivity. At lower doses, intra-mPFC infusions of CPP increased premature responses without affecting accuracy and hyperactivity (Carli et al., 2004, 2006). Also, antagonising CPP-induced glutamate release through pretreatment with the 5-HT_{2A}R antagonist M100907 selectively decreases premature responses without effecting perseverative responses (Ceglia et al., 2004, 2006). A hyperglutamatergic prefrontal state is produced by PCP under the current dosing protocol (Adams and Moghaddam, 1998), which effect upon motor impulsivity could explain the observed reduction in omissions and latencies.

However, elevated dopamine-levels can also cause increased impulsivity. In the rat, amphetamine increases premature responses (Harrison et al., 1997), which is blocked by striatal dopamine depletion (van Gaalen et al., 2006) and the D_2R antagonist eticlopride (Cole and Robins, 1989). In the mouse, amphetamine and the dopamine re-uptake inhibitor GBR12909 increases premature responses (Loos et al., 2010). The effect of acute systemic PCP on premature responses has also been linked to activity within the dorsal striatum. Pretreatment with the 5-HT_{2A}R antagonist M100907 attenuates both PCP-induced increases in premature responses and striatal, but not prefrontal, s-133-creb phosphorylation (Pozzi et al., 2010). A 5 mg/kg dose of PCP causes a significant elevated of striatal dopamine levels 140 min post-treatment, and it may be that this can influence the performance in the current tasks. That said, the increase is relatively small in relation to its effect on prefrontal glutamate (Adams and Moghaddam 1998).

In conclusion, PCP failed to affect learning in the operant task, with no significant effects on trials, correct, or incorrect responses to criterion. However, PCP decreased the number of omissions made as well as latencies for trial-initiation and nosepoke-hole response. These effects could be related to a hyperglutamatergic prefrontal state causing elevated motor impulsivity.

Lastly, the current operant design allows for several possible confounds. First, all three nosepoke-holes remained open at all times. This meant that animals could respond, without effect, in the inactive un-lit nosepoke-hole at all training and testing stages of the experiment. If animals are guided by spatial location to a greater extent than the nosepoke-hole light, animals may still treat the inactive nosepoke-hole as a valid response option. This would also mean that latent inhibition-like effects develop towards the inactive nosepoke-hole as a nosepoke in this hole is without consequence. Second, animals are trained to nosepoke across all nosepoke holes within a single session (Floresco et al., 2008). Hence, within a single training session, all nosepoke-holes are occasionally rewarded. This is a direct contradiction to the idea of discrimination learning. In the current protocol, proactive interference from the training phases could therefore confound learning in the test-phases. An alternative is to train animals to with one response alternative only (Boulougouris et al., 2007, 2008, 2009; Boulougouris and Robbins, 2009, 2010).

4.4.3 *Maze task*

Contrary to the operant task, PCP potently retarded performance in the maze task. This was seen as significant increases in trials, incorrect responses and early-errors across the full reversal, perseverance and learned non-reward conditions. Similar to the operant task, PCP also caused a non-condition dependent decrease in mean trial-times.

It is possible that the retarded learning is due to deficits in novelty recognition, as both the perseverance and learned non-reward tests involve the presentation of a novel response option. Accordingly, although there were no significant drug × test condition interactions, the effect of PCP appeared to be more prominent in the learned non-reward and perseverance tests, which involve the presentation of a novel response-option, than the full reversal test. In support of this, it has previously been shown that PCP retards novelty recognition in the rat (Grayson et al., 2007; MacLean et al., 2010). However, in Experiment 4, PCP failed to influence the proportion of time spent in the

novel arm and the proportion of entries made into the novel arm, indicating equal novelty recognition in PCP and vehicle treated animals. A further possible explanation for the mild effect within the full reversal condition is the greater difficulty, with the relatively high number of trials and incorrect responses of the vehicle treated animals partly masking any PCP-related deficits.

The general impairment in learning was paralleled by decreased mean trial times. When assessing the effect of PCP in reversal paradigms, latency effects are sometimes not reported (Abdul-Monim et al., 2003, 2006, 2010; Gastambide et al., 2012; Idris et al., 2005, 2009, 2010; McLean et al., 2009, 2010), or reported to be without effect (Brigman et al., 2008; Handelmann et al., 1987). Within other paradigms, PCP has disparate effects upon latency indices depending dosing procedures. For example, in a delayed matching to position task, 0.5-2 mg/kg 30 min prior to testing has been shown to decrease response latencies (Smith et al., 2011). In the 5-CSRTT, acute doses of 1-2 mg/kg at a 30 min pretreatment time has been found to decrease correct response latencies in the rat (Smith et al., 2011), while acute doses at 2-3 mg/kg at 10-45 min pretreatment times has been found to increase response latencies (Auclair et al., 2009; Amitai et al., 2007, 2010; Le Pen et al., 2003). Just as with omissions, the decreased latencies are unlikely to be effects of cognitive impulsivity or enhanced motivation for sucrose, but could again be related to motor impulsivity. A further possibility is that the decreased mean trial times of PCP-treated animals are context dependent activations of activity, and therefore observed in the maze but not the locomotor runways.

Impulsivity could also explain the retarded learning. Although PCP failed to affect learning in the operant task where decreased response latencies and omissions also were observed, elevated motor impulsivity is likely to have a stronger impact on choice behaviour in the maze where responses are guided by egocentric motoric cues. Nevertheless, the PCP-induced deficits in learning were associated with an increase in early-errors without affecting the number of late-errors. If a general increase in motor impulsivity was the cause of the retarded learning, the prediction would be of a more general impairment in learning, also affecting late-errors. It may be that as well as causing motor and impulsive disturbances, PCP retards the ability to overcome previous egocentric associations of reward and non-reward.

Most often, the retarding effects of PCP on reversal learning are assumed to be due to increased perseverance and hence model the perseverative deficits of schizophrenic patients (Elliott et al., 1995, 1998). However, NMDAr antagonism has also been shown to have potentiating effects upon non-reinforced associations within latent inhibition paradigms, and it has been suggested that these deficits are related to the cognitive inflexibility induced by PCP and observed in schizophrenia (Gaisler-Salomon et al., 2008; Gaisler-Salomon and Weiner 2003; Klamer et al., 2005; Lipina et al., 2005; Pålsson et al., 2005). In accord with these suggestions, the non-condition dependent retardation of learning would indicate that an acute dose of PCP attenuates reversal learning by decreasing the ability to overcome both perseverance and learned non-reward.

Although the effect of NMDAr-antagonism on learned non-reward has not previously been investigated, the current results may appear to be in discrepancy with the idea of PCP as a model of the cognitive deficits of schizophrenia. Specifically, schizophrenia has traditionally been associated with a decrease in the strength of CS-'no-US' associations in latent inhibition paradigms (Baruch et al., 1988; Williams et al., 1998) while the current result would suggest that PCP increases the strength of CS-'no-US' associations. Yet, decreased latent inhibition has been suggested to be a feature of only acute, positively symptomatic and untreated patients (Cohen et al., 2004) while increased latent inhibition is a feature of chronic patients (Gal et al., 2009) and patients with high negative/positive symptoms ratio (Cohen et al., 2004; Rascle et al., 2001). Hence, increased latent inhibition can be a feature of both acute NMDAr antagonist treatment and schizophrenic symptoms.

Lastly, PCP caused a selective increase in 'early-errors' without affecting 'late-errors'. Although the increase in early-errors was most prevalent in the perseverance test, it was also present in the full reversal and learned non-reward tests. It is widely assumed that early-errors represent perseverance towards the previous CS+ and not related to the ability to overcome previously non-rewarded associations. However, the PCP-induced increase of early-errors in the learned non-reward test suggests that early-errors also are related to the ability to overcome non-reinforced associations. Thus, the assumption of early-errors representing perseverance is contradicted by the observation that PCP also increased early-errors in the learned non-reward test, showing that 'early-errors' also can be related to the ability to suppress non-reinforced associations.

In conclusion, an acute dose of PCP retards egocentric spatial reversal learning in the mouse by increasing the interference from both previous CS-US and CS-'no-US' associations. However, these interpretations remain tentative, as PCP also caused parallel decreases in mean trial-times, indicative of concomitant and possibly confounding non-cognitive effects of motor impulsivity.

4.4.4 *General discussion*

A potential problem when investigating the effects of acute PCP is hyperactivity confounding interpretations of the drugs effect on cognition. Experiment 1 therefore investigated the dose-dependent effects of PCP on activity, showing that 5 mg/kg is without effect on activity levels 3h after treatment. Experiments 2 and 3 subsequently assessed 5 mg/kg using a 3h pre-treatment time on reversal learning, perseverance and learned non-reward in the operant chamber and maze. PCP had no effect on reversal learning in the operant task, while causing both perseverance and learned non-reward related deficits in egocentric reversal learning assessed in the maze procedure. These deficits were primarily related with a non-condition dependent increase in early-errors. Finally, Experiment 4 showed that PCP, at the same dose and pre-treatment time, failed to affect spatial novelty recognition in the maze.

PCP decreased response latencies in both the maze and the operant tasks, as well as decreasing the number of omissions in the operant task. These effects may be due to increased impulsivity through elevated levels of prefrontal glutamate. Interestingly, in the operant task, these disturbances occurred in the absence of any deficits in learning. Increased impulsivity may however have bigger impact upon indices of learning in the maze, where animals make egocentric responses in the dark relative to visuospatially-guided responses in the operant-chamber. However, PCP also caused a non-condition dependent increase in early- but not late-errors. Accordingly, it is tentatively suggested that PCP retards egocentric reversal by impairing the ability to overcome both perseverance and learned non-reward.

A further possibility is that the discrepant effects of PCP in the maze and operant tasks are due to the tasks being dependent upon different brain regions. The currently used dosing protocol causes elevated prefrontal glutamatergic levels, but also, to a lesser extent, increased prefrontal and accumbal levels of dopamine (Adams and Moghaddam, 1998). While elevated levels of prefrontal glutamate and striatal dopamine

is believed to be relevant for an understanding of the symptoms of schizophrenia, elevated levels of prefrontal dopamine can have pro-cognitive effects (Stefani and Moghaddam, 2006). Thus, PCP may not disrupt learning in tasks tapping primarily on prefrontal regions. The next chapter uses these tasks to investigate the effect of prefrontal subregion-specific lesions.

CHAPTER 5

PREFRONTAL CORTEX AND REVERSAL LEARNING

5.1 INTRODUCTION

One consequence of damage to the human prefrontal cortex (PFC) is a failure to adapt decision-making across situations, with patients repeatedly making decisions with adverse consequences despite good knowledge of the negative outcome of that decision (Bechara et al., 2001; Harlow, 1868). Perturbations within the PFC have been interpreted as the cause of the majority of cognitive inflexibility deficits observed in such psychopathology. Also, most pharmacological manipulations affecting cognitive flexibility are believed to act within this circuitry.

That the symptoms of patients suffering from frontal lobe lesions and dementia praecox (or schizophrenia) greatly overlap was noted by Kraeplin (1919) and corroborated by post-mortem studies showing corresponding cerebral atrophy in schizophrenic patients (Kleinman et al., 1988). More recently, schizophrenia has been associated with reduced PFC volume (Turetsky et al., 1995) PFC grey matter density (Koutsouleris et al., 2008) orbitofrontal cortical (OFC) volume (Convit et al., 2001) and decreased cortical cerebral blood flow (Franzén and Ingvar, 1975a, 1975b).

The cognitive symptoms associated with schizophrenia appear to be a consequence of reduced PFC functioning, or hypofrontality. Schizophrenic patients show decreased PFC or dorsal lateral PFC (DLPFC) regional cerebral blood flow when performing the Tower of London task (Andreasen et al., 1992), N-back task (Glahn et al., 2005) continuous performance task (Volz et al., 1999) and the WCST (Kawasaki et al., 1993; Meyer-Lindenberg et al., 2002; Volz et al., 1997). The cognitive inflexibility of schizophrenic patients is also paralleled by similar deficits in frontal lobe patients. Both groups have been tested in a modified version of the CANTAB ID/ED-task allowing separate assessments of perseverance and learned irrelevance by replacing either the previously correct or incorrect dimension with a novel dimension across test trials (Elliot et al., 1995, 1995; Owen et al., 1993). Here, both frontal lobe and schizophrenic patients display prominent perseverative deficits but show little or no

impairment in learned irrelevance. This suggests that hypofrontality is related to deficits in overcoming prior associations with reward rather than irrelevant associations.

Furthermore, different PFC-subregions mediate various forms of cognitive flexibility, and this functional homology is preserved across species (Bissonette and Powell, 2010; Keeler and Robbins, 2010). While the medial wall (the primate DLPFC and rodent medial prefrontal cortex, or mPFC) is critical for attentional set-shifting, the OFC, including the medial (MO), lateral (LO) and ventral orbital (VO) regions, has been shown to be involved in reversal learning (Cools et al., 2002; Hampshire and Owen, 2006; Hornak et al., 2004).

5.1.1 *Orbitofrontal cortex*

Interpretations regarding the cognitive causes of reversal learning deficits following experimental OFC perturbations often stem from analyses of response tendencies during a series of reversal trials. The number of errors made before achieving 50% correct responses in a reversal test, or early-errors, is a typical index used to indicate a perseverative response strategy. Conversely, the number of incorrect responses made after achieving chance level of responding, or late-errors, is considered to be unrelated to perseverance. The prevalent idea is that the OFC is critical for overcoming perseveration, and this appears to be primarily based on findings showing that OFC-lesions, inactivation, or pathology-related aberrations to selectively increase the number of early-errors to criterion. A further method used to dissociate perseverative responses has been to employ successive rather than simultaneous discrimination and reversal tasks, as successive presentations of stimuli allows for separate response analyses when the previous CS+ and the previous CS- are presented independently.

For example, in humans, lesions encompassing the OFC induce deficits in a go/no-go reversal task (Rolls et al., 1994). The deficits are characterised by a decreased ability to supress responding towards the previous CS+ rather than a failure to instigate responding towards the previous CS-. The deficit is therefore argued to be perseverative rather than related to decreased ability to overcome associations of non-reinforcement. Likewise, aspiration lesions of the OFC in the rhesus leads to impairments in both spatial and object discrimination learning *and* reversal learning (Butter, 1969; Jones and Mishkin, 1972), while quinolinic OFC-lesioned marmosets show selective reversal

deficits in the visual ID/ED-task (Dias et al., 1996). These deficits are also interpreted as perseverative as lesioned animals make more errors while responding still is biased towards the previously correct response alternative.

Using a visual touch-screen reversal task in the rat, quinolinic OFC-lesioning has no effect on sessions or trials to criterion yet increases the number of early-errors and is therefore suggested to relate to increased perseveration (Chudasama et al., 2003). In this task, however, lesions of the infralimbic subregion of the mPFC also increase the number of incorrect responses, but selectively after animals have achieved chance levels of responding.

Aspiration lesions of the OFC in the rhesus also leads to deficits in the last four, but not the first reversal, using a serial object go/no-go task (McEnaney and Butter, 1969). While control animals showed a serial-reversal effect, with performance improving across reversal tests, the performance of the lesioned group declined over subsequent tests. Moreover, OFC-animals showed excessive responding to the previously rewarded stimulus, as well as increased omissions when presented with the previously non-rewarded but now rewarded stimulus. This would indicate that an intact OFC is required to overcome both perseveration and learned non-reward (McEnaney and Butter, 1969).

In a serial operant go/no-go odour discrimination and reversal task in the rat, NMDA-induced lesions of the OFC selectively impaired reversal learning but did not affect discrimination learning (Schoenbaum et al., 2002). However, the impairment in this task is directly opposite to the impairment observed by McEnaney and Butter (1969). First, OFC-lesioned, but not controls, showed a serial reversal effect with performance improving across tests. Second, the deficit was only apparent in the first reversal of five presented, suggesting that the effects of OFC-lesioning on reversal learning are transient rather than permanent.

In a four-phase serial operant lever reversal task, quinolinic OFC-lesioned rats showed retarded performance in the first reversal, and facilitated performance in the second reversal, and no effects were observed in the third and fourth reversals (Boulougouris et al., 2007). Pre-surgical reversal training blocked any reversal deficits produced by later OFC-lesioning in this task (Boulougouris et al., 2009), again suggesting that the OFC has a transient role in reversal learning. The OFC-induced impairment in the first reversal was suggested to be due to perseveration, as animals showed an increase in early-errors but not late-errors.

Lastly, muscimol-induced OFC-inactivation impaired both two- and four-choice reversal learning in an olfactory bowl-digging task (Kim and Ragozzino, 2004). OFC-inactivation causes a selective increase in errors committed before attainment of chance level of responding using two-choices, and a general increase in errors throughout the task using four-choices. The OFC was therefore suggested to be selectively involved in overcoming perseveration when task difficulty is low, but also involved in other forms of learning as task difficulty increases (Kim and Ragozzino, 2004).

Nevertheless, in many other paradigms, OFC-lesioning or inactivation has no affect on early-errors, but selectively increases the number of late-errors. In general, the interpretation from these studies has been that although the OFC is involved in reversal learning, the area is not critically involved in perseverance.

For example, Ghods-Sharifi et al. (2008) tested bupivacaine OFC-inactivated rats in a maze visual and egocentric attentional set-shifting and reversal paradigm. Here, OFC-inactivated rats showed a selective deficit in the reversal test through an increased number of late-errors. Based on this, it was speculated that the impairments of OFC-lesioning are unrelated to perseveration.

Presumed OFC-inactivation through baclofen/muscimol also impaired reversal learning in a go/no-go auditory task (Burke et al., 2006). In this paradigm, animals receive successive presentations of a tone or white noise, one of which is associated with reward delivery. The contingencies reverse, and the time spent in the food magazine during each auditory cue are analysed. OFC-inactivation did not affect animals ability to extinguish responding during the previous CS+, but did retard animals ability to start responding during the previous CS-. This suggested that the OFC is necessary for the ability to overcome learned non-reward rather than perseverance (Burke et al., 2006).

Similarly, in a go/no-go odour discrimination and reversal paradigm in the rat, NMDA-induced OFC-lesioning had no effect on two-choice odour discrimination learning but selectively impaired performance on two subsequent reversals (Schoenbaum et al., 2003). The impairment was caused by a selective increase in late-errors, and therefore speculated to be unrelated to perseveration.

Using the ID/ED bowl-digging procedure with three reversal tests in the rat, ibotenic OFC-lesioning failed to affect attentional set-shifting yet potently retarded performance in all reversal phases by increasing trials to criterion (McAlonan and Brown, 2003). This deficit has been further explored in a similar bowl-digging reversal

procedure aimed to separately probe perseverance and learned non-reward. Tait and Brown (2007) used a within-subject design with all animals exposed to a perseverance and learned non-reward test in counterbalanced order. In these tests, the previously correct or incorrect stimulus changed contingency and was paired with a novel stimulus of opposing contingency in two separate tests. OFC-lesioning was found to the impair performance in the learned non-reward test, but to facilitate performance in the perseverance test. The authors concluded that the previously observed reversal deficits following OFC-lesioning in this task were due to elevated interference by learned non-reward and were not related to increased perseveration (Tait and Brown, 2007). Interestingly, OFC-lesioning also caused a selective increase in 'refusals to dig' in the learned non-reward test, suggesting that omissions are produced by previous association of learned non-reward.

In sum, lesioning or inactivating the OFC in the rat and primate most often impairs reversal learning without affecting attentional set-shifting or discrimination learning. These deficits are sensitive to parameters of task difficulty, number of reversals and sensory dimension of discriminanda. The deficits have been shown to be due to a decrease in the ability to overcome learned non-reward (Tait and Brown, 2007) but have also suggested to be effects of increased perseveration due to selective effects on early-errors (Boulougouris et al., 2007; Butter, 1969; Chudasama et al., 2003; Dias et al., 1996; Jones and Michkin, 1972).

5.1.2 *Medial prefrontal cortex*

There is debate as to whether rodents have a prefrontal cortical subregion homologous to the primate DLPFC. The cytoarchitercture of the PFC in the rodent indicate regions corresponding to the primate anterior cingulate, and orbital subregions, but a lack of a medial granular zone distinguishing the primate DLPFC (Preuss, 1995). However, others have suggested that the rodent has a region corresponding to the primate DLPFC based on anatomical connections (Ongür and Price, 2000), and behavioural characteristics following prefrontal lesioning (Uylings et al., 2003).

Indeed, opposite to the effect observed following OFC damage, lesions to the mPFC, encompassing the infralimbic (IL), prelimbic (PL) and anterior cingulate cortices (ACC), often impair attentional set-shifting without affecting reversal learning

in the rodent (Bissonette and Powell, 2010; Brown and Bowman, 2002; Keeler and Robbins, 2011). This effect is analogous to DLPFC-lesioning in the primate.

In the marmoset, quinolinic DLPFC-lesioning had no effect on reversal learning, but impaired attentional set-shifting using the visual ID/ED-task (Dias et al., 1996). In the rat, ibotenic lesions centred on the IL, but also including the PL, had no effect on reversal learning in the bowl-digging task but impaired attentional set-shifting (Birrell and Brown, 2000). In an operant procedure, bupivacaine-induced IL-inactivation did not affect reversal learning in the rat but impaired attentional set-shifting between a visual and a spatial response strategy (Floresco et al., 2008). Others have nevertheless observed reversal impairments following lesions to the medial wall in both the rodent and primate. DLPFC-lesioning retarded egocentric (Pohl, 1979) and allocentric (Butter, 1969) spatial reversal learning in the rhesus, and mPFC lesioning in the rat (Chudasama et al., 2003; Salazar et al., 2004) and mouse (Brigman et al., 2008) impaired visual reversals.

In conclusion, the OFC appears to be critical for reversal learning but not attentional set-shifting, while intact mPFC function is most often is required for attentional set-shifting but not reversal learning. Observed reversal learning impairments are generally considered to be caused by increased perseveration. It appears that this idea primarily is based on observations of lesions selectively increasing the number of early-errors in simultaneous discrimination and reversal tasks, or responding towards the previous CS+ in successive discrimination and reversal tasks. However, data also show that PFC-lesions can increase late-errors in simultaneous discrimination and reversal tasks (Ghods-Sharifi et al., 2008; Kim and Ragozzino, 2004) and impair responding towards the previous CS- in successive discrimination and reversal tasks (Burke et al., 2006; McEnaney and Butter, 1969). Further, the single published study investigating the effect of rodent OFC-lesioning in perseverance and learned non-reward found increased learned non-reward and a concurrent facilitation of perseverance (Tait and Brown, 2007).

Relative to the rat and primate, there have been few studies of cognitive flexibility following prefrontal lesioning in the mouse. One study found that mPFC-lesions induce pattern, but not luminance visual reversal learning deficits (Brigman et al., 2008). Recently, dissociable effects of mPFC- and OFC-lesioning on reversal learning and attentional set-shifting were observed using a bowl-digging procedure in the mouse with NMDA-induced OFC-lesions selectively retarding reversal

learning and mPFC-lesions specifically disrupting attentional set-shifting (Bissonette et al., 2009).

The aim of the current chapter was to investigate the role of the PFC in reversal learning, perseverance and learned non-reward in the mouse. The first experiment assessed OFC-lesioned mice in the egocentric maze task. The second experiment assessed both OFC and mPFC lesioned mice in the visuospatial operant task.

5.2. METHOD

5.2.1 *Experiment 1: Maze reversal learning*

5.2.1.1 Experimental design and statistical analysis

The experiment used 18 C57BL/6J male mice (Charles River, UK) weighing a mean 25.7g at the start of the experiment. After completing the initial spatial discrimination, animals were pair-matched for trials to criterion and randomly assigned to a lesion group. Animals subsequently went through surgery and were allowed a minimum recovery period of one week after which animals again were required to retain criterion on the previously acquired reward contingencies. This was followed by a full reversal test, and subsequent perseverance and a learned non-reward tests. The order of the perseverance and the learned non-reward tests was counterbalanced across the two lesion groups (see Chapter 3). Performance in the reversal test was analysed using one-way between-subjects ANOVAs with lesion as the independent variable. The perseverance and learned non-reward tests were analysed by separate 2 (within-subjects: test condition) × 2 (between-subjects: lesion) × 2 (between-subjects: test order) mixed ANOVAs.

5.2.1.2 Surgery

One animal was omitted from the study after failure to reach criterion on the spatial discrimination phase. In the remaining animals, bilateral stereotaxic lesions were made in the OFC-region (AP, +2.8 mm; ML, ±1.2 mm; DV, +2.2 mm) of anesthetised (isoflurane) animals. The coordinates were chosen as pilot studies suggested them to be the most accurate with reference to Paxinos and Franklin (2001). The skull surface was exposed through a razor incision and the dura was carefully removed. Bilateral burr-holes were drilled directly above the OFC-region, and a 33-gauge needle was slowly inserted at the appropriate coordinates. The needle was left in place for 1 min to allow for the brain to set. 0.3 µl of sterile NMDA (20 mg/ml in 0.9% saline; Tocris, Bristol, UK) was injected at a rate of 0.2 µl/min using a 10 µl Hamilton glass syringe and a syringe pump (802, Univentor, Malta). After the infusion, the needle was left in place for an additional 3 min to allow for dispersion from the infusion-site. Saline vehicle

alone was infused in the sham group. Animals were placed in a warm recovery-box after surgery with water available until they completely regained all motor functions. Testing began after a recovery period of at least one week (7-17 days).

5.2.1.3 Histology

At the end of the experiment, mice were killed through gradually increasing levels of exposure to carbon dioxide and immediately perfused with 4% paraformaldehyde. Brains were then placed in a 4% formalin solution for at least 2 weeks before sectioning. Brains were sectioned (30 μm thick) using a Leica Jung cryostat. Sections were mounted onto gelatin-coated glass slides and air-dried. The tissue was then defatted, Nissl stained with thionine (Sigma-Aldrich, Poole, UK), dehydrated and cover-slipped. Sections and extent of lesions were examined with reference to Paxinos and Franklin (2001) using a Feldt light microscope. Images were obtained with a Leica DMRX microscope and schematic drawings assembled in Gimp (version 2.6).

5.2.2 *Experiment 2: Operant reversal learning*

5.2.2.1 Experimental design and statistical analysis

The experiment used 35 C57BL/6J male mice (Charles River, UK) weighing a mean 27.0g at the start of the experiment. As in the maze experiment, after reaching criterion in the spatial discrimination, animals were matched for trials to criterion and randomly assigned to a lesion group. Animals subsequently went through surgery and were allowed a minimum recovery period of one-week (7-12 days), after which animals again were required to retain criterion on the previously acquired reward contingencies. This was followed by a full reversal test, and subsequent perseverance and learned non-reward test, all proceeded by retention phases on the previously acquired reward contingencies. The order of the perseverance and the learned non-reward tests was counterbalanced across the lesion groups. Performance in the full reversal test was analysed using one-way between-subjects ANOVAs with lesion as the independent variable. The perseverance and learned non-reward tests were analysed by separate 2 (within-subjects: test condition) × 3 (between-subjects: lesion) × 2 (between-subjects:

test order) mixed ANOVAs. With no significant differences in performance between the OFC-sham and mPFC-sham operated animals, the groups were collapsed for behavioural analyses.

5.2.2.2 Surgery and histology

The maze experiment was confounded by two OFC-lesions extending into medial areas. There was also some suggestion that animals showing lesions extending both OFC- and mPFC-regions had greater difficulties in maze reversal learning. The operant experiment therefore included a further group of mPFC-lesioned animals. Moreover, in order to avoid concurrent OFC/mPFC-lesions, the OFC coordinates were moved +0.1 mm laterally from bregma. Thus, bilateral stereotaxic lesions were made in OFC-regions (AP, + 2.8 mm; ML, ±1.3 mm; DV, +2.2 mm) or mPFC regions (AP, +2.1 mm; ML, ± 0.2 mm; DV, +2.2 mm). Again, these coordinates were chosen after pilot lesions showed them to be the most accurate with reference to Paxinos and Franklin (2001). Saline vehicle alone was injected in the two sham control groups. Animals were killed with an overdose of pentobarbitone before being perfused with 4% paraformaldehyde. All other surgical and histological methods remained identical to the maze experiment.

5.2.3 *Neurological screen*

Following surgery and prior to cognitive testing, the sensory and motor abilities of each mouse were assessed in a series of tests derived from Dunnett et al. (1987). The screen included assessments of general posture, startle response, orientation to stimulation of the whiskers, placement reflexes and motor ability on an inclined grid. All animals performed well and there were no differences between the experimental groups in either experiment.

5.3 RESULTS

5.3.1 *Experiment 1: OFC-lesioning and maze reversal learning*

Lesions (Fig. 5.1). Three animals were culled after experiencing post-surgery seizures. Two further animals in the lesion group were omitted from behavioural analyses after showing a limited unilateral lesion and no visible lesion, respectively. Of the remaining five lesioned animals, three had lesions restricted within the lateral, ventral, and medial orbital regions, while two animals showed lesions extending medially into infralimbic, prelimbic and anterior cingulate regions. These medial lesions were largely within the left hemisphere. The final number in each group for behavioural analyses were: sham = 7, OFC = 3, OFC + mPFC = 2. Thus, the small final group sizes made the experiment underpowered, and any inferences made about the effects of PFC-lesioning on egocentric reversal learning have to be considered preliminary.

Behavioural performance. Excitotoxic lesion of the OFC-region had no significant effect on reversal learning, perseverance or learned non-reward in the maze. However, lesioned animals displayed poorer performance in retention of a discrimination acquired pre-surgery (Fig. 5.2). In retention, OFC-lesioned animals made significantly more incorrect responses to criterion ($F_{1,10} = 6.0$, $p < .05$). They also required more trials and more correct responses to criterion, but these differences did not reach significance ($p > .05$). OFC-lesioning failed to affect performance in the full reversal, perseverance and learned non-reward tests (Fig. 5.3; all $p > .05$). However, as two animals had lesions extending into the mPFC, the behavioural data was further analysed using lesion extent as a between-subjects variable (Fig. 5.4).

Lesion extent failed to affect trials and incorrect responses to criterion. There was a significant effect of lesion extent on correct responses to criterion in the full reversal test ($F_{2,9} = 5.9$, $p < .05$). mPFC/OFC-lesioned animals required more correct responses to criterion than both sham ($p < .05$) and OFC-lesioned animals ($p < .01$). OFC/mPFC-lesioned animals also made more late-errors (23.5 ± 1.5) than sham (11.3 ± 3.7) and OFC-lesioned animals (7.7 ± 6.7) in the full reversal test. Again, this difference failed to reach significance.

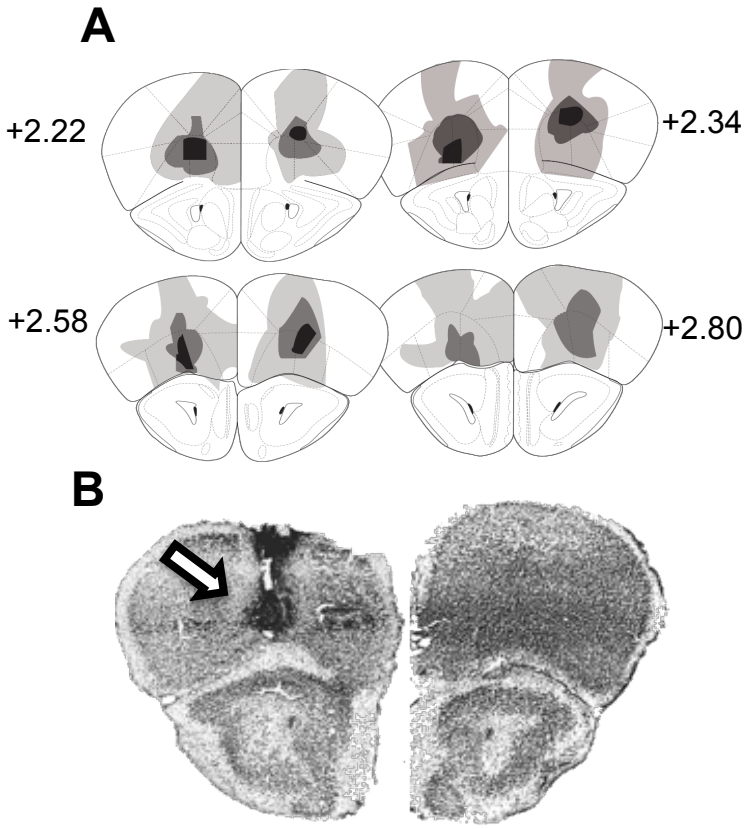

Figure 5.1. Experiment 1: NMDA-induced excitotoxic lesions of the OFC in the maze procedure. (A) Distribution of OFC-lesions with reference to distance from Bregma. The maximum extent of the lesion, present in at least one animal, is shaded in lightest grey. The representative lesion extent, present in ≥ 50% of the animals, is shaded in medium grey. The minimum lesion extent, present in all animals, is shown in black. Drawings adapted from Paxinos and Franklin (2001). (C) Thionine stained sections of OFC-lesion (left) and sham control (right). Arrow point to lesioned area.

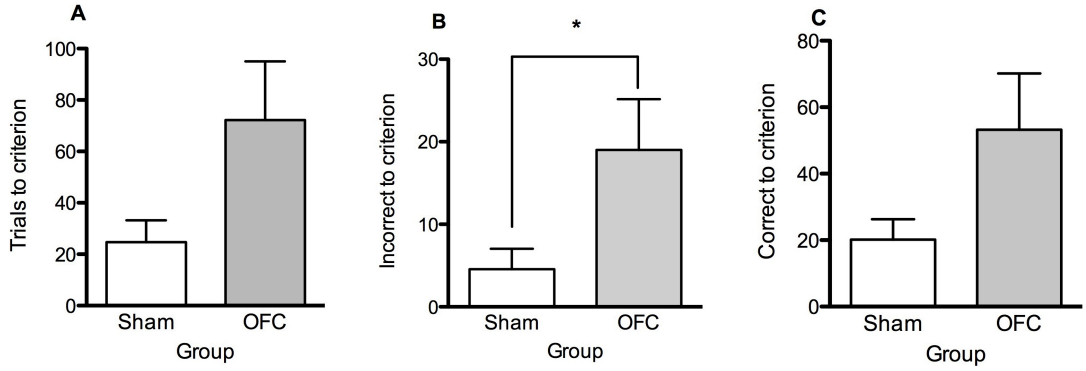

Figure 5.2. Post-operative retention of a two-choice discrimination acquired pre-surgery in the maze procedure. Non-significant effect of lesion on trials ($F_{1,10}$ = 4.9, p = .051) and correct responses to criterion ($F_{1,10}$ = 4.3, p = .064). OFC-lesioned animals made significantly more incorrect responses to criterion ($F_{1,10}$ = 6.0, p = .034).

