Cognitive Dysfunction and Schizophrenia: Modelling Attentional Impairment with Psychotomimetics

Investigating attentional impairment and structural brain abnormalities following phencyclidine administration: Enhancing translatability between preclinical and clinical tests of attention utilising the modified 5-choice task in rats – the 5-Choice Continuous Performance Test

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Submitted for the degree of
Doctor of Philosophy

School of Pharmacy
University of Bradford
2011

To my Parents

Summary

This thesis consisted of experiments designed to explore the construct of attention and investigate the disruptive effects of psychotomimetics, with a specific focus on NMDA antagonists. Phencyclidine (PCP) was administered through a variety of treatment regimens in order to to determine the ability of inducing cognitive-specific disruptions in attentional functioning. The hypothesis that sub-chronic exposure to PCP would result in persistent attentional impairment was tested, using the 5-choice serial reaction time task (5-CSRTT). The 5-CSRTT assesses not only visuospatial attention, but also components of impulsivity, compulsivity, speed of processing and motivation. It was determined that an additional task-related intervention that increased the attentional load was required to elucidate attentional impairment following sub-chronic PCP treatment.

The ability of rats to perform the modified version of the 5-CSRTT, known as the 5-choice continuous performance test (5C-CPT), was investigated. The 5C-CPT was implemented to provide a task that may have greater analogy to the human CPT, than the original 5-CSRTT. The consequence of dopaminergic D_1 system activation was investigated. It was revealed that D_1 partial agonism improved attentional performance in a baseline-dependent manner.

Following successful acquisition of the task, it was shown that repeated PCP treatment induced cognitive disruption that was cognitive-specific, and not confounded by generalised response disruption. Furthermore, a partial attenuation of the PCP-induced performance disruption was achieved following administration of the D_1 partial agonist, SKF 38393. Moreover, sub-chronic PCP treatment was shown to impair 5C-CPT performance in the drug-free state. However, an additional challenge that further increased the attentional load was needed to elucidate a performance deficit. This highlighted that sustained attention/vigilance is sensitive to persistent impairment following sub-chronic PCP administration in a manner consistent with deficits observed in schizophrenia patients.

This prompted the investigation that tested the hypothesis that sub-chronic PCP treatment could induce enduring structural deficits in regions associated with attentional performance. Magnetic resonance imaging (MRI) was conducted, in conjunction with 5-CSRTT and pre-pulse inhibition (PPI). It was revealed that sub-chronic PCP treatment resulted in morphological brain abnormalities in brain regions associated with 5-CSRTT performance. This was coupled with deficits in sustained attentional performance following an increase in attentional load, yet PPI was unaffected. Taken together, these findings suggested sub-chronic PCP treatment impairs attentional functionality, an effect that dissociates between effortful and passive attentional processes.

Keywords

Attention, Vigilance, Schizophrenia, PCP, MRI, 5-CSRTT, 5C-CPT

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Acknowledgments

Firstly, I would like to thank Prof Jo Neill. She has not only been consistent source of support and encouragement, but the opportunities that have arisen from my time at University of Bradford have been amazing. It has been an interesting journey mostly full of ups, a couple of downs, but it wouldn't have begun, or more importantly been completed, without the help and encouragement of Prof Neill.

I would also like to thank Dr Jeff Dalley for welcoming me into the Department of Experimental Psychology for the longest '8-week' project I've ever known. My time at University of Cambridge has been invaluable and I am forever grateful.

Without the input and insight from Dr Jared Young, I don't think rats would be able to perform the 5C-CPT. His consistent and constructive advice and guidance throughout our ongoing collaboration has been thoroughly appreciated.

I would also like to thank Dr Simon Bate for navigating me through the tricky world of statistics. Without his help and advice throughout my PhD, I would no doubt still be grappling with a Student's t-test.

Dr Daniele Caprioli deserves a medal – he not only put up with my constant questioning throughout the progression and of this thesis, but his classic phrase "the problem is..." dramatically enhanced the overall quality of my work.

Further thanks are aimed at Dr Steve Sawiak and David Theobald; Steve for the construction and analysis of the MRI images and David for his technical expertise and time throughout the final phase of the project.

During my time at University of Bradford, a special thanks goes to everyone in M10 (Ben, Sam, Nagi, Laki and Majid), not only for their friendship, input and comments throughout my PhD, but also for the laughs we had at the various conferences attended over the years. All very much appreciated... Additionally, I would like to thank Haley Daniels (Bradford) and Gemini Baven (Cambridge), along with their technical staff, for providing the necessary environment to carry out my studies.

Finally, I would like to thank my family. Without their support (in every sense of the word!), not only throughout my PhD but also during my undergraduate studies, I would never have been able to accomplish this.

Publications

Full Articles

Barnes SA, Young JW, Neill JC (2011) Activation of D1 receptors can improve vigilance in rats as measured by the rodent 5-choice continuous performance test. Psychopharmacology. Under review.

Barnes SA, Young JW, Neill JC (2011) Rats tested after a washout out period from sub-chronic PCP administration exhibited impaired performance in the 5-choice continuous performance when the attentional load was increased. Neuropharmacology. doi:10.1016/j.neuropharm.2011.04.024

Neill JC, Barnes SA, Cook S, Grayson B, Idris NF, McLean SL, Snigdha S, Rajagopal L, Harte MK. (2010) Animal models of cognitive dysfunction and negative symptoms of schizophrenia: Focus on NMDA receptor antagonism. Pharmacology and Therapeutics 128(3): 419 - 432.

Abstracts

Barnes SA and Neill JC. (2010) Signal detection theory reveals sub-chronic PCP induced impairments in the 5-choice continuous performance test in the rat. Journal of Psychopharmacology. 24(3) Abstract supplement: A19

Barnes SA and Neill JC. (2010) Repeated phencyclidine (PCP) increases impulsivity in the 5-Choice Continuous Performance Test in rats. Schizophrenia Research 117 p169 doi:10.1016/j.schres.2010.02.436

Patel AI, Barnes SA, Neill JC (2008) A preliminary investigation into sub-chronic PCP induced deficits in the 5-choice serial reaction time task. Journal of Psychopharmacology 22(5) Abstract supplement: A77

List of Abbreviations

2L/min:	Flow Rate, 2 litres per minute
5-CSRTT:	Five-Choice Serial Reaction Time Task
5C-CPT:	Five-Choice Continuous Performance Test
5-HT:	5-hydroxytryptophan
6-OHDA:	6- hydroxydopamine
ACh:	Acetylcholine
ADHD:	Attention Deficit Hyperactivity Disorder
AMPA:	2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid
ANCOVA:	Analysis of Covariance
ANOVA:	Analysis of Variance
ASST:	Attentional Set-Shifting Task
β:	Beta
BDNF:	Brain Derived Neurotrophic Factor
CB:	Calbindin
***cf*:**	*(conferre)* Bring together
CL:	Correct Latency
CNS:	Central Nervous System
COMT:	Catechol-*O*-methyltransferase
CPP:	3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid
CPT:	Continuous Performance Test
CPT-IP:	Continuous Performance Test-Identical Pairs
CR:	Correct Rejection
CR:	Calretinin
CSF:	Cerebrospinal Fluid
CT:	Computerised Tomography
D':	d-prime
DA:	Dopamine
DARTEL:	Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra
DEn:	Dorsal Endopiriform Cortex
***DISC1*:**	Disrupted-in-Schizophrenia 1
DLPFC:	Dorsal Lateral Prefrontal Cortex
DNAB:	Dorsal Noradrenergic Bundle
DOPAC:	dihydroxy-O-phenyl-acetic acid

DS: Dorsal preSubiculum

DTI: Diffusion Tensor Imaging

DTNBT1: Gene encoding for dysbindin

EAAT2: Excitatory Amino Acid Transporter 2

EDS: Extra-Dimensional Shift

e.g.: *(exempli gratia)* for example

et. al.: *(et allii)* and others

EPSP: Excitatory Post-Synaptic Potential

FA: Fractional Anisotropy

FA: False Alarm

FAR: False Alarm Rate

FDR: False Discovery Rate

Fig: Figure

fMRI: Functional Magnetic Resonance Imaging

FWE: Family-wise Error

g: Gram

GABA: γ-animobutyric acid

GAD_{67}: Glutamic Acid Decarboxylase

GAT1: GABA transporter

GD: Gestation Day

GLT1: Glutamate Transporter

GM: Grey Matter

GPCR: G-protein Coupled Receptor

HR: Hit Rate

IDS: Intra-Dimensional Shift

i.e. *(id est)* that is to say

IL: Incorrect Latency

i.p. interperitoneal

ITI: Inter-trial Interval

KYNA: Kyneurenic Acid

LH: Limited Hold

LTD: Long-term Depression

LTP: Long-term Potentiation

MAM: Methylazoxymethanol

ML: Magazine Latency

MATRICS: Measurement and Treatment Research to Improve Cognition in Schizophrenia

mGluR: Metabotropic Glutamate Receptor

ml/kg: millilitre per kilogram

mg/kg: milligram per kilogram

MK-801: Dizoclipine

µm: micrometer

mPFC: Medial Prefrontal Cortex

MRI: Magnetic Resonance Imaging

NA: Noradrenaline

NIMH: National Institute of Mental Health

NOR: Novel Object Recognition

NMDA: *N*-methyl-*D*-aspartate

***NRG1*:** Gene encoding for Neuregulin 1

NS: not significant

O_2: Oxygen

OCD: Obsessive-Compulsive Disorder

OFC: Orbitofrontal Cortex

PCP: Phencyclidine

PET: Positron Emission Tomography

PFC: Prefrontal Cortex

PND: Post Natal Day

PPI: Pre-pulse Inhibition

PrL: Prelimbic Cortex

PV: Parvalbumin

PV-ir: Parvalbumin immunoreactive

RI: Responsivity Index

RL: Reversal Learning

RM: Repeated Measures

RMW: Relative Molecular Weight

ROI: Region of Interest

SAT: Sustained Attention Task

s.c.: sub-cutaneous

SD: Stimulus Duration

SDT: Signal Detection Theory

SEM: Standard Error of Mean

SI: Sensitivity Index

SKF 38393: (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diolhydrobromide, a D_1 receptor partial agonist

SPECT: Single Photon Emission Computerized Tomography

SPM: Statistical Parametric Mapping

SSC: Spiny Stellate Cell

TO: Time Out

VBM: Voxel Based Morphometry

vSD: Variable Stimulus Duration

vITI: Variable Inter-trial Interval

WCST: Wisconsin Card Sort Task

Chapter 1

General Introduction

1.1 Schizophrenia

Schizophrenia has been described as one of the worst psychiatric disorders to afflict mankind (Tandon et al. 2008). Schizophrenia has been described as such due to the progressive course, social and occupational implications and the tendency to present symptomatology during late adolescence, the time when young adults are forging a life for themselves. Schizophrenia affects approximately 1% of the general population, irrespective of gender or race, however the disease usually presents itself clinically earlier in males (late teens – early twenties) compared to females, where symptoms tend to present in their late twenties. A public misconception is that schizophrenia is a disease that results in split personalities, although the term schizophrenia literally means split (schizein) mind (phren), it was first coined by Bleuler (1908) to describe the patients detachment from reality (Fusar-Poli and Politi, 2008).

Schizophrenia consists of two main symptom domains; positive and negative (van Os and Kapur 2009). Positive symptoms refer to symptoms that are in excess of normal behaviours. These include perceptual disturbances, namely hallucinations (usually auditory but can include visual, taste or tactile hallucinations) bizarre delusions (commonly persecutory or abusive in nature) and thought disturbances that tend to result in disorganized speech (Feldman et al. 1997). The second group of symptoms are known as negative symptoms, referring to the absence of behaviours that are normally present in people of the general population. These include social withdrawal, poverty of speech, blunted affect, reduced motor activity, catatonia or stereotyped behaviour (Feldman et al. 1997). In addition to the positive and negative symptoms, schizophrenia also results in dysfunction in several areas of cognition, which is central to the disorder (Sullivan et al. 1994; Kuperberg and Heckers, 2000). Emil Kraepelin (1921) initially referred to the disorder as dementia praecox due to impairment of cognitive function occurring at such a young age (Meltzer et al. 1999), however due to discrepancies in Kraepelin's initial description, Bleuler described the

condition as schizophrenia (Fusar-Poli and Politi 2008) and is a term now used to describe the condition. Cognitive tests such as the Wisconsin Card Sorting Test, Stroop Colour Word Interference Test and the continuous performance test (CPT) have shown that patients with schizophrenia have deficits across many different domains of cognition, including, working memory, verbal learning, executive function, attention and abstraction (Siever and Davis 2004). It is generally accepted that schizophrenic patients perform, on average, 1.0 – 1.75 standard deviations below healthy people in a wide variety of cognitive tests (Gold 2004) and its been suggested that the severity of the cognitive dysfunction is closely correlated with the functional outcome of the patient (McGurk and Meltzer 2000; Green et al. 2004). Cognitive dysfunction is largely left untreated by current antipsychotic medication and therefore poses a great unmet clinical need (Marder and Fenton 2004).

Schizophrenia has a strong genetic component and although the worldwide prevalence of the disorder is approximately 1%, the prevalence increases to roughly 10% in first-degree relatives of schizophrenia patients. In dizygotic twins, the increase in risk rises to approximately 20%, whereas in monozygotic twins the probability of one twin developing the illness is around 50% if the other twin has the disorder (Gottesman and Wolfgram 1991). There are currently around 43 candidate genes, mutations in which are associated with an increased risk for the development of schizophrenia (Need et al. 2009; Insel, 2010). For example, Harrison and Owen (2003) published a review highlighting several genes that may increase the susceptibility of schizophrenia. In this review they described how the gene coding for neuregulin 1 (*NRG1)* had a significant association with increased risk for schizophrenia. *NRG1* is expressed throughout the central nervous system (CNS) and is involved in the expression and activation of neurotransmitter receptors, including the glutamate receptor (Stefansson et al. 2002). Several other genes have been implicated in the development of the disorder, genes such as *DISC 1* (a protein involved with cell growth, axonal development and neural positioning) (Ozeki et al. 2003), *COMT* (encodes for a protein

responsible for catecholamine degradation) (Williams et al. 2007) and *DTNBP1* (encodes for dysbindin and is suggested to be involved in neuronal plasticity) (Kendler 2004); however, a definitive gene relating to schizophrenia development has yet to be identified. Although it is evident that schizophrenia has a strong genetic component, faulty genes cannot fully explain the disorder as the concordance rates in monozygotic twins is only 50% (Gottesman and Wolfgram 1991). If schizophrenia were a complete genetic disorder, one would expect the concordance rates in monozygotic twins to be 100%. The fact it is not therefore indicates that a combination of both genetic and environmental factors play an important role in the aetiology of the disorder.