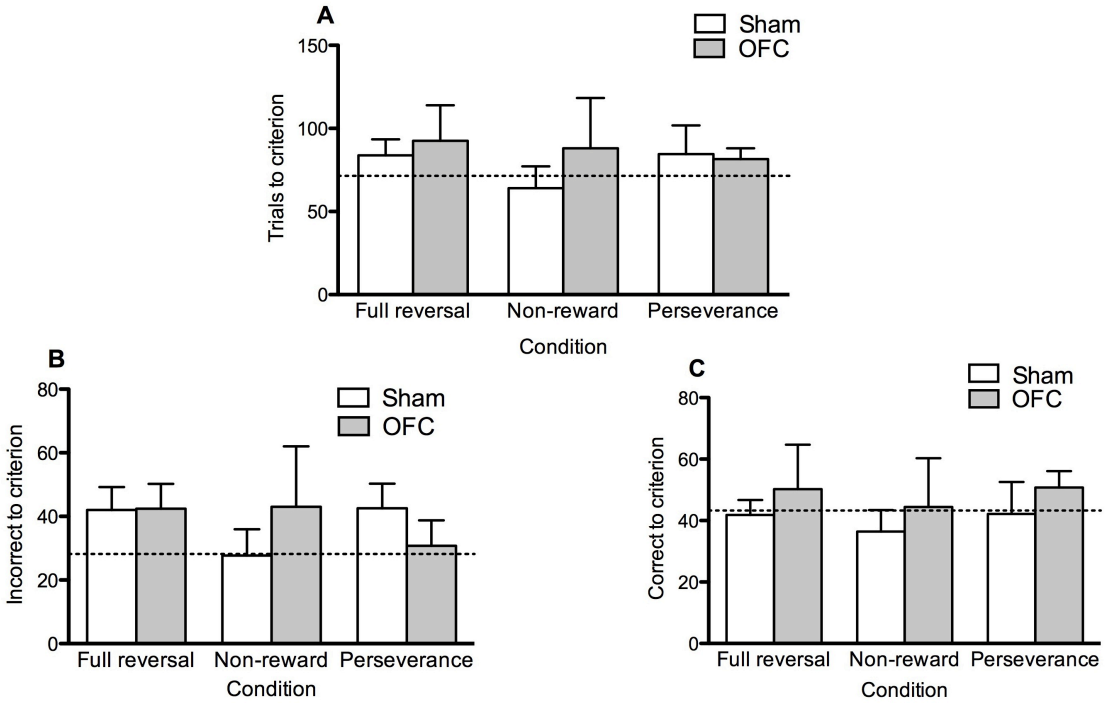

Figure 5.3. Effect OFC-lesioning in the maze procedure. Broken line represents mean performance in the initial spatial discrimination phase. No significant main effects of lesion or lesion × test condition interactions on trials to criterion (A) incorrect responses to criterion (B) or correct responses to criterion (C) in the full reversal, perseverance and learned non-reward tests.

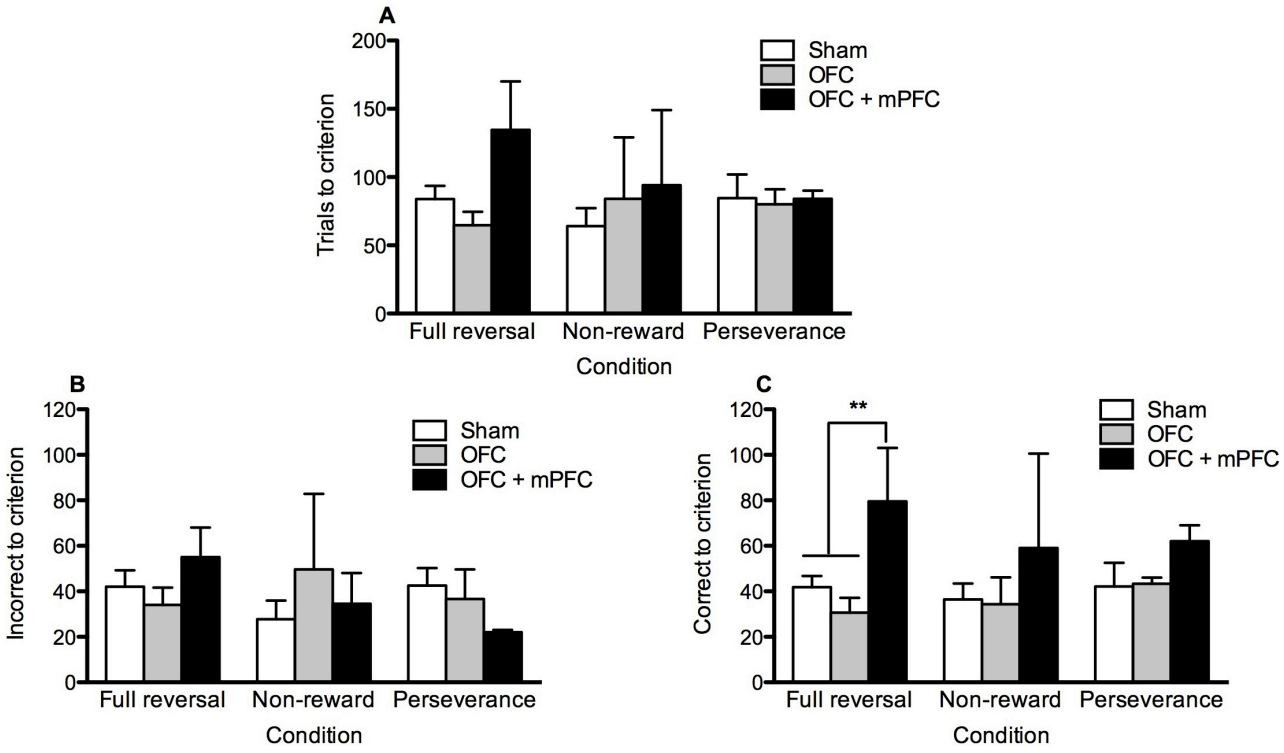

Figure 5.4. Performance of OFC-lesioned and OFC/mPFC-lesioned animals in the maze procedure. Significant effect of lesion extent on correct responses to criterion in the full reversal test ($F_{2,9}$ = 5.9, p = .02). No other significant main effects of lesion extent or lesion extent × test condition interactions.

The maze experiment was underpowered and any inferences remain tentative. There were however some suggestion of differential effects, with lesions including the mPFC producing greater reversal deficits than OFC-lesioning alone. Based on this, two separate experimental groups of independent OFC- and mPFC-lesioned animals were assessed in the operant reversal task.

5.3.2 *Experiment 2: OFC and mPFC-lesioning in operant reversal leaning*

Lesions (Fig. 5.5). Four animals were culled after failing to completely recover motor functions following surgery. One OFC-lesioned animal was omitted from the analysis after failing to complete the reversal test after 25 sessions. Of the remaining 10 animals in the OFC group, nine animals had lesions restricted to the lateral and ventral orbital regions. One animal showed a lesion with limited extension into infralimbic and prelimbic regions. All animals in the mPFC group had lesions restricted to the medial wall concentrated on anterior cingulate and prelimbic regions. The majority of animals in the mPFC-group also showed lesions of the infralimbic-region. The final group sizes were sham-mPFC = 4, sham-OFC = 6, mPFC = 10, OFC = 10.

Behavioural performance. Although there were few significant main effects of lesion or lesion × test condition interactions, OFC-lesioning retarded reversal learning relative to sham-lesioned controls and these deficits where more pronounced in the perseverance test than in the learned non-reward test (Fig. 5.6). There were no significant effects of the lesion on retention of previously acquired reward contingencies.

In the full reversal test, there were no significant effects of lesion on trials ($F_{2,27} = 3.1$, p = ns), correct responses ($F_{2,27} = 3.3$, p = ns), incorrect responses ($F_{2,27} = 1.9$, p = ns) or omissions to criterion ($F_{2,27} = 1.7$, p = ns). The lesion also failed to affect the number of early-errors ($F_{2,27} = 0.6$, p = ns) and late-errors ($F_{2,27} = 2.6$, p = ns). However, separate one-way ANOVAs comparing each lesion group against sham-lesioned controls showed that OFC-lesioning increased trials to criterion ($F_{1,18} = 5.1$, $p < .05$) incorrect responses to criterion ($F_{1,18} = 5.6$, $p < .05$) and late-errors ($F_{1,18} = 4.8$, $p < .05$) in the full reversal test. mPFC-lesioning failed to affect any measure of learning on the full reversal test ($p \geq .35$), although there was a trend for increased late-errors to criterion relative to sham-lesioned controls (p = .07).

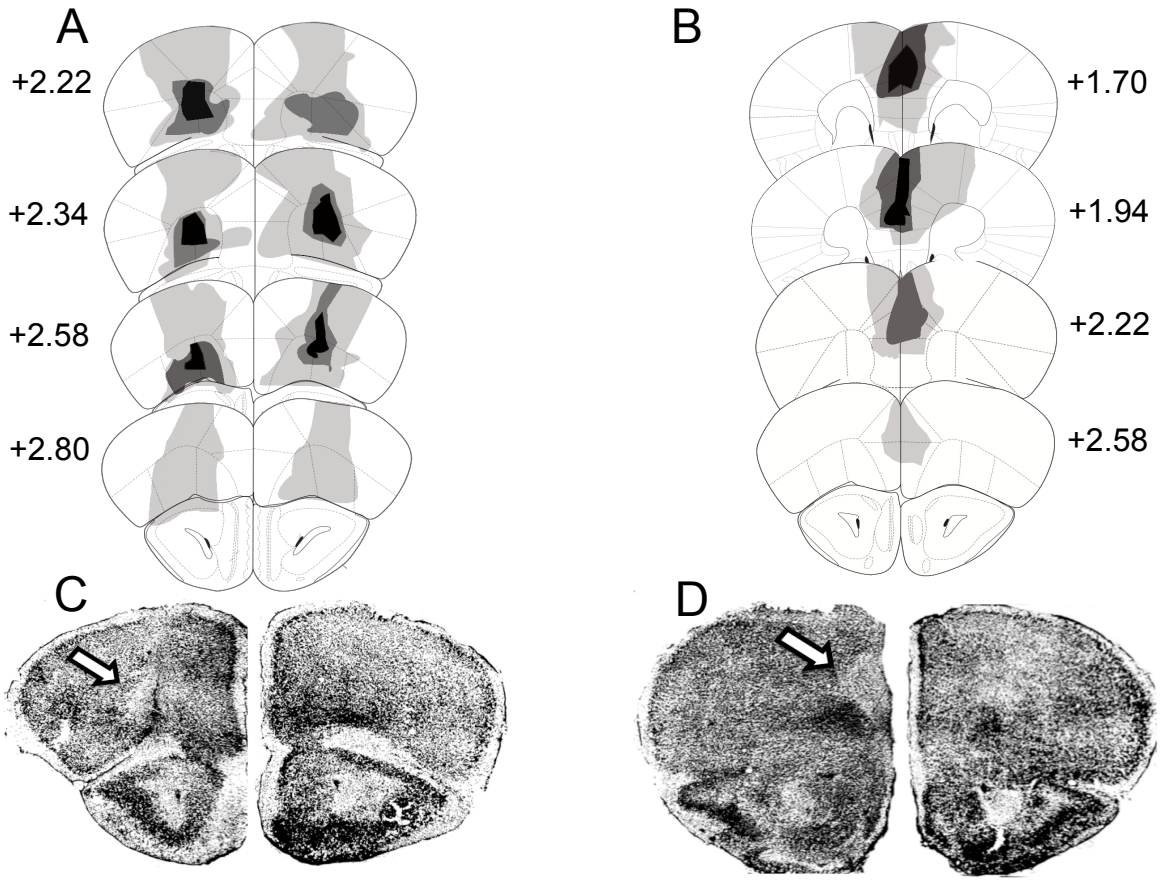

Figure 5.5. Experiment 2: NMDA-induced excitotoxic lesions of the OFC and mPFC in the operant procedure. (A-B) Distribution of OFC (A) and mPFC (B) lesions with reference to distance from bregma. The maximum extent of the lesions, present in at least one animal, is shaded in lightest gray. The representative lesion extent, present in ≥ 50% of the animals, is shaded in medium grey. The minimum lesion extent, present in all animals, is shown in black. Drawings adapted from Paxinos and Franklin (2001). (C) Thionine stained sections of OFC-lesion (left) and sham-control (right). (D) Thionine stained sections of mPFC-lesion (left) and sham-control (right). Arrows point to lesioned area.

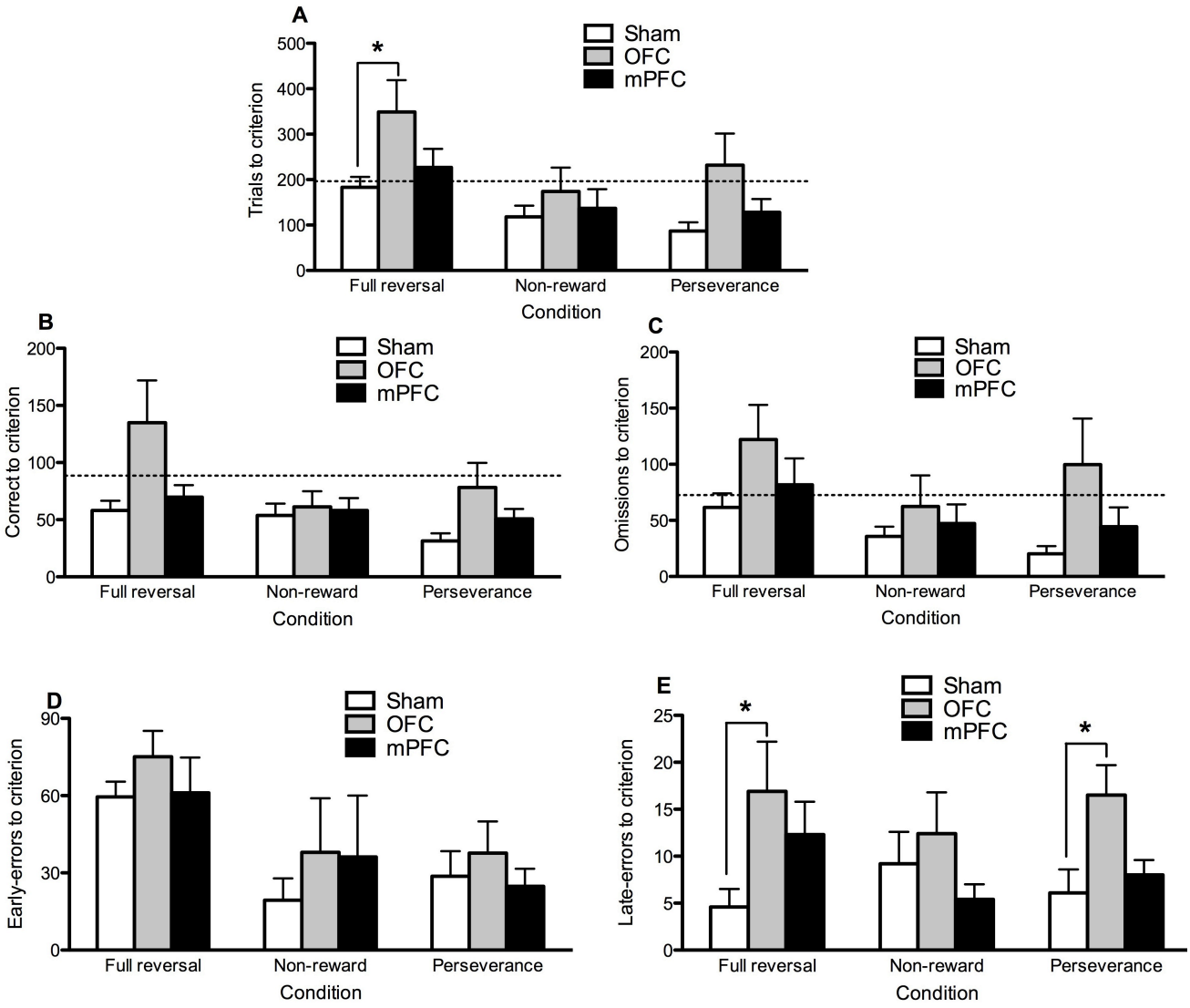

Figure 5.6. Performance of OFC-, mPFC- and sham-lesioned animals in the operant procedure. Asterisk denote differences at which $p < .05$ relative to sham lesioned controls (* $p < .05$). Broken line represents mean performance in the initial spatial discrimination. (A) *Trials to criterion.* No effect of lesion in the full reversal test ($F_{2,27} = 3.1$, $p = .059$). Main effect of lesion ($F_{2,27} = 4.1$, $p = .028$) but no lesion × test condition interaction in the perseverance and learned non-reward tests ($F_{2,27} = 0.4$, $p = .65$) (B) *Correct responses*. No effect of lesion in the full reversal test ($F_{2,27} = 3.3$, $p = .052$). No effect of lesion ($F_{2,27} = 3.1$, $p = .063$) or lesion × test condition interaction ($F_{2,27} = 0.9$, $p = .39$) in the perseverance and learned non-reward tests. (C) *Omissions*. No effect of lesion in the full reversal test ($F_{2,27} = 1.7$, $p = .2$). Main effect of lesion ($F_{2,27} = 3.8$, $p = .035$) but no lesion × test condition interaction ($F_{2,27} = 0.6$, $p = .57$) in the perseverance and learned non-reward tests. (D) *Early-errors*. No effect of lesion ($F_{2,27} = 0.6$, $p = .54$) in the full reversal test. No effect of lesion ($F_{2,27} = 0.5$, $p = .63$) or lesion × test condition interaction ($F_{2,27} = 0.2$, $p = .81$) in the perseverance and learned non-reward tests. (E) *Late-errors*. No effect of lesion ($F_{2,27} = 2.6$, $p = .09$) in the full reversal test. Main effect of lesion ($F_{2,27} = 4.8$, $p = .016$) but no lesion × test condition interaction ($F_{2,27} = 0.7$, $p = .51$) in the perseverance and learned non-reward tests.

Table 5.1 Mean latencies (± SEM) in the operant procedure collapsed over the three test conditions.

	Group			
	Sham	**OFC**	**mPFC**	**p**
Trial initiation	7.01 ± .17	6.87 ± .13	6.90 ± .22	ns
Nosepoke-hole response	4.22 ± .22	4.11 ± .15	4.11 ± .16	ns
Pellet retrieval	2.54 ± .20	2.91 ± .16	2.69 ± .12	ns

No effects of lesion ($p \geq .24$) or lesion × test condition interactions ($p \geq .31$)

In the perseverance and learned non-reward tests, there was a significant main effect of lesion ($F_{2,27} = 4.1$, $p < .05$), but no lesion × test condition interaction ($F_{2,27} = 0.4$, p = ns) on trials to criterion. OFC-lesioned animals required more trials than sham-lesioned controls ($p = .01$) and mPFC-lesioned animals, although the difference between OFC- and mPFC-lesioned animals failed to reach statistical significance ($p = .06$). Similarly, there was a significant main effect of lesion ($F_{2,27} = 4.8$, $p < .05$), but no lesion × test condition interaction ($F_{2,27} = 0.7$, p = ns) on late-errors. OFC-lesioned animals made more late-errors than both sham-controls ($p < .05$) and mPFC-lesioned animals ($p < .01$). There was also a significant main effect of lesion on omissions to criterion ($F_{2,27} = 3.8$, $p < .05$) but no lesion × test condition interactions ($F_{2,27} = 0.6$, p = ns). OFC-lesioned animals made more omissions than sham-lesioned controls ($p = .01$).

Moreover, there were no effects of lesion or lesion × test condition interactions on correct responses to criterion (Lesion: $F_{2,27} = 3.1$, p = ns, Lesion × Condition: $F_{2,27} = 1.5$, p = ns) incorrect responses to criterion (Lesion: $F_{2,27} = 1.1$, p = ns, Lesion × Condition: $F_{2,27} = 0.1$ p = ns) or early-errors (Lesion: $F_{2,27} = 0.5$, p = .ns, Lesion × Condition: $F_{2,27} = 0.2$, p = ns) in the perseverance and learned non-reward tests.

Separate one-way ANOVAs for the perseverance and learned non-reward tests comparing each lesion group against sham lesioned controls showed that mPFC-lesioning failed to affect any measure of learning in either test condition (all $p \geq .33$). Furthermore, OFC-lesioned animals did not differ from sham-lesioned controls in the learned non-reward condition (all $p \geq .34$).

In the perseverance condition, however, OFC-lesioning significantly increased late-errors to criterion ($F_{1,18} = 6.4$, $p < .05$), as well as showing non-significant trends for increased trials to criterion ($F_{1.18} = 4.0$, $p = .06$) correct responses to criterion ($F_{1.18} = 4.3$, $p = .054$) and omissions to criterion ($F_{1.18} = 4.0$, $p = .07$).

5.4 DISCUSSION

The deficits in cognitive flexibility observed in neuropsychiatric disorders, such as schizophrenia, are believed to be due to disruptions within PFC-areas, with the primary cognitive consequence being the inability to supress previous associations of reward, or perseverative responding. In the mouse, there have been few studies investigating anatomical loci of reversal learning, and no published studies looking at the effect upon the separate cognitive components of perseverance and learned non-reward. This chapter therefore assessed animals with sub-region specific prefrontal lesions in egocentric and visuospatial tests of reversal learning, perseverance and learned non-reward.

In the maze, OFC-lesioning had no effects on learning in the three test conditions, although the two animals with lesions extending both the OFC and mPFC showed a transient and largely non-significant trend for a deficit in the initial full reversal test. However, the experiment was underpowered. Therefore group sizes were increased and an mPFC-lesioned group was included in the operant experiment.

In the operant task OFC-lesioned animals were impaired. The impairment was observed as significant increases in trials to criterion and late-errors in the full reversal test. Although no significant lesion × test condition interactions were found, the deficits were more prominently expressed in the perseverance test than the learned non-reward test.

5.4.1 *Maze task*

In the maze, OFC-lesioning had no effect on learning in the full reversal, perseverance or learned non-reward tests. Surprisingly, the lesions impaired retention of a two-choice discrimination acquired pre-surgery by increasing the number of incorrect responses to criterion. It should however be emphasised that the experiment was underpowered and the results, together with any conclusions, are tentative.

Although lesioned animals showed retention deficits in the maze task, both OFC- and mPFC-lesioning failed to affect retention of acquired visuospatial discriminations in the operant procedure. This would indicate that combined OFC/mPFC-lesions have more disruptive effects on post-surgery retention. However, the effect in the maze was independent of lesion extent and observed in animals with

OFC as well as OFC/mPFC lesions, suggesting that the retention deficit not is attributable to mPFC damage. A further possible explanation is different lesion sites across the two experiments. The OFC-lesions in the maze experiment was placed 0.1 mm medially relative to the operant experiment, and therefore covered the MO-subregion to a greater extent than in the operant experiment.

There are, to my knowledge, no published studies looking at retention of egocentric discriminations in the rodent following OFC- or mPFC-lesions. However, manipulations of the PFC in general, and OFC in particular, typically fail to affect retention of previously acquired two-choice discriminations in both rodents and primates. In the rhesus, MO-lesions does not affect auditory successive retention discrimination (Iversen and Mishkin, 1970) and DLPFC- or OFC-lesions are without effect on visual, allocentric spatial, and egocentric spatial two-choice discrimination retention in the marmoset (Dias et al., 1996; Pohl, 1973). Also, OFC-lesions in the rat has no effect on retention of a two-choice visuospatial lever discrimination (Boulougouris et al., 2008) or a two-choice odour discrimination acquired pre-surgery (Shoenbaum et al., 2002, 2003).

That said, OFC-lesioning has been shown to disrupt retention in working memory tasks. In a visuomotor task requiring rhesus monkeys to control a joystick in response to visual stimuli, ventral PFC- and OFC-lesions impaired post-surgery retention (Bussey et al., 2001). OFC-lesions in the rhesus also potently retards retention following surgery in a delayed non-match-to-sample task (Meunier et al., 1997). In the rat, OFC-lesions impaired compound discrimination retention in a task requiring animals to discriminate between two strings based on odour and size (Whishaw et al., 1992), combined PL, MO, and VO-lesioning retarded retention of spatial delayed alternation (Brito et al., 1982), and combined mPFC and MO-lesioning retarded visual object discrimination retention in the rat (Becker and Olton, 1980). Hence, in agreement with the currently observed retention deficit, damage restricted to the OFC or in combination with mPFC-subregions, can delay or disrupt reacquisition of task-performance successfully acquired pre-surgery.

The data presented here would indicate that OFC-lesioning in the mouse fails to affect egocentric reversal learning, learned non-reward and perseverance. Although there are no published studies of egocentric reversals in mouse following PFC-lesions, the current results are in apparent conflict with data showing that excitotoxic OFC-lesioned mice show selective reversal impairments in the bowl-digging paradigm

(Bissonette et al., 2008). Compared to the current study, the lesions in the study by Bissonette et al. (2008) were smaller and largely contained within the VO and LO, although a majority of their animals had lesions extending into lateral parts of the PL. Their lesions also appear to be further anterior and more ventral the current lesions. It is therefore possible that different lesion sites or extents could explain the discrepant findings.

Furthermore, in the study by Bissonette et al. (2008), animals were challenged by a simple olfactory or somatosensory discrimination followed by a compound discrimination and four subsequent intra-dimensional shifts before a single reversal test (Bissonette et al., 2008). This paradigm is clearly very different to the currently used task. A possibility is that the OFC-dependence of reversal learning in the mouse does not generalise across sensory dimensions, and is not critical for egocentric reversal learning. This idea is supported by the deficits produced by OFC-lesioning in the operant procedure.

In agreement with this interpretation, Corwin et al. (1994) tested VO/LO-lesioned rats in an allocentric cheeseboard reversal task and an egocentric delayed alternation task. OFC-lesioning did not affect performance in the egocentric task, but impaired performance in the allocentric cheeseboard reversal task. Moreover, in a egocentric serial maze task, VO-lesioning in the rat has been found to retard reversal performance only in the fifth reversal, but not the first four reversals (Kolb et al., 1974), and differ significantly from sham-lesioned animals only when the five reversals are summed (Nonneman et al., 1974). Taken together, these data suggest that although the OFC is critical for visuospatial allocentric reversal learning, the region has a limited, or no role in egocentric discrimination and reversal learning. This would also be in line with the regions strong connection with temporal and parietal areas involved in visual and visuospatial processing, respectively (Cavada et al., 2000).

Yet, in conflict with this line of reasoning, bupivacaine-induced OFC-inactivation has been shown to selectively retard egocentric maze reversal learning in the rat (Ghods-Sharifi et al., 2008). In this task, animals initially acquired a two-choice visual discrimination and were subsequently challenged by an attentional set-shift to a use of an egocentric response strategy. This was followed by a final egocentric reversal test. Here, OFC-inactivation caused selective deficits in the reversal test by increasing the number of trials to criterion and late-errors. However, during the reversal, the previously relevant visual stimulus was still present in the maze, and it may be that

OFC-inactivation interfere with visuospatial processes rather than with egocentric reversals per se. Indeed, the deficits were specifically due to a reversion back to the use of a visual response strategy rather than affecting the number of responses in the previously correct turning direction (Ghods-Sharifi et al., 2008).

The two animals with lesions covering the OFC as well as the mPFC appeared to show a transient deficit restricted to the initial full reversal test. This deficit is likely to be caused by mPFC rather than OFC damage, as the performance of animals with lesions restricted to the OFC paralleled or were slightly better than the performance of sham-operated controls (Fig 5.4). OFC as well as mPFC-lesioned animals were therefore separately assessed in the operant procedure.

5.4.2 *Operant task*

5.4.2.1 mPFC-lesions

The operant task was better powered, and did not suffer from the small group sizes of the maze experiment. mPFC-lesioned animals showed no deficits in the operant task, although there was a transient trend for a disruption in the initial full reversal test seen as a non-significant increase in late-errors.

mPFC-lesioning has previously produced disparate results on reversal learning in the rodent. Combined lesions of the IL-, PL-, and ACC-subregions retarded visuospatial lever reversal learning by increasing the number of days to criterion (Salazar et al., 2004). Similar lesions also impaired performance in a three-lever operant task (Kosaki and Watanabe, 2012), two-odour go/no-go reversal task (Ferry et al., 2000) and a two-choice maze reversal task (Kolb, 1974; Nonneman et al., 1974). mPFC-lesions, centred on the ACC and PL-regions, retarded visual pattern reversal learning in the mouse by increasing the number of late-errors but not early-errors (Brigman et al., 2008), and induced visual reversal learning deficits observed as a specific increases in late-errors in the rat (Bussey et al., 1997). In the water maze, complete mPFC-lesions induced deficits in the first out of three reversals (de Bruin et al., 1994), and in maze object and spatial tasks, complete mPFC-lesioned rats showed deficits in the first two out of five reversals (Becker et al., 1981).

However, much recent work in the rat has shown mPFC-lesions to be without effect on reversal performance. For example, PL- or IL-lesions or inactivations have no

effects on visuospatial operant (Boulougouris et al., 2008; Floresco et al., 2008) or bowl-digging reversal learning (Birrell and Brown, 2000; Ragozzino et al., 2003) and ACC-lesions had no effect on touch-screen visual reversal learning (Bussey et al., 1997).

In the mouse, lesioning of the IL- and PL-subregions did not affect bowl-digging (Bissonette et al., 2008) or visual luminance reversal learning (Brigman et al., 2008). The current results are in agreement with these studies, suggesting that mPFC-lesions, centred on the PL and ACC, but also extending into the IL, have little effect on visuospatial reversal in the mouse.

5.4.2.2 OFC-lesions

OFC-lesioned mice showed retarded visuospatial reversal learning in the operant task. Although no significant lesion × test condition interactions, these deficits were most prominently expressed in the full reversal and perseverance tests. As such, the effects appear to be analogous to the perseverative cognitive flexibility impairments seen in schizophrenic (Elliot et al., 1995, 1998) and PFC-lesioned patients (Owen et al., 1993) and commonly assumed to be the underlying mechanism of retarded reversal learning following experimental perturbations.

However, the deficits in the full reversal and perseverative tests were characterised by an increase in incorrect responses after animals had achieved chance level of responding (late-errors), rather than incorrect responses when responding still was biased towards the previous correct response alternative (early-errors). Notably, late-errors have a stronger impact than early-errors on other measures of learning when the criterion is calculated over 10-trial bins, as late-errors are made when animals are nearer to achieving criterion.

The prevalent method for indexing a perseverative response strategy is to analyse early-errors, as it is assumed that the number of early-errors strongly reflects the stability of the CS-US association, or perseveration. Later phases and incorrect responses made during those phases are not believed to be related to perseveration, but to measure more general cognitive abilities related to attention and acquiring alternative CS-US associations (Boulougouris et al., 2008; Brigman et al., 2008, 2010; Clarke et al., 2008; Kim and Ragozzino, 2004). Importantly, a primary reason for OFC-lesions being considered to induce perseverative deficits, and thus model the perseverative

deficits seen in prefrontal patients, are studies showing selective increases in early-errors (Boulougouris et al., 2008; Chudasama and Robbins 2003; Kolb et al., 1974; Nonneman et al., 1974). Others have nevertheless found OFC inactivation or lesions to increase late-errors without affecting early-errors, and these studies typically reject the idea that OFC-lesions cause perseverative deficits (Ghods-Sharifi et al., 2003; Schoenbaum et al., 2003).

The assumption that early-errors, but not late-errors, represent perseveration is contradicted by the current experiment. This is the first study to analyse early-errors and late-errors following separate assessments of reversal learning, perseverance and learned non-reward in PFC-lesioned animals. In this experiment, OFC-lesioning caused an increase in late-errors in the perseverance test, suggesting that late-errors also can be a product of elevated perseverance. These ideas are discussed further in Chapter 9.

5.4.3 General discussion

The current chapter indicates that, as in rats and primates, the ability to reverse an established visuospatial two-choice discrimination is dependent upon the integrity of the OFC in the mouse. This confirms a recent finding from the bowl-digging task (Bissonette et al., 2008). The current work also extends these findings by showing that these deficits are more closely related to perseveration than learned non-reward, and thus analogous to the deficits displayed by prefrontal (Owen et al., 1993) and schizophrenic patients (Elliot et al., 1995, 1998). Yet, these deficits do not generalise across tasks, with excitotoxic OFC-lesions failing to affect egocentric spatial reversal learning, perseverance and learned non-reward. The maze experiment was nevertheless underpowered, and this conclusion remains tentative.

Moreover, previous work has produced disparate results on reversal learning following mPFC-lesioning in the rodent. Here, mPFC-lesions, centred in the ACC and PL but also extending into the IL area, were without significant in the operant reversal task. mPFC-lesioned animals did however appear to show a transient deficit in the egocentric task, although the small group sizes in this experiment prevents any definite conclusions.

Finally, deficits after OFC-lesioning where characterised by increases in late-errors rather than early-errors. Often, these errors are not considered to be indexing perseverance, but rather general failures in attention or to form associations based on

stimuli-reward contingencies. However, the currently observed increase in late-errors in the perseverance and full reversal tests suggest that these also can be indicative of perseverative response tendencies.

CHAPTER 6

5-HT2C RECEPTOR ANTAGONISM AND REVERSAL LEARNING

6.1 INTRODUCTION

The suggestion that serotonin (5-hydroxytryptamine, or 5-HT) is involved in the pathology of schizophrenia originates in the disturbances resembling the disorder created by 5-HT agonists such as LSD (Claridge, 1978; Woolley and Shaw, 1954).

Altered serotonin levels have since been linked to a range of cognitive and anatomical abnormalities in schizophrenic patients. For example, schizophrenics show lower brain levels of 5-HT which correlate with cerebral atrophy (Jennings et al., 1985; Nybäck et al., 1983; Potkin et al., 1983), severity of cognitive impairment (Powchik et al., 1998), severity of negative symptoms (Bowers et al., 1978; Csernansky et al., 1990), response to clozapine (Lieberman et al., 1994), hypofrontality during the WCST (Weinberger et al., 1988), and poor long-term outcome including decreased social contact, increased hospitalisation and unemployment (Wieselgren et al., 1998).

While classical neuroleptics such as haloperidol and chlorpromazine primarily act through the mechanism of D_2 receptor (D_2R) antagonism, most newer atypical antipsychotics, such as clozapine and olanzapine, show additional more potent antagonistic affinities at the 5-$HT_{2A}R$ and the 5-$HT_{2C}R$. Their superior therapeutic efficacy has been related to their high-affinity antagonism at the 5-$HT_{2A}R$ relative to the D_2R, or large 5-$HT_{2A}R/D_2R$ binding ratios (Ceulemans et al., 1985; Kane et al., 1988; Meltzer et al., 1989; Meltzer and McGurk, 1999). However, some have found no cortical 5-$HT_{2A}R$ abnormalities in schizophrenic patients (Trichard et al., 1998a), and that chlorpromazine, at higher clinically relevant doses, display greater affinity for the 5-$HT_{2A}R$ than clozapine (Trichard et al., 1998b). While this suggests that the superior efficacy of atypical antipsychotics may be unrelated to 5-$HT_{2A}R$ antagonism, a further clinically relevant property of most atypicals is 5-$HT_{2C}R$ antagonism (Meltzer, 2010).

6.1.1 *The 5-HT_{2C}R and schizophrenia*

The 5-HT_{2C}R is a G-protein-coupled receptor (GPCR) signaling through G_q, activating phospholipase C (PLC), causing elevated levels of the second messengers inositol triphosphate (IP) and diacylglycerol (DAG) leading to Ca^{2+} influx and neuronal depolarisation. Greatest expression levels of the 5-HT_{2C}R is found in the choroid plexus, but high levels are also found in the PFC, nucleus accumbens (NAc), amygdala, dorsal striatum (DStr), ventral tegmental area (VTA), substantia nigra (SNc), and hippocampus (Barnes and Sharp, 1999; Clemett et al., 2000; Julius et al., 1988).

The 5-HT_{2C}R undergoes post-transcriptional mRNA editing which is likely to be of relevance for both the pathology and treatment of schizophrenia as well as learning and memory. 5-HT_{2C}R mRNA is edited though adenosine-to-inosine substitutions at five positions rendering 24 different potential protein isoforms (Burns et al., 1997; Price and Sanders-Bush 2000). Although humans, rats and different strains of mice express different numbers of protein isoforms, both the unedited and edited isoforms are similar in structure across species (Dracheva et al., 2009; Du et al., 2006; Englander et al., 2005; Hackler et al., 2006). Compared to the edited isoforms, the unedited isoform can show a four-fold increase in constitutive production of IP (Herrick-Davis et al., 1999) and a 5-fold or greater elevation in response to binding of the agonists mCPP, DOI, 5-HT, LSD, and MK212 (Fitzgerald et al., 1999; Niswender et al., 1999). However, fluoxetine, mianserin, clozapine, spiperone and ketanserin, all non-selective 5-HT_{2C}R antagonists, either show equal affinity for all 5-HT_{2C}R isoforms, or greater affinity for the edited isoforms, thus opposing the characteristics of 5-HT_{2C}R agonists (Niswender et al., 1999; Quirk et al., 2001). 5-HT_{2C}R mRNA editing may also be relevant for learning and memory, as rats challenged in the water maze, relative to rats exposed to swimming alone, show altered 5-HT_{2C}R mRNA editing (Du et al., 2007).

Disrupted 5-HT_{2C}R mRNA editing, altered 5-HT_{2C}R expression levels, and 5-HT_{2C}R single nucleotide polymorphisms (SNPs) are all features of schizophrenia. In post-mortem studies, schizophrenic patients show decreased 5-HT_2R binding in the PFC (Bennett et al., 1979) and DLPFC (Arora and Meltzer 1991; Mita et al., 1986), although a significant upregulation has been observed in the OFC (Whitaker et al., 1981). There is also a 1.5-fold decrease of 5-HT_{2C}R mRNA in the PFC of schizophrenic patients (Castensson et al., 2003, 2010), an effect attributed to the pathology rather than treatment, as chronic neuroleptic treatment fails to affect 5-HT_{2C}R mRNA levels in the

in the PFC of the rat brain (Buckland et al., 1997). However, subregion-specific analyses show that chronic clozapine treatment can decrease 5-HT_{2C}R mRNA levels in several regions, including the hippocampus (Buckland et al., 1997) and choroid plexus (Hietala et al., 1992).

5-HT_{2C}R gene SNPs and haplotypes also correlate with disease outcome and treatment response. The Cys23Ser allele SNP show higher levels of constitutive activity (Okada et al., 2004) and has been linked to increased length of hospitalisation (Segman et al., 1997), and well as good response to clozapine (Sodhi et al., 1995; Veentra-VanderWeele et al., 2000). Other 5-HT_{2C}R SNPs, alone or in combination, are associated with clinical response to olanzapine, clozapine, risperidone and chlorpromazine (Arranz et al., 2000; Reynold et al., 2005).

Schizophrenic patients also show decreased 5-HT_{2C}R pre-mRNA editing corresponding to an upregulation of the more potent unedited 5-HT_{2C}R isoform in the PFC (Sodhi et al., 2001). However, other have found no differences between patients and controls (Dracheva et al., 2003; Iwamoto et al., 2003) and one study found that schizophrenic suicide victims show increased levels of 5-HT_{2C}R pre-mRNA edited isoforms in the PFC (Niswender et al., 2001). Thus, schizophrenia is associated with abnormal activity at the 5-HT_{2C}R, although studies vary with regard to a corresponding up- or downregulations of receptor activity, perhaps due to heterogeneity of patient populations, brain area of investigation, and previous neuroleptic use (Arora and Meltzer, 1991).

6.1.2 *5-HT and reversal learning*

Acute tryptophan depletions in healthy subjects impair performance in the reversal stages of the CANTAB ID/ED-task (Park et al., 1994; Rogers et al., 1999, but see Evers et al., 2005; Finger et al., 2007; Talbot et al., 2005), and 5,7-DHT-induced PFC 5-HT depletions in the marmoset selectively impairs reversal learning in the visual ID/ED-task (Clarke et al., 2004, 2005). Hence, lowering brain 5-HT content generally impairs reversal performance and this may be due to disrupted 5-HT signalling within the OFC (Roberts et al., 2011; Boulougouris and Robbins, 2010).

Accordingly, OFC-specific 5,7-DHT-induced 5-HT depletions, but not OFC-specific 6-OHDA-induced DA depletions, impair reversal learning in the ID/ED-task (Clarke et al., 2007). This reversal impairment has further been shown to be caused by

increased perseverance rather than increased learned non-reward. 5-HT depleted animals show deficits in a perseverance test, where the previously correct stimulus becomes incorrect and is paired with a novel correct stimulus, but perform as well as controls in a learned non-reward test, where the previously incorrect stimulus becomes correct and is paired with a novel incorrect stimulus (Clarke et al., 2007).

Lowering brain 5-HT content also retards reversal learning in the rat. PCPA-induced 5-HT depletions induce deficits in both go/no-go discrimination acquisition and reversal learning (Masaki et al., 2006). However, while 5-HT concentrations in the mPFC and amygdala correlate both with discrimination and reversal performance, 5-HT levels in the OFC correlate with reversal performance only (Masaki et al., 2006). Moreover, PCPA- and stress-induced 5-HT depleted rats show deficits in the first of three reversals in the bowl-digging procedure (Danet et al., 2009). This stress-induced impairment can also be rescued through an acute 5 mg/kg dose of the selective serotonin re-uptake inhibitor (SSRI) citalopram, causing a putative increase in OFC 5-HT-levels (Invernizzi et al., 1992).

Similarly, a pharmacologically induced transient PFC 5-HT decrease through a lower 1 mg/kg dose of citalopram (Adell and Artigas, 1991; Hjorth and Auerbach, 1994) impairs performance in a probabilistic lever reversal task in the rat (Bari et al., 2010) while repeated, subchronic or an acute high 10 mg/kg dose of citalopram, all increasing PFC 5-HT content (Invernizzi et al., 1992), improves reversal performance in this task (Bari et al., 2010).

The role of 5-HT systems in reversal learning has also been explored in the mouse using a visual touch-screen task (Brigman et al., 2010). Contrary to the effect in the primate and rat, 5-HT loss through systemic PCPA has no effect reversal performance in this task. Similarly, deletion of the Pet-1 transcription factor controlling 5-HT neuronal development, causes a 89% loss of cortical and hippocampal 5-HT content (Hendricks et al., 2003), do not alter reversal learning performance (Brigman et al., 2010).

However, elevating brain 5-HT content through 5-HT transporter (5-HTT) KO or subchronic treatment with the SSRI fluoxetine leads to improved performance. While the 5-HTT KO mouse show decreased trials and errors to criterion over the complete reversal test, the fluoxetine-induced improvement in trials and incorrect responses to criterion is only observed in the early-phase of learning when responding still is biased

towards the previously correct stimuli, and no effects from fluoxetine are seen when the complete reversal test is summed (Brigman et al., 2010).

Similarly, the 5-HTT KO rat shows facilitated performance in a two-choice auditory go/no-go task (Nonkes et al., 2011). This improvement appears to be due to enhanced suppression of learned non-reward rather than perseverance. The 5-HTT KO develops faster responding towards the previous CS- (opposed by learned non-reward) but do not differ from wild-types in responding towards the previous CS+ (opposed by perseverance). The improvement may be related to the elevated cortical 5-HT levels observed in 5-HTT KO animals (Mathews et al., 2004). However, notably, the 5-HTT KO rat also show perseverative-like responding in a Pavlovian reinforcer devaluation paradigm, suggestively due to OFC and amygdalar overactivation (Nonkes et al., 2010).

The reversal learning effects of global or subregion-specific manipulations of 5-HT levels has been suggested to be related to altered activity at the 5-HT_{2C}R (Roberts 2011; Boulougouris and Robbins 2010). In the rat, the 5-HT_{2C}R antagonist SB243213 attenuates the disruptive psychotomimetic reversal learning deficits of subchronic PCP (McLean et al., 2009), and the 5-HT_{2C}R antagonist SB242084 facilitates performance in an operant visuospatial reversal learning task (Boulougouris et al., 2008). As the SB242084 induced improvement is associated with a selective decrease in the number of early-errors, the authors suggest that the effects are related to decreased perseverance. These effects have more recently been shown to be due to activity in the OFC, as intra-OFC, but not intra-mPFC or intra-NAc infusions, facilitates performance in the same task (Boulougouris and Robbins 2010). However, in this study, higher doses of OFC-specific infusions of SB242084 also decreased the number of late-errors.

6.1.3 *The neuropharmacology of the 5-HT_{2C}R*

Most interest has centered on the receptors control of striatal dopamine (DA) signaling though constitutive inhibitory influence on GABAergic cells in the VTA and SNc (Eberle-Wang et al., 1997). Systemic, intra-VTA, or intra-PFC infusions of the 5-HT_{2C}R antagonists SB206553 and SB242084 potently elevate VTA DA-neuronal firing and DA dialysate levels in the NAc (Di Giovanni et al., 1999; Di Matteo et al., 1999). This is believed to be the cause of the elevated motor impulsivity and hyperactivity effects observed following 5-HT_{2C}R antagonist administrations (Fletcher et al., 2007, 2009; Higgins et al., 2003; Winstanley et al., 2004).

Unlike the ventral striatum, the DStr is critically involved in reversal learning with lesions retarding reversal learning in the rat and primate (Castañé et al., 2009; Clarke et al., 2008; Kirkby, 1969). In the DStr, reversal learning is controlled by DA, with subregion-specific DA but not 5-HT depletions retarding visual reversal performance in the marmoset (Clarke et al., 2011). Although a role of the 5-HT_{2C}R in control of mesolimbic DA is well established, the role of the 5-HT_{2C}R in nigrostriatal DA has been a subject of debate (Deurwaerdère and Spampinato, 2001; Di Matteo et al., 2001).

A 5-HT_{2C}R involvement in nigrostriatal DA-signalling is supported by its high expression levels in the DStr (Clemett et al., 2000) and the elevated striatal DA-levels and SNc DA-neuron firing observed in the 5-HT_{2C}R KO mouse (Abdallah et al., 2008). Subregion specific infusions of the 5-$HT_{2B/2C}$R antagonist SB206553 also affects striatal DA, although the direction of effect can vary depending on site of infusion (Alex et al., 2005; Lucas et al., 2000). The 5-HT_{2C}R agonists Ro60175, MK212, and mCPP have nevertheless no or limited effect on nigrostriatal DA activity (Di Giovanni et al., 2000; Di Matteo et al., 1999).

Most relevant are studies using the selective 5-HT_{2C}R antagonist SB242084. Although elevated DA-signalling has been observed at some higher doses in anesthetised animals (De Deurwaerdere et al., 2004; Di Matteo et al., 1999; Navailles et al., 2006), SB242084 is most often without effect on nigrostriatal DA-signalling. In the anaesthetised rat, systemic doses of SB242084 at 0.3, 5, and 10 mg/kg do not affect DA or DOPAC levels in the DStr (De Deurwaerdere et al., 2004; Navailles et al., 2005; Di Matteo et al., 1999). In freely moving rats, a 10 mg/kg systemic dose (Gobert et al., 2000) or systemic doses between 0.16 and 0.64 mg/kg similarly fails to affect SNc basal or burst firing and DStr DA-levels. Thus, although 5-HT_{2C}R appears to be involved DA-regulation within the nigrostriatal pathway, SB242084 has little effect. The limited effect of SB242084 on nigrostriatal DA has been suggested to be due to a stimulatory role of the 5-HT_{2C}R in the DStr, which is opposed by an inhibitory role of the receptor at the level of the SNc (Di Matteo et al., 2001; Lucas et al., 2000).

The 5-$HT_{2A/2C}$R also constitutively inhibits PFC DA-levels. Systemic SB206553 elevates DA-dialysate levels in the PFC, but do not affect levels of 5-HT (Gobert et al., 1999, 2000). Long-lasting and immediate elevations of DA, but not 5-HT, are also produced by systemic SB242084 at both higher (10 mg/kg; Millan et al., 1998) and lower doses (0.63 mg/kg; Gobert et al., 2000). These effects are not dependent upon

activity within the PFC, as local infusion of SB206553 (Alex et al., 2005) Ro-60-0175 (Pozzi et al., 2002) and SB242084 (Pozzi et al., 1999) all fail to affect PFC DA-levels.

Furthermore, the 5-HT_{2C}R agonists DOI and MK212 increase PFC release of acetylcholine (ACh; Nair and Gudelsky, 2004). 5-HT, mCPP, and clozapine also increase striatal and hippocampal ACh level, effects that can be blocked by the 5-HT_{2C}R antagonists RS102221 and mesulergine (Bonsi et al., 2007; Chung et al., 2004; Zhelyazkova-Savova et al., 1997). These effects may be of relevance for cognitive flexibility, as rats challenged in an egocentric reversal task show striatal ACh-elevations (Ragozzino and Choi, 2004) and ACh depletion through excitotoxic lesioning of the basal forebrain impair object (Ridley et al., 1985) and visual reversal learning in the marmoset (Roberts et al., 1992) and bowl-digging reversals in the rat (Tait and Brown, 2009). There is however as yet no evidence for any effects of independent 5-HT_{2C}R antagonism within these systems.

5-HT_{2C}R mRNA is also present at higher densities in raphe nuclei (Hoffman and Mezey, 1989; Molineaux et al., 1989). Here, targeted administrations of the non-selective 5-HT_{2C}R agonists WAY100635, DOI, DOB, Ro 60-0175 and co-administrations with the antagonists SB206553, ritanserin and SB242084 indicates that the 5-HT_{2C}R has constitutive inhibitory control over 5-HT neuronal firing within the dorsal raphe (Boothman et al., 2003, 2006; Quérée et al., 2009). Furthermore, SB242084 alone is without affect within this circuitry, and there is no evidence for its relevance to executive functioning. The dorsal raphe is however interconnected with the OFC (Goncalves et al., 2009; Morecraft et al., 1992; Porrino and Goldman-Rakic, 1982), allowing a potential mechanism whereby 5-HT_{2C}R antagonism could augment OFC 5-HT levels within tasks of executive functioning.

Although the 5-HT_{2C}R appears to be involved in reversal learning, little is known about the effect of 5-HT_{2C}R antagonism on the separate cognitive components of reversal learning. The 5-HT_{2C}R has been suggested in to be involved in processes related to perseverance with its effect on early-errors (Boulougouris et al., 2008; Boulougouris and Robbins, 2010). There is no published data on the role of the 5-HT_{2C}R in learned non-reward or the closely related paradigm of latent inhibition. However, decreasing 5-HT signalling through medial raphe nucleus lesions (Asin et al., 1980; Lorden et al., 1983; Loskutova et al., 1990; Solomon et al., 1980) as well as global (Solomon et al., 1978) accumbal (Loskutova, 2001) and hippocampal (Cassaday et al., 1993b) 5-HT depletions attenuate latent inhibition. Expression of latent inhibition

is also associated with increased striatal and limbic 5-HT (Loskutova et al., 1990; Molodtsova, 2003). This would suggest that decreasing 5-HT signalling releases behaviour suppressed by previous non-reinforcement and indicates that facilitated reversal learning through SB242084 could be achieved though attenuated learned non-reward.

The current chapter explored the role of the 5-HT_{2C}R in reversal learning perseverance and learned non-reward using the selective 5-HT_{2C}R antagonist SB242084. It should be noted that most 5-HT_{2C}R agonist and antagonist also have significant affinities for the 5-HT_{2A}R, and indeed the 5-HT_{2B}R. Yet, the restricted brain expression of the 5-HT_{2B}R (Choi and Maroteaux, 1996) would suggest a lack of involvement of this receptor in these compounds effect on neurotransmitter regulation and cognition. However, SB242084 has a 100-fold and 158-fold selectivity for the 5-HT_{2C}R over the 5-HT_{2B}R and 5-HT_{2A}R, respectively, and more than 200-fold selectivity for the 5-HT_{2C}R over 5-HT_{1}R, 5-HT_{4}R, 5-HT_{6}R, 5-HT_{7}R, D_2R, and D_3R (Kennett et al., 1997). There is nevertheless some suggestion that SB242084 could have inverse agonist rather than antagonist affinity at the 5-HT_{2C}R (Herrick-Davis et al., 2000), but there is as yet no evidence to support this.

Experiment 1 explored the activity levels of mice following SB242084 on its own and against the 5-$HT_{2A/2C}$R agonist mCPP induced hypoactivity in order to assess the behavioural effectiveness of the given dose and pre-treatment time. Experiment 2 and 3 investigated the effects of SB242084 in the operant visuospatial and maze egocentric reversal tasks. In order to explore possible mechanism for the effect of SB242084 in Experiment 3, Experiment 4 looked at SB242084 and maze novelty recognition.

6.2 METHOD

6.2.1 *Drug*

SB242084 (Tocris, Bristol, UK) was initially dissolved in PEG400 (Sigma-Aldrich, Poole, UK) at 20% of the final required volume, which was then made up by 10% (w/v) hydroxypropyl-beta-cyclodextrin (Fluka, Poole, UK). mCPP (Tocris, Bristol, UK) was dissolved in saline. Stock solution was aliquoted and frozen at -80°C in vials of quantities required for each test day. SB242084 was administered subcutaneously (s.c.) in the nape of the neck at a dose of 0.5 mg/kg in a volume of 4 ml/kg 30 min prior to testing. mCPP was administered intraperitoneally (i.p.) at a dose of 1 mg/kg in a volume of 10 ml/kg 5 min prior to testing.

6.2.2 *Experiment 1: Locomotor activity*

The experiment used 32 C57BL6/J male mice (Charles River, UK) weighing a mean 26.5g at the start of the experiment. Animals received SB242084, mCPP, or vehicle giving four experiment groups (vehicle vs. vehicle, SB242084 vs. vehicle, SB242084 vs. mCPP, vehicle vs. mCPP). Activity was monitored over a 90 min test session, and data analysed by a two-way 4 × (between-subjects: drug) 6 (within-subjects: time) mixed ANOVA. Significant interactions were explored through LSD post-hoc analyses.

6.2.3 *Experiment 2: Operant reversal learning*

The experiment used 43 C57BL/6J male mice (Charles River, UK) weighing a mean 24.6g at the start of the experiment. After completing the spatial discrimination drug-free, animals were matched for trials to criterion and randomly assigned to a drug and test condition. Animals subsequently completed three test phases, each proceeded by a drug-free retention-phase. Data for the SB242084 experiment was analysed using 3 × (between-subjects: test condition) 3 × (within-subjects: test phase) 2 (between subjects: drug) mixed ANOVAs. Significant interactions were followed-up by separate ANOVAs to establish simple effects.

6.2.4 *Experiment 3: Maze reversal learning*

The experiment used 72 C57BL/6J male mice (Charles River, UK) weighing a mean 24.9g at the start of the experiment. Animals were run in two batches of 36, with each animal tested every other day. After completing the initial spatial discrimination drug-free, animals were matched for trials to criterion and assigned to a drug and test condition. Animals which failed to reach criterion within 250 trials where assigned a trial-score of 250 for that test and removed from further testing. The data was analysed by 2 × (drug) 3 (test condition) between-subject ANOVAs. Significant interactions were followed-up by separate ANOVAs or LSD post-hoc analyses to establish simple effects.

6.2.5 *Experiment 4: Maze novelty recognition and attraction*

The experiment used 28 C57BL6/J male mice (Charles River, UK) weighing 26.5g at the start of the experiment. The experimental design and statistical analysis was exactly as described in Chapter 2. In brief, animals were initially habituated to a T-maze or a Y-maze for 3×12 min/day for three day. Testing took place on the fourth day over 2×15 min intervals. In the first 15 min interval, the maze was maintained in the same configuration as during maze habituation. In the second 15 min interval, one of the previously open arms was closed while an arm 45° to the north or south was opened.

Animals were dosed with 0.5 mg/kg of SB242084 15 min before testing. Thus the novel-arm was introduced 30 min after dosing, similar to the experiments of cognitive flexibility. Proportion of time and proportion of arm entries in each arm was scored before and after the 45° change in arm location. An arm-entry was scored when the animal placed its back-paws behind the small regress separating the central platform from the extending arm. The data was analysed by 2 × (within-subject: test phase) 2 (between subjects: drug) mixed ANOVAs.

6.3 RESULTS

6.3.1 *Experiment 1: Effect of SB242084 on mCPP-induced hypoactivity*

SB242084 at 0.5 mg/kg increased activity on its own as well as in animals pre-treated with the non-selective 5-HT_{2C}R agonist mCPP, roughly doubling the activity levels over the 90 min test phase (Fig 6.1). There were significant main effects of time ($F_{5, 140} = 73.7$, $p < .0001$) and drug ($F_{5, 140} = 21.0$, $p < .0001$) and a significant time × drug interaction ($F_{5, 140} = 73.7$, $p < .0001$). SB242084 significantly increased ($p < .0001$) while mCPP significantly decreased activity levels ($p < .05$) relative to animals treated with vehicle only. Animals pre-treated with SB242084 and challenged with mCPP displayed heightened activity similar to SB242084 alone.

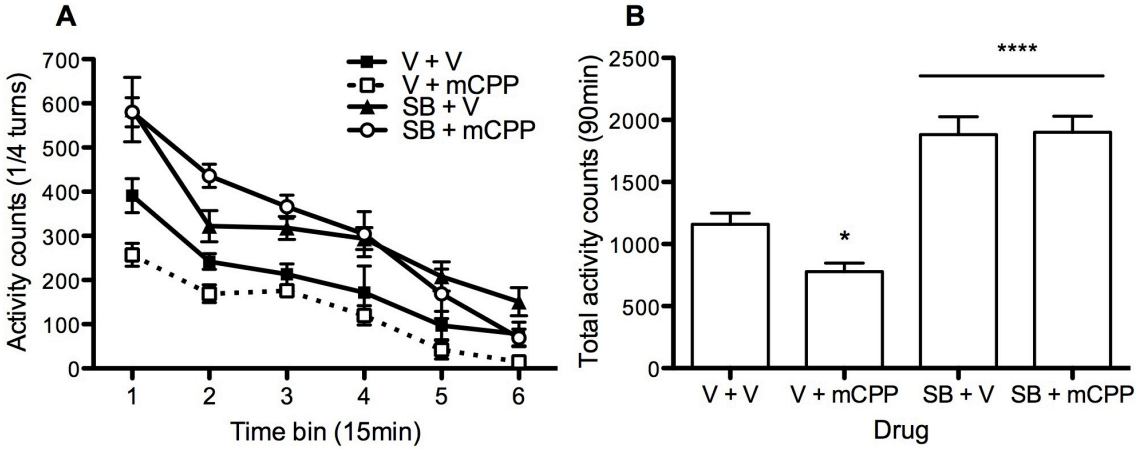

Figure 6.1 Effect of SB242084 on locomotor activity in animals pre-treated with mCPP. Data expressed in 15 min time bins (A) and total activity counts over the 90 min session (B). Asterisk denote differences at which $p < .05$ (*$p < .05$, ****$p < .0001$).

6.3.2 *Experiment 2: SB242084 and operant reversal learning*

SB242084 facilitated performance in the full reversal and learned non-reward tests but did not significantly affect learning in the perseverance test (Fig. 6.2-6.4).

Performance improved across each test phase, seen as significant decreases in trials to criterion ($F_{2,74} = 8.8$, $p < .0001$), correct responses ($F_{2,74} = 5.9$, $p < .01$) and omissions ($F_{2,74} = 12.6$, $p < .0001$) but not incorrect responses to criterion ($F_{2,74} = 1.9$, p = ns). There were no significant differences between groups on drug-free retentions of a learned response, or the number of early-errors or late-errors.

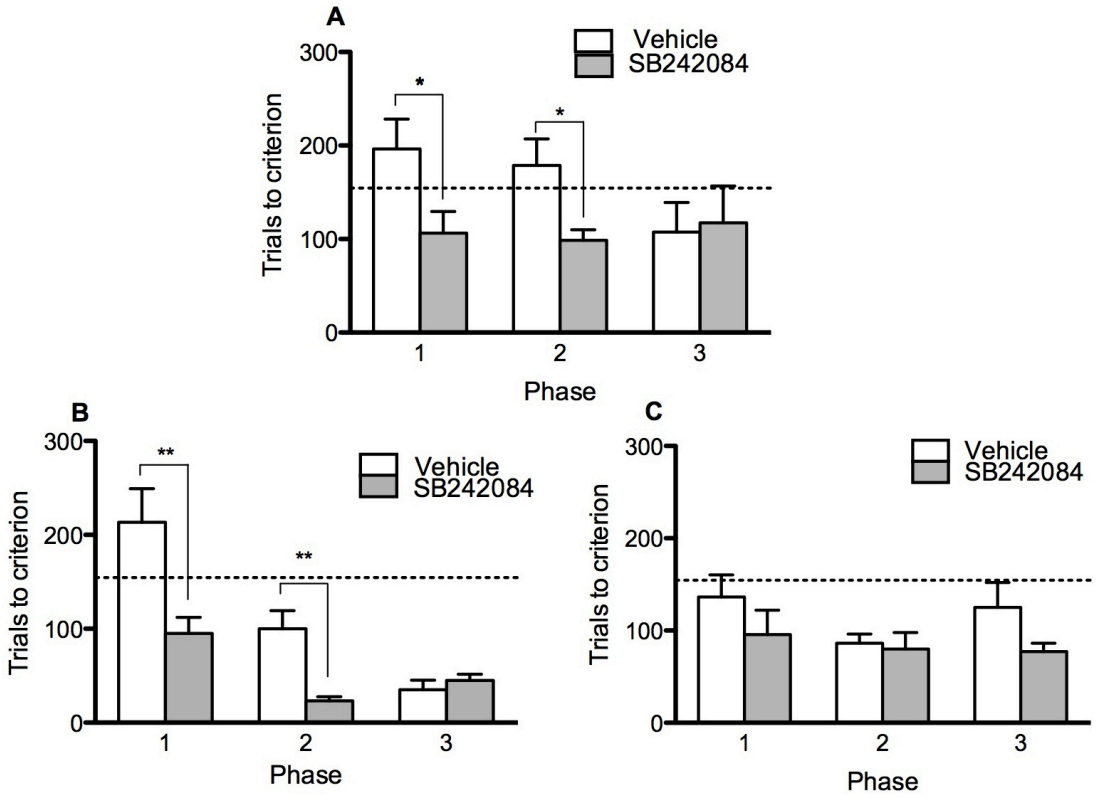

Figure 6.2. Effect of SB242084 on trials to criterion in the three test phases of the full reversal (A) learned non-reward (B) and perseverance tests (C) in the operant procedure. Broken line represents mean performance in the initial spatial discrimination. Asterisk denote differences at which $p < .05$ (*$p < .05$, **$p < .02$). Significant main effects of test phase ($F_{2,74} = 8.8$, $p < .0001$) test condition ($F_{1,37} = 6.6$, $p = .004$) drug ($F_{1,37} = 18.3$, $p = .0001$) and significant test phase × drug ($F_{2,74} = 3.4$, $p = .039$) and test phase × test condition interactions ($F_{4,74} = 2.6$, $p = .044$). SB242084 decreased trials in the first ($F_{1,37} = 15.8$, $p < .0001$) and second ($F_{1,37} = 17.7$, $p = .002$) but not the third test phase ($p > .70$). ANOVA restricted to the two first test phases where SB242084 had an effect showed a significant drug × test condition interaction ($F_{2,37} = 4$, $p = .027$).

SB242084 significantly decreased trials to criterion in the first and second test phase (Fig. 6.2; test phase × drug interaction: $F_{2,74} = 4.2$, $p < .05$). An ANOVA restricted to the first two test phases where the drug had an effect showed a significant drug × test condition interaction ($F_{2,37} = 4$, $p < .05$) where SB242084 decreased trials to criterion in the full reversal ($F_{1,14} = 10.7$, $p < .01$) and learned non-reward tests ($F_{1,10} = 30.5$, $p < .001$) but not in the in the perseverance test ($p = .16$).

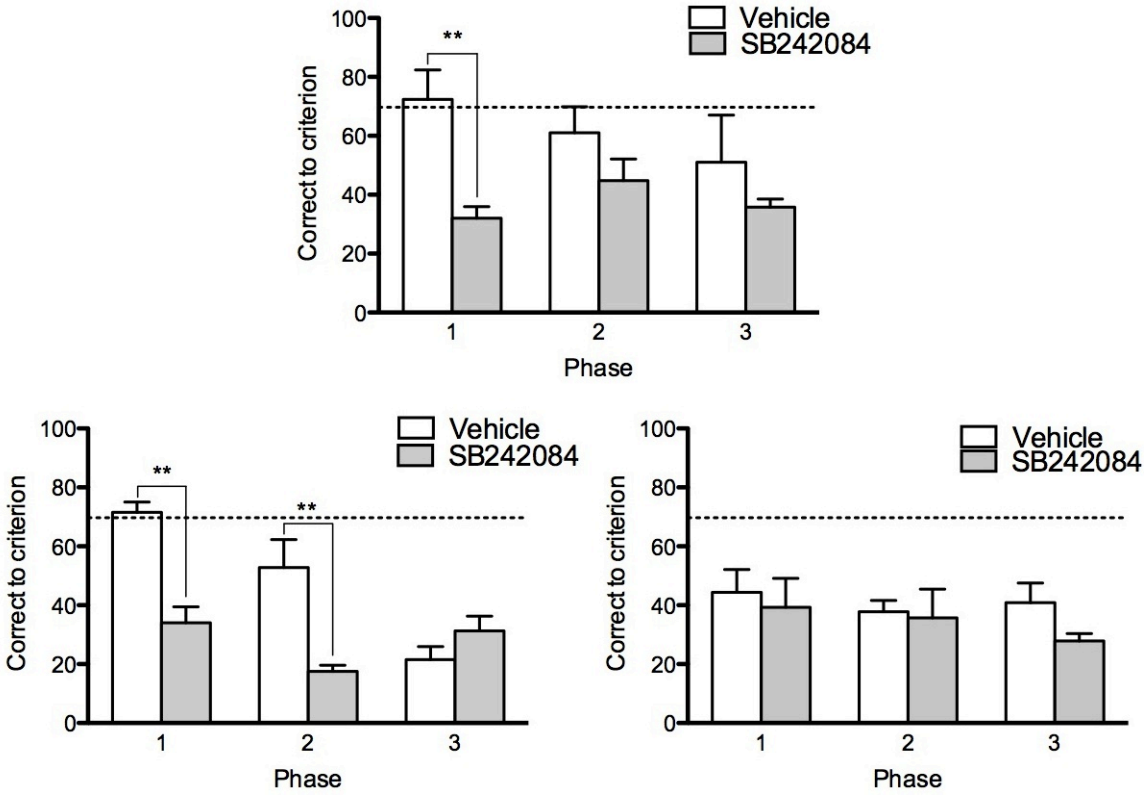

Figure 6.3. Effect of SB242084 on correct responses to criterion in the three test phases of the full reversal (A) learned non-reward (B) and perseverance tests (C) of the operant procedure. Broken line represents mean performance in the initial spatial discrimination phase. Asterisk denote differences at which $p < .05$ (*$p < .05$, **$p < .02$). Significant main effects of test phase ($F_{2,74} = 5.9$, $p < .004$) drug ($F_{1,37} = 15.4$, $p < .0001$) test condition ($F_{2,37} = 3.3$, $p < .046$) and significant test phase × drug ($F_{2,74} = 3.4$, $p < .038$) and test phase × drug × test condition interactions ($F_{4,74} = 2.8$, $p = .032$). SB242084 decreased correct trials the full reversal (Phase 1: $F_{1,14} = 14.2$, $p = .002$) and learned non-reward tests (Phase 1: $F_{1,10} = 33.2$, $p < .0001$; Phase 2: $F_{1,10} = 13.4$, $p = .004$) but not in the perseverance test ($p \geq .6$).