The National Institute of Mental Health (NIMH), in combination with educational institutions, implemented an initiative called the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS.ucla.edu) (Green et al. 2008; Kern et al. 2008; Nuechterlein et al. 2008). The MATRICS initiative was formed in an attempt to accurately identify areas of cognition that are afflicted in schizophrenia, furthering the development of new treatment strategies to improve the cognitive and social dysfunction evident in the disorder. The primary aim of the MATRICS initiative was to identify a series of clinical and preclinical test batteries which addressed the cognitive disturbances in terms of specific domains that are affected, rather than cognitive dysfunction as a whole (Marder and Fenton 2004). The MATRICS initiative implemented a series of clinical tests that identified seven separate areas of cognition that are affected to some degree in schizophrenia. These include speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, and social cognition (Nuechterlein el al. 2004; Floresco et al. 2005). Thus, the MATRICS initiative introduced an element of standardisation to cognitive assessment when determining the severity of cognitive deficits in schizophrenia, enabling meaningful comparisons to be made between studies conducted within different laboratories and attempting to enhance the translation

from animals to humans. The separation of cognitive dysfunction into distinct categories enables researchers to develop pre-clinical tests that address the specific cognitive domain with greater ease then before, and will eventually lead to the development of reliable animal models that can be used to assess drug efficacy in alleviating cognitive dysfunction.

1.2 Attention

Attention is an area of cognition that is defined as a system that allows an individual to detect, select and process relevant sensory information, while simultaneously filtering out any irrelevant stimuli from the environment, enabling an individual to attend appropriately to certain situational demands (Maunsell and Treue, 2006). Attention is not a single entity (Muir 1996), but more of a functional system that is comprised of several different cognitive constituents that operate in conjunction with one another to form a functioning attentional system. The attentional system is comprised of several specific forms of attention, which include sustained, selective and divided attention (Robbins 2002; Chudasama and Robbins 2004). Sustained attention allows the individual to maintain attention and attend to a specific task over a prolonged period. This form of attention ensures that attentional goals are upheld over a period of time (Parasuraman 1998; Sturm and Willmes 2001). Although sustained attention is sometimes also referred to as vigilance, subtle differences exist between the two constructs (Robbins, 1998) (described in more detail in chapter 4). The ability to sustain one's attention in a vigilant manner enables an individual to focus attentional processing to a specific task for a prolonged duration, facilitating the detection of relevant or salient signals from the surrounding environment that may be of importance. Parasuraman et al. (1987) stated that the difference in protocols of vigilance tasks between laboratories impose fundamental differences in how the construct is measured. Therefore, a task classification system was developed investigating the effects of four experimental dimensions and the effect they have on assessing vigilance. Signal discrimination type, event

rate, modality and source complexity were all considered (Parasuraman et al. 1987; Muir 1996) and it has been determined that the most effective method of assessing vigilance involves a task that presents successive signal and non-signal events, high event-rates, the use of low level salient signals and event asynchrony, thus producing a task assessing vigilance with a high level of power and reproducibility (McGaughy and Sarter 1993).

Selective attention enables sensory information to be given informational priority by limiting the number of sensory channels available (Muir 1996). Informational priority is important due to the finite computational capacity of the brain; therefore, selective attention is a means by which important sensory information is processed, whilst also acting as an 'attentional filter' ensuring irrelevant information is ignored facilitating the resistance of informational distraction (Muir 1996; Parasuraman 1998). A classic example demonstrating selective attention is known as the 'cocktail party effect', facilitating the focus of attentional processing to a specific conversation, whist simultaneously and selectively filtering out the background 'noise' of irrelevant conversations proposed by Broadbent (1958). However, Broadbent's model of attentional selectivity cannot account for hearing someone mention your name across the room in this scenario, as according to the model, incoming irrelevant sensory information is filtered *before* being processed, thus it would be impossible to determine if your name had been mentioned. An adaptation of the Broadbent selective filter was proposed and suggested unintentional sensory information is held in a temporary buffer zone before being processed or discarded, therefore facilitating the selective filtering of irrelevant stimuli but also enabling the detection of unexpected sensory cues, providing a model that can account for the 'cocktail party' effect (Treisman 1964).

Divided attention enables the individual to divide attentional capabilities across several temporal or spatial channels, allowing the performance of several tasks at once. This allows the individual to allocate attentional processing power to various tasks at the same time, despite the limited attentional capacity of the individual (Broadbent 1958; Parasruaman

1998; Robbins 2002). Impairments in tasks assessing divided attentional capabilities may result from exhaustion of this capability due to simultaneous fractionation of processing power (parallel processing) or exhaustion from continuous shifts between multiple attentional activities (serial processing - Muir 1996). Musicians routinely utilise divided attentional capacities, focusing on reading sheet music, playing the instrument along with listening to the musical output. Focus must be divided to play the instrument to an appropriate standard, but also in synchrony with other musicians, with attention being allocated to the conductor. Taken together, these three forms of attention function collectively forming an operational attentional system, enabling the individual to select and attend to various tasks and ignore irrelevant stimuli. This facilitates the detection and appropriate response to salient information from the surrounding environment, while at the same time providing the underlying substrate for several higher order cognitive processes including learning, memory and executive functioning (Chudasama and Robbins 2004; Riedel et al. 2006).

1.3 Neuroanatomy of Attention in Humans

A number of studies have been designed in order to elucidate regions of the human brain that are involved in attentional processes with various attentional models being proposed. Despite the many and varied models of the attentional system, many cortical (frontal, prefrontal, parietal) and sub-cortical regions (limbic system, basal ganglia, reticular activating system), along with connectivity between regions are consistently suggested to be involved with attentional processing (Riccio et al. 2002).

In order to elucidate this, many studies subject volunteers to attentional tasks and measure brain activity by a variety of imaging techniques, including single photon emission tomography (SPECT), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), allowing insight into the neuroanatomy of the attentional network

in vivo (Hager et al. 1998). Ojeda et al (2002) highlighted that previous investigations in this area showed specific prefrontal regions and the cingular gyrus are involved in attentional tasks, as well as being intricately involved in other cognitive processes such as working memory (Grady et al. 1997; Halpern and Zatorre 1999). Ojeda et al. (2002) utilised an auditory adaptation of the continuous performance test (CPT – described in detail below), measuring relative cerebral blood flow by utilising PET and radiolabelled water molecules in order to demonstrate possible regions of interest involved in sustained attention in humans. Results indicated that the dorsal lateral prefrontal cortex (DLPFC) is involved in the effective performance of tasks that require not only attentional processes but also elements of working memory. Additionally, it was reported that schizophrenia resulted in a failure of activation of the parietal cortex, which is in agreement with the posterior attentional model proposed by Posner and Peterson (1990). Ojeda et al. (2002) however reported inconsistencies in data collected by previous groups concerning the involvement of the cingulate region in attentional tasks. It appears that the cingulate region's involvement in attention is determined by the type of attention assessed (e.g. selective, sustained etc.) or the sensory modality being processed. The group concluded that the anterior cingulate cortex is indeed involved in attentional processing, indicating involvement of the structure in some but not all of the cognitive elements involved in attention, specifically the regions involvement in divided attention. An fMRI study was conducted encompassing a simple task that involved the assessment of sustained attention (Lewin et al. 1996). The group assessed visual sustained attention by asking volunteers to concentrate on a small dim central dot centred on a black background. During the session, the dot dimmed repeatedly and the subjects were asked to maintain their attention on the dot and count the number of times the dot dimmed over the course of the session. Inclusion of the element of maintenance over a prolonged session increases the attentional load and is therefore a valid means of measuring sustained attention. This investigation indicated that the right middle frontal

gyrus and parietal lobe were activated in human volunteers when asked to perform a task that imposed demands on sustained attention. These data were in agreement with the findings demonstrated by Pardo and colleagues (1991), in which they conducted a similar study using the PET technique, which identified the previously mentioned regions of the brain and the involvement in sustained attention. Other laboratories have conducted studies that have also implicated several subcortical structures such as the thalamus and basal ganglia in the modulation and maintenance of attentional processes and arousal (Buchsbaum and Haiger 1987; Hager et al. 1998). Further imaging studies (Hager et al. 1998) demonstrated using fMRI, that the mediodorsal and anterior nuclei of both hemispheres of the thalamus have increased activity in test subjects when the attentional load is increased. The group then go on to suggest that the thalamus may act as a relay station, providing afferent input to the DLPFC and anterior cingulate cortex, areas that are also implicated in the attentional system. Considering the extensive reciprocal connections with various cortical brain regions (Kamishina et al. 2009), the suggestion that thalamic nuclei serve as a relay centre in attentional processes is a likely situation. Lawrence et al. (2003) has demonstrated that a network of multiple neuronal regions, including frontal and parietal cortices and thalamic and caudate nuclei, are involved in attention. The regions of the brain involved in the attention system proposed by Lawrence and colleagues (2003) are in agreement with those suggested by Wigen et al. (2008), who conducted a pharmaco-fMRI study. These findings suggested that an extensive sub-cortical network is involved, along with frontal and parietal cortices (Poser and Peterson 1990), in several of the cognitive elements that are directly involved with attentional processing.

1.4 Attentional Impairment and Schizophrenia

Attentional impairment is evident in a number of psychiatric disorders and has been identified as a core component of schizophrenia symptomatology ever since the disease was

first scientifically recognised at the turn of the 20th century (Kraepelin 1921; Bleuler 1951). Attentional impairments may be core to the disorder, may precede the presentation of psychotic symptoms, outlast psychotic symptoms, may be a useful indicator of the susceptibility to the disorder and indicate functional outcome. Additionally, impaired attentional performance is often present in non-symptomatic relatives of schizophrenia patients (Cornblatt and Keilp 1994; Jones et al. 1994; Green 1997; Parasuraman 1998; an der Heiden and Hafner 2000; Chen and Faraone 2000; Wang et al. 2007). Attentional impairments in schizophrenia typically result in the inability to select and interpret incoming sensory information, and as a result can lead to inappropriate responding in various situations (Nestor and O'Donnell 1998). As the attentional system is intimately linked to the function of several higher-order cognitive processes (Riedel et al. 2006), impairment of the attentional system can also lead to a number of subsequent consequences, including increased distractibility, memory impairment, learning impairment, confusion, perseveration (compulsive behaviour) or behavioural disinhibition (increased impulsivity). These symptoms are regularly seen to some degree in schizophrenia patients (American Psychiatric Association 1989, p 1217; Nestor and O'Donnell 1998), as well as in a variety of other psychiatric conditions. One clinical test that can be used to measure an important aspect of attention, namely attention/vigilance, is the continuous performance test (CPT). The CPT was developed in the late 1950's by Rosvold and colleagues (1956) and the original task involves the patient reporting the rare occurrence of a single target letter that is visually presented amongst a number of irrelevant letters over a prolonged duration of approximately ten minutes. A correct response to the target stimulus generates a hit, whereas an omission is recorded as a miss. Conversely, an incorrect response to the presentation of a non-target stimulus is deemed a false alarm, with the contrary measure being a correct rejection and represents the individual correctly inhibiting a response to the non-target stimulus.

The term 'continuous performance test' is actually a generalised term used to describe a group of tests assessing attentional functioning. A number of variants of the basic task exist, varying what constitutes a target stimulus, potentially tapping into different constructs of the attentional system depending on the basic protocols of the task. For example, during the AX-CPT (Cornblatt and Keilp 1994) the target letter (X) is only correct when it is immediately preceded by the letter A, and therefore recruits an element of working memory in addition to the attentional component of the task. As such, the difficulty of the task can be further increased by 'priming' for errors; presentation of 'AY' stimuli probe for deficits in inhibitory control, whereas 'BX' stimuli generally tax working memory capabilities of the individual. Similarly, within the CPT-identical pairs (CPT-IP, Green et al. 2004; Holmen et al. 2010), a correct target stimulus consists of the second identical letter presented in succession and errors can be probed by presenting 'XY' stimuli instead of 'XX', for example. Alternatively, Conners CPT (Egeland et al. 2009) presents large numbers of target trials within the session and the non-target trials constitute the rare and unpredictable element of the task, in contrast to other CPTs that generally have a greater bias towards the non-target trial being presented throughout the session.

Schizophrenia patients perform poorly in all variants of the CPT (Straube et al. 2002). The MATRICS initiative included the CPT-IP in their cognitive battery in order to assess attention/vigilance in the clinical population, with schizophrenia patients demonstrating impaired performance (Cornblatt and Malhorta 2001; Groom et al. 2008). Impairment in attentional capabilities may result in increased omissions, which will result in a reduction in choice accuracy. Along with the increase in omissions, attentional impairment (or behavioural disinhibition) may also result in an increase in the number of 'false alarms', whereby the subject incorrectly reports the detection of the stimulus when the non-target stimulus was presented (Cornblatt and Keilp 1994). Due to the ability of the CPT to generate and record false alarms, investigators find it advantageous to use signal detection theory

(SDT) to give an indication of signal discrimination (Rutschmann et al. 1977), while at the same time giving insight into the strategy of responding (i.e. liberal or conservative responding). Signal detection analysis (described in greater detail in Chapter 4) typically provides two additional indices, D prime (d') and β, where d' is a measure of the subjects' ability to detect the target stimulus from background noise and β is the measure of strategy of responding. Impaired CPT performance, exhibited by schizophrenia patients typically consists of a reduction in accuracy and an increased frequency of false alarm responding. Patients consequentially produce d' values that are ~2.5 – 2.8 standard deviations below the population mean, suggesting schizophrenia impairs the ability to discriminate between the target signal from background noise presented in the CPTs (Liu et al. 2002). Along with schizophrenia patients performing poorly in CPTs, the many variants of the task can also give indications of vulnerability to schizophrenia to those at risk in the population, as non-symptomatic relatives of patients with schizophrenia often have a reduction in task performance compared to healthy volunteers (Cornblatt and Keilp 1994; Chen and Faraone 2000; Cornblatt and Malhotra 2001).