On correct responses to criterion (Fig. 6.3), there was a significant main effect of drug ($F_{1,37} = 15.4$, $p < .0001$) as well as significant test phase × drug ($F_{2, 74} = 3.41$, $p < .05$) and test phase × drug × test condition interactions ($F_{4, 74} = 2.8$, $p < .05$) over the three test phases. SB242084 decreased correct trials to criterion in the full reversal (Phase 1: $F_{1,14} = 14.2$, $p < .01$) and learned non-reward tests (Phase 1: $F_{1,10} = 33.2$, $p < .0001$; Phase 2: $F_{1,10} = 13.4$, $p < .01$) but not in the perseverance test ($p \geq .6$).

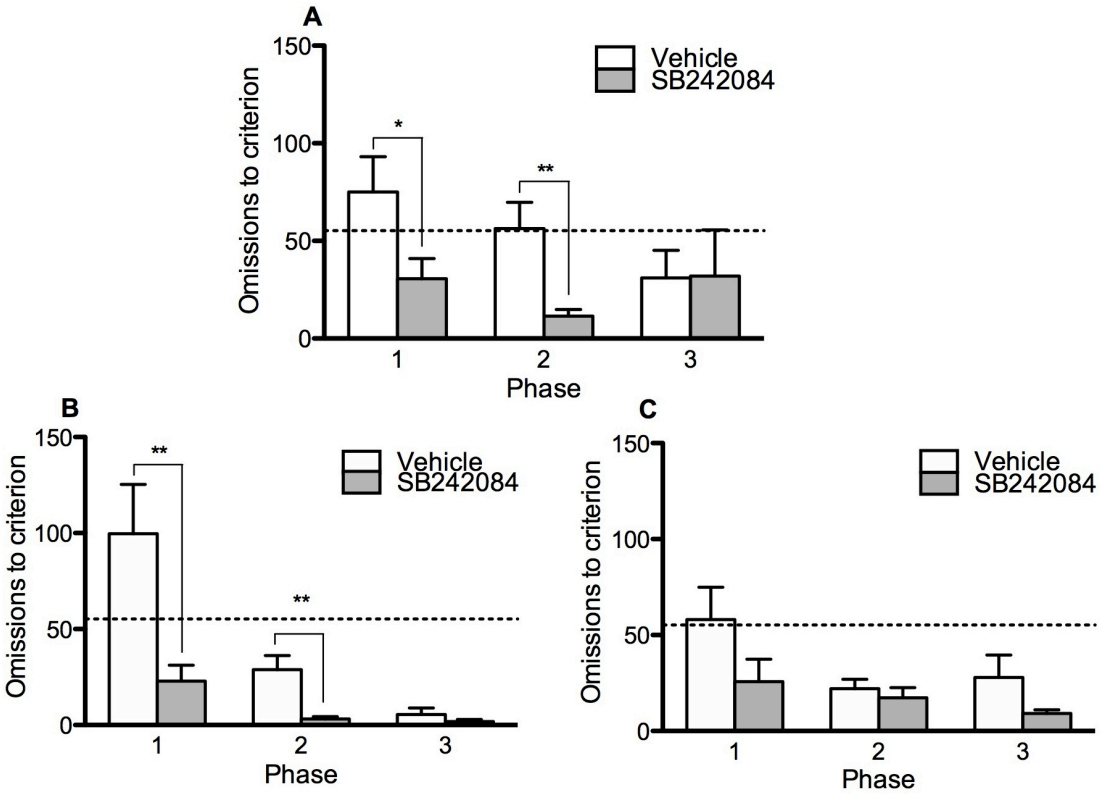

Figure 6.4. Effect of SB242084 on omissions to criterion in the three test phases of the full reversal (A) learned non-reward (B) and perseverance tests (C) of the operant procedure. Broken line represents mean performance in the initial spatial discrimination phase. Asterisk denote differences at which $p < .05$ (*$p < .05$, **$p < .02$). Significant main effects of test phase ($F_{2, 74} = 22.6$, $p < .0001$) test condition ($F_{2,37} = 3.7$, $p = .033$) and drug ($F_{1,37} = 32.2$, $p < .0001$) as well as a significant test phase × test condition interaction ($F_{4,74} = 2.8$, $p = .031$).

SB242084 decreased omissions to criterion, most prominently in the full reversal and learned non-reward tests (Fig. 6.4). ANOVA showed a significant main effect of drug ($F_{1,37} = 16.8$, $p < .0001$) and test phase × drug interaction ($F_{4,74} = 4.5$, $p < .05$). SB242084 significantly decreased omissions to criterion in the first ($F_{4,41} = 13.9$, $p < .001$) and second ($F_{4,41} = 13.6$, $p < .001$) but not the third test phase ($p = .53$). However, the drug × test condition interaction did not reach significance.

SB242084 did not affect incorrect responses to criterion (Fig. 6.5). However, there was a significant effect of test condition ($F_{2,37} = 12.4$, $p < .0001$) and test phase × condition interaction ($F_{4,74} = 4.5$ $p < .01$). Animals made more incorrect responses in the full reversal test than in the learned non-reward ($p < .0001$) and perseverance tests ($p = .05$), and more incorrect responses in the perseverance test than in the learned non-reward test ($p < .01$). In the learned non-reward test, animals made more incorrect responses in the first than the second ($p = .002$) and third test phases ($p = .001$). There were no effects of test phase on incorrect responses in the full reversal and perseverance tests.

Finally, SB242084 significantly decreased all latency indices (Table 6.1; pellet retrival: $F_{1, 36} = 16.6$, $p < .0001$, nosepoke-hole response: $F_{1,36} = 10.6$, $p = .002$, trial initiation: $F_{1, 36} = 11.4$, $p = .002$). There were no significant effects of test condition (all $p \geq .188$) or drug × test condition interaction on any latency measures (all $p \geq .288$).

Table 6.1. Mean latencies (± SEM) in the operant task collapsed over the three test phases and three test conditions.

	Group		
	Vehicle	**SB242084**	**p.**
Trial initiation	6.18 ± .19	5.25 ± .20	.002
Nosepoke-hole response	4.24 ± .20	3.67 ± .13	.002
Pellet retrieval	2.02 ± .05	1.70 ± .05	.0001

No effects of condition ($p \geq .19$) or drug × condition interactions ($p \geq .29$).

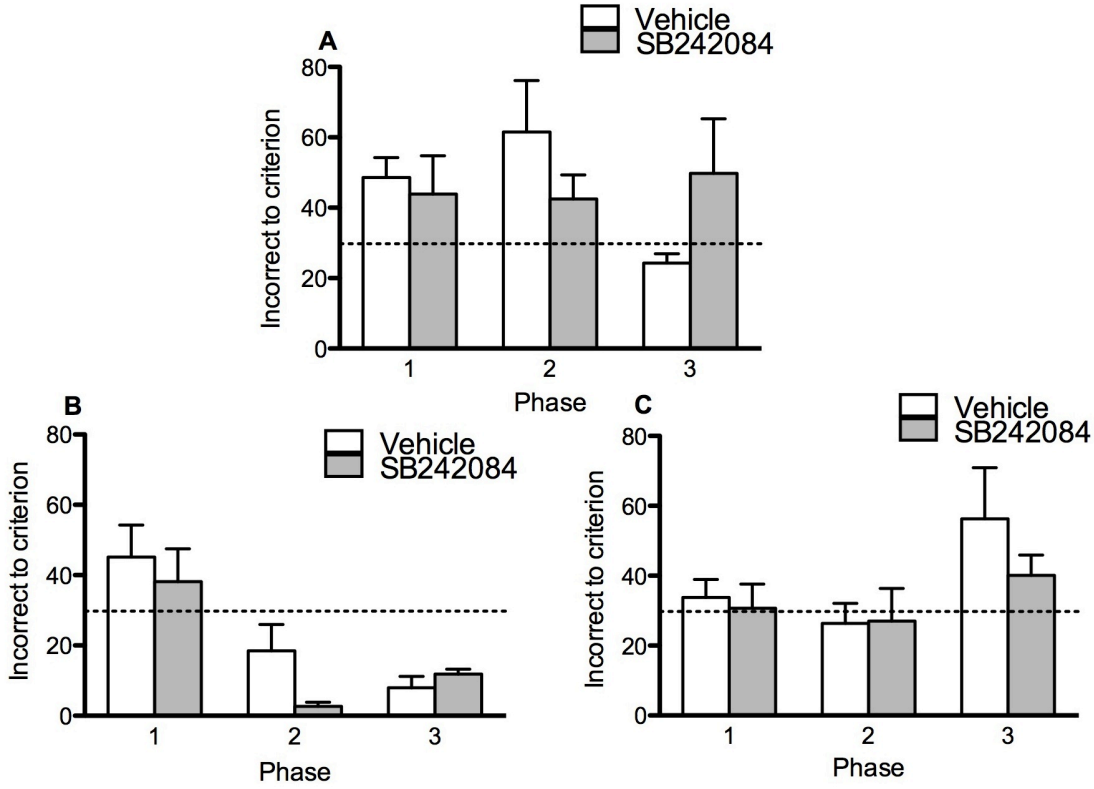

Figure 6.5. Effect of SB242084 on incorrect responses to criterion in the three test phases of the full reversal (A) learned non-reward (B) and perseverance tests (C) of the operant procedure. No effect of drug or drug × test condition interactions. Significant effect of test condition ($F_{2,37}$ = 12.4, $p < .0001$) and phase × test condition interaction ($F_{4,74}$ = 4.5, $p < .01$).

6.3.3 *Experiment 3: SB242084 and maze reversal learning*

Four animals were excluded after failing to respond in the spatial discrimination test, and one animal was excluded when becoming ill due to dehydration after a problem with the water-dispenser. Two animals failed to complete the full reversal test within 250 trials (one in each drug condition). There were no significant main effect of drug or drug × test condition interaction on probe-trials to criterion, early-errors or late-errors.

SB242084 facilitated and retarded performance in perseverance and learned non-reward tests, respectively, while failing to affect learning in the full reversal test (Fig. 6.6). There was a significant main effect of test condition ($F_{2,61}$ = 13.1, $p <. 0001$) and drug × test condition interaction ($F_{2, 61}$ = 3.1, $p = .05$) on trials to criterion (Fig.

6.5A). Animals required more trials to reach criterion in the full reversal test than in the perseverance ($p < .001$) and learned non-reward tests ($p < .0001$).

Separate one-way ANOVAs showed that SB242084 decreased trials to criterion in the perseverance test ($F_{1,20} = 4.5$, $p < .05$), while increasing trials to criterion in the learned non-reward test ($F_{1,22} = 4.4$, $p < .05$). SB242084 did not effect trials to criterion in the full reversal test ($F_{1,19} = 1.8$, p = ns).

There was also a significant main effect of test condition ($F_{2, 61} = 9.6$, $p < .0001$) and drug × test condition interaction ($F_{2, 61} = 3.5$, $p < .05$) on incorrect trials to criterion (Fig. 6.5B). Animals made more incorrect responses to criterion in the full reversal test than in the perseverance ($p < .01$) and learned non-reward tests ($p < .0001$). SB242084 decreased the number of incorrect responses made in the perseverance test ($F_{1, 20} = 6.0$, $p < .05$). Conversely, SB242084 treated animals made more incorrect responses in the learned non-reward test, although the difference failed to reach significance ($F_{1, 22} = 3.0$, p = ns).

There were a significant effect of test condition on correct responses to criterion (Fig. 6.5C; $F_{2,61} = 13.5$ $p < .0001$) but no significant effects of drug or drug × test condition interaction. Animals required more correct responses to criterion in the full reversal test than the perseverance and learned non-reward tests ($p < .0001$).

A concomitant facilitation of learning in the perseverance test where the novel response option is correct, and impairment of learning in the learned non-reward test were the novel response option is incorrect, is indicative of an effect on novelty attraction. Novelty place recognition and attraction of SB242084 treated animals was therefore assessed in the maze.

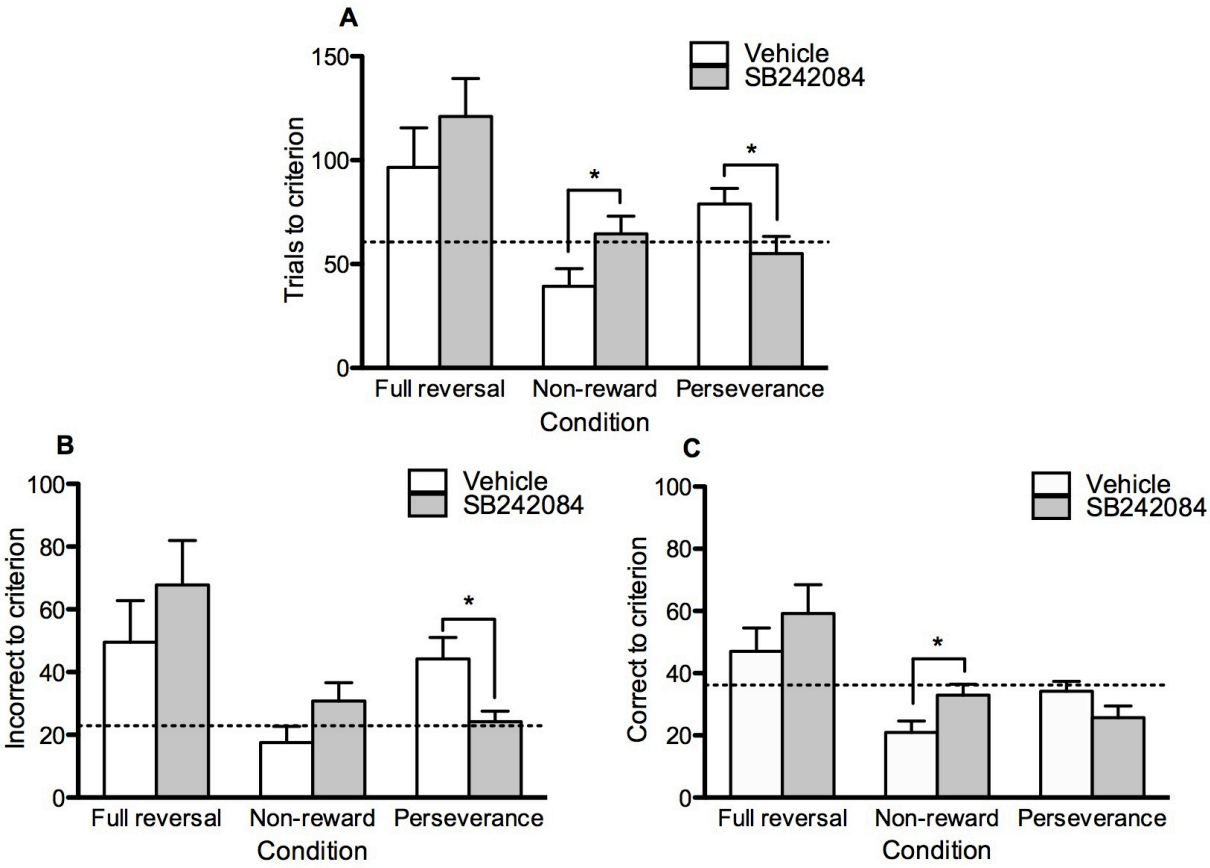

Figure 6.6. Effect of SB242084 on trials (A), incorrect responses (B), and correct responses (C) to criterion in the three test conditions of the maze procedure. Asterisk denote differences at which $p < .05$. Broken line represents mean performance in the initial spatial discrimination phase. (A) *Trials to criterion*. Significant effect of test condition ($F_{2,61} = 13.1$, $p < .0001$) and test condition × drug interaction ($F_{2,61} = 3.1$, $p = .05$). SB242084 decreased trials to criterion in the perseverance test ($F_{1,20} = 4.5$, $p = .046$), and increased trials to criterion in the learned non-reward test ($F_{1,22} = 4.4$, $p = .047$). (B) *Incorrect responses.* Significant effect of test condition ($F_{2,61} = 9.6$, $p < .0001$) and test condition × drug interaction ($F_{2,61} = 3.5$, $p = .04$). SB242084 decreased incorrect responses to criterion in the perseverance test ($F_{1,20} = 6.0$, $p = .02$). (C) *Correct responses*. Significant effect of test condition ($F_{2,59} = 10.4$, $p < .0001$).

6.3.4 *Experiment 4: Effect of SB242084 on maze novelty place attraction*

Animals spent more time in the novel arm and made more arm-entries into the novel arm, but SB242084 failed to affect both of these measures (Table 6.2).

There were no effects of maze-configuration or drug × maze-configuration interaction on entries into the novel or old arms. There was significant effects of phase on proportion of time ($F_{1,26} = 12.3$, $p < .01$) and proportion of arm-entries made into the novel arm ($F_{1,26} = 3.8$, $p < .0001$). There were no effects of drug ($p \geq .16$) or drug × phase interactions ($\geq .19$) on proportion of time spent in the novel arm or arm entries into the novel arm.

SB242084 did not affect the total time spent in the novel arm (SB242084 M: 254.0 ± 16.9, Vehicle M: 244.8 ± 22.9) or old arms (SB242084 M: 175.18 ± 13.7, Vehicle M: 150.4 ± 15.9). However, SB242084 increased the total number of arm entries (SB242084 M: 62.8 ± 4.6; $p < .05$, Vehicle M: 41.3 ± 2.1) presumably due to increased activity levels (see Experiment 1).

Table 6.2. Effect of SB242084 on novelty recognition and attraction. Proportion of time and proportion of entries (± SEM) before a 45° shift (pre-shift) and after a 45° shift (post-shift) in vehicle and SB242084 treated animals.

	Proportion of time (%)			**Proportion of entry counts (%)**		
	Pre-shift	*Post-shift*	*Change*	*Pre-shift*	*Post-shift*	*Change*
Vehicle	30.0 ± 2.8	45.6 ± 4.2	+ 15.6	34.2 ± 1.4	47.6 ± 2.7	+13.3
SB242084	35.5 ± 3.5	43.0 ± 3.1	+7.4	33.7 ± 1.4	42.0 ± 2.0	+8.3
Total	32.8 ± 2.3	44.3 ± 2.6	+11.5**	34.0 ± 1.0	44.8 ± 1.7	+9.5**

** $p < .01$

6.4 DISCUSSION

The experiments in this chapter show that SB242084 has variable effects on reversal learning, perseverance and learned non-reward depending on task parameters. SB242084 was found to improve operant visuospatial reversal learning in the mouse, consistant with recent observations from a similar operant procedure in the rat (Boulougouris et al., 2008). Additionally, these effects are shown to be due to decreased interference from learned non-reward, rather than perseverance.

However, these effects did not generalise across testing paradigms. SB242084 failed to influence maze egocentric reversal learning, apparently to be due to opposing facilitating and impairing affects on perseverance and learned non-reward. One potential explanation for this pattern of results is that 5-HT_{2C}R antagonism enhances choice for a novel response option in the maze. However, SB242084 failed to affect performance in a novelty place recognition test, suggesting that the results are related to learning.

6.4.1 *Locomotor activity*

SB242084 increased activity levels at 0.5 mg/kg, roughly doubling the activity over a 90 min test session. It had similar effects on activity in animals pre-treated with the 5-$HT_{2A/2C}$ R antagonist mCPP. The hypoactivity effects of mCPP are consistent with previous work in the rodent showing that mCPP, at doses relevant to the current study, decreases activity levels in the rat (Kennett and Curzon, 1988; Lucki et al., 1989) and mouse (Gleason et al., 2001) although the effect is not always significant (Dalton et al., 2004; Fletcher et al., 2009; Heisler and Tecott, 2000). In the mouse, SB242084 at 0.3 and 1 mg/kg causes 30-40% elevations in activity over 90 min test sessions (Fletcher et al., 2009. The more potent elevation observed here may be related to the extensive habituation prior to drug-challenge in the study by Fletcher et al. (2009) and lack of habituation in the current experiment.

6.4.2 *Operant task*

In the operant procedure, SB242084 facilitated learning in the full reversal and learned non-reward tests, but did not affect performance in the perseverance test. The

facilitation was observed as decreases in trials, correct responses and omissions to criterion. SB242084 did not affect incorrect responses to criterion, late-errors, or early-errors. This suggests that SB242084 facilitates operant visuospatial reversal learning in the mouse by a specific increase in the ability to overcome non-rewarded, rather than rewarded, associations.

The facilitated learning observed in the learned non-reward test after SB242084 might be explained by a decrease in novelty attraction. This is unlikely, as a decrease in novelty attraction would cause a parallel retardation of learning in the perseverance test, where the novel response option is correct. Moreover, a decrease in novelty attraction is not sufficient to explain the manipulations facilitating effects on learning, as learning also was improved in in the full reversal test, where no novel stimulus was introduced.

A common phenomenon observed in the current study as well as by many others is a 'serial reversal effect' (Boulougouris et al., 2008; Clarke et al., 2007; Mackintosh, 1983), i.e. an increased speed of learning across repeated reversals. This 'serial reversal effect' was shown to be restricted to the learned non-reward and full reversal tests while absent in the perseverance test. This indicates that facilitated reversal learning with repeated testing results from an increasing ability to overcome learned non-reward.

SB242084 decreased omissions to criterion in the learned non-reward and full reversal test. The lack of effect in the perseverance test may result from a small number of omissions, and raises the possibility of floor effects. The observed effects of SB242084 on omissions are similar to its effect in the 5-CSRTT (Fletcher et al., 2007; Higgins et al., 2003; Winstanley et al., 2004). Decreasing activity at the 5-HT_{2C}R has also previously been linked to enhanced motivation, with systemically SB242084 treated mice showing delayed break-points on a progressive ratio schedule for food reinforcement (Simpson et al., 2011), an effect believed to be related to the striatal hyperdopamineragic state induced by SB242084 (De Deurwaerdère et al., 2004). However, SB242084 at 2 mg/kg and 6 mg/kg does not affect food intake in the rat (Kennett et al., 1997) and 0.5 mg/kg of SB242084 does not affect performance on progressive ratio schedules for food and sucrose reinforcers in the mouse (Fletcher et al., 2010). 0.5 mg/kg of SB242084 also fails to affect food and sucrose intake (Fletcher et al., 2009; Hewitt et al., 2002; Dalton et al., 2006). This suggests that SB242084, at the current dosing protocol, is without affect on motivation to feed and work for food reinforcers.

Moreover, if omissions index motivation for sucrose, at least three predictions can be made. Just as motivation, omissions should remain stable, and not differ significantly across the three test phases, the three test conditions, or tests that require new learning and retention phases that require no new learning. None of these predictions are supported by the data. On the other hand, if omissions were related to learning and learned non-reward, the predictions are that omissions should decrease across subsequent test phases in the SB242084 experiment (a serial reversal effect), be made in tests that require new learning but not retention phases requiring no new learning, and be greater in tests that has a component of learned non-reward (full reversal and learned non-reward tests) than tests that has no component of learned non-reward (perseverance and retention tests). All of these predictions are supported by the data (Fig 6.4). This indicates, as previously suggested, that omissions can be considered is a component of learning and learned non-reward, produced by a reluctance to approach previously non-rewarded response options (Tait and Brown, 2007).

SB242084 decreased correct responses to criterion in the full reversal and learned non-reward tests while failing to influence incorrect responses to criterion. Correct and incorrect responses can both be produced by either avoidance or approach of previously non-rewarded and rewarded stimuli. However, incorrect responses to criterion could be thought of as measure of responding at the previously rewarded CS and hence linked to perseverance, while correct responses to criterion could be thought of as a measure of responding at the previously non-rewarded CS and thereby linked to learned non-reward. This is also suggested by the data, with animals requiring more incorrect responses to criterion in the full reversal and perseverance tests than in the learned non-reward test, and more correct responses to criterion in the learned non-reward and full reversal tests than the perseverance test (Fig. 6.3). A decreased number of correct rather than incorrect responses could thus be interpreted as indicative of an effect on learned non-reward rather than perseverance.

SB242084 also decreased pellet collection, response and magazine latencies in all test conditions. As shown in Experiment 1, as well as by others (Fletcher et al., 2009; Martin et al., 2002), SB242084 induces hyperactivity in the rodent. Thus, hyperactivity, or a related effect on motor performance, could explain the effect of SB242084 on magazine, response, and pellet retrieval latencies.

The facilitating effects of systemic 5-HT_{2C}R antagonism on reversal learning may appear to contradict the retarding effects of 5-HT depletions (Clarke et al., 2007;

Danet et al., 2009, 2010). This discrepancy has previously been explained by incomplete 5-HT depletions causing 5-HT_{2C}R supersensitivity (Roberts 2011; Boulougouris and Robbins 2010). This is supported by observations of PCPA-induced 5-HT depletions causing a downregulation of 5-HT_{2C}R pre-mRNA editing resulting in a 30% elevation in expression of the unedited receptor isoform with at least a four-fold increase in affinity for 5-HT relative to edited isoforms (Gurevich et al., 2002). It is also supported by the observation that PCPA-induced 5-HT depletions potentiates mCPP-induced self-grooming in the rat (Graf et al., 2003).

The SB242084-induced facilitation of reversal learning though decreased learned non-reward also contradict findings of 5,7-DHT induced OFC 5-HT depletions causing perseverative reversal deficits, but not learned non-rewarded related deficits, using the ID/ED-task in the marmoset (Clarke et al., 2007). The dissimilar effects in this study are likely to be related to the use of a different species in a rather different test paradigm.

However, the reversal learning deficits produced by 5-HT depletions may not be related to altered activity at the 5-HT_{2C}R. Within the PFC, higher levels of the 5-HT_{2A}R than the 5-HT_{2C}R have been detected (Pompeiano et al., 1994), as well as high to moderate levels of the 5-HT_6R (Gérard et al., 1996; Lacroix et al., 2004) and the 5-HT_7R (Béïque et al., 2004) which like the 5-HT_{2C}R are involved in reversal learning (Fone et al., 2008; McLean et al., 2009). Altered activity at these receptors rather than the 5-HT_{2C}R could therefore account for the reversal deficits observed following 5-HT depletions. This idea is further supported by electrophysiological responses to 5-HT within the PFC of the 5-HT_{2C}R KO mouse. Microiontophoretically-applied 5-HT potently inhibits pyramidal-neuron firing in the OFC of both anesthetised wild-type and 5-HT_{2C}R KO mice (Rueter et al., 2000). This suggests that the impairing effects of OFC-specific 5-HT depletion on reversal learning are related to altered activity at other receptors than the 5-HT_{2C}R.

Moreover, the reversal learning improvement following 5-HT_{2C}R antagonism may not by related to altered 5-HT activity. Systemic SB242084 facilitates reversal learning but fails to influence dialysate levels of 5-HT within the PFC (Gobert et al., 2000; Millan et al., 1999) and has no affect on raphe nuclei cell firing when administered on its own (Boothman et al., 2003, 2006). This suggests that 5-HT_{2C}R antagonism facilitates reversal learning through non-5-HT related mechanisms.

Alternatively, SB242084 could enhance reversal learning by increasing DA-signaling within the ventral striatum. This is however unlikely, since NAc-lesions have no effect on operant visuospatial (Castañé et al., 2009; Burke and Mair, 2001) and olfactory reversal learning in the rat (Schoenbaum et al., 2003) or visual, spatial and motor reversal learning in the macaque (Stern and Passingham, 1985). Importantly, SB242084, which facilitate reversal learning when administered systemically, has no affect on reversal learning when administered into the NAc (Boulougouris and Robbins, 2010).

Furthermore, the 5-HT_{2C}R constitutively inhibits DA-signaling within the DStr (Abdallah et al., 2008; Alex et al., 2005) and an SB242084-induced elevation in DStr DA could possibly affect reversal performance. However, elevated DA-levels in the DStr are associated with retarded rather than improved reversal learning (Clatworthy et al., 2009). Also, SB242084 at doses relevant to the current study has no effect on SNc neuron firing or dialysate levels of DA in the DStr (Gobert et al., 2000; Millan et al., 1999).

Systemic SB242084 also increases PFC DA-content, which could have pro-cognitive consequences (Stefani and Moghaddam, 2006), and it may be that this is related to the improved reversal performance. This is however contradicted by work showing that OFC-specific infusion of SB242084, which is likely to be without effect on PFC DA-levels (Pozzi et al., 1999), facilitates reversal learning (Boulougouris and Robbins, 2010).

In sum, 5-HT_{2C}R antagonism through SB242084 facilitates serial operant visuospatial reversal learning in the mouse by decreasing the interference from previously non-rewarded rather than rewarded associations. These effects are observed as decreases in trials, correct responses and omissions to criterion. The effects of SB242084 on learning are likely to be unrelated to altered DA and 5-HT signalling within the PFC and striatum.

6.4.3 *Maze task*

SB242084 had opposing effects on perseverance and learned non-reward in the egocentric maze task. SB242084 decreased trials and incorrect responses to criterion in the perseverance test, but increased trials to criterion in the learned non-reward test. Additionally, the opposing effects of SB242084 on perseverance and learned non-reward appear to summate into no overall effects on egocentric full reversal learning.

An increase in novelty attraction would predict a performance facilitation in the perseverance test, where the novel response option is correct, and a parallel performance impairment in the learned non-reward test, where the novel response option is incorrect. The observed opposing effects of SB242084 in the perseverance and learned non-reward tests could therefore be explained by elevated novelty attraction.

There are as yet no evidence for a role of the 5-HT_{2C}R in novelty attraction. The 5-HT_{2C}R is however involved in anxiety, with SB242084 increasing punished responding in the Geller-Seifer and Vogel conflict tests and time spent in the open-arms in the elevated plus-maze (Kennett et al., 1994, 1997; Martin et al., 2002). It might be that anxiolysis could influence performance in the current task by decreasing the anxiety related to entering a novel arm and thereby explain the opposing effects of SB242084 in the perseverance and learned non-reward tests. However, in Experiment 4, SB242084 failed to affect both the proportion of entries and proportion of time spent in the novel-arm. Hence, with no evidence supporting a role of the 5-HT_{2C}R or an effect of SB242084 on novelty attraction, as well as a lack of effect of SB242084 on novelty place recognition and attraction, the observed results in the perseverance and learned non-rewarded tests are unlikely to be related to increased novelty attraction.

SB242084 selectively decreased the number of trials and incorrect responses in the perseverance test. The SB242084-induced facilitation of operant lever reversal in the rat has previously been discussed in terms of anticompulsive effects (Boulougouris et al., 2008; Boulougouris and Robbins 2010; Roberts, 2011) as both perseverative responding and compulsivity can be thought of as behaviours maintained despite no longer being predictive of reward (Dalley et al., 2011; Izquierdo and Jentsch, 2012).

Accordingly, mCPP-induced compulsive grooming (Graf et al., 2003) and retardation of maze spatial alternation (Tsaltas et al., 2005, 2009) have both been found to be reduced through pretreatment with SB242084, and systemic or intra-OFC infusions of the 5-HT_{2C}R antagonist RS102221 decreases excessive lever-pressing in

the signal-attenuation paradigm (Flaisher-Grinberg et al., 2008). The effect of systemic and intra-OFC infusions of SB242084 in operant visuospatial reversal learning are believed to be related to similar anticompulsive effects (Boulougouris et al., 2008; Boulougouris and Robbins 2010). The data on the role of the 5-HT_{2C}R in compulsivity is however equivocal. Similar to SB242084, the 5-HT_{2C}R agonists can attenuate compulsive behaviour in the marble burying and schedule induced polydipsia paradigms (Bös et al., 1997; Martin 1998), and these effects can be blocked by SB242084 (Egashira et al., 2011).

6.4.4 *General discussion*

The current results indicate discrepancies between tasks in terms of mode of problem solving. That is, while responding in the operant task appear to be guided primarily by the use of non-reinforced associations, responding in the maze loads more strongly upon associations of reward. In the operant serial task, animals required more trials to criterion in the learned non-reward and full reversal tests than in the perseverance test. In the maze task, however, animals required more trials in the full reversal test than both the perseverance and learned non-reward test. These differences may be related to some of the differences in tasks parameters referred to in Chapter 2, Discussion.

The chapter also presents clear differences in the effect of SB242084 on maze egocentric and operant visuospatial reversal learning, perseveration and learned non-reward. First, SB242084 facilitated reversal learning in the operant task, but had no effect on reversal learning in the maze task. Second, SB242084 decreased perseverance in the maze task, but had no effect on perseverance in the operant task. Third, SB242084 facilitated the ability to overcome learned non-reward in the operant task, but retarded the same measure in the maze task.

The most obvious difference was observed in the learned non-reward test. There are no published data on 5-HT_{2C}R specific compounds and learned non-reward. There is however a rich literature on non-specific 5-HT_{2C}R antagonist in the closely related paradigm of latent inhibition, which like learned non-reward assesses the ability to overcome associations of non-reinforcement. Here, SB242084 was observed to cause an increase of low baseline learned non-reward in the maze task, but a decrease of high

baseline learned non-reward in the operant task. These opposing baseline dependent effects are similar to the effect of 5-HT_{2C}R antagonists on latent inhibition.

First, compounds with 5-HT_{2C}R antagonist properties can induce or potentiate latent inhibition at low baseline levels where limited or no latent inhibition is displayed by vehicle-treated controls. These effects have been observed following treatment with the atypical antipsychotics clozapine (Shadach et al., 2000; Trimble et al., 1998) and olanzapine (Dunn et al., 1994), the SSRI fluoxetine (Jakob 1995) and the putative antipsychotic S16924 (Millan et al., 1999). These compound all display potent antagonist affinity at the 5-HT_{2C}R (Meltzer, 2010; Middlemiss and Tricklebank, 1992; Millan et al., 1999; Ni and Miledi, 1997; Pälvimäki et al., 2003).

Second, 5-HT_{2C}R antagonists can also reduce or block latent inhibition at high baseline levels where strong latent inhibition is displayed by vehicle-treated controls. In this situation, the non-selective 5-HT_{2C}R antagonists clozapine, fluperlapine, sertraline, risperidone, amisulpride, ritansarin and amperozide all decreases latent inhibition (Barrett et al., 2004; Cassaday et al., 1993a; Dunn et al., 1991; Loskutova et al., 1990; Shadach et al., 2000).