Another aspect of the attentional domain, selective attention, can be assessed using the Stroop task developed by Stroop (1935), in which schizophrenia patients demonstrate impaired performance (Barch et al. 2009). The task consists of words spelling various colours, printed in different coloured ink (e.g. Yellow, Blue, Red etc.). Either the participant has to name the word written or the colour of the ink the word is printed in. Reading of the word is governed by a pre-potent response, requiring minimal cognitive control. To read the colour of the ink, however, requires higher levels of 'top-down' cognitive control involving the recruitment of the PFC in order to bias responding inhibiting the pre-potent 'word reading' response enabling the colour of the ink to be named (Lesh et al. 2010), indicative of the higher levels of selective attentional capacity necessary (Pardo et al 1990). Schizophrenia

patients demonstrate impairment in the Stroop task, exhibiting longer reaction times (Wapner and Krus, 1960; Everitt et al. 1989) and increased error rates (Perlstein et al. 1998) compared to healthy controls, suggesting impaired selective attention is associated with the disease.

1.5 Behavioural Inhibition and Inappropriate Responding

Along with the various attentional processes that are dysfunctional in schizophrenia, the disorder also results in impairment in aspects of behavioural inhibition resulting in an increase in inappropriate responding (Paine and Carlezon Jr 2009). One type of task that can reveal an increase in behavioural disinhibition includes Go/No-Go tasks as they require the participant to respond to certain stimuli (go trial) and withhold a response to a different type of stimuli (no-go trials) throughout the task. Coupled with assessing attentional processing, the CPT is a classic example of a task that can incorporate elements of Go/No-Go tasks. Previous studies have demonstrated that schizophrenia patients perform poorly in these types of task and the impairment may not be mediated solely by the attentional element of the task. The reduction in performance can also be attributed to the inability of the patient to withhold from responding when a response is not required, characterised by an elevated false alarm rate (Green 1997; Ng 2002). Weisbrod and colleagues (2000) conducted an auditory version of a Go/No-Go task in healthy volunteers and schizophrenia patients, while at the same monitoring brain activity using the fMRI technique. In the Go condition, the subjects were required to press a button upon hearing an infrequent tone. In the No-Go situation, the subjects were to continuously respond when a frequent tone was presented, but withhold from responding when the infrequent tone appeared. The group demonstrated that there was no difference in performance between schizophrenia and non-schizophrenia subjects in trials requiring a 'go' response, but there was a significant reduction in the ability of schizophrenia patients to perform the task when instructed to withhold from responding in the No-Go trials. This implied that schizophrenia results in the inability to withhold from

inappropriate responding to irrelevant stimuli or discriminate between trial types. This may contribute to impaired performance during CPTs. Using the fMRI data, Weisbrod and colleagues (2000) indicate that the anterior cingulate gyrus, bilateral dorsolateral prefrontal cortex and left premotor cortex in healthy volunteers became activated when performing the No-Go task. Previous studies (Pardo et al. 1990; Berman et al. 1992; Petrides et al. 1993; D'Esposito et al. 1995; Carter et al. 1997) also implicated that these brain regions are involved in executive control and behavioural inhibition. The study conducted by Weisbrod and colleagues (2000) demonstrated a reduction in cerebral activation in schizophrenia patients compared to healthy volunteers in these regions when attempting to perform the No-Go component of a task, further supporting the previous findings.

Thus, it is evident that schizophrenia patients demonstrate attentional dysregulation, disruption that may be pivotal in the development of additional cognitive impairment. It is now pertinent to discuss the potential neurobiological processes that may be implicated in the pathophysiology of schizophrenia.

1.6 Dysfunctional Neurotransmitter Systems and Schizophrenia

Although there is a clear genetic component to the development of schizophrenia (discussed previously), highlighted by the increased concordance rate resulting from family history, decades of research has attempted to establish causality between abnormal neurotransmission and schizophrenia pathology. Many neurotransmitters have been implicated in the development of schizophrenia; however, significant attention has focused on the involvement of monoaminergic neurotransmitters or the amino acid neurotransmitters, including glutamate and gamma-aminobutyric acid (GABA).

1.6.1 Dopamine

One of the first hypotheses describing a dysfunctional neurotransmitter system and the development of schizophrenia involved dopamine, specifically the hyperdopaminergic hypothesis of schizophrenia. Dopamine is an essential monoaminergic neurotransmitter within the brain and is thought to be involved in the pathophysiology of a number of conditions, including Parkinson's disease (Brown and Marsden, 1990), drug addiction (Jentsch and Taylor, 2003; Dalley et al. 2007), attention-deficit hyperactivity disorder (ADHD) (Berridge and Devilbiss, 2010) and also schizophrenia (Howes and Kapur, 2009). The suspected involvement of dopamine in a number of neurological and psychiatric disorders demonstrates the key importance of this neurotransmitter system and highlights the impact of dysregulation of the dopaminergic system on the functionality of an individual. The basis for the hypothesis emerged from observations that psychostimulant drugs, such as amphetamine, which act by facilitating dopaminergic transmission (Ferris et al. 1972), can induce episodes of psychosis similar to that seen in the clinical population (Connell 1958; Bell 1973). Furthermore, schizophrenia patients given psychostimulant drugs show signs of increased psychosis at doses that are sub-psychotogenic in normal controls (Lieberman et al. 1987). Moreover, drugs that primarily act as dopamine receptor antagonists (specifically D_2 antagonists) were introduced in the 1950's as the first class of drugs to be marketed as antipsychotics as they showed efficacy in attenuating psychotic symptoms in psychiatric patients (Singh and Kay, 1975). Taken together, these findings suggest that there is a strong involvement of an over active dopaminergic system in the pathology of schizophrenia, playing a pivotal role in the development of psychotic, positive symptoms. A number of shortfalls exist in the hyperdopaminergic hypothesis, however. While amphetamine (and related psychostimulants) is effective at inducing positive-like symptoms, it is relatively ineffective at producing the negative symptomatology and cognitive deficits evident in the disorder. Furthermore, antipsychotic medication (particularly classical antipsychotics) is

typically ineffective in attenuating cognitive dysfunction exhibited in the disorder, ruling out dopamine D_2 involvement in this aspect of the disorder.

1.6.2 Glutamate

Research into the glutamatergic system and schizophrenia yielded the potential involvement of excitatory amino acids in the development of the disorder when it was discovered that cerebrospinal fluid (CSF) contained low levels of glutamate in patients with schizophrenia (Kim et al. 1980). However, this theory failed to gain credit for several reasons. Firstly, the findings were difficult to replicate in subsequent studies and at the time of the suggested hypothesis, scientific knowledge of the glutamatergic system was limited. As a result it was thought that dysfunction of the glutamate system would result in neurotoxicity and gross developmental abnormalities, which are not seen in schizophrenia patients (Luby et al. 1959; Moghaddam 2005). Glutamate is the major excitatory neurotransmitter in the CNS and approximately 40% of neurons utilise glutamate. There are two main classes of glutamate receptors; ionotropic and metabotropic. Ionotropic receptors, when activated, open a central channel in the receptor and allow the passage of charged ions across the cell membrane (Goff and Coyle 2001). The ionotropic receptors include kainite, NMDA and AMPA subtypes and, due to the fast passage of ions when activated, these are responsible for rapid cellular depolarisation (Moghaddam 2005). Metabotropic receptors on the other hand are G-protein coupled receptors (GPCR). Once glutamate binds to and activates the receptor complex, a signal cascade of 2^{nd} messengers is initiated and this has a number of cellular effects, depending on the class of glutamatergic metabotropic receptor activated. There are several subtypes of metabotropic glutamate receptor and they are classified as mGlu1 through to mGlu8 and modulate slower neurotransmission, compared to the ionotropic glutamate receptors (Moghaddam 2005).

The NMDA subtype of glutamate receptors are (amongst others) responsible for the slow excitatory postsynaptic potentials (EPSPs) and long term potentiation/depression (LTP/LTD). One of the most important consequences following activation of the NMDA receptor (thus generating EPSPs) is long-lasting change in synaptic function, known as synaptic plasticity e.g. LTP/LTD (Nestler et al. 2001). It has been postulated that these processes are intricately involved in various complex behaviours, including associative learning, working memory, behavioural flexibility and attention (Moghaddam 2005), all of which are affected to some degree in schizophrenia. Since the work of Kim et al. (1980), the scientific community's knowledge of glutamate and the NMDA receptor subtype has vastly increased and evidence has accumulated indicating dysfunctional NMDA receptor function in the pathophysiology of schizophrenia (Olney and Faber, 1995; Olney et al. 1999).

Administration of non-competitive NMDA receptor antagonists (phencyclidine, ketamine, MK-801) can induce a psychotomimetic state in normal humans (Luby et al. 1959; Allen and Young 1978) that resembles characteristics of schizophrenia. Importantly aspects of the negative and cognitive symptomatology of the disorder are also exhibited (Javitt and Zukin 1991; Krystal et al. 1994; Goff and Coyle 2001), and interestingly symptoms are exacerbated in stabilised schizophrenia patients (Lahti et al. 1995). Following these findings, behavioural research into NMDA receptor blockade and the possibility that it can successfully model a more complete picture of schizophrenia gained momentum (Javit and Zukin 1991). This resulted in the suggestion that the NMDA receptor is dysfunctional in schizophrenia and led to the development of the glutamatergic hypofunction hypothesis of schizophrenia (Abi-Saab et al. 1998; Javit and Zukin 1991; Olney and Faber 1995; Tsai and Coyle 2002). The glutamatergic hypofunction hypothesis suggests that dysfunctional NMDA receptor(s) and/or abnormal downstream NMDA receptor effects are core to the pathophysiology of the disease (Olney et al. 1999; Goff and Coyle 2001), potentially providing a more compelling model than the hyperdopaminergic hypothesis of schizophrenia (Coyle 2006). Therefore, it is thought

that blockade of the NMDA receptor system may reliably replicate aspects of schizophrenia symptomatology in humans and thus, the condition may reflect a dysfunctional glutamate system.

1.6.3 γ-animobutyric acid (GABA)

Neocortical neurons are organised in layers (layers I – VI), with approximately 70 – 80% consisting of excitatory (glutamatergic) pyramidal neurons (DeFeilpe and Farinas 1992), with the remaining neocortical neurons consisting mainly of GABAergic inhibitory interneurons (Markram et al. 2004). Gamma-aminobutyric acid (GABA) functions as the principle inhibitory amino acid neurotransmitter in cortical interneurons. The major exception is a class of interneuron called the spiny stellate cell (SSC) which is excitatory and contains glutamate. The SSC is only found in a specific cortical region; layer IV of the primary sensory area, receiving afferents from thalamic nuclei, with information being relayed to cortical layer II/III (Lund 1973; LeVay, 1973; White 1989; Thomson 1997; Feldmeyer et al. 2002).

Interneurons containing different calcium-immunoreactive proteins have unique morphometry (fig 1.1); calbindin (CB)-immunoreactive (ir) neurons are described as a double bouquet cell (primates) and provide vertical inhibition to pyramidal cells located within the same minicolumn (DeFelipe et al. 1989; Raghanti et al. 2010). Interneurons containing calretinin (CR) extend vertically to synapse with pyramidal dendrites located in separate cortical layers within a narrow minicolumn, with a morphometry consisting of bipolar, double bouquet and Cajal-Retzius cells (DeFelipe 1997; Raghanti et al. 2010). Finally, there are interneurons that are immunoreactive for parvalbumin (PV), namely the basket cells and chandelier cells. Both are predominantly multipolar. Basket cells have long-range axons that extend horizontally across the cortical layer, terminating with dendrites of pyramidal cells located in a different minicolumn (Lund and Lewis 1993; DeFeilpe 1997; Raghanti et al. 2010). Chandelier cells also project horizontally through the cortical layer, with a shorter range than

the basket cells, but again terminating with dendrites of pyramidal neurons (DeFelipe 1997; Raghanti et al. 2010).