These opposing baseline-dependent effects on latent inhibition have nevertheless been suggested to be unrelated to activity at the 5-HT_{2C}R, and instead produced by the compounds concurrent antagonism at the 5-HT_{2A}R and the D_2R (Schadach et al., 2000). The suggestion has been that D_2R antagonism prevails at low baseline levels, causing potentiated latent inhibition, but 5-HT_{2A}R antagonism prevails at high baseline levels, causing attenuated latent inhibition (Schadach et al., 2000). This is however contradicted by the ability of fluoxetine to increase latent inhibition without showing affinity for the D_2R (Pälvimäki et al., 2003). It is also contradicted by findings that 5-HT_{2A}R antagonism through SR46349B and ICI169369 potentiates, rather than attenuates, latent inhibition using a protocol yielding latent inhibition in controls (McDonald et al., 2003). Hence, while there are no studies of 5-HT_{2C}R selective compounds within paradigms of latent inhibition, non-selective 5-HT_{2C}R antagonists can have opposing effects on the strength of non-reinforced associations, with 5-HT_{2C}R antagonists elevating latent inhibition at low baseline levels (paralleling the observed effect of SB242084 in the maze task) and attenuating latent inhibition at high baseline levels (paralleling the observed effect of SB242084 in the operant task).

A further cross-task discrepancy is the effect of SB242084 on full reversal learning. Here, SB242084 facilitated reversal learning in the operant task, yet failed to

affect reversal learning in the maze task. If anything, the direction of effect in the maze was in the opposite direction with SB242084-treated animals requiring more trials to criterion in the full reversal test. The effects of SB242084 on operant visuospatial reversal learning has been shown to be OFC-dependent with intra-OFC infusions, but not intra-NAc or intra-mPFC infusions, improving the performance in the rat (Boulougouris and Robbins, 2010). Chapter 4 indicates that operant visuospatial reversal learning, but not maze egocentric reversal learning, is dependent upon the integrity of the OFC. Hence, the effect and lack of effect upon reversal learning in the operant and maze tasks, respectively, could be explained by the tasks relative loading upon the OFC.

The effect in the operant paradigm would suggest that 5-HT_{2C}R antagonism is of limited therapeutic efficacy for executive functioning in schizophrenia. SB242084 caused a specific decrease in learned non-reward, and schizophrenic patient show no learned irrelevance deficits within attentional set-shifting (Elliot et al., 1995, 1998), and has in other paradigm been shown to express attenuated latent inhibition (Baruch et al., 1988; Williams et al., 1998) and learned irrelevance (Young et al., 2005). Further pharmacologically produced decrements in learned non-reward through 5-HT_{2C}R antagonism could therefore be of detrimental effect. The effect of SB242084 in the operant task nevertheless suggests that 5-HT_{2C}R antagonism may be therapeutic in disorders showing cognitive flexibility impairments believed to be related to deficits in overcoming irrelevant or non-reinforced associations, such as Parkinson's disease (Slabosz et al., 2007) and OCD (Kaplan et al., 2006; Swerdlow et al., 1999).

Although SB242084 failed to affect reversal learning in the maze procedure, its effects in the learned non-reward and perseverance tests would be indicative of therapeutic efficacy in schizophrenia. Thus, both the pathology related attenuation of non-reinforced associations, as well as the pathology related augmentation of perseverance may, be opposed by 5-HT_{2C}R antagonism through SB242084.

In sum, it appears that SB242084 facilitates operant serial visuospatial reversal learning in the mouse by decreasing the influence of previously non-rewarded associations. In the maze task, however, SB242084 was without affects on reversal learning, apparently due to opposing facilitation in the perseverance test and impairment in the learned non-reward test.

Most work investigating the role of the 5-HT_{2C}R in relation to the pathology and treatment of the cognitive deficits of schizophrenia has used subtype-selective

compounds. An alternative approach is to use to the 5-HT_{2C}R KO mouse. Although constitutive loss of the 5-HT_{2C}R has some different cortical and striatal consequences to acute antagonism, additional electrophysiological work has been done in this mutant mouse which may give clues for the mechanism behind altered reversal performance following reduced function at the 5-HT_{2C}R. Studies of such mice are the focus of the experiments reported in Chapter 7.

CHAPTER 7

REVERSAL LEARNING IN 5-HT2C RECEPTOR KNOCK-OUT MICE

7.1 INTRODUCTION

The great majority of studies investigating the role of the 5-HT_{2C}R in cognition have been done pharmacologically, with relatively few studies using the 5-HT_{2C}R knock-out (KO) mouse. Although the neurochemical and low-level behavioural consequences of 5-HT_{2C}R deletion show substantial overlap with the effects of 5-HT_{2C}R antagonism through SB242084, inconstancies are observed, which may be related to additional loss of constitutive receptor activity in the mutant, developmental compensations, or to potential inverse agonist activity of SB242084.

The 5-HT_{2C}R KO has so far not been discussed as a possible instrument for assessing behaviours relevant to the pathology of schizophrenia. The 5-HT_{2C}R KO do however show dopaminergic and glutamatergic signalling abnormalities within the PFC and striatum that may be of relevance for reversal learning and the cognitive deficits of schizophrenia.

7.1.2 *The 5-HT_{2C}R KO mouse*

The 5-HT_{2C}R KO mouse was generated by inserting a nonsense mutation into exon 5 of the 5-HT_{2C}R gene (*htr2c*), producing a stop codon within the receptors fifth putative transmembrane segment and deleting the C-terminal end of the protein. Introducing the mutation into the corresponding position of the rat 5-HT_{2C}R complementary DNA resulted in mRNA failing to produce functional receptors in *Xenopus* oocytes. The mutation was subsequently introduced into the mouse genome via homologous recombination in 129-ES (embryonic stem) cells (Tecott et al., 1995). The 5-HT_{2C}R is X-linked (Yu et al., 1991). Breeding of wild-type (WT) males with heterozygous females therefore produce male offspring either hemizygous KO or hemizygous WT, with the benefit of single litters of age-matched WT and KO male mice.

However, mutations causing constitutive loss of specific components in 5-HT systems often cause adaptations additional to the mutation. For example, 5-HT_{1B}R KO

mice show reduced response to 5-HT_{2C}R agonists (Clifton et al., 2003), and 5-HTT KO mice show region-specific up- and down-regulation of the 5-HT_{1A}R and 5-HT_{1B}R (Fabre et al., 2000; Li et al., 2000), as well as decreased levels and altered expression patterns of the 5-HT_{2A}R in the PFC and striatum (Li et al., 2003; Rioux et al., 1999), and increased 5-HT_{2C}R density in the amygdala, but decreased 5-HT_{2C}R mRNA in the habenula (Li et al., 2003).

There is, nevertheless, little evidence for adaptive changes in the 5-HT_{2C}R KO mouse. Quantitative receptor autoradiography of the 5-HT_{2C}R KO mouse brain show no regional differences in density or mRNA expression of 5-HTT protein and 5-HT receptors (López-Giménez et al., 2002). The 5-HT_{2C}R KO mouse does however show slight but significant global reductions in expression of the 5-HTT, the 5-$HT_{1B/1D}$R and the 5-HT_{7}R (López-Giménez et al., 2002). Furthermore, the inhibition OFC pyramidal neuron cell firing produced by sub-region specific infusions of the 5-$HT_{2A/2C}$R agonist DOI is supressed by the 5-HT_{2A}R antagonist M100907 in wild-type but not 5-HT_{2C}R KO mice, indicating that the 5-HT_{2C}R KO mouse may show a compensatory down-regulation in 5-HT_{2A}R antagonist affinity (Rueter et al., 2000).

7.1.3 *Neurophysiology and behaviour of the 5-HT_{2C}R KO mouse*

Similar to the effects of SB242084 in WT mice, the 5-HT_{2C}R KO mouse shows increased locomotor activity levels. The mutants also exhibit elevated food anticipatory activity (Hsu et al., 2010), as well as novelty- and cocaine-induced locomotor activity (Rocha et al., 2002). Others have found that the 5-HT_{2C}R KO mouse show elevated activity when assessed over 24-hours (Nonogaki et al., 2003), during the first 30 min of a 60 min test session (Hill et al., 2010), and a 30% elevation in activity over a 90 min test session is observed when data from five experiments are summed (Fletcher et al., 2009). The hyperactivity of 5-HT_{2C}R KO mice is milder than that produced by SB242084, which is in accordance with the weaker elevation of mesoaccumbal DA-signalling observed in the 5-HT_{2C}R KO mouse relative to that produced by SB242084 (Abdallah et al., 2009; Gobert et al., 2000).

Although standard in vivo microdialysis revealed no baseline abnormalities in DA-dialysate levels within the DStr and NAc of 5-HT_{2C}R KO mice (Rocha et al., 2002), differences are evident using no-net flux quantitative microdialysis (Abdallah et al., 2008). In the 5-HT_{2C}R KO mouse, SNc neuron tonic firing and bursting are

increased by 20% and 50%, respectively, and there is a near 100% elevation in DStr DA-dialysate levels. Tonic and burst firing of VTA DA-cells are however unaffected, but there is a modest but significant increase in DA-levels within the NAc which is likely to be related to observed increases in activity (Abdallah et al., 2008). As reversal learning is dependent upon both the integrity (Kirkby, 1969; Ragozzino et al., 2001) and DA-signalling within of the DStr (Clarke et al., 2011; Clatworthy et al., 2009), these aberrations are likely to be of consequence for learning in tasks requiring cognitive flexibility.

5-HT has an inhibitory effect on neuronal activity within the OFC, mPFC and DStr, with microiontophoretic application of 5-HT or the 5-$HT_{2A/2C}$R agonists DOI and mCPP supressing neuronal firing in the rat (Ashby et al., 1990; Ashby and Wang, 1990; El Mansari and Blier, 1997; Zghoul and Blier, 2003). These effects have been suggested to be mediated by 5-HT_{2C}Rs within the OFC. Accordingly, the mCPP and DOI-induced OFC-inhibition are not blocked by ritanserin (Bergqvist et al., 1999), which show higher affinity for the 5-HT_{2A}R over the 5-HT_{2C}R. However, mCPP-induced inhibition, but not DOI-induced inhibition, is blocked by clozapine and risperidone. As mCPP, clozapine and risperidone all show greater affinity for the 5-HT_{2C}R over the 5-HT_{2A}R, the effects are suggested to be related to activity at the 5-HT_{2C}R (Bergqvist et al., 1999). This suggestion is nonetheless contradicted by work in the 5-HT_{2C}R KO mouse. 5-HT_{2C}R KO mice do not differ from WTs in the inhibitory response to microphonoretic 5-HT, DOI and higher levels of mCPP when applied in the OFC or the head of the caudate nucleus, indicating that the inhibitory effects of 5-HT primarily are mediated by the 5-HT_{2A}R (Rueter et al., 2000).

However, constitutive loss of the 5-HT_{2C}R results in glutamatergic supersensitivity in the OFC and DStr (Reuter et al., 2000). In the 5-HT_{2C}R KO, significantly less of the AMPAr agonist quisqualate is required to activate pyramidal glutamatergic OFC neurons. Glutamatergic supersensitivity may also be related to the elevated DA-levels in the DStr of the 5-HT_{2C}R KO mouse, as quisqualate infusions increase levels of dialysate DA in the caudate nucleus of the rat (Imperato et al., 1990) and cat (Barbeito et al., 1990). AMPAr supersensitivity within the OFC and DStr is likely to have consequences for performance in tasks of cognitive flexibility, as these areas are activated in human fMRI studies of probabilistic reversal learning (Remijnse et al., 2005; Rogers et al., 2000) and the degree of OFC activation positively correlates with reversal performance (O'Doherty et al., 2001).

The 5-HT_{2C}R is also expressed in the hippocampus (Wright et al., 1995), where serotonergic afferents from the raphe nuclei facilitate long-term potentiation (LTP; Bliss et al., 1983) that supports spatial learning (Davis et al., 1992; Jeffery and Morris, 2004). The 5-HT_{2C}R KO mouse shows diminished LTP induction in dentate gyrus synapses of the medial perforant path, but normal LTP induction in synapses between dentate mossy fibers and CA3, CA3 and CA1, and CA1 and the subiculum (Tecott et al., 1998). However, the 5-HT_{2C}R KO mouse also show increased expression of BDNF within the dentate gyrus (Hill et al., 2010), which could have pro-cognitive effects (Bekinschtein et al., 2011).

Despite these differences, relatively few cognitive abnormalities have been found in in the 5-HT_{2C}R KO mouse. The mutant shows normal context discrimination acquisition, and normal acquisition of appetitive lever-pressing (Tecott et al., 1998) and also do not differ from WT controls in the forced swim test, prepulse-inhibition, or working and reference memory in the radial arm maze (Hill et al., 2011). However, similar to SB242084 treated animals, the 5-HT_{2C}R KO mouse shows an anxiolytic profile in the elevated zero-maze, elevated plus-maze, mirrored chamber, open-field, emergence-neophobia and novel-object tests (Heisler et al., 2007; Tecott et al., 1998). Others have nevertheless observed no differences between 5-HT_{2C}R KO mice and WT controls in the elevated plus-maze and emergence neophobia (Hill et al., 2011).

The 5-HT_{2C}R KO mouse does however show some impairments of performance in the water maze (Tecott et al., 1998). Here the mutant displays normal acquisition of a visuospatial discrimination. However, when removing the platform and monitoring preferences in swimming location, 5-HT_{2C}R KO animals fail to show a preference for the previous platform location (Tecott et al., 1998). Furthermore, the 5-HT_{2C}R KO mouse has been suggested a model the compulsive symptoms of OCD, as the mutant shows compulsive-like behaviour of chewing non-nutritive clay and failure to habituate head-dipping behaviour in a cheese-board task (Chou-Green et al., 2003). There have as yet been no studies of executive functioning in the 5-HT_{2C}R KO mouse. The current chapter therefore investigated the effects of 5-HT_{2C}R deletion on cognitive flexibility. Experiment 1 assessed the 5-HT_{2C}R KO mouse in the operant visuospatial task, while Experiment 2 used the egocentric maze task.

7.2 METHOD

7.2.1 *Genotyping and breeding*

The animals were bred at the University of Sussex with the original progeny of 5-HT_{2C}R KO mice being a gift from L. Tecott. Wild-type male mice were crossed with females heterozygous for the X-linked 5-HT_{2C}R mutation of a C57BL6/J background generating male WT and KO offspring. Genotyping was achieved using PCR on tissue samples from ear punches. The wild-type allele was detected using primers of the 5-HT_{2C}R gene sequences flanking the Neo insertion: m5h2c (5′-AGTTGATGTTCATCTCAGGTGGC-3′) and 3N2 (5′-GGGTCCTATAGATCGAGGTACC-3′). The mutant allele was detected using primers complimentary to neomycin resistance gene (Neo) sequences: NeoD (5′-CACCTTGCTCCTGCCGAGAAA-3′) and NeoH (5′-AGAAGGCGATAGAAGGCGATG-3′). Breeding animals had been backcrossed for more than 20 generations. Animals were 10-24 weeks old (age-matched for genotype) at the beginning of the experiments.

7.2.2 *Experiment 1: Operant experimental design and statistical analyses*

The experiment used 24 male mice (11 WTs; 13 KOs) weighing a mean 26.7g at the start of the experiment. The experiment used a within-subjects design in which each animal completed a full reversal test, followed by a perseverance and learned non-reward test. The order of the perseverance and learned non-reward tests were counterbalanced across genotypes. Performance in the simple discrimination and reversal phases were analysed using one-way between-subjects ANOVAs with genotype as the independent variable. The perseverance and learned non-reward tests were analysed using 2 × (within-subjects: test condition) 2 × (between-subjects: genotype) 2 (between-subjects: test order) mixed ANOVAs.

7.2.3 *Experiment 2: Maze experimental design and statistical analyses*

The experiment used 33 male mice (18 WTs; 15 KOs) weighing a mean 25.9g at the start of the experiment. Animals were run in two batches, with each animal being

tested every other day. The experiment used a repeated measures design, with each animal completing an initial spatial discrimination followed by a full reversal test. Animals subsequently completed a learned non-reward and a perseverance test, with the order of the perseverance and learned non-reward tests counterbalanced across genotypes. Animals failing to reach criterion within 250 trials were assigned a trial-score of 250 for that test condition and not given further trials.

A large number of predominantly KO animals failed to complete criterion across all test conditions within the 250 trial-limit. Therefore, genotype differences in proportion achieving criterion within each stage was initially investigated by analysing the distribution of animals failing or passing through chi-square distributed analysis. The data from each test condition was subsequently analysed through one-way between-subjects ANOVAs with genotype as the independent variable. Behavioural analyses only included animals attempting a given stage (Leeson et al., 2009; Jazbec et al., 2007).

7.3 RESULTS

7.3.1 *Experiment 1: 5-HT$_{2C}$KO and operant reversal learning*

5-HT$_{2C}$R KO animals showed facilitated performance in the full reversal and learned non-reward tests but did not differ from wild-type controls in the perseverance test (Fig. 7.1A-D).

In the full reversal test, 5-HT$_{2C}$R KO animals made significantly fewer trials (Fig. 7.1A; $F_{1,22} = 4.5$, $p < .05$) and omissions to criterion (Fig 7.1C; $F_{1,22} = 4.8$, $p < .05$) than WT animals.

There was also a significant genotype × test condition interaction on trials to criterion in the perseverance and learned non-reward tests ($F_{1,20} = 4.6$, $p < .05$). 5-HT$_{2C}$R KO animals required significantly fewer trials to criterion in the learned non-reward test (Fig. 7.1A; $F_{1,22} = 6.0$, $p < .05$) but not in the perseverance test ($F_{1,22} = 0.4$, p = ns). There were no significant main effects or genotype or genotype × test condition interactions on any latency indices (Table 7.2).

There were no significant differences between genotypes on retention of a learned response. 5-HT$_{2C}$R KO animals required significantly less correct responses to criterion in spatial discrimination (Table 7.1; $F_{1,22} = 5.3$, $p < .05$). However, genotype failed to effect the number of trials ($F_{1,22} = 3.9$, p = ns) incorrect responses ($F_{1,22} = 0.24$, p = ns), and omissions to criterion ($F_{1,22} = 3.8$, p = ns).

Table 7.1. Mean responses of WT and 5-HT$_{2C}$R KO animals in the spatial discrimination of the operant task.

	Group		
	WT	**5-HT$_{2C}$R KO**	**p**
Trials	245.4 ± 41	152.3 ± 26.1	ns
Correct	120.6 ± 19.5	70.5 ± 11.4	.031
Incorrect	36.4 ± 4.7	33.2 ± 4.3	ns
Omissions	106.6 ± 27.3	48.6 ± 14.6	ns

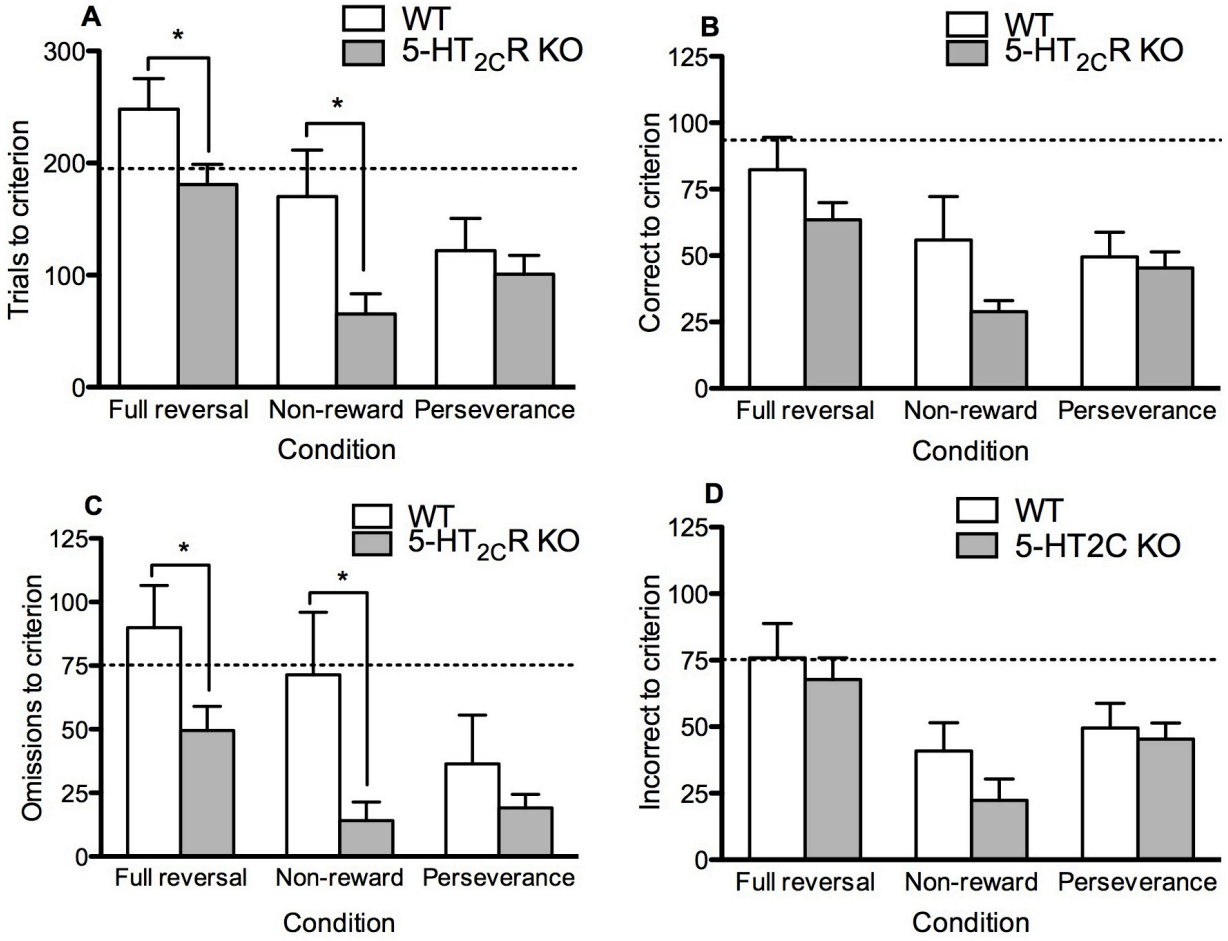

Figure 7.1. Effect of 5-HT_{2C}R KO in the operant procedure. (A) *Trials to criterion*. Significant main effect of genotype in the full reversal test ($F_{1,22} = 4.5$, $p < .046$) and genotype × test condition interaction in the non-reward and perseverance tests ($F_{1,20} = 4.6$, $p < .045$). 5-HT_{2C}R KO mice required fewer trials in the non-reward test ($F_{1,22} = 6.0$, $p = .023$). (B) *Correct responses*. No effect of genotype in the full reversal test ($F_{1,22} = 2.0$, $p = .17$). No effect of genotype ($F_{1,20} = 1.7$, $p = .2$) or the genotype × test condition interaction ($F_{1,20} = 4.0$, $p = .06$) in the perseverance and learned non-reward tests. (C) *Omissions*. Significant effect of genotype in full reversal test ($F_{1,22} = 4.8$, $p = .04$). No effect of genotype ($F_{1,20} = 4.0$, $p = .059$) or genotype × test condition interaction ($F_{1,20} = 3.1$, $p = .094$) in the learned non-reward and perseverance tests. (C) *Incorrect responses.* No effect of genotype in the full reversal test ($F_{1,22} = 0.3$, $p = .59$). No effect of genotype ($F_{1,20} = 1.5$, $p = .23$) or genotype × test condition interaction ($F_{1,20} = 1.2$, $p = .28$) in the perseverance and learned non-reward tests.

Table 7.2. Mean latencies (± SEM) in the operant task collapsed over the three test phases and three test conditions.

	Group		
	WT	**5-HT$_{2C}$R KO**	**p**
Trial initiation	6.29 ± .33	6.38 ± .30	ns
Nosepoke-hole response	3.75 ± .26	3.59 ± .22	ns
Pellet retrieval	2.31 ± .08	2.39 ± .12	ns

No significant effects of condition ($p \geq .76$) or genotype × condition interactions ($p \geq .72$).

7.3.2 *Experiment 2: 5-HT$_{2C}$R KO and maze reversal learning*

In the maze, 5-HT$_{2C}$ KOs showed retarded performance relative to WT animals. This was particularly evident in the perseverance test, but also observed in spatial discrimination acquisition.

Attrition rates. Significantly more 5-HT$_{2C}$R KO mice (N = 8) than WT mice (N = 2) failed to complete the four test conditions (Fig. 7.2; $x^2 = 7.5$, $p < .01$). Although no animals failed to complete the spatial discrimination or the learned non-reward tests, more 5-HT$_{2C}$R KO (N = 4) than WT animals (N = 1) failed to complete the perseverance test ($x^2 = 4.2$, $p < .05$). Further, more 5-HT$_{2C}$R KO mice (N = 4) than WT mice (N = 1) failed to complete the full reversal test, although this difference remained non-significant ($x^2 = 2.8$, p = ns).

Trials and responses. 5-HT$_{2C}$R KO animals required more trials ($F_{1,31} = 6.1$, $p < .05$) and made more incorrect responses to criterion in the initial spatial discrimination (Table 7.3; $F_{1,31} = 6.1$, $p < .05$). There was also a trend for 5-HT$_{2C}$R KO animals to perform worse in the subsequent full reversal test, showing increased trials and incorrect responses to criterion, although both these differences failed to reach significance (Fig. 7.3; trials, $F_{1,31} = 4.0$, p = ns; incorrect, $F_{1,31} = 3.7$, p = ns).

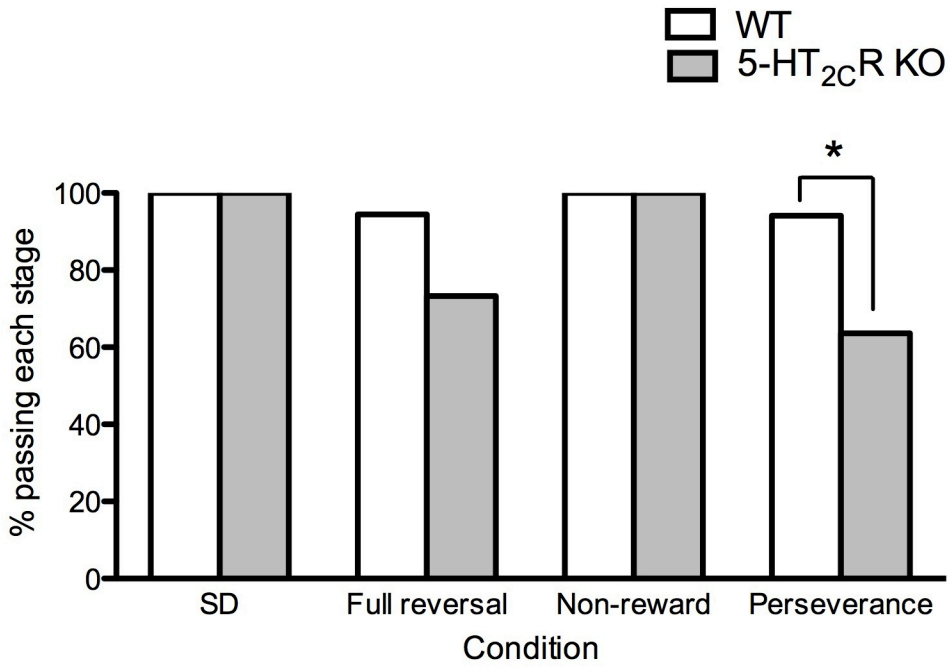

Figure 7.2. Attrition at each learning stage of WT and 5-HT_{2C}R KO animals in the maze procedure. Asterisk denote group difference at $p < .05$.

5-HT_{2C}R KO mice showed deficits in the perseverance test, requiring more trials ($F_{1,31} = 5.8$, $p < .05$) and as well as making more incorrect responses to criterion compared to WT animals ($F_{1,31} = 4.4$, $p < .05$). There were no effects of genotype in the learned non-reward test (Fig. 7.3; $p \geq .20$)

To explore whether the deficits observed in the perseverance and full reversal test could be accounted for by differences within discrimination learning, the performance within these two test conditions were analysed using the initial discrimination data as covariates. When accounting for initial egocentric discrimination performance, the effect of genotype in the perseverance test remained significant (trials, $F_{1,25} = 7.6$, $p < .01$; incorrect responses, $F_{1,25} = 4.2$, $p = .05$). The genotype differences in the full reversal test could however be accounted for by the initial spatial discrimination performance (trials, $F_{1,30} = 0.6$, p = ns; incorrect, $F_{1,30} = 0.6$, p = ns).

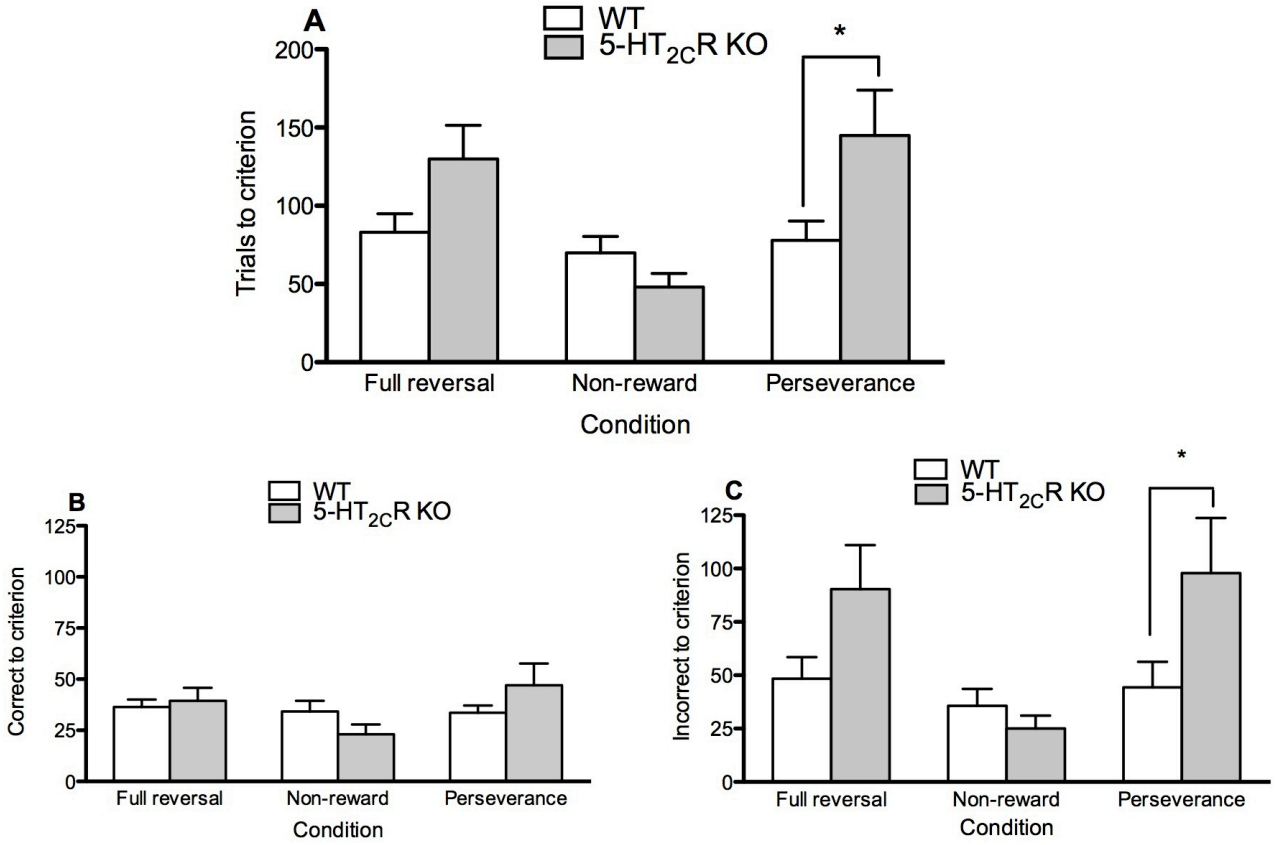

Figure 7.3. Performance of WT and 5-HT_{2C}R KO animals in the full reversal, perseverance and learned non-reward tests of the maze procedure. (A) *Trials to criterion.* Significant effect of genotype in the perseverance test ($F_{1, 26}$ = 5.8, p = .023) and near-significant effect in the full reversal test ($F_{1,31}$ = 4.0, p = .055). No effect of genotype in the learned non-reward test ($F_{1,23}$ = 1.8, p = .20). (B) *Correct responses.* No effects of genotype (p ≥ .20). (C) *Incorrect responses*. Significant effect of genotype in the perseverance test ($F_{1, 26}$ = 4.4, p = .045) and near-significant effect in the full reversal test ($F_{1,31}$ = 3.7, p = .062). No effect of genotype in the learned non-reward test ($F_{1,23}$ = 0.7, p = .40).

Table 7.3. Mean responses of WT and 5-HT_{2C}R KO animals to criterion in the spatial discrimination of the maze task.

	WT	**5-HT_{2C}R KO**	**p**
Trials	55.2 ± 7.2	85.5 ± 10.3	.019
Correct	37.2 ± 4.6	49.5 ± 5.7	ns
Incorrect	17.9 ± 3.0	35.9 ± 7.1	.019

7.4 DISCUSSION

The experiments in this chapter shows that constitutive loss of the 5-HT_{2C}R has variable effects on reversal learning, perseverance and learned non-reward depending on the nature of the task. The 5-HT_{2C}R KO mouse exhibited facilitated visuospatial operant reversal learning. This facilitation was associated with decreased interference from previously non-rewarded rather than rewarded associations, and parallels the decreased trials, correct responses and omissions observed in SB242084 treated mice using the same procedure (Chapter 6).

Conversely, the 5-HT_{2C}R KO mouse showed discrimination and perseverative impairments in the maze. These deficits were expressed as increases in trials and incorrect responses to criterion. The performance in the full reversal condition did not differ from WT controls. Generally, the performance of the 5-HT_{2C}R KO in the maze task was the opposite to that observed from SB242084 treated animals using the same procedure (Chapter 6).

7.4.1 *Operant task*

In the operant task, 5-HT_{2C}R KO mice showed facilitated performance in the full reversal and learned non-reward tests but did not differ from WTs in the perseverance test.

The facilitated reversal learning was associated with a decrease in omissions to criterion, indicating that it might be explained as a product of elevated motivation. The 5-HT_{2C}R KO mouse is hyperphagic by 5 weeks of age, and develops obesity at 5-6 months of age (Nonogaki et al., 1998; Tecott el al., 1995). These animals also show elevated break-points on progressive ratio schedules when responding for cocaine (Rocha et al., 2002). Nevertheless, 5-HT_{2C}R KO mice do not differ from WT controls in lever-press extinction for cocaine (Rocha et al., 2002), on wet-mash intake over a 90 min test session (Fletcher et al., 2009) or on progressive ratio schedules when responding for food and sucrose reinforcers (Fletcher et al., 2010). The similar latencies for trial-initiation, nosepoke-hole response and pellet retrieval in KO and WT animals further indicate that motivation is unaffected. The large number of omissions in test conditions that comprise a component of learned non-reward (the full reversal and learned non-reward tests) as opposed to test conditions including no component of learned non reward (the perseverance test) indicates, as previously suggested, that a

high number of omissions are related to associations of learned non-reward (Tait and Brown, 2008) and that a motivational explanation can be discounted.