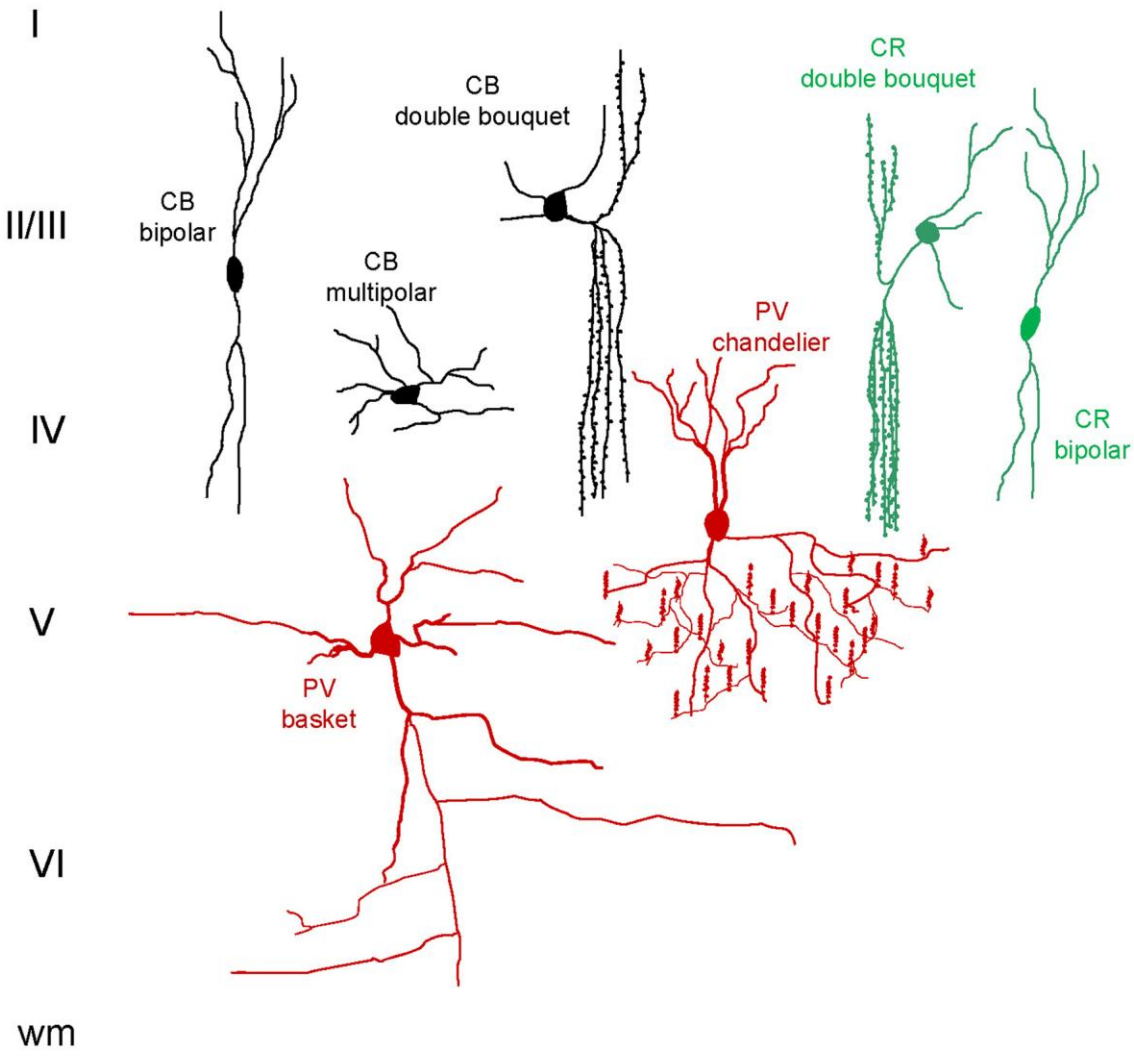

Fig 1.1 Schematic of the various layers of the neocortex, along with the major interneuron subtypes indicating the different cortical layers they populate. Different calcium-binding proteins are localised in individual interneurons, such as calbindin (CB), calretinin (CR) and parvalbumin (PV)-immunoreactive interneurons and each interneuron is located in the specific cortical layers, with a unique morphometry and all utilising GABA as an inhibitory neurotransmitter. Image taken from Raghanti et al. 2010

There is substantial evidence for dysfunctional GABAergic interneuron function in the pathology of schizophrenia, which was first proposed by Roberts in 1972. Following the original suggestion by Roberts (1972) a number of post-mortem studies have confirmed

abnormalities relating to GABAergic interneurons in schizophrenia patients (Blum and Mann 2002), with deficits in PV containing interneurons in the prefrontal cortex (Beasley and Reynolds, 1997; Beasley et al. 2002) and the hippocampus (Zhang and Reynolds 2002). Coupled with the deficits in GABAergic containing interneurons in schizophrenia patients, there is also a consistent reduction in the expression of the mRNA and protein for glutamic acid decarboxylase$_{67}$ (GAD_{67}), a synthesizing enzyme for GABA in the prefrontal cortex of schizophrenia patients (Guidotti et al. 2000; Volk et al. 2000; see Neill et al. 2010 for review). Furthermore, it has been demonstrated that mRNA expression for GAD_{67} along with GAT1 (GABA transporter) is reduced in schizophrenia in PV-ir containing neurons within the PFC (Hashimoto et al. 2003). These findings clearly implicate a dysfunctional GABAergic system in the pathogenesis of schizophrenia, and due to their location, deficits that may contribute to the development of cognitive dysfunction associated with the disorder (Lewis et al. 2005).

The primary function of GABAergic interneurons (located within the PFC or hippocampus) is to provide local inhibitory control over cortical excitatory pyramidal neurons, regulating their activity and excitatory output (Benes and Berretta 2001). Furthermore, the regulation of the GABAergic interneurons is determined by afferents arising from glutamatergic neurons. Taken together, there is evidence to suggest that a reciprocal GABAergic – glutamatergic interaction in prefrontal and hippocampal regions exist, and this interaction may be dysfunctional in schizophrenia patients (Coyle 2004) potentially leading to cognitive impairment in patients with schizophrenia (Lewis et al. 2005).

1.7 Animal Models of Schizophrenia Symptomatology

As the previous section described, schizophrenia is an extremely complex disease with an even more complex neuropathology and aetiology. Attempts to develop animal models of schizophrenia have met certain difficulties, due to the complexity of the disorder lending to difficulties assessing the validity of the model under scrutiny. Furthermore, certain aspects

of the disorder are inherently human constructs (verbal learning and fluency etc) and thus, impossible to assess in experimental animals. Additionally, aspects such as delusional thoughts and auditory hallucinations are equally difficult to quantify in a rodent model. However, it is imperative to model aspects of schizophrenia in experimental animals enabling the processes behind the neurobiology and symptomatology to be investigated, enhancing our overall knowledge of the disease. Once the understanding of the biological process behind the symptomatology is improved, efficacious pharmacotherapy targeting cognitive dysfunction and social deficits can be developed more readily.

1.7.1 Neurodevelopmental Models

Although the precise mechanisms underlying neurobiological features of schizophrenia are not completely understood, there is a hypothesis that the disorder may arise as a result of "early neurodevelopmental" insults (Pantelis et al. 2005), contributing to atypical brain development. Abnormalities in brain development are seen to begin prenatally and may continue throughout childhood, the changes that have been observed during these periods must have consequences for development of neuronal circuitry and connectivity (Gourion et al. 2004). It was noted that monkeys reared in isolation demonstrated a number of behavioural deficits, including impaired grooming, stereotypic movements and social interaction defects in adulthood (Kornetsky and Markowitz 1978). These observations were reminiscent of some of the negative symptomatology presented in schizophrenia (Andreasen 1982). Further investigation led to the implementation of the isolation rearing neurodevelopmental model of schizophrenia (Paulus et al. 1998; Varty and Geyer 1998). Coupled with aspects of negative symptomatology, elements of cognitive impairment are also evident, with deficits in prepulse inhibition present in isolation-reared rats (Geyer et al. 1993; Fone and Porkess 2008). Additionally, isolation-rearing induced cognitive deficits with

animals displaying disrupted performance within a novel object recognition task and attentional set-shifting (McLean et al. 2010).

A number of methods have been used to model the developmental origin of schizophrenia symptomatology in animals. These models attempt to replicate some of the factors that may be casual factors to the development of schizophrenia, such as early stressful experience (Lehmann et al. 2000). One developmental model is based on the postulation that schizophrenia is the result of a temporary reduction of glutamatergic neurotransmission (Goff and Coyle 2001). Studies have shown that repeated neonatal PCP (10 mg/kg s.c. on PND 7, 9 and 11) administration may lead to some behavioural disturbances in adult animals, namely, reduction of baseline prepulse inhibition (PPI) of the acoustic startle response and retardation of acquisition of spatial alteration task in female and male Sprague-Dawley rats (Wang et al. 2001) and delayed spatial learning task (Yuede et al. 2010). Moreover, a similar treatment regime, i.e. PND 7, 9 and 11 resulted in cognitive deficits, impairing performance in an attentional set-shifting task (Broberg et al. 2008). Yet another study showed that administering the DNA methylating agent, methylazoxymethanol (MAM), to the pregnant dams on gestational day (GD) 17 induced neurodevelopmental deficits (Lodge and Grace 2007). Administration of MAM during GD 17 resulted in cortical thinning with increased neural packing density in adult rats (Moore et al. 2006), disrupted PPI, increased sensitivity to PCP (Flagstad et al. 2004; Moore et al. 2006), executive behavioural impairment (Gourevitch et al. 2004), social impairment (Talamini et al. 2000) and impaired rhythmic activity of frontal cortical regions (Goto and Grace 2006), all of which have been observed in schizophrenia patients to some degree (Lodge and Grace 2007). Some studies have also shown impairments in working memory in adulthood in Sprague-Dawley rats with perinatal sub-chronic MK-801 administration (0.1 mg/kg for four days beginning from PND 7, twice a day) (Stefani and Mogaddam 2005) and disturbances in psychomotor activity (Schiffelholz et al. 2004). Moreover, postnatal PCP administration has been shown to induce a number of

neuropathological abnormalities in brain regions implicated in schizophrenia, including the hippocampus and frontal cortex (Ikonomidou et al. 1999; Wang and Johnson 2005). Also, recent neurochemical evidence suggests that blockade of NMDA receptors during brain development using MK-801 results in postpubertal emergence of hippocampal brain-derived neurotrophic factor (BDNF) upregulation, which is associated with the behavioural abnormality in animals, tested using locomotor activity in adolescent Sprague-Dawley rats (Guo et al. 2010). This has been implicated with the development of schizophrenia-like behaviour and pathophysiology, thus demonstrating face validity to the development of clinical symptomatology in humans in early adulthood.

1.7.2 Pharmacological Models

The previously described models of schizophrenia symptomatology are neurodevelopmental and involve pharmacological or environmental insult either prior to birth or shortly afterwards. A number of pharmacological models exist which involve treating *adult* animals, investigating the potential of drug treatment to mimic aspects of the behavioural and neurobiological effects observed in schizophrenia. Administration of psychostimulant drugs (e.g. amphetamine) is an obvious method, based on the hyperdopaminergic hypothesis of schizophrenia. Following several days of continuous amphetamine administration (Gaylord and Eison 1983), both monkeys and rats display a number of 'hallucinatory' behaviours i.e. 'wet-dog' shakes or parasitotic-like grooming episodes. Although these measures are an indirect assessment of hallucinations (for obvious reasons), they are suggested to serve as a means of identifying psychotic-like behaviour arising from amphetamine treatment. Another method of assessing aspects of positive symptomatology is by the quantification of pre-pulse inhibition (PPI) of a startle response (described in detail in section 1.13). It is suggested PPI deficits reflect deficits in sensorimotor gating and potentially pivotal in fragmentation of thought seen in schizophrenia (McGhie and Chapman 1961), and is evident in schizophrenia

patients (Braff et al. 2001). Drugs such as amphetamine, cocaine, apomorphine, along with a variety of other drugs that act by increasing dopaminergic transmission have been shown to impair PPI (for review see Geyer et al. 2001). Furthermore, antipsychotics have been shown to attenuate PPI impairment induced by dopaminergic compounds (Mansbach et al. 1988; Geyer et al. 2001). Therefore, utilising this methodology, it is possible to screen novel compounds to assess their efficacy in attenuating aspects of positive symptomatology in the clinical population. However, no matter how effective psychostimulant drugs are at inducing aspects of the positive symptomatology in animals, they are relatively *ineffective* in modelling the full range of symptoms present in the disorder (i.e. negative and cognitive symptoms – previously discussed in detail). Therefore, the primary aim of this thesis was to investigate the ability of PCP treatment in adult rats to model aspects of the cognitive dysfunction present in schizophrenia patients. However, the validity and the treatment regimen used must be considered, which is discussed in detail below.

1.8 Modelling Aspects of Schizophrenia Symptomatology

NMDA receptor antagonists (e.g. PCP, MK-801, ketamine) have been used on a number of occasions to investigate aspects of schizophrenia ever since PCP was described as a schizophrenomimetic agent by Luby and colleagues (1959). Different laboratories use a variety of treatment regimens to administer NMDA receptor antagonists in order to assess the behavioural and/or cognitive effects of the drug. However, there is debate as to what is regarded as the most effective method of drug administration during preclinical testing. Which regimen results in impairments, in not only cognition, but also neurochemical and morphological changes that are most relevant to those seen in schizophrenia (Jentsch and Roth 1999; Morris et al. 2005; Amitai and Markou 2010; Neill et al. 2010)? Many studies make use of acute administration of the drug, whilst others favour sub-chronic or chronic dosing schedules and employ a strategy of repeated exposure of the drug before behavioural

testing is conducted following a significant wash-out period. Both regimens can induce behavioural symptomatology resembling that presented in schizophrenia (Jentsch and Roth 1999), but both also appear to have their merits and downfalls, differences that will be discussed below.

1.8.1 Acute PCP Treatment

Acute exposure to PCP can result in a variety of behavioural responses in rodents that are indicative of schizophrenia-like symptoms, and are a useful way of inducing dysfunction in paradigms used to assess behaviour that attempt to mimic aspects of schizophrenia symptomatology (Jentsch and Roth 1999). These include impairment in motivation, motor function, frontal cortex function, temporal cortex function social behaviour and sensorimotor gating (Heale and Harley 1990; Hoehn-Saric et al. 1991; Aguardo et al. 1994; Sams-Dodd 1996; Verma and Moghaddam 1996; Jentsch and Roth 1999). While acute administration can induce cognitive impairment mimicking that observed in schizophrenia, Jentsch and Roth (1999) have highlighted the fact that acute administration impairs many faculties that are not affected in the disorder. Administration of PCP has been shown to impair acquisition of conditioned emotional response (Hoehn-Saric et al. 1991), conditioned cue preference (Stevens et al. 1997) and brightness discrimination (Tang and Franklin 1983), tasks that involve sensory processes and associative learning (Jentsch and Roth 1999) and these processes remain intact in schizophrenia. Additionally, following acute NMDA antagonism it has been demonstrated that there is a significant increase above basal levels of glutamate, dopamine, serotonin and acetylcholine within the PFC (Deutch et al. 1987; Hertel et al. 1996; Verma and Moghaddam 1996; Jentsch et al. 1997 a, b; Adams and Moghaddam 1998; Abekawa et al. 2006), which may give rise to the behavioural or cognitive disruption observed following acute NMDA antagonist exposure. However, schizophrenia is associated with a *reduction* of frontal cortical dopamine transmission (Weinberger et al. 1988; Daniel et

al. 1989; Davis et al. 1991), not an increase. Additionally, acute administration of PCP produces a biphasic response to glucose metabolism, with an increase 3 h after exposure, a reduction 24 h following exposure and a return to baseline approximately 48 h after acute administration (Gao et al. 1993; Cochran et al. 2003). In accordance with the discrepancies regarding cortical dopamine transmission, the increase in glucose utilisation following acute NMDA antagonism is in contrast to that observed in schizophrenia, with patients demonstrating 'hypofrontalitiy', characterised by a reduction in frontal blood flow and glucose utilisation (Weinberger et al. 1986; Weinberger and Berman 1996; Jentsch and Roth 1999). Moreover, the behavioural and physiological alterations associated with acute exposure to PCP, or other NMDA antagonists, are generally transient and short lived and thus, are only apparent as a result of direct exposure to the drug, being present only while the drug is still within the system, or for a short duration after metabolism and excretion (Jentsch and Roth 1999). Therefore, acute PCP exposure perhaps only offers a temporary method of impairing cognitive performance, but in a manner that is not completely analogous to the physiological consequences of the disorder. Furthermore, and possibly of greater consequence to the interpretation of behavioural performance, relates to the effect of acute NMDA receptor antagonism and the emergence of generalised behavioural disruption – acute administration often results in ataxia, stereotypy and/or sedation (Verebey et al. 1981; Melnick et al. 2002). Locomotor impairments may result in a non-specific inability to perform the task at hand, obscuring the interpretation of impaired performance resulting from cognitive dysfunction. Additionally, acute NMDA antagonism has been suggested to result in motivational impairment (Jentsch and Roth 1999; Paine and Carlezon 2009). Thus, if the animal has a reduced drive to perform a given task, it could further confound the behavioural interpretation. To circumvent these potentially confounding consequences of acute NMDA antagonist administration, experimenters often assess cognitive function following repeated, sub-chronic or chronic exposure to NMDA

receptor antagonists, conducting cognitive testing without the generalised behavioural disturbances potentially hindering interpretation.

1.8.2 Sub-chronic PCP Treatment

The review article published by Jentsch and Roth (1999) stated that long-term, repeated exposure to PCP (or other NMDA antagonists) may provide an enhanced model of some of the cognitive impairments in animals that resemble those that are seen clinically in schizophrenia. Research has suggested that sub-chronic PCP treatment regimens are able to induce a more selective set of deficits, including social impairment, locomotor sensitisation and specific cognitive impairments indicative of the symptomatology clinically presented in the disorder (Jentsch and Roth 1999; Morris et al. 2005; Neill et al. 2010). Studies have shown that rats sub-chronically treated with PCP demonstrate less social interaction and actively avoid social contact with unfamiliar rats, in comparison to interaction levels of rats treated with saline (Snigdha and Neill 2008a, b). This is reminiscent of symptoms seen in the clinic (Bobes et al. 2010) and one of a few models that demonstrate the ability of PCP (and potentially other NMDA receptor antagonists) to induce certain aspects of the negative symptomatology of the disorder.

Sub-chronic PCP treatment has also been shown to selectively disrupt the ability of animals to perform in a variety of cognitive tasks following cessation of drug treatment (see Neill et al. 2010 for review), disturbances that are of relevance to the cognitive domains putatively affected in schizophrenia (Young et al 2009a). Domains of cognitive function that are susceptible to disruption include episodic-like memory (Grayson et al. 2007; Karasawa et al. 2008), cognitive flexibility (Idris et al. 2005; McLean et al. 2009), and executive functioning deficits (Rodefer et al. 2005; Rodefer et al. 2008; McLean et al. 2008; Broberg et al. 2009). PCP-induced deficits in these tasks occurred following a substantial washout period (several months in some cases). These findings indicate performance deficits were not due to 'acute

drug effects' or withdrawal effects, suggesting that long-term exposure to PCP produced long-lasting neurobiological changes resulting in an impaired ability to perform in a variety of cognitive paradigms. Furthermore, repeated or sub-chronic exposure to NMDA antagonists significantly reduced or completely ameliorated the ataxic or stereotyped behaviour that may potentially confound interpretation following acute administration (Podhorna and Didriksen 2005; Didriksen et al. 2007; Amitai et al. 2007). The lack of generalised motoric impairment following these treatment regimens suggests a degree of tolerance to the generalised behavioural impairment observed following acute NMDA administration, thus eliminating this complication when interpreting performance. Along with the profound behavioural and cognitive impairments, sub-chronic PCP treatment also induces various neurochemical and neuroanatomical changes that resemble those presented clinically in schizophrenia patients. Mouri and colleagues (2007) described several studies that revealed following chronic PCP administration, basal or stress-induced utilisation of dopamine in the PFC was decreased for several days after drug treatment in mice, rats and monkeys (Jentsch et al. 1997a, b; Noda et al. 2000), mimicking the reduction of frontal dopaminergic transmission observed in schizophrenia (Weinberger et al. 1988; Daniel et al. 1989). Furthermore, in addition to disrupted PFC dopamine utilisation, Noda et al. (2000) showed that following sub-chronic PCP treatment, 5-HT utilisation increased in the PFC under stressful conditions. These data indicate that prolonged blockade of NMDA receptors results in disruption of the balance between prefrontal dopaminergic and serotonergic neurotransmission (Mouri et al. 2007). Several reports have also suggested that repeated exposure to PCP reduces the density of parvalbumin containing neurons in the hippocampus and prefrontal cortex of the rat (Cochran et al. 2003; Reynolds et al. 2004; Abdul-Monim et al. 2007; Mouri et al. 2007). This is consistent with the reduction of GABAergic interneurons seen in the hippocampus and frontal cortex of schizophrenia patients (Beasley and Reynolds 1997; Beasley et al. 2002; Zhang and Reynolds 2002). In addition to the PCP-induced

dysfunctional GABAergic transmission, a dramatic reduction in the number of prefrontal dendritic spine synapses (41% reduction) coupled with an increase in astroglial process density (59% increase) has also been demonstrated following sub-chronic PCP treatment in rats (Hajszan et al. 2006). This effect is also consistent with the reduction of PFC dendritic spine density demonstrated in schizophrenia patients (Glantz and Lewis 2000). In light of these findings, the suggestion that long-term, repeated exposure to NMDA receptor antagonists may provide a valid means of pharmacologically inducing cognitive, neurochemical and morphological changes in experimental animals, reminiscent to those seen in schizophrenia patients, certainly looks promising.

1.9 Dopamine and Cognition

It has recently become evident that dopamine plays a regulatory role in aspects of the many domains of cognition that are compromised in schizophrenia (Sawaguchi and Goldman-Rakic 1991; Cohen and Servan-Schreiber 1993; Goldman-Rakic et al. 2004), including attention (Nieoullon 2002). Dopaminergic projections arise from the midbrain and project to cortical and sub-cortical brain regions. Fibres arising from the substantia nigra project to areas of the brain associated with movement and motor control and are likely the projections that are involved in the neurodegenerative disorder, Parkinson's Disease (Fearnley and Lees 1991). Conversely, projections ascending from the ventral tegmental area (VTA) of the midbrain project to cortical brain regions (fig 1.2) and are thought to be associated with higher-order cognitive processing, in particular those that terminate in the frontal cortical regions (Davis et al. 1991).

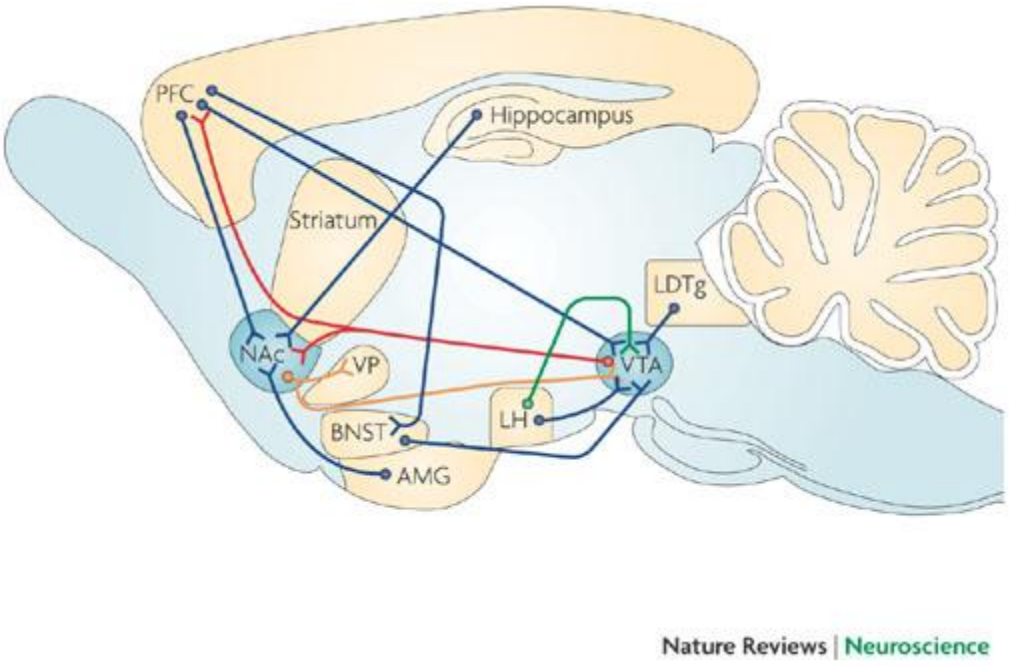

Fig 1.2. Simplified schematic of the circuitry and projections of the mesolimbic dopamine system in the rat brain highlighting the major inputs to the nucleus accumbens (NAc) and ventral tegmental area (VTA) (glutamatergic projections, blue; dopaminergic projections, red; GABAergic projections, orange; orexinergic projections, green). Glutamatergic synapses excite postsynaptic neurons and GABAergic synapses inhibit postsynaptic neurons. Dopamine release exerts more complex modulatory effects. AMG, amygdala; BNST, bed nucleus of the stria terminalis; LDTg, laterodorsal tegmental nucleus; LH, lateral hypothalamus; PFC, prefrontal cortex; VP, ventral pallidum. Image and figure legend taken from Kauer and Malenka, 2007.

This led to the suggestion that dopamine may have a dual dysfunction in the pathophysiology in schizophrenia (Davis et al. 1986). Dopaminergic over-activation in sub-cortical regions gives rise to the positive symptoms of the disease, whilst reduced function of dopaminergic innervation of cortical regions ('hypofrontalitiy') may contribute to the negative and cognitive aspects of the disorder (Davis et al. 1986; Davis et al. 1991; Grace, 1991; Howes and Kapur 2009). This provides a possible explanation to why classical neuroleptic drugs that are potent dopamine D_2 antagonists, acting on receptors predominantly located in sub-cortical regions (Guillin et al. 2007), show efficacy in treating auditory hallucinations and bizarre thought (i.e. positive symptoms). However, these drugs are relatively ineffective in treating

the cognitive or negative symptoms of the disease (Scatton and Sanger, 2000; Ibrahim and Tamminga, 2010), symptoms which are postulated to be mediated by cortical brain regions. Dopamine D_1-like receptors are increasingly being implicated in cognitive processing (Sawaguchi and Goldman-Rakic 1991; Sawaguchi and Goldman-Rakic 1994; Goldman-Rakic et al. 2004). Dopaminergic D_1 receptors are located within the PFC (Sawaguchi and Goldman-Rakic, 1991; Guillin et al. 2007), a region with extensive reciprocal innveration with various sub-cortical structures (Elman et al. 2006) and has been suggested to be associated with the functionality of a number of cognitive domains, including working memory (Arnsten et al. 1994; Sawaguchi and Goldman-Rakic 1994), visual learning and memory (McLean et al. 2009) and reasoning and problem solving (Mclean et al. 2009). Furthermore, D_1 dopaminergic neurotransmission and the PFC is also suggested to be involved in attentional processing (Nieoullon, 2002; Lesh at al. 2010), in particular the involvement of providing 'top-down' cognitive control to coordinate behaviour (Miller 2000; Miller and Cohen, 2001). Further evidence to implicate dysfunctional dopaminergic D_1 receptors within frontal cortical regions in schizophrenia comes from PET studies, demonstrating a down-regulation of dopamine D_1 receptors in schizophrenia patients within the PFC (Okubo et al. 1997). It should also be noted that the reduction in D_1 receptor expression demonstrated by Okubo et al. (1997) occurred in patients naive from drug treatment, eliminating the potential confound of altered receptor expression induced by long-term antipsychotic medication. Thus, down regulation of frontal cortical dopaminergic receptors is likely to be due to the pathology of the disorder, and given their location in a region implicated in cognitive control, may also be associated with impaired cognition in the disorder. Consequently, the MATRICS initiative identified the dopaminergic D_1 system as a promising target for future pharmacological focus and prompted the development of several new compounds which may prove efficacious in the treatment of schizophrenia (Tamminga 2006).

1.10 Preclinical Models of Attentional Processes

As previously discussed, it has been identified that cognitive dysfunction, including attentional impairment, is core to the pathophysiology of the disorder (Cornblatt and Keilp 1994; Marder et al. 2004; Ibrahim and Tamminga 2010). Cognitive impairment is suggested to be inherently associated with the functional outcome of schizophrenia patients (Green, 1996), and is one of the driving factors influencing a patients' inability in successfully reintegrating back into society, even once psychotic symptoms have been managed by pharmacotherapy. It is therefore of vital importance that the underlying mechanisms of the cognitive processes affected in schizophrenia are properly understood, thus enabling elucidation of the dysfunctional processes involved that result in the emergence of cognitive deficits exhibited in the disorder. It is clear that attentional processing is dysfunctional in schizophrenia patients. Furthermore, attention has been described as 'the gateway to cognition' (Riedel et al. 2006), reflecting the possibility that attention forms the underlying substrate for several higher-order cognitive processes. As a result, several preclinical tasks have been developed that assess attentional function in experimental animals, which therefore facilitate the investigation of the fundamental neurobiological processes that govern the control of the multifaceted construct of attentional processing.