The neurophysiological effects of reduced activity at the 5-HT_{2C}R are well explored using 5-HT_{2C}R selective compounds. Although these studies show that the 5-HT_{2C}R is implicated in the regulation of DA, 5-HT, ACh, and NA in the PFC, striatum and brainstem (Gobert et al., 2000; Boothman et al., 2006; Pozzi et al., 1998), there is little to suggest that any of these mechanisms are involved in the facilitation of reversal learning following reduced activity at the 5-HT_{2C}R (see Chapter 6, Discussion).

A possible mechanism is however suggested by electrophysiological studies in the OFC of the 5-HT_{2C}R KO mouse. In the mutant, significantly less of the AMPAr agonist quisqualate is required to activate pyramidal glutamatergic neurons within the OFC (Rueter et al., 2000), and it may be that facilitated glutamatergic signalling through the AMPAr within the OFC is the mechanism behind improved cognitive flexibility. Potentiation of AMPAr transmission through treatment with positive modulators of the AMPAr has wide-ranging pro-cognitive effects (Black, 2005). In the rat, these include a selective enhancement of LTP-formation within the PFC (Black et al., 2000), an attenuation of the attentional set-shifting deficits produced by subchronic PCP (Broberg et al., 2009), and a selective improvement of reversal learning in the bowl-digging procedure (Woolley et al., 2009). It may be that the facilitated reversal learning observed following reduced activity at the 5-HT_{2C}R is mediated by a similar mechanism. This would be in accordance with work showing that the degree of OFC activation positively correlates with reversal learning performance (O'Doherty et al., 2001). It would also be line with work showing that the 5-HTT KO rat, which show enhanced reversal learning through decreased learned non-reward (Nonkes et al., 2011), also show an OFC-overactivation (Nonkes et al., 2010). However, there are as yet no published studies looking at the effects of positive modulation at the AMPAr on the ability to overcome non-reinforced associations.

7.4.2 *Maze task*

In the maze task, the 5-HT_{2C}R KO mouse showed perseverative impairments, observed as increased attrition rates, trials to criterion, and incorrect responses to criterion in the perseverance test. 5-HT_{2C}R KO mice did not differ from controls in the full reversal test. The direction of effect of 5-HT_{2C}R KO in the learned non-reward test

of the maze procedure was similar to the effect of 5-HT_{2C}R KO in the learned non-reward test of the operant procedure.

The 5-HT_{2C}R KO mouse shows decreased anxiety, and spends more time in the open quadrant of an elevated zero maze and in the centre of an enclosed open field. These mice also spend more time investigating a novel object and have reduced latencies to enter a brightly lit novel environment (Heisler et al., 2007; Tecott et al., 1998). The anxiolytic profile could potentially affect performance in the perseverance and learned non-reward conditions by increasing the tendency to enter a novel arm. If an anxiolytically mediated increase in novelty-attraction affected the performance in this task, the prediction would be that 5-HT_{2C}R KO animals should show a concomitant facilitation of learning in the perseverance test, where the novel response option is correct, and a retardation of learning in the learned non-reward test, where the novel response option is incorrect. However, the performance of the 5-HT_{2C}R KO mice in the learned non-reward and perseverance tests in the maze task are, if anything, in the opposite direction predicted by an augmentation of novelty attraction. In sum, a 5-HT_{2C}R KO induced increase in novelty-attraction is unlikely to explain effects upon learning in the perseverance and learned non-reward tests.

In the present experiment, the 5-HT_{2C}R KO mouse also showed deficits in two-choice egocentric spatial discrimination acquisition, observed as an increase in trials and incorrect responses to criterion. Although an analysis of covariance suggested that this deficit could account for the non-significant increases in trials and incorrect responses to criterion in the full reversal test, this impairment did not account for the deficits observed in the perseverance test.

Impaired spatial learning in the 5-HT_{2C}R KO mouse has previously been observed in the water-maze and explained by altered function in the dentate gyrus (Tecott et al., 1998). The 5-HT_{2C}R is expressed in this area (Klempin et al., 2010) and spatial learning is dependent upon its integrity (Conrad and Roy, 1993; McNaughton et al., 1989; Schuster et al., 1997; Tilson et al., 1988; Walsh et al., 1986). LTP-induction in the medial perforant path of the dentate gyrus appears to be supported by 5-HT and 5-HT_{2C}R activation, as LTP-formation is suppressed both by global 5-HT depletions through intraventricular 5,7-DHT (Bliss et al., 1983) and in the 5-HT_{2C}R KO mouse (Tecott et al., 1998). Since LTP-formation within the perforant path of the dentate gyrus correlate with spatial learning in the water maze (Jeffery and Morris, 2004) and blocking LTP-formation in the medial perforant path retards water maze performance

(Davis et al., 1992), the observed retardation of discrimination learning in 5-HT_{2C}R KO mice could be related to the suppressed hippocampal LTP-formation.

The 5-HT_{2C}R KO mouse also showed marked perseverative impairments in the maze task. Constitutive loss of the 5-HT_{2C}R resulted in a selective increase in attrition rates, trials to criterion, and incorrect responses to criterion in the perseverance test. However, this perseverative impairment failed to influence learning in the full reversal test, where 5-HT_{2C}R KO mice performed as well as controls. This might be related to a slight non-significant performance facilitation in the learned non-reward test.

The perseverative impairment is in agreement with some studies of the 5-HT_{2C}R KO mouse and 5-HT_{2C}R selective compounds in paradigms of compulsive behaviour. Compulsive behaviour, as assessed in the marble-burying paradigm, is attenuated by 5-$HT_{2A/2C}$R agonists such as mCPP, DOI, Ro60-0175, Ro60-0332, and WAY161503 (Bös et al., 1997; Njung'e et al., 1991), and this attenuation can be blocked by SB242084 (Egashira et al., 2011). The selective 5-HT_{2C}R agonists Ro 60-0332 and Ro 60-0175 also reduce schedule-induced polydipsia, which has been explained in terms of compulsivity (Martin et al., 1998). It is also consistent with the 5-HT_{2C}R KO mutants compulsive-like chewing of non-nutritive clay and failure to habituate head-dipping behaviour in a cheese-board task. The observed increases in compulsive responding have been suggested to relate to the elevated striatal dopaminergic-tone seen in the 5-HT_{2C}R KO mouse (Chou-Green et al., 2003).

Hence, the perseverative deficits of the 5-HT_{2C}R KO could be related to increased dopaminergic activity in the DStr. The caudate nucleus is activated by reversal learning (Rogers et al., 2000) and electrolytic lesions retard visual maze reversal learning in the rat (Kirkby 1969). However, unlike other brain regions, there is ample evidence for a role of the caudate nucleus in rodent egocentric spatial learning. Lidocaine-induced inactivation impairs egocentric but not visuospatial discrimination in the T-maze (Packard and McGaugh, 1996). Moreover, caudate-lesions or caudate-inactivation leave allocentric two-choice spatial discrimination and working memory intact, while causing deficits in egocentric two-choice spatial discrimination, working memory and reversal learning (Brasted et al., 1997; Cook and Kesner, 1988; De Leonibus et al., 2005; Kesner et al., 1993; Mitchell and Hall, 1988; Packard and McGaugh, 1996; Palencia and Ragozzino, 2004; Potegal et al., 1969; Ragozzino et al., 2002). The 5-HT_{2C}R is strongly expressed in the caudate nucleus (Clemett et al., 2000; Eberle-Wang et al., 1997; Pazos and Palacios 1985) and the 5-HT_{2C}R KO mouse shows

elevated levels of SNc neuronal firing and DA-dialysate levels in the DStr (Abdallah et al., 2008), potentially produced by AMPAr supersensitivity in the head of the caudate nucleus (Barbeito et al., 1990; Imperato et al., 1990; Reuter et al., 2000). Selective DA-depletion in the head of the caudate nucleus retards visual reversal learning in the marmoset (Clarke et al., 2011), and it has been suggested that increased striatal dopaminergic activation may lead to perseveration (Clarke et al., 2011). In the marmoset, visual reversal learning is also impaired by amphetamine (Mason et al., 1992) and by the D_3R agonist 7-OH-DPAT (Smith et al., 1999). In the rat, visuospatial reversal learning is impaired by the $D_{2/3}R$ agonist quinpirole (Boulougouris et al., 2009) as well as by amphetamine (Idris et al., 2005). In humans, DA-agonist treated but not unmedicated Parkinson patients show impaired performance in a probabilistic reversal learning task (Cools et al., 2001; Swainson et al., 2000). Using a similar probabilistic reversal task, increased DA-activity at D_2R and D_3R in the caudate nucleus, observed as an increase in methylphenidate induced [11C]-raclopride displacement, is negatively correlated with reversal performance (Clatworthy et al., 2009). Thus, it may be that the perseverative impairment of the 5-$HT_{2C}R$ mutant could be explained by elevated DA-levels in the caudate nucleus.

Interestingly, the elevated DA-signalling within the DStr of the 5-$HT_{2C}R$ KO mouse is paralleled by similar DA-elevations in schizophrenic patients (Bird et al., 1979; Crow et al., 1979). Also, the potentiated striatal DA-release observed following amphetamine in schizophrenic patients is most strongly observed in the head of the caudate nucleus (Kegeles et al., 2006), where the 5-$HT_{2C}R$ KO mouse also shows potent glutamatergic supersensitivity (Rueter et al., 2000)

7.4.3 *General discussion*

Similar to the effects of SB242084 descibed in Chapter 6, the current chapter presents discrepant effects of 5-$HT_{2C}R$ KO on operant visuospatial and maze egocentric reversal learning, perseverance and learned non-reward. That the 5-$HT_{2C}R$ KO mouse both shows improved reversal performance in the former and increased perseveration in the latter is consistant with pharmacological data showing that both 5-$HT_{2C}R$ agonists and antagonists can attenuate (Graf et al., 2003; Tsaltas et al., 2005, 2009; Flaisher-Grinberg et al., 2008) or augment (Njung'e et al., 1991; Egashira et al., 2011; Martin et al., 1998; Bos et al., 1997) compulsive behaviour. It may be that the discrepant finding

across the two tasks is related to the tasks tapping different brain systems and subpopulations of the 5-HT_{2C}R.

In this chapter, three potential abnormalities in the 5-HT_{2C}R mutant which could affect discrimination and reversal learning performance have been discussed. The 5-HT_{2C}R KO mouse display glutamatergic pyramidal neuron supersensitivity within the OFC as well as glutamatergic striatal supersensitivity and elevated DA-levels in the caudate nucleus of the DStr. The 5-HT_{2C}R KO mouse also shows reduced LTP-induction in the medial perforant path of the dentate gyrus. These abnormalities could differentially contribute to the manipulations effect on learning in the two tasks.

The first possibility is that both the facilitated operant reversal learning and perseverative maze impairment is related to altered activity in the OFC. Thus, increased activity in the OFC through glutamatergic pyramidal neuron supersensitivity could be beneficial in the operant task, which is dependent on the integrity of the OFC, but may cause an 'overactivation' in the maze task, which shows less dependence upon the OFC (see Chapter 5). This interpretation would be in accordance with previous findings from the 5-HTT KO rat. Similar to SB242084 treated and 5-HT_{2C}R KO mice, the 5-HTT KO rat show improved reversal learning through decreased interference from learned non-reward in a two-choice auditory go/no-go task (Nonkes et al., 2011). This suggestion would also be in agreement with work showing a positive correlation of OFC activation reversal performance (O'Doherty et al., 2001).

The 5-HTT KO rat does however also show perseverative-like responding in a Pavlovian reinforcer devaluation paradigm, a deficit associated with a OFC-overactivation as indicated by elevated subregion-specific cFos-activity (Nonkes et al., 2010). OFC-hyperactivations have also been associated with psychopathologies showing reversal learning deficits, such as ADHD (Rubia et al., 2009), cocaine addiction (Bolla et al., 2003), major depression (Drevets et al., 1992), and OCD (Saxena et al., 1999; Swedo et al., 1989). Thus, the facilitated reversal learning as well as the increased perseveration could both be related to increased OFC-activity.

A second possibility is that the egocentric maze task is more dependent on the DStr and DStr DA-signalling than the operant task. As the 5-HT_{2C}R KO mouse show elevated DA-signalling within the DStr, and increased DA-levels in the caudate nucleus might be related to perseveration (Clarke et al. 2011; Clatworthy et al., 2009; Chou-Green et al., 2003), the elevated DA-levels could account for the perseverative deficits

in the maze. This could also explain the egocentric-specific retardation of spatial discrimination learning of 5-HT_{2C}R KO mice.

Lastly, it has been suggested that the reduced LTP-formation in the dentate gyrus observed in 5-HT_{2C}R KO mice produces an altered spatial search strategy in tasks of discrimination learning (Tecott et al., 1998). One possibility is that this abnormality causes increased use of allocentric cues, or an alternative decrease in the use of an egocentric response strategy. This would be in agreement with the facilitated and retarded learning, respectively, of the 5-HT_{2C}R KO mouse in the operant and maze tasks.

The idea that different subpopulations of 5-HT_{2C}Rs have different and opposing effects on discrimination and reversal learning, perseverance, and learned non-reward could be further investigated using subregion-specific infusions of SB242084. These ideas are discussed further in Chapter 9.

In sum, the 5-HT_{2C}R KO mouse displays facilitated operant visuospatial reversal learning through decreased interference from learned non-reward, but show perseverative and discrimination learning deficits in the egocentric maze task. These differences may relate to the tasks different loading upon glutamatergic- and dopaminergic-signalling in the OFC and striatum.

CHAPTER 8

5-HT2C ANTAGONISM AND VISUAL REVERSAL LEARNING IN THE RAT

8.1 INTRODUCTION

Clinical tests of cognitive functioning in schizophrenia, including reversal learning, generally use visual cues and are computer-administered (Ceaser et al., 2008; Jazbec et al., 2007; Leeson et al., 2009; Murray et al., 2008; Pantelis et al., 1997, 1999, 2004, 2009; Tyson et al., 2004). Prevalent preclinical assays are however often very different, which may result in the cost of lost predictive and construct validity and ultimately decreased translation between animal and human. One approach to increasing the validity of preclinical tasks could be to design and use assays closely resembling the neuropsychological tests used with human participants (Bussey et al., 2008).

On this view, visual touch-screen tests have been developed for cognitive testing in the rodent, paralleling the computerised visual reversal tasks used with schizophrenic patients. In such procedures, computer graphic stimuli are presented on a touch-screen and infrared beams surrounding the screen detect nosepoke responses towards stimuli. Reversal learning protocols in this set-up are sensitive to PFC-lesioning in the mouse (Brigman et al., 2008; Graybeal et al., 2011) and rat (Bussey et al., 1997) and pharmacological and genetic manipulations elevating 5-HT signalling in the mouse (Brigman et al., 2010).

Recently, a 3-stimulus serial discrimination, reversal learning, and attentional set-shifting protocol has been developed using the touch-screen apparatus and tested with rats treated with the 5-HT_{2C}R antagonist SB242084, the 5-HR_{2C}R agonist WAY163909, and the 5-HT_{2A}R antagonist M100907 (Alsiö et al., 2011). The protocol originates from a procedure initially used to assess cocaine treated vervet monkeys in the Wisconsin Test Apparatus, and involves simultaneous discriminations between three separate visual stimuli (Jentsch et al., 2002; Lee et al., 2007). In an initial discrimination, one stimulus is designated as CS+ while two other stimuli are designated as CS-. Following reversal, the previous CS+ become a CS-, and one of the previous CS-‘s becomes the new CS+. The third stimulus remains as a CS- during both discrimination and reversal learning. Animals are exposed to several of these reversals,

and novel stimuli replace the previous stimuli at the beginning of each new discrimination. In this paradigm, 1 mg/kg of SB242084 increased the number of incorrect responses to criterion in both reversal learning and attentional set-shifting, while M100907 improved reversal learning by decreasing the number of incorrect responses to criterion in reversal two only. M100907 also improved attentional set-shifting, while WAY163909 was without affect on any measure of learning (Alsiö et al., 2011).

It has been suggested that the simultaneous presentation of three stimuli have at least four added benefits over standard two-choice tasks. First, rats are prone to adopting spatial response strategies in visual tasks. These spatial biases could be reduced by rewarding repetitive responding towards a spatial location at 33% in a 3-stimulus task, relative to 50% in a 2-stimulus task. Second, relative to a 2-stimulus task, a 3-stimulus task allows less of a possibility of problem solving through configural or non-discriminatory forms of learning. Third, a 3-stimulus task is more difficult than a 2-stimulus task and therefore provides increased room for detection of pro-cognitive effects. Fourth, it makes analyses of response strategies based on perseveration and learned non-reward easier (Gilmour et al., 2012).

Moreover, the touch-screen apparatus and visual dimension may also be superior to the spatial dimension for assessing reversal learning. When using nosepoke-holes, levers or mazes, animals are trained using the same stimuli that are used for testing. In the touch-screen apparatus, interference from training on testing is minimal as animals are trained using different stimuli from those used in later testing.

The experiment in the current chapter investigated the effects of SB242084 on touch-screen visual reversal learning in the rat. Experiment 1 used a modified version of the 3-stimulus serial protocol developed by Alsiö et al. (2011) to assess rats treated with three doses of SB242084 (0.1, 0.5, and 1.0 mg/kg) in discrimination and reversal learning. Experiment 2 used a 2-stimulus task with one full reversal only in a protocol more closely resembling the operant procedure in this thesis as well as previous protocols where SB242084 has been found to improve reversal performance in the rat (Boulougouris et al., 2008; Boulougouris and Robbins, 2010).

8.2 METHOD

8.2.1 *Drug*

SB242084 (Eli Lilly, Indianapolis, IND, USA) was initially dissolved in PEG400 (Fisher Scientific, Loughborough, UK) at 20% of the final required volume, which was then made up by 10% (w/v) hydroxypropyl-beta-cyclodextrin (Sigma-Aldrich, Poole, UK). Stock solution was aliquoted and frozen at -80°C in vials of quantities required for each test day. SB242084 was administered intraperitoneally (i.p.) at doses of 0.1, 0.5, 1.0 mg/kg in a volume of 10 ml/kg 20 min prior to testing.

8.2.2 *Animals*

Experiment 1 and Experiment 2 used separate groups of 46 male Lister hooded rats (Harlan, UK). Animals were housed in groups of four within individually ventilated plastic cages containing sawdust with ad libitum access to water under a 12:12h light-dark period (lights on: 07:00h). Animals were food deprived and their body-weights were maintained at about 85% of their free feeding weight. Feeding was done each day 1h after testing. Animals were weighed each day of drug-administration, and all animals were weighed once weekly. The experiments were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

8.2.3 *Apparatus*

The experiments used 16 operant chambers (Med Associates, Georgia, VT, USA; 30 cm × 39 cm × 29 cm) placed in sound-attenuating wooden boxes with fans for the purpose of ventilation and masking external noise. A central magazine connected to an external pellet dispenser delivering 45mg sucrose pellet (Sandown Scientific, Middlesex, UK). A houselight was located near the ceiling directly above the magazine. A tone-generator was placed near the ceiling in the farther end of the chamber next to the houselight. The opposite side of the chamber contained a touch-sensitive screen (IT150-IR, Craft Data Ltd, Chesham UK; 30 cm × 22.5 cm), which was covered with a 3-hole (Experiment 1) or a 2-hole (Experiment 2) Perspex mask creating three or two response-windows (8 × 8 cm) through which the animals could respond. The response-

windows were spaced 2.5 cm apart in the 2-hole mask and 1.5 cm apart in the 3-hole mask. Responses were detected by the breaking of infrared beams lining the screen and were associated with a computer-generated tone. Below the monitor (1.5 cm) was a protruding metal 'shelf' (30 cm × 5 cm). This shelf was attached by a hinge at 90° relative to the chamber, and could swing downwards at 90° but was counter-weighed to automatically return to its position. The shelf was intended to force animals to stop and rear-up towards the stimuli head first, and thereby facilitate attention towards the stimuli and decrease accidental choices. The schedules were programmed in and run by Ratos in-house software. Animals were observed using cameras located in the ceiling of each operant chamber.

8.2.4 *Procedure*

8.2.4.1 Experiment 1: 3-stimulus visual reversal learning

Training stage 1. The training and testing was conducted in accordance with a 3-stimuli reversal learning protocol from Cambridge University (Adam Mar). Animals initially received a single 45 min session of Pavlovian and instrumental training. The session began with the delivery of a 45mg sucrose pellet paired with a 1s tone and the onset of the magazine-light. The tone and onset of magazine-light were coupled with delivery of pellet reward throughout all phases of both experiments. The tone was longer and higher in frequency than the tone associated with a touch-screen response. Collection of pellet reward caused the magazine-light to turn off, and the presentation of a white square (6 cm × 6 cm) positioned 1 cm from the bottom of the screen in one of three response-windows. Across each bin of 15-trials, the white square was presented equal number of times in each response-window but the same response-window was never used for more than two consecutive trials. Touching the white-square caused the stimulus to disappear immediately, and led to pellet delivery coupled with the 1s tone and the onset of the magazine-light. If the animal failed to touch the white-square within 30s, a pellet was delivered coupled with the 1s tone and the onset of the magazine-light, the white-square disappeared, and an omission was recorded. When the animal collected the pellet reward the magazine-light was extinguished and a new trial was initiated.

Training stage 2. As stage 1, a session began with the delivery of a pellet reward coupled with a 1s tone and the onset of the magazine-light. A nosepoke in the magazine turned the magazine-light off and caused the presentation of the white square one out of three response-windows position 2 cm from the bottom of the screen. Touching the stimulus resulted in pellet delivery, and a new trial started when the pellet reward was collected. The criterion was ≥ 100 correct responses in a single 45 min session.

Training stage 3. A 5s ITI was now introduced. Collection of pellet reward caused the magazine light to turn off and the initiation of a 5s ITI when the chamber remained dark. When the ITI had elapsed, the magazine-light began flashing at a rate of 1 Hz to distinguish it from the steady magazine-light associated with pellet delivery. A nosepoke in the magazine turned the flashing light off and initiated a new trial. The session ended after 45 min or 100 trials, and the criterion was 100 correct trials in 45 min.

Training stage 4. As stage 3, however, responses in unlit response-windows were now non-rewarded. If the animal touched an unlit response-window, the stimulus was immediately removed, the houselight was turned on for a 5s time-out, and an incorrect response was recorded. After the animal collected the pellet reward after a correct trial, or after the 5s time-out has elapsed following an incorrect trial, a 5s ITI was initiated when the chamber remained dark. After the ITI had elapsed, the magazine-light began flashing at 1 Hz and new trial started when the animal nosepoked in the magazine. The session ended after 45 min or 100 trials, and criterion was ≥ 75 correct responses in a 45 min session.

3-stimulus visual discrimination and reversal learning. After trial initiation, three different stimuli (one stimulus designated as CS+, and the other two stimuli designated as CS-‘s) were now presented in the three response-windows. The six possible spatial stimuli configurations occurred an equal number of times over every 30 trials but never reoccurred for more than two consecutive trials. If the animal touched the CS+, all stimuli disappeared and a pellet reward was delivered. If the animal touched a CS-, the stimuli were removed, the houselight switched on during a 5s time-out, and an incorrect response was recorded. After the animal collected the pellet reward after a correct trial, or after the 5s time-out had elapsed following an incorrect trial, a 5s ITI was initiated. After the ITI had elapsed, the magazine-light began flashing at 1 Hz and a new trial started when the animal nosepoked in the magazine. The session ended after 45 min or 100 correct trials. The criterion in this and all subsequent tests was ≥ 9

correct responses over 10 trials twice. Contrary to previous experiments in this thesis, the experiments in this chapter used a rolling trial-count for criterion. Thus, rather than following the completion of a 10-trial bin, a new chance of reaching criterion began as soon as an animal no longer could reach criterion over the next 10 trials.

8.2.4.2 Experiment 2: 2-stimulus visual reversal learning

In a second experiment the procedure was adapted to more closely parallel the protocols in this thesis and the protocol used by Boulougouris et al. (2007, 2008, 2009, 2010). In the test phase, animals were required to respond at the stimuli within 10s and trials per session were limited to 100. A comparison of the different parameters in the two experiments is shown in Table 8.1. The training was done using a protocol of previous reversal tasks at Eli Lilly. The initial Pavlovian and instrumental training of Experiment 1 was omitted. On the first day, animals were exposed to the apparatus for 40 min in the dark with the fan on and the magazine loaded with pellets. Magazine training began on day 2.

Training stage 1. A session began with the houselight and magazine-light on and the delivery of a pellet reward. Collection of pellet reward caused the magazine-light to go off and the initiation of a 5s ITI. After the ITI, the magazine lit-up, coupled with a 0.5s tone and pellet reward. Collection of pellet reward initiated the 5s ITI. The touchscreen remain off throughout the session. Animals were given 50 trials, and the criterion was 50 correct responses in 50 min.

Table 8.1. Comparison of experimental parameters of Experiment 1 and Experiment 2

	Experiment 1	**Experiment 2**
	3-stimulus serial reversal	*2-stimulus reversal*
Trials per session	Unlimited	100
Correct per session	100	100
Incorrect per session	Unlimited	100
Omissions	NA	> 10s
Criterion	2 ×9 correct over 10 trials twice in one session	2 ×9 correct over 10 trials twice in one session

Training stage 2. The houselight was again kept on throughout the session. A trial began with the onset of the magazine-light. A nosepoked in the magazine turned the magazine-light off and lead to the appearance of two white squares in each of the two touch-screen response-windows. Touching a white-square stimulus caused immediate removal of the stimuli, delivery of pellet reward coupled with a 0.5s tone and the illumination of the magazine-light. Pellet collection caused the magazine-light to turn off and the beginning of a 5s ITI. The magazine-light was lit-up following the 5s ITI, and a nosepoke in the magazine initiated a new trial. The criterion was ≥ 40 correct responses for two consecutive days.

Training stage 3. Now the houselight was turned off throughout the session and a single white-square stimulus was presented. The white-square was presented in one pseudorandom response-window, with the location across trials being generated by the software. Touching the white-square lead to delivery of pellet-reward, magazine-light turning on, and a 0.5s tone. Responses at in the blank second response-window were associated with a 5s time-out when the houselight was kept on. Animals received two days of training on this schedule.

Training stage 4. Animals could now omit responses. Failure to respond on the touch-screen within 10s caused the immediate removal of the stimulus and the onset of a 5s ITI when the chamber was kept dark. After this ITI, the magazine was lit-up and a trial was initiated when the animal nosepoked in the magazine. Everything else was identical to the previous training stage. Animals received a further two days of training on this schedule.

2-stimulus visual discrimination and reversal learning. After trial initiation, two stimuli (one stimulus designated as CS+, and one stimulus designated CS-) were presented in the two response-windows. The sequence of spatial stimuli configuration across trials were randomly generated by the software but remained identical across sessions. If the animal touched the CS+, all stimuli were removed and a pellet reward was delivered. If the animal touched the CS-, all stimuli were removed, the houselight was switched on for a 5s time-out and an incorrect response was recorded. If the animal failed to respond within 10s, the stimuli were removed, the 5s ITI was initiated, and an omission was recorded. After the animal nosepoked to collect the pellet reward after a correct trial, or after the 5s time-out had elapsed following an incorrect trial, a 5s ITI was initiated. After the ITI had elapsed, the magazine-light was turned on and a new trial started when the animal nosepoked in the magazine. The session ended after 45 min or 100 trials. As in Experiment 1, the criterion was ≥ 9 correct responses over 10 trials twice. When the criterion was reached, animals were challenged with a reversal on the next day.

8.2.5 *Experimental design and statistical analysis*

Experiment 1 used a serial design with new stimuli-triplets presented in each new discrimination phase (Fig. 8.1). The stimuli in each discrimination and reversal challenge were identical to the stimuli used by Alsiö et al. (2011). After completing an initial three-choice discrimination drug-free, animals were matched for trials to criterion and assigned to a drug dose (vehicle, 0.1, 0.5, or 1 mg/kg). Animals subsequently completed two more three-choice visual discriminations followed by reversals. Animals were dosed in reversal 1, reversal 2 and visual discrimination 3. Animals completed visual discrimination 1, visual discrimination 2, and reversal 3 drug-free.

In Experiment 2, animals initially completed a two-choice discrimination drug-free, and were subsequently matched for trials to criterion and assigned to a drug dose for reversal testing (vehicle, 0.1, 0.5, or 1 mg/kg). The stimuli used in Experiment 2 were chosen as rats previously have been shown to have minimal spontaneous visual biases for this stimuli-pair (Bussey et al., 2008).

The dependent variables for both experiments were trials to criterion, incorrect responses to criterion, correct responses to criterion, latency to respond towards the stimuli and latency for retrieval of pellet reward. In Experiment 1, incorrect responses

towards the previous CS+ were classified as perseverative errors, while incorrect responses toward the constant CS- were classified as learning errors. In the reversal phases of both Experiment 1 and Experiment 2, incorrect responses were further coded as early-errors and late-errors corresponding to before and after animals had reached random responding. Thus, in Experiment 1, early-errors were the number of incorrect responses made before achieving ≥ 3 correct responses over 10 trials twice. In Experiment 2, early-errors were the number of incorrect responses made before achieving ≥ 5 correct responses over 10 trials twice. Late-errors were errors made after reaching < 3 or < 5 correct responses over 10-trials twice in Experiment 1 and Experiment 2, respectively. Lastly, the proportion of responses in each response-window and towards each visual stimuli in the first and last day of each visual discrimination were analysed for spatial and visual biases. The data for the three reversal phases of Experiment 1 were analysed by 3 (within-subjects: reversal phase) × 4 (between-subjects: drug dose) mixed ANOVAs. The data for the third visual discrimination test in Experiment 1, and the reversal phase of Experiment 2, were analysed by one-way between-subjects ANOVAs with drug dose as the independent variable. Significant interactions were followed by LSD post-hoc comparisons.

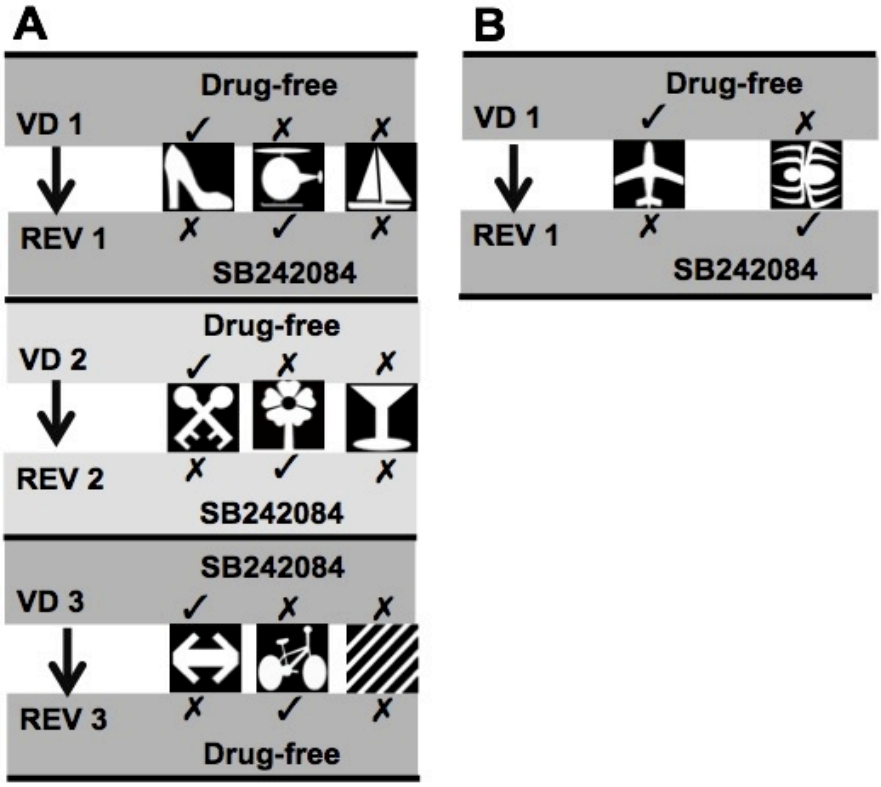

Figure 8.1. The experimental procedures of the 3-stimulus serial reversal learning (A) and 2-stimulus reversal learning (B) experiments. The stimuli were of the same contingencies for each phase of the experiments for all animals.

8.3 RESULTS

8.3.1 *Experiment 1: SB242084 and 3-stimulus visual reversal learning*

SB242084 did not affect learning in a visual discrimination, but caused a general disruption of performance in the reversal phases. SB242084 decreased stimuli-response times, consistent with previous experiments in this thesis. The SB242084-induced impairment was associated increased trials, incorrect responses and correct responses to criterion. SB242084 also increased late-errors, but decreased early-errors. Moreover, no serial-reversal effects were observed, and SB242084 had dose dependent effects in reversal 2, but not in reversal 1. Animals previously dosed with SB242084 also showed reversal impairments in the drug-free third reversal, particularly animals previously treated by the highest 1 mg/kg dose of SB242084.

There were no main effects of SB242084 when dosed in the third visual discrimination (trials: $F_{3,29} = 0.8$, p = ns, correct responses: $F_{3,29} = 0.7$, p = ns, incorrect responses: $F_{3,29} = 0.8$, p = ns, response latency: $F_{3,29} = 2.3$, p = ns, pellet latency: $F_{3,29} = 0.9$, p = ns), and drug dose did not affect total days of testing ($F_{3,29} = 0.8$, p = ns).

SB242084 did however disrupt performance in the reversal phases. There were significant main effects of reversal phase ($F_{2,58} = 105.9$, $p < .0001$) and dose ($F_{3,29} = 5.2$, $p < .01$) and a significant dose × reversal phase interaction ($F_{6,58} = 2.7$, $p < .05$) on trials to criterion (Fig. 8.2A). At 0.1 mg/kg, SB242084 increased trials to criterion in the first reversal only ($p < .05$). 0.5 mg/kg increased trials to criterion in the second reversal only ($p < .05$). 1 mg/kg increased trials to criterion in all reversals, including the drug-free third reversal ($p \leq .012$).