1.10.1 Sustained Attention Task

McGaughy and Sarter (1995) developed an operant based task to assess attentional abilities with focus on the construct of vigilance in rats, known as the sustained attention task (SAT). The task consists of two discrete trials (summarised in fig 1.3), one where a signal is presented (signified by the presentation of a visual stimulus) and one where the signal is not presented (with the absence of a light stimulus).

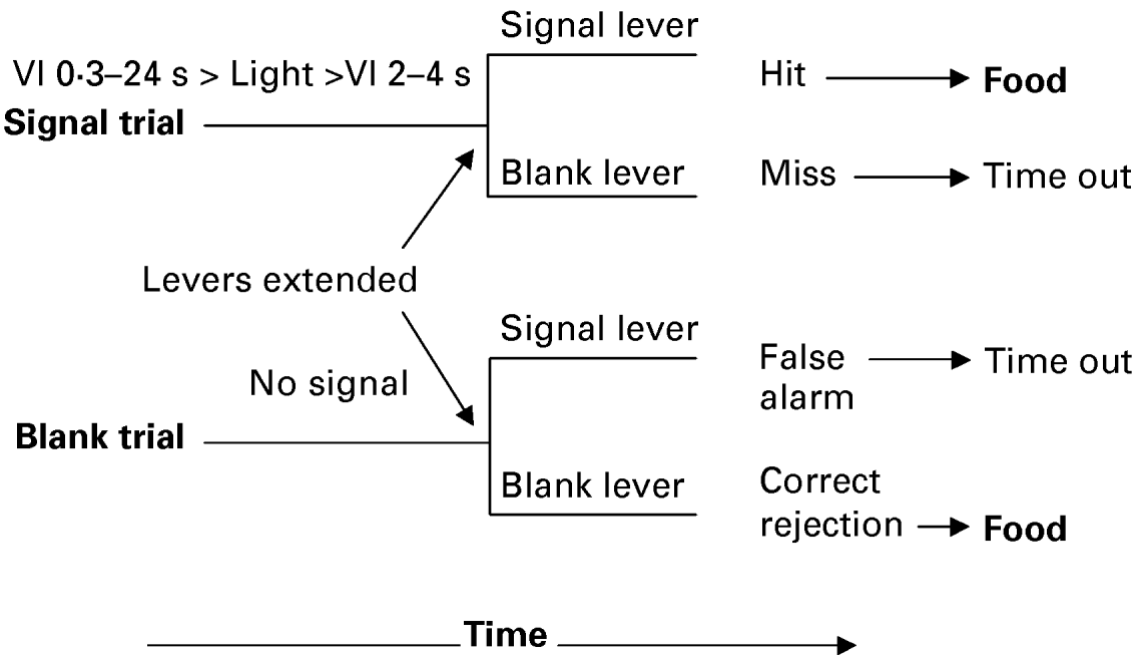

Fig 1.3. Schematic diagram of the two trial types presented in the sustained attention task (SAT). VI signifies a variable interval, following which the signal is presented (or absent), following the signal event and another VI the response levers are presented and the animals' response is recorded. Correct responses result in food reward and incorrect responses are penalised by a 2 s time out period. Image taken from Rezvani et al. 2008.

Following the signal or non-signal and post-stimulus interval, two response levers are presented and depending on whether the signal was presented or not, lever presses result in hits or misses (signal trial), or correct rejections or false alarms (non-signal trial), with hits and correct rejections resulting in the delivery of a food reward. The signal and non-signal trials are presented to the animal in a pseudo-randomised order so that both trial types are presented a number of times throughout the whole session. In the original study, McGaughy and Sarter (1995) varied the stimulus duration (SD) of the signal (25, 50 or 500 ms) and the session consisted of 27 signal trials (of each SD) and 81 non-signal trials in a session totalling 162 trials. As the SAT produces measures similar to that generated by human CPTs (i.e. hits, misses, correct rejections and false alarms) depending on what action the animal took when presented with each of the two trial types, SDT can be accurately employed. SDT was used

to generate an index of discriminability (termed the vigilance index; VI), facilitating the quantification of vigilance levels (McGaughy and Sarter 1995; Sarter at al. 2001). Antagonism of the NMDA receptor system has been demonstrated to impair performance in the SAT, following systemic administration of dizocilpine (MK-801) (Rezvani and Levin 2003; Rezvani et al. 2008a, b). Acute drug treatment impaired attentional processing characterised by a reduction in hits and a reduction in correct rejections, suggesting an inability to discriminate between signal and non-signal events. The experimental design conducted by Rezvani and Levin (2003) grouped the trials from the session into block and it was identified that dizocilpine treatment impaired the ability to correctly respond to the target stimulus in only the middle and last group of trials. However, the reduction in correct rejections was evident throughout the session, although more pronounced in the latter stages of the session. *In-vivo* microdialysis demonstrated that acetylcholine (ACh) efflux within the mPFC is involved in attentional performance, characterised by a 140% increase above basal levels upon initiation of the behavioural procedure (Kozak et al. 2006). Additional evidence to suggest that NMDA antagonism disrupts attentional functioning is provided by Kozak et al. (2006). These findings demonstrated that bilateral infusion of DL-2-amino-5-phosphonovaleric acid (APV) into the basal forebrain (BF) resulted in an impaired ability to perform the SAT, exemplified by a reduction in hits (although, in contrast to Rezvani and Levin 2003, APV in this case did not induce a reduction in correct rejections). Moreover, it was demonstrated that infusion of APV resulted in increased release of acetylcholine (Ach) efflux in the mPFC. This increase was over and above the 140% rise from basal level elicited from the behavioural task alone with ACh efflux levels reaching ~200% above baseline (Kozak et al. 2006), supporting previously demonstrated increases in cortical ACh in response to NMDA antagonists (Kim et al. 1999; Nelson et al. 2002). These findings not only confirm that NMDA antagonism disrupts performance of an attentional operant task, but suggests that cholinergic innervations of the mPFC, arising from the BF is intrinsically involved in the ability

of an individual to engage in the task at hand and suggests its involvement in attentional processing, particularly under challenging attentional conditions.

1.10.2 Five-Choice Serial Reaction Time Task

The 5-choice serial reaction time task (5-CSRTT) is one of the most commonly used tasks of attentional function in experimental animals. The 5-CSRTT is based on Leonard's five-choice serial reaction task (Leonard, 1959; Wilkinson, 1963), and has been said to share many features with the human CPT (Bari et al, 2008). The 5-choice task is conducted in an operant chamber that uses light stimuli instead of levers. The chamber contains five functional apertures, which contain incandescent bulbs or LEDs that provide the brief visual stimulus, and the rodent must respond to the visual stimulus by nose poking the aperture in which the stimulus was presented. Correct responses result in delivery of a food reward, while incorrect responses result in a time out (for a more detailed description of the task see chapter 2 and chapter 3). Although the description of the task makes it appear simplistic in nature, optimal performance in the task requires a number of cognitive processes (Robbins, 2002). During the inter-trial interval (ITI) the animal must divide and sustain its attention across the five spatial locations in order to detect the brief visual stimulus. In addition, during the ITI, the rat must focus its attention and withhold from responding before the stimulus is presented as this would result in a premature response (Chudasama and Robbins 2004a; Bari et al. 2008). In an adaptation of the basic protocol, the 5-choice task can also measure elements of selective attention. If the task interpolates busts of distracting white noise at the time the visual stimulus is presented, or just prior to, the animal must selectively filter out the white noise in order to detect presentation of the stimulus. Therefore the 5-choice task is capable of measuring the three main components of attention, sustained, divided and in certain circumstances, selective attention (Robbins 2002; Chudasama and Robbins 2004a). As there are five apertures for the rat to respond to, following the visual

stimulus, the chance performance is therefore ~20% correct response, whereas operant tasks using levers (typically two) often have chance performance levels of 50% (Robbins 2002). This value gives a useful baseline level of performance, as rats that are highly trained in the task will be performing at approximately 70 - 80% correct responding.

1. 11 Possible Neural Circuits Involved in the 5-CSRTT

Many regions of the rat brain have been implicated in successful performance of the 5-choice task and therefore hypothesised to be involved in attentional processing. There are a variety of methods that can be used to elucidate regions of the brain and their involvement in behavioural processes, whether it is revealing involvement in attentional functionality or behavioural inhibition. One methodology includes the use of neurotoxic lesion, in which selected regions of the brain or connectivity are disrupted, followed by assessment of 5-CSRTT performance.

1.11.1 Neurotoxic Lesions and the 5-CSRTT

Chudasama et al. (2003) conducted extensive work into dissociating and elucidating sub-regions of the PFC involved in task performance. The PFC can be divided into specific sub-regions, depending on sub-cortical efferent projections and involvement of each sub-region in specific aspects of 5-choice task performance can be dissociated. Lesions to the medial PFC (mPFC) primarily results in a reduction in choice accuracy, attributed to impaired attentional processing. Medial PFC lesions also increased premature responding, suggesting that this region is involved in impulse control. Neurotoxic lesions of the prelimbic cortex also resulted in a reduction in accuracy and an increase in premature responding, whereas infralimbic cortex lesion tended to only result in an increase in omissions (Passetti et al. 2002). Along with attentional processing and impulse control, frontal cortical regions are also implicated in cognitive flexibility, demonstrated by an increase in perseveration

following lesion of mPFC and orbitofrontal cortex (Chudasama et al. 2003). Furthermore, along with the involvement of frontal cortical regions, it is evident that various sub-cortical regions are involved in 5-choice task performance. Lesion of striatal regions have resulted in impaired 5-choice performance (Rogers et al. 2001), along with thalamic regions (Chudasama and Muir 2001). However, these sub-cortical brain regions have been demonstrated to receive substantial afferent input from cortical regions (Divac and Diemer 1980; McGeorge and Faull 1989; Sesack et al. 1989; Rogers et al. 2001), and perhaps the impairment in task performance following a sub-cortical lesion is due to the reduction in top-down control originating from frontal cortical regions and not due to the involvement of the sub-cortical region, *per se* (Miller 2000; Miller and Cohen 2001).

1.12 Psychotomimetics and the 5-CSRTT

Performance in the 5-choice task can be modified by pharmacological agents. Of specific interest to the work presented in this thesis is the effect of psychotomimetic administration on the behaviours measured in the task, and the potential elucidation of their impact on attentional processing. One method involved intracerebral injections of drugs directly into specific brain regions suggested to be involved in performance of the task. Following drug administration, performance can then be assessed in the 5-choice task to determine drug effects and impact of the specific brain region on task performance.

Baviera et al. (2008) used the competitive NMDA receptor antagonist 3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid (CPP), injected directly into the medial PFC of rats trained in the 5-choice task. The frontal cortex is densely populated with NMDA receptors (Cotman and Iversen 1987; Ulus et al. 1992) and this experiment demonstrated that selective blockade of NMDA receptors within this region produced a profound impairment in the rat's ability to perform in the task. Bilateral injection of CPP (50ng/side) in the mPFC region of the rat brain resulted in a reduction in choice accuracy, increased the

number of omissions, increased the correct latency and produced behavioural disinhibition in the form of increased perseverative and premature responding. These data clearly implicate the mPFC and a functioning NMDA glutamatergic system in successful performance of the task. These data were in agreement with the results produced by Murphy et al. (2005) who also demonstrated that bilateral central infusion of CPP into the mPFC produced performance deficits in the 5-choice task. However, Murphy and colleagues (2005) investigated the effects of CPP infusion in the prelimbic and the infralimbic cortices, which are specific sub-regions of the PFC. This study demonstrated that blockade of NMDA receptors within the PFC produced dissociable effects depending on the region. Infusions of CPP produced a reduction in choice accuracy and an increase in omissions across both cortical regions within the medial PFC; however, an increase in premature responding was only evident when NMDA receptors contained within the infralimbic cortex, but not the prelimbic cortex, were blocked. Murphy et al. (2005) suggested that these data demonstrated a dissociable role for the prefronto-cortical glutamatergic system concerning behavioural disinhibition, localised to the ventromedial infralimbic region of the mPFC.

1.13 Sensorimotor Gating

Sensorimotor gating refers to the pre-attentive abilities of the individual, crucial in the ability to filter out irrelevant sensory stimuli and can be construed as a form of passive attention or a construct of automatic attentional processing of incoming sensory information (Graham 1975). If an individual is exposed to a sudden, unexpected noise (for example, 120db burst of white noise) a startle response is generated, in which muscles involuntarily contract, and this is conserved across species, and present in all mammals (Braff et al. 2001). If the startle stimulus is preceded by a stimulus that falls below the threshold of that which elicits the startle response, there is a diminished reaction when the startling stimulus is presented. This is known as pre-pulse inhibition (PPI) of the startle response and is thought to represent a

method of quantifying the process of sensorimotor gating (Braff et al. 2001; Geyer et al. 2001; Young et al. 2009a). The startle stimulus and the sub-threshold stimulus needn't always be auditory, as it has been shown that various stimulus modalities can be used to elicit a startle response or induce PPI of the startle response (Swerdlow et al. 2002). However it is extremely time-dependent with a tight time effect window (50 – 300 ms) between the prepulse and startle stimulus onset for startle inhibition to occur (Blumenthal and Gescheider 1987; Swerdlow et al. 1999; Young et al. 2009a). Schizophrenia patients demonstrate a deficit in PPI; a startling stimulus elicits a full response even when preceded with a pre-pulse of a lower magnitude (Braff et al. 2001). It has been suggested that schizophrenia patients have a deficit in the early stages of information processing and the ability to filter and integrate incoming sensory information resulting in downstream disorganisation, hypothesised to be involved in the cognitive fragmentation, clinical symptomatology and functional impairment seen in the disorder (Venables 1960; Braff and Geyer 1990; Braff et al. 2001). Interestingly, impairments in the PPI of the startle response are also seen in humans and animals following treatment with NMDA receptor antagonists (Mansbach and Geyer 1989; Geyer et al. 1990; Keith et al. 1991; Johansson et al. 1995; Martinez et al. 1999; Jones and Shannon, 2000; Heekeren et al. 2007), thereby highlighting the putative face validity of this pharmacological challenge to model passive attentional componets of schizophrenia. This suggests an involvement of NMDA receptors in passive attentional processing, filtering and organising incoming sensory information and therefore implicates NMDA receptor dysfunction in the pathogenesis of schizophrenia.

1.14 Neuroimaging and Schizophrenia

Along with computerized tomography (CT) and positron emission tomography (PET), magnetic resonance imaging (MRI) has revolutionised the medical field by providing a means of visualising internal tissue and organs, including neurobiological structures *in vivo* without

the need for invasive surgical procedures. The non-invasive nature of imaging techniques enables the comparison of neurobiological structure or function in both healthy and patient groups (Feldman et al. 1997), facilitating the identification of key differences in biology that may have a mechanistic bearing in the pathophysiology of schizophrenia.

MRI is a widely used neuroimaging technique which relies on the behaviour of charged atoms in a strong magnetic field. In this setting nuclei resonate and emit a radio frequency at unique wavelengths. The frequency of this radio wave emission is dependent on what type of atom it originates from, as well as what chemical or physical environment the atom is located in (Feldman et al. 1997). Additionally, depending on the atom and its environment (i.e. the tissue it's located in), the relaxation period, which is the time taken for the atom to return to its previous state when the magnetic field is removed, differs and is characteristic of the atom type and location. Based on these measures, different tissue types and compositions can be deduced and an image of the brain, detailing various structures and functionality, can be constructed.

One MRI technique that can help elucidate the structural composition of the brain is voxel based morphometry (VBM), which is a technique that compares different brains on a voxel-by-voxel basis following a process that has compensated for macroscopic differences which occur naturally between samples (Mechelli et al. 2005). VBM is generally used to identify regional differences in grey and white matter and cerebrospinal fluid as opposed to general global differences in brain structure; therefore VBM imaging provides greater sensitivity in detecting abnormalities in volumetric structure in specific target regions of the brain. A particular advantage of VBM is that it is essentially hypothesis-free and is sensitive to the detection of morphometry differences that lie outside a particular region of interest (Job et al. 2002). Structural imaging has identified a number of abnormalities present in the brains of schizophrenic patients that are absent from normal controls, and these differences may account for the pathology present in the disorder. In first-episode schizophrenia patients,

morphometric analysis demonstrated that patients show reductions in grey matter primarily in the left and right caudate nuclei, cingulate gyri, parahippocampal gyri, superior temporal gyri, right thalamus and prefrontal cortex (Chua et al. 2007). Additionally, in this study volumetric analysis demonstrated there was an increase in the volume of CSF, particularly in the right lateral ventricle (Chua et al. 2007), likely due to an enlargement of the ventricles, which is a consistent finding among schizophrenia patients (Van Horn et al. 1992; Pomarol-Chotet et al. 2010). Wolf et al. (2008) also demonstrated grey matter deficits in schizophrenia, located within the left hippocampal gyrus and the right superior frontal cortex utilising VBM. In addition, they showed impaired cognitive abilities were correlated with structural abnormalities in a task that assesses divided attention (Wolf et al. 2008). Furthermore, it has been demonstrated that alterations in grey matter density observed in schizophrenia patients in the left thalamic nucleus, left angular, and supramarginal gyrus and left inferior frontal and postcentral gyri are positively correlated to the impaired performance of the CPT-IP assessing sustained attentional abilities (Salagado-Pineda et al. 2003). Additionally, this study was carried out in treatment-naive patients, ruling out the potential confound that attentional impairment or structural abnormalities were the result of chronic pharmacotherapy. Thus, structural disturbances are not only associated with schizophrenia pathophysiology, they may play a pivotal role in the cognitive impairment demonstrated in the disorder.

A recent development of MRI is a technique known as diffusion tensor imaging (DTI) and rather than determining the structural density or volume of tissue, DTI can reveal abnormalities in the connectivity between various regions of the brain (Filler 2009). The methodology behind DTI involves the movement of water molecules through varying mediums of brain tissue. In regions where water molecules can move unrestricted (i.e. the ventricles) water diffuses in an isotropic fashion (i.e. in all directions). However, when water molecules are confined within the axons of neurons, movement is restricted by the axonal

membranes and so diffusion is anisotropic (i.e. flows along the axis of the axon). This enables the production of a measure of fractional anisotropy (FA); high FA suggests a non-spherical tensor in which water is moving in a particular direction (i.e. along an axon), whereas a reduced FA indicates more isotropic movement which can be the result of compromised white matter (Beaulieu, 2002). By using the information obtained from DTI (isotropic regions, anisotropic regions and flow direction), it is possible to implement a technique known as fibre tractography (Kubicki et al. 2005), which visualises the neuronal tracts within the brain, thus enabling the comparison between normal and diseased brains to determine if the pathology is associated with deficits in white matter connectivity. Since its implementation, DTI has revealed white matter abnormalities in schizophrenia (Kubicki et al. 2002; Kubicki et al. 2005) that correlate with cognitive impairment (Dwork et al. 2007). Consistent findings have demonstrated lower anisotropy in schizophrenia patients, compared to controls, indicating white matter abnormalities or a reduction in connectivity in frontal and prefrontal regions (Buchsbaum et al. 1998; Lim et al. 1999). Furthermore, Camchong et al. (2009) demonstrated a reduced FA in frontal white matter in non-symptomatic relatives of schizophrenia patients, demonstrating the heritability of white matter abnormalities. Recently, white matter abnormalities have been demonstrated in the medial frontal cortex of schizophrenia patients (Pomarol-Clotet et al. 2010). Thus, these studies highlight that the integrity of myelination of frontal regions of the brain are compromised and may play an integral role in the pathophysiology of schizophrenia.

1.15 Experimental Aims

The aim of the work presented within this thesis was firstly to characterise the pre-clinical test of attention, the 5-choice serial reaction time task, and to establish the effect of psychotomimetic drugs on task performance. Importantly, the differing effect of treatment regimens and task disruption were assessed.

To further investigate attentional processing, a modified version of the 5-CSRTT was implemented, which may have greater analogy to its human counterpart, known as the 5-choice continuous performance test (5C-CPT). This task has previously only been validated in mice, and therefore it was important to determine if rats can be successfully trained to perform this task and necessarily discriminate between target and non-target trials. Moreover, the effect of D_1 dopamine activation on task performance was assessed, with the ability to attenuate impaired performance following a behavioural challenge investigated.

The primary aim of this thesis was to characterise the attentional-disruptive effect of PCP on 5C-CPT performance. Firstly the ability of repeated PCP administration to induce cognitive-specific deficits in the 5C-CPT was investigated. Secondly, as a paucity of information regarding persistent attentional impairment following NMDA receptor antagonism exists, the ability of a sub-chronic PCP treatment regimen to induce 5C-CPT deficits in the drug-free state was explored.

Finally the morphology of the brain was assessed, utilising structural magnetic resonance imaging (MRI), to determine if sub-chronic exposure to PCP induced long-term structural abnormalities in the brain. Neuroimaging was conducted in conjunction with the assessment of attentional processing investigating the dissociation between sustained and passive attentional impairment.

Chapter 2

General Methods

The following chapter will describe the general methodology used throughout the experimental chapters contained within this thesis. Detailed methods relating to specific aspects of the hypothesis under investigation are described in the relevant experimental chapters. The experiments carried out in the two 5-CSRTT chapters (3 and 7) were performed at different institutes (University of Bradford and University of Cambridge, respectively) and headed as such.

2.1. Subjects

2.1.1 University of Bradford

All subjects used in behavioural testing carried out at University of Bradford were female Lister-hooded rats (chapters 3, 4, 5, and 6; Charles River; approx 250 $\pm$ 10 g at the start of experiment) were housed in groups of five on a 12 hour reversed light cycle (lights on at 7:00pm – with the exception of chapter 3 in which animals were house under standard lighting; lights on at 7:00am). All animals at University of Bradford were housed in a temperature (21 $\pm$ 2^{o}C) and humidity (55 $\pm$ 5%) controlled environment. All experiments took place in the dark cycle (except experimental chapter 3), under red lighting, and animals had free access to food (Special Diet Services, UK) and water until one week prior to the beginning of training, when food restriction reduced animal weight to 90% of their free-feeding body weight (approximately 14g rat chow/rat/day). Food restriction continued throughout training and testing, however water was available *ad libitium* whilst in the home cage. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) 1986 Act and local University of Bradford ethical guidelines.

2.1.2 *University of Cambridge*

Male hooded-Lister rats (chapter 7; Charles River, UK; weighing approx 350 ± 10 g at the start of the experiment) were housed in groups of four on a 12 hour reversed light cycle (lights on at 7:00pm) in a temperature (21 ± 2°C) and humidity (55 ± 10%) controlled environment. Behavioural testing took place during the dark cycle, under red lighting. Animals had free access to food (Special Diet Services, UK) and water until one week prior to the beginning of training, where food restriction reduced animal weights to 90% of their free-feeding body weight (approximately 16 - 18g rat chow/rat/day). Food restriction continued throughout training and testing, however water was available *ad libitium* whilst in the home cage. All experiments were conducted in accordance with the UK Animals (Scientific Procedures) 1986 Act and local University of Cambridge ethical guidelines.

2.2 Drugs

All drugs were administered at a volume of 1ml/kg via the interperitoneal (i.p.) route and drug dose is expressed as free-base. Phencyclidine hydrochloride (PCP, Sigma-Aldrich, UK) was dissolved in 0.9% sterile saline (conversion factor, CF, 1.15). d-Amphetamine sulphate (Amphetamine, Sigma-Aldrich, UK) was dissolved in 0.9% saline (CF 1.36). SKF 38393 (Sigma-Aldrich, UK) was dissolved in distilled H_2O (CF 1.14). The CF was calculated by the following:

$$\text{Conversion factor} = \frac{\text{Relative Molecular Weight (RMW)}}{\text{(RWM – RWM of the salt)}}$$

Information regarding the specific treatment regimen and the dose the animal received can be found in the relevant experimental chapters.

2.3 Apparatus

2.3.1 University of Bradford

The test apparatus (for chapters 3, 4, 5, and 6) consisted of eight 25 cm x 25 cm aluminium chambers, each enclosed within a wooden sound attenuating box (fig 2.1 - constructed in-house for GlaxoSmithKline, GSK Harlow, UK, gifted to the University of Bradford). The rear wall of the testing chamber was concavely curved and contained nine individual apertures, four of which were occluded, leaving apertures 1, 3, 5, 7 and 9 free for exploration. Each aperture was 2.5 cm^2, 4 cm deep and set 2 cm above floor level. Located at the rear of each aperture was a yellow incandescent bulb (chapter 3) or white LED (chapters 4, 5, and 6), which provided the visual stimulus. An infrared photocell beam fixed vertically, at the entrance of each aperture, registered nose-poke responses. Located on the front wall of the test chamber was a food magazine that allowed retrieval of the food reward (45 mg sucrose Rodent Pellet, Sandown Scientific). A hinged panel covered the food magazine and a micro-switch reported the collection of food rewards (see fig 2.1 and 2.2 for outline of chamber layout). In addition to the sound attenuating box, there was also a low-level fan that not only provided ventilation, but also provided a means to mask extraneous background noises. The floor of the test chamber consisted of a wire grid, under which was a removable tray that was covered with sawdust. All eight chambers were connected to a PC and data collection and initial analysis was controlled by K-Limbic software (Conclusive Solutions) which generated an Excel spreadsheet (Microsoft) containing raw data.

Fig 2.1. A 5-choice serial reaction time task chamber at University of Bradford

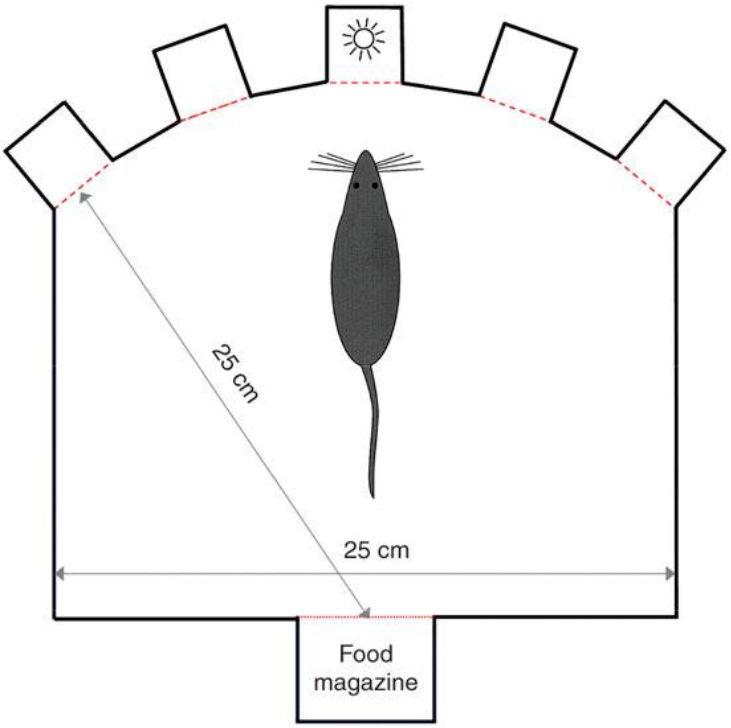

Fig 2.2. A schematic diagram of the 5-CSRTT chamber indicating the arrangement of the five response apertures and the food magazine. Image taken from Bari et al. 2008.

2.3.2 *University of Cambridge*

The apparatus set-up used in experimental chapter 7 was similar to that described for the previous experimental chapters, with the following exceptions: twelve 5-hole boxes were used (Med Associates, Saint Albans, Vermont, US) with the visual stimulus being provided by a yellow LED. Reward pellets were delivered to the magazine at the front of the chamber, and food collection was reported via infrared photobeam. All twelve chambers were connected to a PC and data collection and initial analysis was controlled by Whisker control system v2.5 (Cambridge Cognition, Cambridge, UK).