Similarly, there were significant main effects of reversal phase ($F_{2,58} = 90.1$, $p < .0001$) and dose ($F_{3,29} = 5.2$, $p < .01$) and a significant dose × reversal phase interaction ($F_{6,58} = 2.3$, $p < .05$) on correct responses to criterion (Fig. 8.2B). 0.1 mg/kg of SB242084 had no effects on correct responses to criterion. 0.5 mg/kg increased correct responses to criterion in the second reversal only ($p < .05$). 1.0 mg/kg increased correct responses to criterion in all reversals, including the drug-free third reversal ($p \leq .017$).

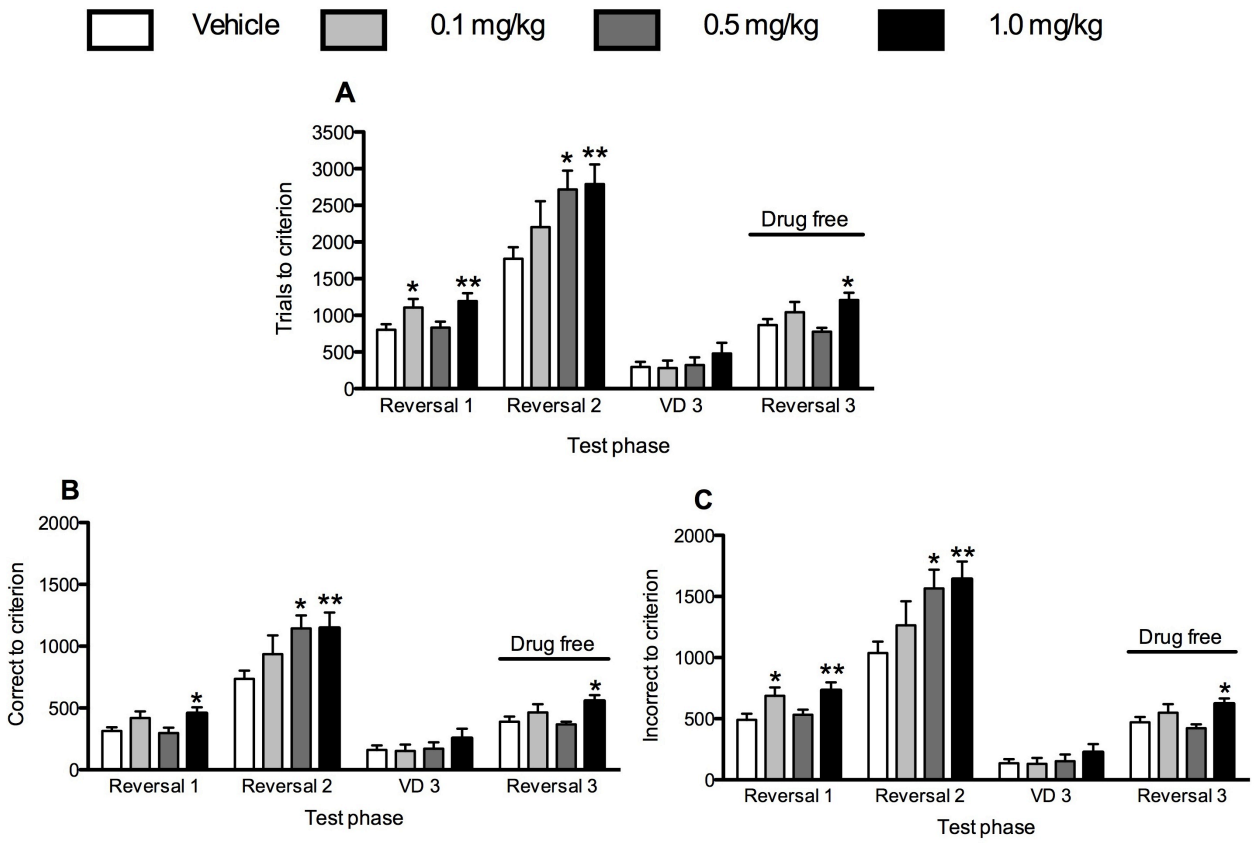

Figure 8.2. Effect of SB242084 on trials, correct, and incorrect responses to criterion in 3-stimulus serial touch-screen visual reversal learning in the rat. Asterisks denote significant difference from vehicle (*p < .05, **p < .01). (A) *Trials to criterion.* Main effects of reversal phase ($F_{2,58}$ = 105.9, p < .0001) and dose ($F_{3,29}$ = 5.2, p = .004) and dose × reversal phase interaction ($F_{6,58}$ = 2.7, p = .02). (B) *Correct responses.* Main effects of reversal phase ($F_{2,58}$ = 90.1, p < .0001) and dose ($F_{3,29}$ = 5.2, p = .005) and dose × reversal phase interaction ($F_{6,58}$ = 2.3, p = .046). (C) *Incorrect responses.* Main effects of reversal phase ($F_{2,58}$ = 117.3, p < .0001) and dose ($F_{3,29}$ = 5.6, p = .004) and dose × reversal phase interaction ($F_{6,58}$ = 3.0, p = .013).

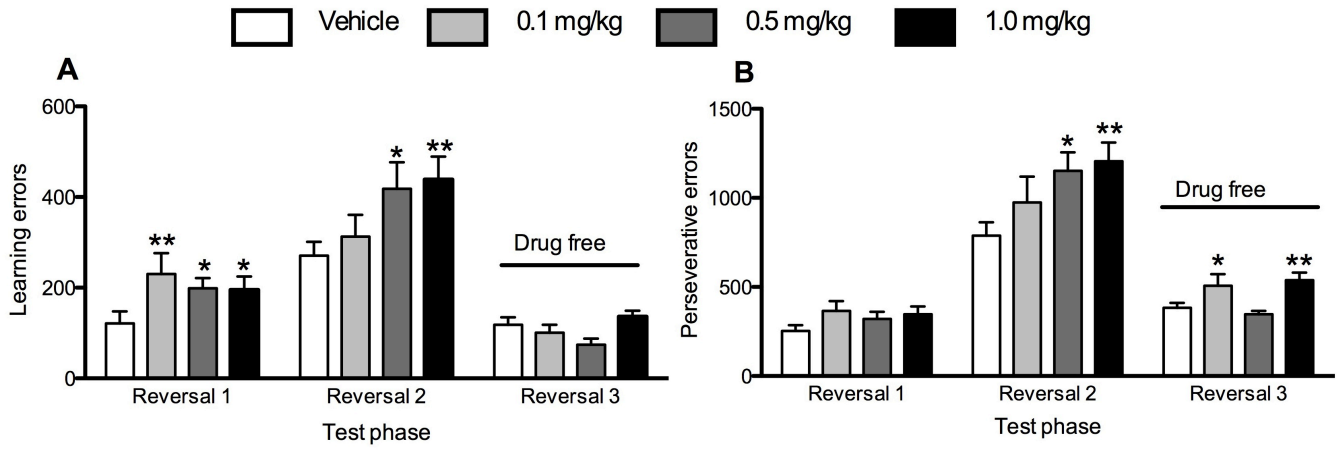

Figure 8.3. Effect of SB242084 on learning errors and perseverative errors to criterion in 3-stimulus serial touch-screen visual reversal learning in the rat. Asterisks denote significant difference from vehicle (*p < .05, **p < .01). (A) *Learning errors.* Main effect of reversal phase ($F_{2,58}$ = 93.3, p < .0001) and dose × reversal phase interaction ($F_{6,58}$ = 3.9, p = .002). (B) *Perseverative errors.* Main effects of reversal phase ($F_{2,58}$ = 153.1, p < .0001) and dose ($F_{3,29}$ = 4.1, p = .015) and a significant dose × reversal phase interaction ($F_{6,58}$ = 3.0, p = .014).

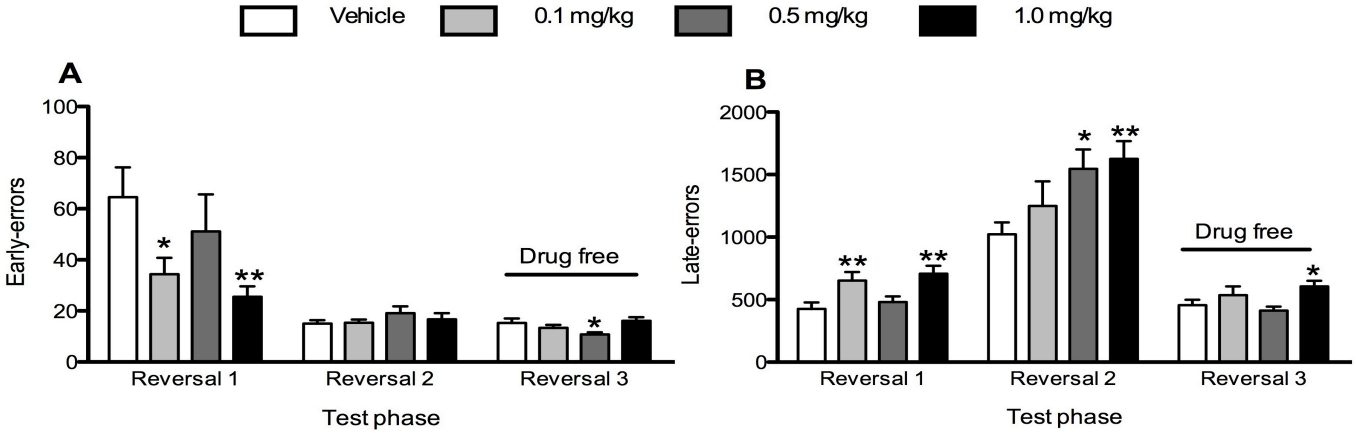

Figure 8.4. Effect of SB242084 on early-errors and late-errors in 3-stimulus serial touch-screen visual reversal learning in the rat. Errors made before achieving ≥ 3 correct responses over 10 trials twice were coded as early-errors, and errors made after achieving < 3 correct responses over 10 trials twice were coded as late-errors. Asterisks denote significant difference from vehicle (*p < .05, **p < .01). (A) *Early errors.* Significant reversal phase ($F_{2,58}$ = 31.4, p < .0001) and dose × reversal phase interaction ($F_{6,58}$ = 3.5, p = .005). (B) *Late errors.* Significant reversal phase ($F_{2,58}$ = 120.4, p < .0001) dose ($F_{3,29}$ = 6.0, p = .003) and dose × reversal phase interaction ($F_{6,58}$ = 2.9, p = .015).

There were also significant main effects of reversal phase ($F_{2,58} = 117.3$, $p < .0001$) and dose ($F_{3,29} = 5.6$, $p < .01$) and a significant dose × reversal phase interaction ($F_{6,58} = 3.0$, $p < .05$) on incorrect responses to criterion (Fig. 8.2C). 0.1 mg/kg of SB242084 increased incorrect responses in the first reversal only ($p < .05$). 0.5 mg/kg increased incorrect responses in the second reversal only ($p < .05$). 1 mg/kg of SB242084 increased incorrect responses to criterion in all reversals, including the drug-free third reversal ($p \leq .024$).

On learning errors to criterion (Fig. 8.3A), there was a significant main effect of reversal phase ($F_{2,58} = 93.3$, $p < .0001$) and a significant dose × reversal phase interaction ($F_{6,58} = 3.9$, $p < .01$). 0.1 mg/kg of SB242084 increased learning errors to criterion in the first reversal only ($p < .01$). 0.5 mg/kg increased learning errors to criterion in the first and second reversals ($p < .05$). Similarly, 1.0 mg/kg increased learning errors in the first ($p < .05$) and second ($p < .01$) reversals. There were no effect of SB242084 on learning errors to criterion in the drug-free third reversal.

On perseverative errors to criterion (Fig. 8.3B), there were significant main effects of reversal phase ($F_{2,58} = 153.1$, $p < .0001$) and dose ($F_{3,29} = 4.1$, $p < .05$) and a significant dose × reversal phase interaction ($F_{6,58} = 3.0$, $p < .05$). SB242084 failed to affect perseverative errors to criterion in the first reversal. 0.1 mg/kg of SB242084 increased perseverative error in the third drug-free reversal only ($p < .05$). 0.5 mg/kg increased perseverative errors in the second reversal only ($p < .05$). 1 mg/kg increased perseverative errors in the second reversal ($p < .01$) as well as the drug-free third reversal ($p < .01$).

Next, incorrect response before (early-errors) and after (late-errors) achieving random responding was analysed for the three reversals. SB242084 decreased the number of early-errors, but increased the number of late-errors. For early-errors (Fig. 8.4A), there was a significant main effect of reversal phase ($F_{2,58} = 31.4$, $p < .0001$) and dose × reversal phase interaction ($F_{6,58} = 3.5$, $p < .01$). In reversal one, SB242084 decreased the number of early errors at 0.1 mg/kg ($p < .05$) and at 1 mg/kg ($p < .01$). 0.5 mg/kg decreased early-errors in the drug-free reversal three only ($p < .05$). For late-errors (Fig. 8.4B), there was significant main effects of reversal phase ($F_{2,58} = 120.4$, $p < .0001$) and dose ($F_{3,29} = 6.0$, $p < .01$) and a dose × reversal phase interaction ($F_{6,58} = 2.9$, $p < .05$). 0.1 mg/kg of SB242084 increased late-errors in the first reversal only ($p =$

.01). 0.5 mg/kg increased late-errors in the second reversal only ($p < .05$). 1 mg/kg increased late-errors in all three reversals ($p \leq .025$).

There was also a significant main effect of reversal phase ($F_{2,58} = 3.6$, $p < .05$) and a significant dose × reversal phase interaction ($F_{6,58} = 5.2$, $p < .0001$) on stimuli response latencies (Fig. 8.5A). 0.1 mg/kg and 0.5 mg/kg of SB242084 decreased response latencies in the second reversal ($p < .05$). 1 mg/kg of SB242084 decreased response-latencies in the first and second reversals ($p < .05$). There were no effects of dose in the third drug-free reversal. SB242084 failed to affect pellet retrieval latencies (Fig. 8.5B; $p \geq .24$).

Animals previously treated with SB242084 were impaired in the drug-free third reversal, and the impairment was most apparent in animals previously treated with 1 mg/kg. This deficit was further explored through a separate one-way ANOVA using performance when dosed in the third visual discrimination as a covariate. Prior discrimination performance could not account for this reversal deficit, with the 1 mg/kg group still showing a significant increase in trials ($p < .05$) and correct responses to criterion ($p < .05$). However, the effect on incorrect responses to criterion fell just above significance ($p = .07$).

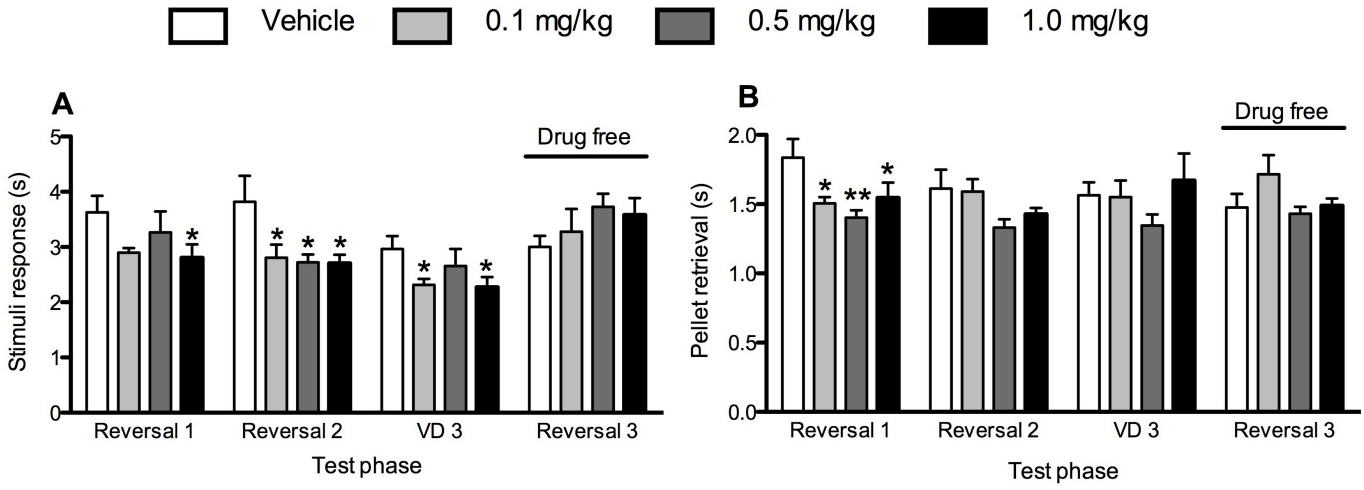

Figure 8.5. Effect of SB242084 on stimuli response and pellet retrieval latencies in the 3-stimulus serial touch-screen visual reversal learning task in the rat. Asterisks denote significant difference from vehicle (*$p < .05$, **$p < .01$). (A) *Stimuli response latencies.* Main effect of reversal phase ($F_{2,58} = 3.6$ $p = .034$) and significant dose × reversal phase interaction ($F_{6,58} = 5.2$ $p < .0001$). (B) *Pellet retrieval latencies*. No effect of dose or dose × reversal phase interaction ($p \geq .24$).

8.3.2 *Experiment 2: SB242084 and 2-stimulus visual reversal learning*

In a similar way to its effect to its effect in the 3-stimulus experiment, SB242084 also impaired 2-stimulus reversal learning. The impairment was associated with increased trials, correct responses, and late-errors to criterion, as well as decreased early-errors and decreased stimuli response and pellet retrieval latencies.

On trials to criterion, there was no overall effect of dose (Fig. 8.6A; $F_{3,42} = 2.4$, p = ns) but a significant dose linear effect ($F_{1,42} = 7.1$, $p = .01$) with trials to criterion increasing with increasing dose. On correct responses to criterion (Fig. 8.6B), there was a significant effects of dose ($F_{3,42} = 3.4$, $p < .05$) as well as a significant dose linear effect ($F_{1,42} = 10.1$, $p < .01$). Only 1.0 mg/kg of SB242084 increased the number of correct responses to criterion relative to vehicle treated controls ($p < .05$). There were no effects of SB242084 on incorrect responses to criterion (Fig. 6C; $F_{3,42} = 0.1$, p = ns) or omissions to criterion (Fig. 6D; $F_{3,42} = 0.9$, p = ns).

On early-errors (Fig. 8.6E), there was no effect of dose ($F_{3,42} = 2.2$, p = ns) but a significant dose linear effect ($F_{1,42} = 5.8$, $p < .05$), with early-errors decreasing with increasing dose. On late-errors (Fig. 8.6F), there was a significant effect of dose ($F_{3,42} = 2.9$, $p < .05$) as well as a dose linear effect ($F_{1,42} = 8.1$, $p < .01$). 0.5 mg/kg and 1 mg/kg of SB242084 increased the number of late-errors relative to vehicle-treated controls ($p < .05$).

There was also a significant main effect of dose on stimuli response latencies (Fig. 8.6G; $F_{3,42} = 5.7$, $p < .01$), with all doses decreasing the time taken to respond ($p \leq .01$). There was also a significant effect of dose on pellet retrieval latencies (Fig. 8.6H; $F_{3,42} = 3.8$ $p < .05$), with 0.5 mg/kg ($p < .05$) and 1 mg/kg ($p < .01$) of SB242084 decreasing the time taken for collection of pellet reward.

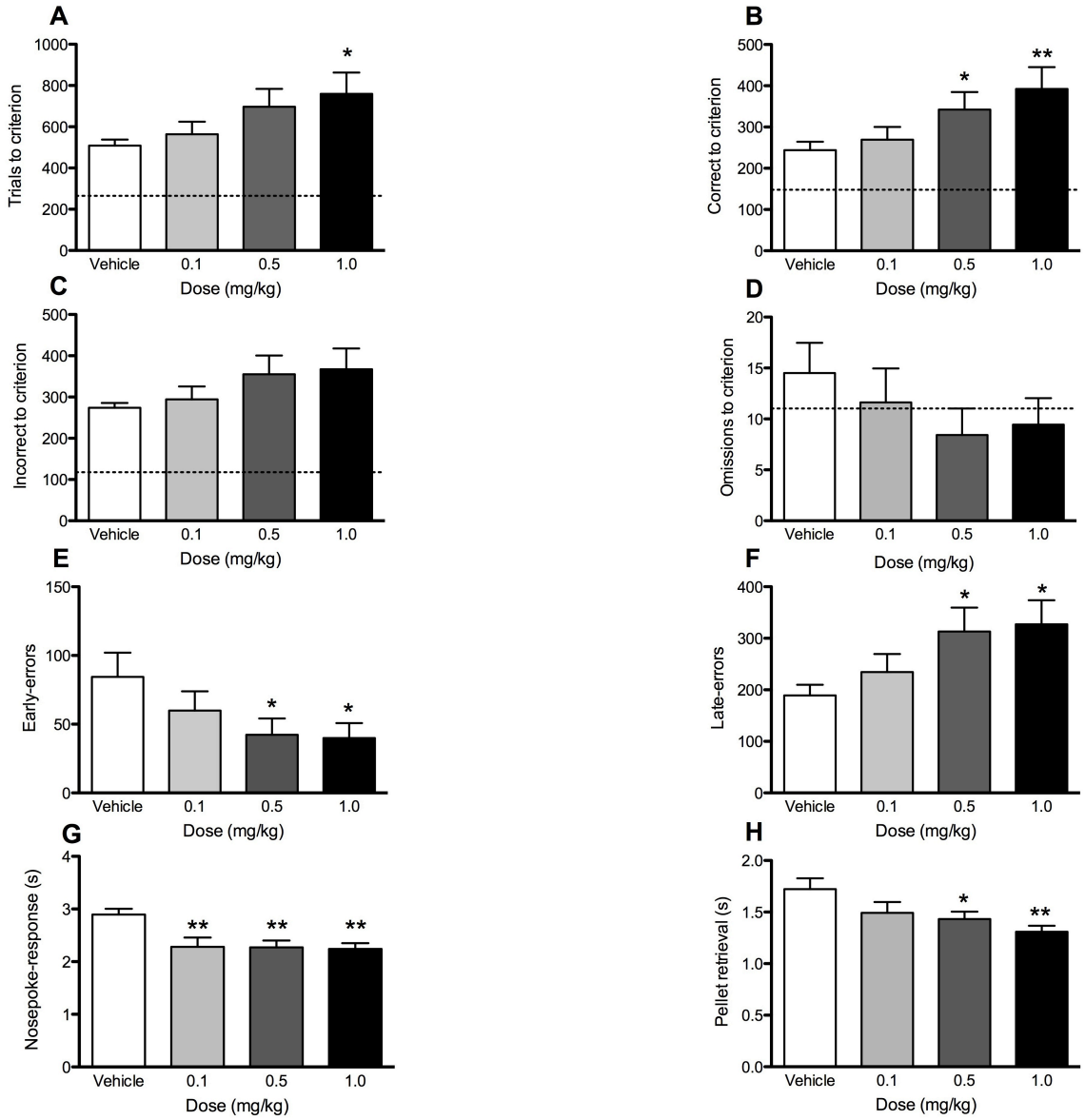

Figure 8.6. Effect of SB242084 on 2-choice touch-screen visual reversal learning in the rat. Asterisks denote significant difference from vehicle (* $p < .05$, ** $p < .01$) Broken line represents mean performance in the visual discrimination phase. (A) *Trials to criterion*. No effect of dose ($F_{3,42} = 2.4$, $p = .079$) but a significant dose linear effect ($F_{1,42} = 7.1$, $p = .01$). (B) *Correct responses.* Significant dose ($F_{3,42} = 3.4$, $p = .03$) and dose linear effects ($F_{1,42} = 10.1$, $p = .003$). (C) *Incorrect responses.* No dose ($F_{3,42} = 0.1$, $p = .95$) or dose linear effects ($F_{1,42} = 0.2$, $p = .66$). (D) *Omissions.* No dose ($F_{3,42} = 0.9$, $p = .45$) or dose linear effects ($F_{1,42} = 2.0$, $p = .16$). (E) *Early-*errors. No effect of dose ($F_{3,42} = 2.2$, $p = .10$) but a significant dose linear effect ($F_{1,42} = 5.8$, $p = .02$). (F) *Late-errors.* Significant dose ($F_{3,42} = 2.9$, $p = .045$) and dose linear effects ($F_{1,42} = 8.1$, $p = .007$). (G) *Stimuli response latencies.* Significant effect of dose ($F_{3,42} = 5.7$, $p = .002$). (H) *Pellet retrieval latencies.* Significant effect of dose ($F_{3,42} = 3.8$, $p = .016$).

8.3.3 *Spatial and visual biases*

In Experiment 1, animals showed a consistent spatial bias towards the central response-window throughout the three visual discriminations (Table 8.2). In visual discrimination 1, animals had an initial bias to respond towards the incorrect Helicopter stimulus. In visual discrimination 2 and 3, animals had biases for the correct Key and Arrow stimuli, respectively. Relative to Experiment 1, animals in Experiment 2 had limited or no overall spatial or visual biases (Table 8.3).

Table 8.2. Spatial and visual biases on the first and last day of visual discrimination 1 to 3 in Experiment 1. The correct stimulus is shown in the central column (bias > 33%).

	Visual discrimination 1		
	Left	**Centre**	**Right**
First day	31%	**55%**	13%
Last day	29%	**48%**	22%
	Boat	**Shoe**	**Helicopter**
First day	27%	21%	**41%**
Last day	29%	**59%**	11%
	Visual discrimination 2		
	Left	**Centre**	**Right**
First day	20%	**57%**	23%
Last day	28%	**42%**	24%
	Flower	**Key**	**Goblet**
First day	35%	**41%**	24%
Last day	28%	**56%**	10%
	Visual discrimination 3		
	Left	**Centre**	**Right**
First day	33%	**43%**	23%
Last day	35%	**42%**	23%
	Bike	**Arrow**	**Zebra**
First day	35%	**54%**	11%
Last day	30%	**60%**	10%

Bold denote significant biases ($p < .01$)

Table 8.3. Spatial and visual biases on the first and last day of the visual discrimination in Experiment 2. The correct stimulus was the plane (bias > 50%)

	Visual discrimination 1	
	Left	**Right**
First day	45%	55%
Last day	43%	**57%**
	Plane	**Spider**
First day	53%	47%
Last day	**70%**	30%

Bold denote significant biases ($p < .05$)

8.4 DISCUSSION

Using the visual touch-screen procedure, SB242084 was found to impair performance in both 3-stimulus serial reversal learning and 2-stimulus reversal learning in the rat. These deficits were expressed as increased trials, correct and incorrect responses to criterion. SB242084 decreased early-errors to criterion, but increased late-errors to criterion, as well as inducing faster response latencies and pellet retrieval times. Together, this suggests that SB242084 impairs performance by elevating motor impulsivity. It may be that the touch-screen apparatus has a greater sensitivity to manipulations of impulsivity than other protocols used in this thesis.

8.4.1 *3-stimulus and 2-stimulus visual reversal learning*

The 3-stimulus reversal task has been suggested to have four added benefits over traditional 2-stimulus tasks. First, as rodents are prone to adopting spatial response strategies when challenged with visual discriminations, adding a third stimulus could attenuate spatial biases by lowering reinforcement rates for repetitive responding towards a specific spatial location (Gilmour et al., 2012). Thus, spatially biased responding is rewarded at 50% in a 2-stimulus task, but only at 33% in the 3-stimulus

task. Converse to this prediction, greater spatial biases were observed in the 3-stimulus task (Table 8.2) than in the 2-stimulus task (Table 8.3). One potential explanation is that spatial biases are more readily determined by task difficulty than spatial reinforcement rates. Thus, with increasing task demands, animals revert to the use of spatial response strategies. A further possible method for decreasing reinforcement rates for spatial biases without using a third stimulus would be to use two stimuli randomly appearing in two of three available response-windows.

Next, a 3-stimulus task should allow less of a possibility of adopting configural or non-discriminatory response strategies. It is difficult to interpret if animals in the 3-stimulus task use less configural approaches than animals in the 2-stimulus task. However, the stimuli biases in Tables 8.2 and 8.3, would suggest that non-discrimination based responding may be present in the 3-stimulus task. Animals show high and low responding towards the non-rewarded Helicopter stimulus on the first and last day, respectively, of visual discrimination 1. In the same discrimination, responses towards the second non-rewarded Shoe stimulus are largely unaffected between the first and last day. This suggests that animals primarily learn to avoid the Helicopter stimulus, and that animals may reach criterion by other strategies than discriminating between the three stimuli.

In visual discriminations 2 and 3 of Experiment 1, rats directed most responses towards two of the three available stimuli, effectively making these discriminations 2-choice rather than 3-choice (Table 8.2). Following trial initiation, animals either quickly turn to the right, placing their paws on the shelf between the central and left visual stimuli, or quickly turn to the left placing their paws on the shelf between the central and right visual stimuli. Discrimination learning therefore often involves only two of the three available response-windows, and two of the three visual stimuli, as animals seldom stretch out to view the third distant response-window and stimuli.

This is also the likely cause of the low numbers of early-errors in in the second and third reversals of Experiment 1 (Figure 8.4). As animals respond towards two of the three stimuli, one of which is rewarded, they quickly reach a level of random responding. In order to avoid stimuli biases in the 3-stimulus task, further studies investigating stimuli-triplets that are equally discriminable are required, as previously has been done with stimuli-pairs (Bussey et al., 2008).

Moreover, it has been suggested that the 3-stimulus task adds task difficulty with the benefit of increased room for detection of compounds cognitive enhancing effects.

The current results suggest that this is dependent on the stimuli presented. As the correct Helicopter stimulus in the first reversal of the 3-stimulus task is the most easily discriminated, the difficulty of this reversal do not differ greatly from the difficulty of the reversal in the 2-stimulus task. However, the second reversal is more difficult, presumably as the correct Flower stimulus is difficult to discriminate from the incorrect Key stimulus, as well as a possible bias for responding towards the incorrect Key stimulus. Again, work is required to determine triplets of stimuli that are equally discriminable. Of further note is that added task difficulty has effects beyond leaving room for cognitive improvement, as it also can alter the functional role of brain-regions involved in reversal learning, such as the OFC (McDonald et al., 2007; Kim and Ragozzino et al., 2005).

Lastly, the primary reason for using three rather than two stimuli is that it should give a better discernment of stimulus perseveration versus stimulus avoidance (Gilmour et al. 2012). However, as both previously non-rewarded but now rewarded, previously rewarded but now non-rewarded, and stimuli remaining non-rewarded, are presented simultaneously within each trial, there are few indications of responses being guided by stimuli avoidance versus stimuli approach strategies.

It should be noted that others have suggested that responses towards the third consistently non-rewarded stimulus provides a control for random responses that would normally occur during a search for an alternative response strategy, while responses towards the previously rewarded but now non-rewarded stimulus are strictly perseverative (Lee et al., 2007). In view of this, adding a third stimulus gives a better measure of perseverative responding with little consideration of learned non-reward. Nevertheless, as three stimuli of previously acquired contingencies are presented simultaneously, any one of them may still guide stimulus response selection.

The touch-screen visual dimension has previously been used to assess perseverance and learned non-reward by replacing the previously correct or incorrect stimuli across reversal trials in the marmoset (Clarke et al., 2007). It may be that this approach is superior to the 3-stimuli simultaneous procedure used in the current chapter for testing perseverance and learned non-reward. The protocol used by Clarke et al. (2007) to test perseverance and learned non-reward in the visual dimension also carries an advantage over the spatial dimension. In the spatial dimension, stimuli occur on a continuum. For example, the left nosepoke-hole is only left in relation to the right nosepoke-hole. This is not relevant in the touch-screen apparatus when visual stimuli

are used.

8.4.2 *Effect of SB242084 on visual reversal learning in the rat*

SB242084 did not affect performance when administered in the visual discrimination phase of Experiment 1 but impaired the performance in the reversal phases of both experiments. The lack of effect in the third visual discrimination may be related to stimuli biases. As animals show a bias towards the correct Arrow stimulus in the third visual discrimination, the few trials required to reach criterion may mask any effects of SB242084.

SB242084 impaired most indices of learning in the reversal phases, including trials to criterion, incorrect responses to criterion and correct responses to criterion. In the 3-stimulus task, SB242084 increased both perseverative and learning errors to criterion, and these impairments were associated with faster response times and pellet collection latencies. SB242084 treated animals also made fewer early-errors, but more late-errors. This suggests that SB242084 induces random responding within these tasks. It also indicates that the SB242084-induced impairment not primarily relates to deficits in overcoming previous associations of reward or non-reward, but is rather a product of impulsive responding.

In the 5-CSRTT, SB242084 causes elevated motor impulsivity observed as increased premature responding in both rats and mice. Doses of 0.1 mg/kg and 0.5 mg/kg increase premature responses and decrease correct response latencies (Winstanley et al., 2004) as well as decreasing omissions and reward retrieval latencies in the rat (Fletcher et al., 2007; Higgins et al., 2003). Although the effects are more modest in the mouse, 0.3 mg/kg of SB242084 increase premature responses at longer ITIs (Fletcher et al., 2007). These effects are believed to be related to the facilitating effects of SB242084 on mesoaccumbal DA-signalling (Fletcher et al., 2007).
It is not however not clear if an SB242084-induced elevation of motor impulsivity can be dissociated from an SB242084-induced elevation in activity. Premature responses in the 5-CSRTT are always coupled with decreased response latencies and/or pellet retrieval latencies (Winstanley et al., 2004; Higgins et al., 2003; Fletcher et al., 2009). Moreover, SB242084 has dose-dependent effects on activity levels that overlap and match the dose-dependent effects of SB242084 on impulsive responding in the 5-CSRTT (Fletcher et al., 2009). Also, both the SB242084-incuded elevation of impulsivity and the SB242084-induced hyperactivity are believed to be caused by the

increased DA-levels in the NAc.

The touch-screen procedure may have a greater sensitivity to manipulations of impulsivity than other procedures in this thesis. After trial initiation in the touch-screen procedure, rats quickly turn around and rear by placing their paws on the shelf with their noses approximately 1-2 cm from the screen. Correct responses are typically preceded by side-to-side head movements as animals scan stimuli repeatedly before making a response. In relation to the size of the animals, the rat touch-screen chambers are smaller than the mouse nosepoke-hole chambers, and animals make faster responses in the visual touch-screen task (≈ 3s) compared to the visuospatial nosepoke-hole task (≈ 5s). It is possible that the visual procedure has a greater sensitivity to manipulations of accumbal dopaminergic levels and impulsivity, which would mask any effects of SB242084 on prefrontally mediated executive functioning and reversal learning.

Interestingly, animals previously treated with SB242084 showed altered performance in the third drug-free reversal phase of Experiment 1. Here, animals previously treated with the highest 1 mg/kg dose showed increased trials, correct, incorrect, perseverative errors and late-errors to criterion, while 0.1 mg/kg increased perseverative errors only. 0.5 mg/kg on the other hand, significantly decreased early-errors compared to vehicle treated controls. The effects on trials and correct responses to criterion were independent of the performance when dosed in the preceding third visual discrimination. The deficits were not paralleled by decreased response latencies or pellet retrieval latencies. Furthermore, these deficits were selective to perseverative-errors and late-errors, without effect on early-errors and learning-errors to criterion. This would suggest that the impaired performance in the third reversal not primarily relate to increased impulsivity.

Prior to the last drug-free reversal test, animals had received a mean 23 (± 0.9) drug treatments. These effects may therefore relate to subchronic treatments with SB242084. As yet, there are no studies of subchronic or chronic treatments with 5-HT_{2C}R selective compounds and cognition or 5-HT_{2C}R expression. In the rat, subchronic treatment with the non-selective 5-HT_{2C}R antagonist clozapine has no effect on whole brain or PFC levels of 5-HT_{2C}R mRNA in the rat (Buckland et al., 1997), although 30-50% decreases in 5-HT_{2C}R mRNA or 5-HT_{2C}R binding in the hippocampus, cerebellum, cortex (Buckland et al., 1997) and choroid plexus (Hietala et al., 1992; Kuoppamäki et al., 1993, 1994) have been observed. Behaviourally, chronic treatment with the non-selective 5-HT_{2C}R citalopram (Pälvimäki et al., 1996) has

facilitating effects on probabilistic lever reversal learning in the rat, opposite to the effect observed here (Bari et al., 2010). Also, chronic treatments with the non-selective 5-HT_{2C}R antagonist fluoxetine (Pälvimäki et al., 1996) decreases early-errors to criterion in a 2-stimulus visual touch-screen reversal task in the mouse, which is in accordance with the current effects of SB242084. However, performance in the late-phase of the experiment was unaffected (Brigman et al., 2010). Thus, although no studies have investigated the effect of 5-HT_{2C}R selective compounds on cognition and 5-HT_{2C}R functioning, chronic treatment with non-selective 5-HT_{2C}R antagonists can affect 5-HT_{2C}R expression and reversal performance.

It should be noted that the 5-HT_{2C}R also appears to be involved in LTP-formation within visual cortex. 5-HT promotes LTP-formation in areas of high 5-HT_{2C}R expression of the rat visual cortex (Kojic et al., 2000), while the 5-$HT_{2A/2C}$R antagonist ketanserin and the 5-HT_{2C}R antagonist mesulergine block LTP-induction in visual cortical slices of the rat (Komatsu, 1996; Kojic et al., 1997). These effects could potentially influence the performance in the current tasks.

Lastly, a primary advantage of the touch-screen apparatus has been suggested to be its face validity in relation to clinical tasks (Bussey et al., 2008, 2011). However, as opposed to human subjects, visual response strategies are not prepotent in the rodent. The prepotent visual strategy used by human subject is paralleled by prepotent spatial response strategies in the rodent. This is supported by the relatively high number of trials required to reach criterion in the current tasks compared to other tasks in this thesis. It may therefore be that the increased face validity of the touch-screen apparatus come at a cost of decreased predictive and construct validity.

To sum up, SB242084 impairs 3-stimulus and 2-stimulus touch-screen reversal learning in the rat. These deficits may be related to increased motor impulsivity. More work is required to determine stimuli-triplets which are equally and readily discriminable. It has been suggested that the 3-stimulus task give indications of response strategies based on perseverance and learned non-reward (Gilmour et al., 2012). However, a better method may be to replace previously correct or incorrect stimuli across reversal trials, an approach previously used in the marmoset (Clarke et al., 2007).

CHAPTER 9

DISCUSSION AND CONCLUSIONS

9.1 INTRODUCTION

In Chapter 1, two main aims of the experiments presented in this thesis were stated:

(3) Develop assays for investigating reversal learning, perseverance and learned non-reward in the mouse.

(4) Use these tasks to assess the cognitive mechanisms for altered performance after manipulations to brain systems recognised to be involved in reversal learning and relevant for human psychopathology, with a specific focus on schizophrenia.

The purpose of this chapter is to review the results presented in Chapter 4-8 of this thesis and discuss the implications of these results for the issues raised in Chapter 1. It will also discuss the implications of the results for understanding reversal learning, its clinical relevance, the limitations of the paradigms used in this thesis, as well as future directions for using reversal learning as a measure of cognitive flexibility in the rodent.

9.2 REVERSAL LEARNING, PERSEVERANCE AND LEARNED NON-REWARD

As described in Chapter 1, interpretations of the data obtained in reversal paradigms often assume that appetitive, previously rewarded, associations have exclusive control over response behaviour in tasks of cognitive flexibility. Previous work, however, suggests that non-reinforced associations also have a prominent role in discrimination and reversal paradigms. In this thesis, I have developed paradigms for assessing reversal learning, perseverance and learned non-reward in the mouse. The data presented from these paradigms support the idea that learned non-reward can have a strong influence on reversal learning in the mouse (see Table 9.1 for a summary of the experimental results in this thesis). In Chapter 4, NMDAr antagonism through acute

PCP induced a reversal learning impairment in the maze task. This impairment was associated with decreased ability to overcome both learned non-reward and perseverance. In Chapter 6, prior administration of the selective 5-HT_{2C}R antagonist SB242084 facilitated reversal learning in the operant task, and this facilitation was associated with a selective increase in the ability to overcome the effects of learned non-reward. In the maze task, SB242084 decreased perseverance but increased learned non-reward, perhaps therefore explaining the lack of observable effects on the full reversal learning. In Chapter 7, 5-HT_{2C}R KO mice showed improved reversal learning in the operant task, an improvement associated with a selective facilitation in the ability to overcome learned non-reward.

It is therefore likely that reversal learning in these procedures is sensitive to manipulations that influence either the ability to overcome previously established reinforced or well as non-reinforced associations, or a mixture of these two effects.

Table 9.1. Summary of the experimental results presented in chapters 4-8.

	Acute PCP	**OFC lesion**	**mPFC lesion**	**5-HT_{2C}R KO**	**5-HT_{2C}R antagonist**
Operant visuospatial reversal learning					
Discrimination	n/a	n/a	n/a	No effect	n/a
Full reversal	No effect	Impaired	Impaired	Improved	Improved
Perseveration	No effect	Impaired	Impaired	No effect	No effect
Non-reward	No effect	Impaired	Impaired	Improved	Improved
Maze egocentric reversal learning					
Discrimination	n/a	n/a$^{\Psi}$	n/a	Impaired	n/a
Full reversal	Impaired	No effect	n/a$^{\phi}$	No effect	No effect
Perseveration	Impaired	No effect	n/a	Impaired	Improved
Non-reward	Impaired	No effect	n/a	No effect	Impaired
Operant visual reversal learning					
Discrimination	n/a	n/a	n/a	n/a	No effect
Full reversal	n/a	n/a	n/a	n/a	Impaired

$^{\Psi}$ = OFC-lesioning retarded retention of a two-choice discrimination acquired pre-surgery. $^{\phi}$ = Animals with lesions extending both the mPFC and OFC displayed a transient impairment. n/a = not tested.

9.3 VISUOSPATIAL, EGOCENTRIC SPATIAL, AND VISUAL REVERSAL LEARNING

A common assertion is that reversal learning shows pronounced homology of functional neuroanatomy, pharmacology and transmitter systems across species and test paradigms (Bissonette and Powell, 2012; Keeler and Robbins, 2011). This position is challenged by the experiments reported in this thesis. The detailed pattern of effects failed to translate between the operant visuospatial, maze egocentric, and operant visual assays. In Chapter 4, PCP retarded maze egocentric reversal learning but had no effect on operant visuospatial reversal learning. In Chapter 5, OFC-lesions led to impaired visuospatial operant reversal learning but had no effect on egocentric maze reversal learning. In Chapters 6 and 8, SB242084 facilitated operant visuospatial reversal learning, failed to effect maze egocentric reversal learning, and impaired touch-screen visual reversal learning. In Chapter 7, 5-HT_{2C}R KO mice showed improved operant visuospatial reversal learning but did not differ from control animals in the full reversal test of the egocentric maze task, paralleling the results of studies in Chapter 6.

Relatively few studies have used mice in reversal learning, with fewer protocols using mice within the spatial dimension and fewer still using egocentric tasks. As discussed in the empirical chapters, the discrepant effects observed across the egocentric, visuospatial and visual tasks are nevertheless in accordance with the relatively common and diverse reports of dimension-specific olfactory, visual, and visuospatial reversal learning effects following manipulations of serotonergic, prefrontal and glutamatergic-systems in the rat and primate. Different protocols and sensory dimensions are likely to be sensitive to manipulations of different brain regions and transmitter systems in different species, which on a behavioural level may produce different effects on components relevant for successful reversal learning, including perseverance, learned non-reward, impulsivity and motor functioning.

9.4 PREVALENT METHODS FOR ASSESSING PERSEVERATIVE RESPONSE TENDENCIES

A common method for measuring tendencies of a perseverative response strategy is to analyse ‘early-errors’ and ‘late-errors’. Early-errors are though to represent incorrect responses made while responding is still biased towards the previous

CS+. It is believed that the number of incorrect responses made during this phase reflects the stability of the CS-US association, or perseveration. Incorrect responses made when responding no longer is biased towards the previous CS+, or late-errors, are considered to reflect general cognitive abilities related to attention and the acquisition of an alternative CS-US association. Although never made explicit, it may also be that late-errors are believed to involve learned non-reward. Hence, the prevalent view is that early-errors measures perseveration while late-errors are unrelated to perseveration.

Experiments reported in this thesis show that late-errors can be prominent in a perseverance test, while early-errors can be a feature of a leaned non-reward test. In Chapter 4, PCP increased the number of early-errors to criterion in both the learned non-reward and perseverance tests of the maze task. However in Chapter 5, OFC lesions induced a perseverative reversal impairment by increasing the number of late-errors to criterion in the operant task. This could either be due to the perseverance and learned non-reward tests lacking in construct validity, or that early-errors and late-errors fail to measure perseverance and learned non-reward.

In experiments cited in this thesis, early-errors and late-errors had been analysed in full reversal only, where previously correct and incorrect CSs are presented simultaneously across reversal trials. It is therefore plausible that both previously excitatory and inhibitory conditioning influences choice-behaviour in both early and late phases of learning. The paradigms developed in this thesis add to these protocols by analysing early-errors and late-errors in tests where the previously correct or incorrect CS is paired with novel stimulus.

If a manipulation affects late-errors in one of the tests where novelty is a feature, as well as in a full reversal test which lack novel stimuli, a fitting interpretation would be that the effects on late-errors are related to shared features of these tests and unrelated to test differences. In Chapter 5, OFC-lesioning increased late-errors to criterion in both the perseverance and full reversal tests. The effect on late-errors is therefore most likely related to perseverance and unrelated to novelty. In Chapter 4, acute PCP caused a non-condition dependant increase in the number of early-errors. The increase in early-errors is therefore most likely related to both perseverance and learned non-reward and unrelated to novelty.

These results suggest that early-errors and late-errors may not be good measures of the ability to overcome previous associations of reward or non-reward. However, early-errors and late-errors have other values, unrelated to perseveration and learned

non-reward. For example, matching patterns of responding during early and late phases of learning in two different reversal tasks may still indicate that the two tasks are solved using similar approaches or depend on similar underlying brain mechanisms.

9.5 OMISSIONS

In discrimination and reversal tasks, omissions are often included and discussed as controls for motivational or motor disturbances. Results reported in this thesis argue that omissions also can be a product of learning, most readily produced by associations of non-reward.

If omissions primarily reflect motivational or motor effects, the number of omissions should be independent of the tasks cognitive components, with similar number of omissions being made in full reversal, perseverance, learned non-reward and retention tests. Moreover, there should be a stable number of omissions across subsequent reversal tests in serial designs. Both predictions are contradicted by results in this thesis, as well as by data from other reversal assays.

In a serial operant lever reversal task, omissions decrease across subsequent reversal tests (Boulougouris et al., 2008). Similar effects were reported in Chapter 5 of this thesis. This shows that the number of omissions can be modulated by the number of reversals previously completed.

Furthermore, in a bowl-digging paradigm, omissions are more prominent in a learned non-reward test, where the previous CS- is paired with a novel CS, than in a perseverance test, where the previous CS+ is paired with a novel CS (Tait and Brown, 2008). This effect was also observed in the operant procedure of Chapters 5 and 6. This suggests that the number of omissions depends on cognitive components of the reversal test, and that a greater number of omissions can be made in tests with a component of learned non-reward. This indicates that omissions in tasks of reversal learning can be related to learning and learned non-reward.

Notably, omissions are a primary measure of learning in other tasks of cognitive flexibility, including successive reversal learning paradigms (Burke et al., 2006; McEnaney and Butter, 1969; Nonkes et al., 2011; Schoenbaum et al., 2003) and latent inhibition (Bonardi et al., 2010; Lubow, 1989). Omissions should also be considered relevant for learning in simultaneous discrimination and reversal tasks.

9.6 IMPLICATIONS FOR TASK DESIGN, VALIDITY AND TRANSLATION

Previous work, and experiments reported in this thesis, suggest that perseverance and learned non-reward constitute independent domains of reversal leaning and that the effect of a manipulation on the first cognitive domain may bear little relationship to the effect on the second cognitive domain. In Chapter 1, Discussion, it was suggested that this could have implications for how an effect on reversal learning is interpreted.

For example, many studies discussed in this thesis assess full reversal only, but regard perseverance of main interest. Yet a manipulation affecting perseverance could also be without effect or have additional effects in the opposite direction on learned non-reward, potentially masking any perseverative effects on full reversal learning. This type of effect was observed in Chapters 6 and 7. In Chapter 6, SB242084 decreased perseverance but increased learned non-reward, but had no effect on full reversal learning in the maze task. In Chapter 8, 5-HT_{2C}R KO mice showed perseverative deficits as well as a non-significant improvement in learned non-reward in the maze task, again producing no effects on full reversal learning. In both cases Occam's Razor would suggest accepting the argument that opposing effects on perseveration and learned non-reward have led to no effect in the full reversal condition.

Thus, if perseverative responding in a task of cognitive flexibility is to be assessed, a test of perseverance rather than a full reversal test where perseveration and learned non-reward are combined may be more suitable (Garner et al. 2006). This could also be viewed as comparable to the tests preceding the attentional set-shifting phase of the CANTAB ID/ED and rodent bowl-digging tasks.

A second issue discussed in Chapter 1 is that the pathological psychiatric deficit and a putative therapeutic effect of treatment may act on separate cognitive mechanisms. For example, animal models of schizophrenia often show reversal learning impairments believed to be caused by increased perseveration. A variety of compounds, including the 5-HT_{2C}R antagonist SB243213 (McLean et al., 2009) show predictive validity within these paradigms by attenuating the reversal impairment. It is therefore assumed that the compound blocks the models perseverative response deficits in such models.

However, the impairment could also be blocked by an effect within a cognitive domain unrelated to the original deficit in the model. As a specific example, in a test of

full reversal learning only, 5-HT_{2C}R antagonism may indicate efficacy against the assumed perseverative reversal learning deficits induced by the psychotomimetic PCP (McLean et al., 2009).

Additional tests of perseverance and learned non-reward, however, suggest that stand-alone 5-HT_{2C}R antagonism may have no effects or further detrimental effects on cognition in schizophrenia. 5-HT_{2C}R antagonism improves reversal learning by decreasing the interference from previously non-rewarded associations (Chapter 5), and this mechanism appear to be unrelated to pathological deficit (Elliot et al., 1995, 1998).

9.7 NEUROANATOMY AND NEUROCHEMISTRY

There is evidence for reversal learning being dependent upon the integrity of the OFC, DStr, and hippocampus, and 5-HT-signalling within the OFC and DA-signalling in the DStr. Previous work suggests that these mechanisms may have different roles in discrimination learning, reversal learning, perseverance, and learned non-reward (Burke et al., 2009; Clarke et al., 2007, 2011; Nonneman et al., 1974; Tait and Brown, 2007). The manipulations used in the thesis can have multiple and variable effects on several of these brain regions and transmitter systems.

Electrophysiological data show that the 5-HT_{2C}R KO mouse have diminished LTP-formation in the medial perforant path of the dentate gyrus, and this mechanism has been used to explain the aberrant visuospatial learning of 5-HT_{2C}R KO mice in a water maze task (Tecott et al., 1998). Specifically, the 5-HT_{2C}R KO mouse fails to show a preference for the previous platform location when the platform is removed. Although these results cannot be inferred as demonstrating facilitated or impaired learning, it has been suggested that the 5-HT_{2C}R KO mouse may show an altered spatial search strategy due to reduced LTP-formation in the dentate gyrus (Tecott et al., 1998). A tentative possibility is that the reduced hippocampal LTP-formation causes increased use of allocentric cues, or an alternative decrease in the use of an egocentric response strategy. This would be in agreement with the facilitated and retarded learning, respectively, of the 5-HT_{2C}R KO mouse in the operant and maze tasks.

A second interpretation of the data from Tecott et al. (1998) is that 5-HT_{2C}R KO mice more readily investigates previously non-reinforced areas when the previously reinforced spatial location becomes non-reinforced. The behaviour of the 5-HT_{2C}R KO

mouse in the water maze task would therefore be analogous to the mutants improved operant reversal learning and decreased learned non-reward observed in Chapter 7.

However, there is currently little evidence for a role of hippocampal 5-HT or hippocampal 5-HT receptors in cognitive flexibility, and any abnormality in this area may be unrelated to the behaviours of the 5-HT_{2C}R KO mouse in the water maze and the current maze and operant tasks. Nevertheless, as the integrity of the hippocampus is necessary for reversal learning (Nonneman et al., 1974), coupled with a role of hippocampal 5-HT in latent inhibition (Cassaday et al., 1993b) and the involvement of the 5-HT_{2C}R in hippocampal LTP-formation associated with spatial learning (Jeffrey and Morris, 2004), further assessment of its role in cognitive flexibility is warranted. This would be most readily done using subregion-specific infusion of 5-HT subtype-selective compounds in a reversal paradigm.

Reversal learning is also dependent upon the DStr and DA-signalling in the DStr. Previous work in the rodent suggests that the DStr has limited or no involvement in allocentric spatial learning but is prominently involved in egocentric spatial learning, including egocentric reversals (e.g., Cook and Kesner 1988; Kesner et al. 1993; Mitchell and Hall 1988; Palencia and Ragozzino 2004; Ragozzino et al. 2002). This would indicate that DStr perturbations have greater consequence for learning in the maze task than in the operant task. In this region, increased DA-levels are seen in pathologies associated with reversal learning deficits, such as schizophrenia (Kegeles et al., 2006; Owen et al., 1978; Crow et al., 1978, 1979) and may cause perseverative impairments in the primate (Clarke et al., 2010; Clatworthy et al., 2005). This would be in agreement with the currently observed egocentric perseverative impairments of 5-HT_{2C}R KO and acutely PCP-treated mice (see also Abdallah et al., 2008; Carboni et al., 1989).

Thus, increased DA-signalling in the DStr is produced by constitutive loss of the 5-HT_{2C}R or PCP, it is also observed in schizophrenic patients, and it is associated with potential perseverative reversal impairments. Although little work has been done in the rodent, studies of how increased DA-signalling in the DStr could contribute to perseverative behaviours in the rat or mouse would be valuable. Based on the studies described here, an egocentric assay may be the most suitable to assess such impairments.

The most thoroughly investigated brain region in reversal learning is the OFC, which has been shown to mediate the SB242084-induced improvement of visuospatial

reversal learning (Boulougouris and Robbins, 2010). The mechanism has been speculated to be altered OFC 5-HT signalling, as the facilitating effects of OFC-specific SB242084-infusions and global 5-HT depletions are apparently contradictory (Boulougouris and Robbins 2009; Roberts, 2011). These findings are only inconsistent if one assumes that both 5-HT_{2C}R antagonism and 5-HT depletion affects reversal learning by reducing 5-HT signalling in the OFC. However, reduced activity at the 5-HT_{2C}R does not seem to affect OFC 5-HT signalling (Reuter et al. 2000). Alternatively, Chapter 7, Discussion, speculates that the prefrontal mechanism controlling for the 5-HT_{2C}R-related facilitation of reversal learning may be increased sensitivity at glutamatergic AMPAr's in the OFC.

Furthermore, other studies suggest that either increased OFC-activation (O'Doherty et al. 2003) or elevated 5-HT OFC-levels (Masaki et al. 2006) may facilitate reversal learning. However, it should be noted that these two mechanisms appear directly contradictory, as 5-HT inhibits OFC-activity (El Mansari and Blier, 1997; Rueter et al., 2000; Zghoul and Blier, 2003). The idea that increased OFC-activation facilitates reversal learning is also challenged by the increased OFC-activity observed in pathologies showing reversal impairments (Bolla et al. 2003; Drevets et al. 1992; Rubia et al. 2009; Saxena et al. 1999). Thus, although it seems likely that the facilitating effects of reduced 5-HT_{2C}R-activity on visuospatial reversal learning is due to altered activity in the OFC, it is not clear if this is due to increased or decreased activity in this region. The role of the OFC and of OFC 5-HT_{2C}Rs in reversal learning should be explored using subregion-specific SB242084-infusions in separate tests of perseverance and learned non-reward. This could be done with parallel observations of PFC c-fos mRNA activation to assess the role of this receptor subtype on prefrontal activity.

A further relevant region is the ventral striatum, and DA-signalling in the NAc. Although this region appears to have a minor role in the ability to overcome associations of reward and non-reward, it regulates locomotor activity and motor impulsivity, which could affect performance in reversal tasks. This may be most relevant for the currently observed effects of PCP in the maze task and of SB242084 in the touch-screen task. Mechanisms of altered signalling in the ventral striatum should also be considered when targeting the dorsal striatum. Interestingly, the major striatal dopaminergic abnormalities of the 5-HT_{2C}R KO mice appear to be in the dorsal striatum (Adballah et al. 2008), while the main effect of systemically administered SB242084 is in the ventral striatum (Di Matteo et al. 1999). This may be due to differences in the

effect of chronic loss of receptor function and acute antagonism or additional inverse agonist activity of SB242084. These two models could potentially be used for separate analyses of the role of the two dopaminergic pathways in cognitive flexibility.

In sum, the manipulations used in the thesis often have several and variable effects on brain mechanisms involved in discrimination learning, reversal learning, perseverance, and learned non-reward. This is most evident in the case of reduced activity at the 5-HT_{2C}R. For example, if a manipulation that reduces activity at the 5-HT_{2C}R is assessed in a paradigm most sensitive to altered accumbal functioning, impulsivity or hyperactivity effects are likely to prevail and retarded learning may be observed. However, if the paradigm predominantly assays executive functioning, DStr or prefrontal mechanisms may prevail and altered ability to overcome previously non-rewarded (OFC) or rewarded (DStr) associations could be observed.

9.8 CLINICAL RELEVANCE

As discussed in Chapter 1, impaired reversal learning has been observed in most neuropsychiatric disorders but most commonly in schizophrenia. However, conclusions regarding the relevance of the studies described in this thesis for understanding the pathology and treatment of the cognitive symptoms of schizophrenia are prevented foremost by the lack of studies assessing the specific cognitive causes of impaired reversal learning in clinical populations.

Some work has nevertheless been done within the related paradigms of attentional set-shifting, learned irrelevance, and latent inhibition. Within attentional set-shifting, schizophrenic patients show perseverative impairments without apparent deficits in learned irrelevance (Elliot et al. 1995, 1998), but often also show decreased learned irrelevance and latent inhibition when assessed in stand-alone tests (Gal et al., 2005; Lubow and Gewirtz, 1995; Schmidt-Hansen et al., 2009; Young et al., 2005). Thus, the cognitive inflexibility deficits of schizophrenia are most likely perseverative, although an additional decrease in the interference from previously non-rewarded or irrelevant associations may be present.

If this is accepted, the SB242084-induced attenuation of perseverance and augmentation of learned non-reward in the maze task would suggest that 5-HT_{2C}R antagonism may have efficacy against the cognitive deficits of schizophrenia, as the two effects opposes both deficits associated with the condition. However, the selective

SB242084-induced attenuation of learned non-reward in the operant task would indicate that 5-HT_{2C}R antagonism might have either no or even a detrimental effects in the treatment of schizophrenia, as irrelevant associations fail to contribute to patients cognitive inflexibility deficits (Elliot et al. 1995, 1998) or are already diminished in the pathology (Gal et al., 2005; Lubow and Gewirtz, 1995; Schmidt-Hansen et al., 2009; Young et al., 2005).

Similarly, the perseverative reversal impairment of the 5-HT_{2C}R KO mouse in the maze procedure suggests that constitutive loss of the receptor models the cognitive impairments of the disease. However, the attenuation of learned non-reward in 5-HT_{2C}R KO mice in the operant task suggests that this mutant strain has little relevance as a model of the cognitive deficits of the disease.

Moreover, OFC-lesioning mimics the perseverative reversal deficits of schizophrenic patient when assessed in the operant task, but does not seem to be relevant in the maze task, were it appears to be without effects.

Acute PCP mimics the perseverative deficits of schizophrenic patients when assessed in the maze task, but also causes additional deficits in learned non-reward not yet observed in schizophrenic patients. Conversely, the behaviour of PCP-treated animals in the operant task would indicate that acute NMDAr antagonism has no relevance to schizophrenia, as few effects on learning were observed.

Clearly, the clinical relevance of the experimental manipulations used in the present studies relation to the cognitive symptoms of schizophrenia can only be inferred with a better understanding of the kind of reversal learning deficit displayed by patients as well as the kind of task that carries the greatest resemblance in problem solving strategies to those used by humans.

Experiments reported in this thesis show that different tasks and training methods can have differential effects on perseverance and learned non-reward, which in turn may affect the role of involved brain regions and transmitter systems. An example is 5-HT_{2C}R antagonism. SB242084 facilitates reversal learning if the protocol primarily measures learned-non reward, as in the operant procedure, but has opposing effects on perseverance and learned non-reward if the task emphasises perseverance-related effects, as in the maze procedure. SB242084 can also impair reversal learning, especially if the task is sensitive to changes in impulsivity, as in the touch-screen procedure. For optimal translation, it might be important to match prevalent response strategies in human and animal tasks. For example, a preclinical task where response

behaviour primarily is guided by non-rewarded association is likely to have limited validity if responses in the clinical test procedure are primarily guided by reward.

9.9 LIMITATIONS AND FUTURE DIRECTIONS

The spatial tasks of reversal learning, perseverance and learned non-reward described here contain at least three confounds. The first is stimulus generalisation. In the spatial dimension, the left nosepoke-hole or left turning-direction is to the left in relation to the right nosepoke-hole or right turning-direction. This makes perseverance and learned non-reward testing in the spatial dimension susceptible to stimulus generalisation, so that all three tests might be considered tests of full reversal learning.

A further component of the tasks is the converse of generalisation, novelty. Manipulations of novelty-attraction or novelty-recognition could affect performance in the perseverance and learned non-reward tests where the now correct or incorrect CS, respectively, is novel. It should nevertheless be noted that the procedures in this thesis control for novelty though both intrinsic features of the tests and additional experiments. An effect on novelty attraction or recognition should produce opposing effects in the perseverance and learned non-reward tests. Performance in a full reversal test can also control for the effect of novelty, as no novel stimuli is introduced in this test condition. Where these measures suggested that novelty may have influenced learning, animals were tested in a further place novelty recognition and attraction test.

Moreover, when using two nosepoke-holes or two turning-directions, animals were habituated or pre-trained using the same stimuli that later figure with opposing contingencies in testing. This may cause interference additional to the contingencies learned in the initial spatial discrimination. It is also likely to be of further relevance in serial spatial protocols, where in later reversals, previous excitatory and inhibitory conditioning has taken place towards both the current CS+ and CS-.

The limited availability of stimuli in the operant visuospatial procedure also creates a discrepancy between the full reversal, perseverance and learned non-reward tests when a serial design is used (Fig. 9.1). In the third full reversal test, animals are challenged by a contingency-shift previously experienced in the first full reversal test. In the third learned non-reward and perseverance tests, however, animals are challenged with shifts not previously experienced. Thus, it may not be appropriate to make interpretations about learning in the third full reversal test in relation to learning in the third perseverance and third learned non-reward tests.

Some of these limitations could be overcome by using more complex stimuli in the visual dimension. Generalisation would be a lesser issue if using equally discriminable visual stimuli. There would be fewer limitations on the number of available stimuli. Novel stimuli can replace old stimuli in the beginning of each new visual discrimination, and animals can be trained and tested on separate stimuli. This should decrease the influence of training and previous discriminations and reversals on later testing. It also makes the discrepancies of the full reversal, perseverance, and learned non-reward tests in operant visuospatial serial designs irrelevant. Two preliminary studies were described in Chapter 8 and could provide the foundation for experiments that used the same range of experimental tests, including those for perseveration and learned non-reward, that were described in earlier chapters using visuospatial paradigms.

In Chapter 1, it was also discussed how novelty-attraction and avoidance has been assessed in attentional set-shifting (Chapter 1, Table 4). With reduced restrictions on the number of available stimuli, these types of novelty tests could also be introduced in a serial visual reversal learning protocol, as shown in Figure 9.2.

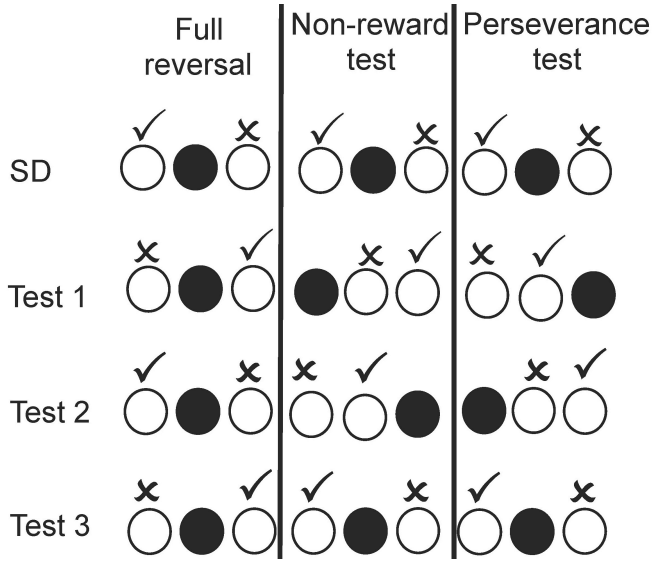

Figure 9.1. Example of contingency shifts in the operant serial reversal paradigm. In Test 1 and Test 2 of the full reversal condition, animals are challenged with novel contingency shifts. The contingency shift of Test 3 of the full reversal condition is identical the contingency shift of Test 1 of the full reversal condition. All contingency shifts in the perseverance and learned non-reward conditions are novel.

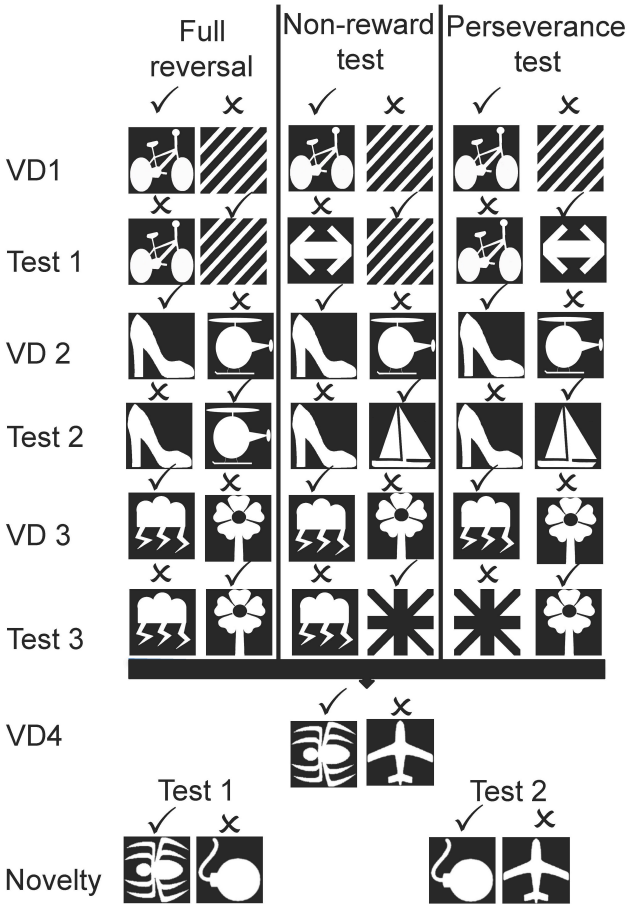

Figure 9.2. Visual between-subjects design for assessing full reversal, perseverance, learned non-reward, paralleling the visuospatial design in Figure 9.1. In a final novelty test, either the previously correct or incorrect stimulus remain of the same contingency and is paired with a novel stimulus of the opposite contingency. Enhanced novelty attraction should retard learning in novelty test 1 and facilitate learning in novelty test 2. A manipulation of perseverance should affect leaning in novelty test 1, but not novelty test 2. A manipulation of learned non-reward should affect learning in novelty test 2, but not novelty test 1.

9.10 SUMMARY

The experiments presented in this thesis have provided evidence to support the general assertions that:

1) Learned non-reward influences reversal learning in the mouse;

2) The NMDAr, 5-HT_{2C}R and PFC are involved in controlling spatial reversal learning, perseverance and learned non-reward in rodents;

3) Few effects of PFC-lesioning, and NMDAr and 5-HT_{2C}R antagonism in reversal learning generalise across visuospatial, egocentric spatial, and visual tasks.

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