2.4 Behavioural Procedure – 5-CSRTT

Rats were trained to detect and report the occurrence of a brief visual stimulus (1 s) (Mirza and Stolerman, 1998; Fletcher et al. 2007; Young et al. 2007) presented pseudo-randomly in one of the five available apertures following an inter-trial interval (ITI, 5 s), conducted with the house light on. The pseudo-random presentation of the visual stimulus was banked. Each bank consisted of a stimulus being presented once in a random order in each of the five apertures. Once the visual stimulus had been presented in each of the five apertures, the next bank of stimulus presentations began, therefore ensuring only a maximum of two consecutive stimuli presentation in the same aperture was possible throughout the session. The alternative would have been a completely random sequence in which large numbers of consecutive stimuli presentations in a single aperture could potentially occur. A summary of performance of each trial presented in the 5-CSRTT is described in figure 2.3.

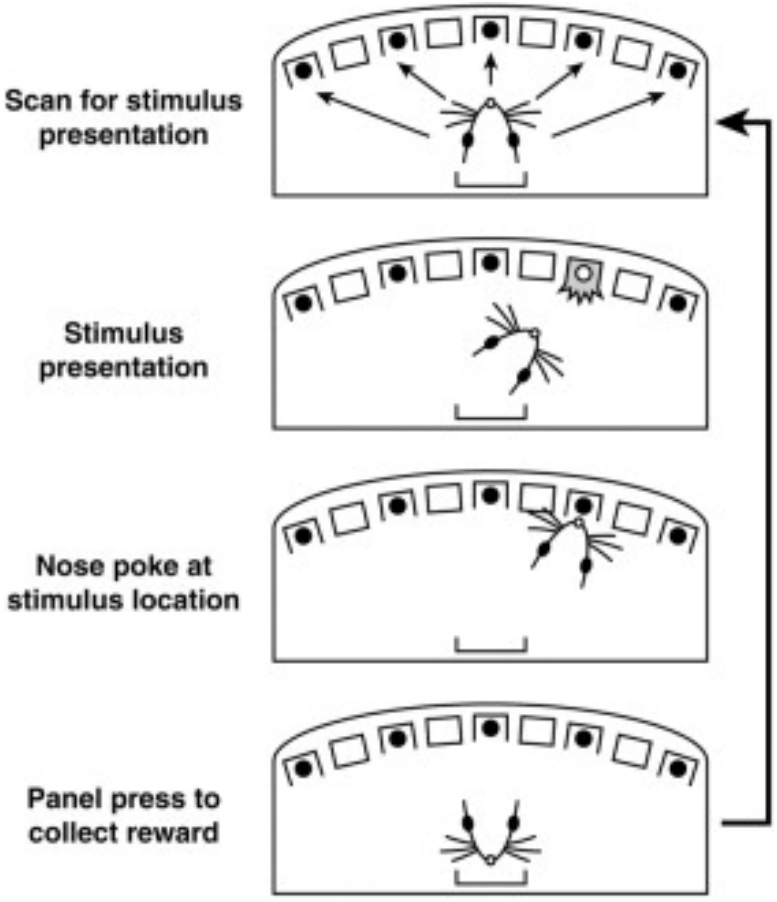

Fig 2.3. Schematic diagram of the correct performance of a trial presented to the rat during the 5-CSRTT. Image taken from Amitai and Markou 2010

Correct detection of the visual stimulus within the limited hold (LH; time period following the stimulus during which animals can make a response, 5 s) was reported via a nose-poke in the aperture the stimulus was presented in **(correct response)**, and resulted in a food reward. Reward collection immediately initiated the next ITI during which the animal had to attend to the array of visual apertures and detect the presentation of the next visual stimulus. Failure to respond to the stimulus **(error of omission)** or responding to an aperture where the light was not presented **(incorrect response)** resulted in the animal being exposed to a 5 s time out (TO) period, whereby the house light was extinguished and no food reward was delivered. A repeat response during the TO period restarted the 5 s TO period. Failure to wait for the stimulus presentation, i.e. a nose-poke during the ITI, was recorded as a

premature response and was also followed by a TO period. Following the TO after a premature response, the ITI for the previous trial began automatically. Animals also received a TO period following a single repeat response in any of the apertures (**perseverative response**). The whole session was completed within 100 trials (20 banks of 5 trials) or 30 minutes, whichever occurred first. In previous 5-CSRTT studies, accuracy is also referred to as percent correct responses and the terms are used interchangeably. However, an important distinction exists and one that is often overlooked; accuracy is calculated independently of omissions (Muir 1996), whereas percent of correct responding is calculated from the total number of responses within the session (correct, incorrect and omissions). Throughout this thesis, this method of calculating accuracy and percent correct responses has been adopted, as previously described by others (Amitai et al. 2007; Amitai and Markou 2009; Amitai and Markou 2010). The following table (table 2.1) describes the behavioural measures of the 5-CSRTT and how they were calculated:

Table 2.1 Description of the behavioural measures used in the 5C-CPT

Measure & Description
Accuracy: Correct responses/(correct + incorrect)*100 – a measure of selective attention
Percent Correct: Correct responses/total number of trials*100 – a measure of overall performance
Percent omission: Omissions/(correct + incorrect + omissions) *100 – a measure of sustained attention/motivation
Correct Latency, CL: Time taken to make a correct response; used to assess psychomotor speed
Incorrect Latency, IL: Time taken to make an incorrect response to assess general response speed
Magazine Latency, ML: Time taken to collect the food reward to assess motivational state
Total trials: Number of trials completed within the session
Premature responses: Inappropriate responses made within the ITI period to assess motor impulsivity
Perseverative responses: Inappropriate repeat response following a correct response

2.5 Training Schedule – 5-CSRTT

2.5.1 University of Bradford

Firstly, rats were habituated to the test boxes for two days prior to training; during which the magazine tray and response apertures were lit and baited to encourage exploration. Each rat was assigned to an operant box, which remained constant throughout training and testing. Additionally, the time of day the animals were trained, tested and fed remained constant throughout the entire experiment. The training schedule (for chapter 3) started with both the stimulus duration (SD) and limited hold (LH) duration lasting for 60 s. Based on rats' individual performance the SD was progressively reduced (see table 2.2) to the test criteria of 1 s SD. Animals were moved up a stage when they reached a stable >80% accuracy and <20%

omissions for three consecutive days. The LH was also progressively reduced as the animal acquired the task in the same manner as the SD, with the exception that the LH reduction stopped at 5 s therefore giving testing criteria of 1 s SD and 5 s LH. Throughout the training period, the ITI and time out period remained at 5 s. Rats progressed through the stages of training when they had reached the criteria of >80% correct and <20% omissions, which was stable over three consecutive days. Rats were deemed trained when they had reached this level of performance utilising the testing conditions (1 s SD, 5 s ITI, 5 s LH) for five consecutive days. When rats had reached criterion, they were only trained 3 days per week, enabling the rest of the cohort to reach criterion and avoiding the quicker rats becoming over-trained. The animals were trained Mondays – Fridays and animals took approximately 16 weeks to acquire the task.

Table 2.2: Description of the 5-CSRTT training schedule

Training Stage	Stimulus Duration (SD)	Limited Hold (LH)	Inter-trial Interval (ITI)	Time Out (TO)
0	60	60	5	5
1	30	30	5	5
2	15	15	5	5
3	10	10	5	5
4	8	8	5	5
5	5	5	5	5
6	2.5	5	5	5
7	2	5	5	5
8	1	5	5	5

Summary of the training schedule used in chapter 3. Animals progressed through training stages once they attained the desired performance criterion for three consecutive days. All durations shown are seconds.

2.5.2 *University of Cambridge*

Animals used in chapter 7 were habituated to the 5-CSRTT chambers as previously described (section 2.5.1). Following the habituation period, animals were trained (6-days per week) in the 5-CSRTT procedure by progressively increasing the task difficulty when animals reached a predetermined criterion. Details of the training schedule can be found in table 2.3. Animals progressed to the next training stage once they reached the desired criterion for each stage and were deemed trained once they reached stage 7 and performed consistently for a period of 3 days. Training to criterion took approximately 50 – 60 sessions.

Table 2.3: Description of the 5-CSRTT training schedule

Training Stage	Stimulus Duration (SD)	Limited Hold (LH)	Inter-trial Interval (ITI)	Criterion to move to the next stage
0	120	30	2	> 30 Trials
1	30	20	2	> 30 Trials
2	20	20	2	> 30 Trials
3	10	10	5	> 50 Trials
4	5	5	5	> 50 Trials >80% Accuracy < 20% Omissions
5	2.5	5	5	> 50 Trials > 80% Accuracy < 20% Omissions
6	1.25	5	5	> 50 Trials > 80% Accuracy < 20% Omissions
7	1	5	5	> 50 Trials > 80% Accuracy < 20% Omissions

Summary of 5-CSRTT training schedule used in chapter 7. All durations shown are measured in seconds.

Below is a summary of the response sequence used within the University of Cambridge (fig 2.4), describing the possible response outcomes of the 5-CSRTT. Although it is largely similar to the previous methods (used in Chapter 3), one important difference exists; following a premature response the animal had to initiate the subsequent trial following the TO period and therefore a premature response was counted as a trial. Additionally, while perseverative responding was recorded, they did not initiate a 5 s TO period.

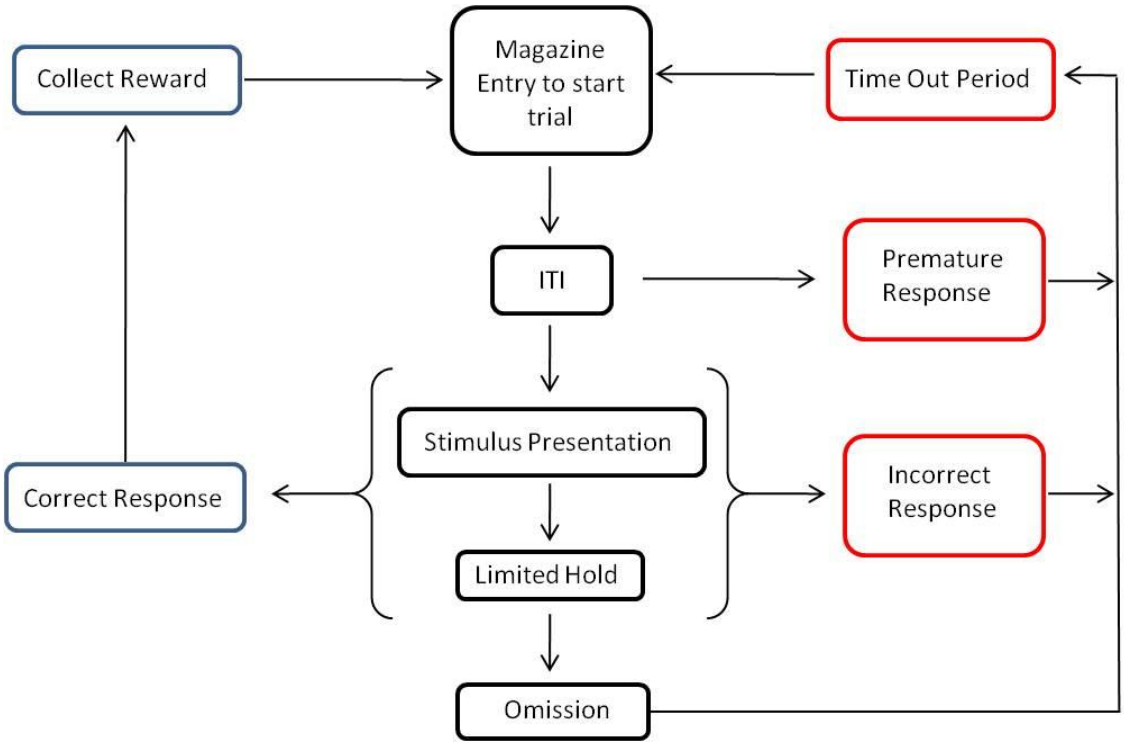

Fig 2.4. A schematic summary of the response sequence used in the 5-CSRTT described in detail above. Image adapted from Bari et al. 2008.

2.6 Five-Choice Continuous Performance Test (5C-CPT)

2.6.1 Behavioural Procedure

The 5C-CPT is similar in some aspects to the 5-CSRTT which is described in detail above. However, a number of differences in the methodology exist. Instead of a fixed ITI of 5 s described previously, chapters 4, 5, and 6 utilised a variable ITI (4.0, 4.5, 5.5, and 6.0 s) which had a mean of 5 s throughout training. The variable ITI was implemented in order to

minimise a temporally mediated strategy being used by the rats' when responding during the session (Spratt et al. 2000). The 5C-CPT encompasses the measures typically generated by the original 5-CSRTT and correct responses result in a reward being delivered, whilst incorrect responding initiates a TO. Responses made during the TO period restarted the 5 s TO period and were recorded as **time out responses**.

Importantly, the 5C-CPT procedure also included no-go trial stimuli in which lights in all apertures are presented, signifying that the animal must inhibit responding (**correct rejection**) in order to obtain a reward (fig 2.5). An incorrect response made during non-target trials (either during the stimulus presentation or during the LH period) was recorded as a **false alarm** and resulted in a TO period. In a similar manner to 5-CSRTT training, presentation of target and non-target stimuli was banked and presented in a pseudo-random order throughout the session. Details of the ratio of stimuli presentation can be found below (section 2.6.3). The session consisted of 120 trials, lasting no more than 30 minutes. Table 2.4 describes the additional behavioural measures assessed by the 5C-CPT.

Table 2.4 Description of the behavioural measures used in the 5C-CPT

Measure & Description
Correct Rejections: Correctly withheld /(correct rejections + false alarms)*100 – a measure of correct rejection of no-go trials
Hit rate, p[HR]: The proportion of target trials correctly detected
False alarm rate, p[FA]: The proportion of non-target trials incorrectly responded to: to assess response disinhibition
Sensitivity index, SI: Non-parametric measure of the ability to discriminate between target and non-target trials – a measure of vigilance
Responsivity index, RI: Non-parametric measure of the response bias or strategy of the animal

Summary of additional measures available in the 5C-CPT.

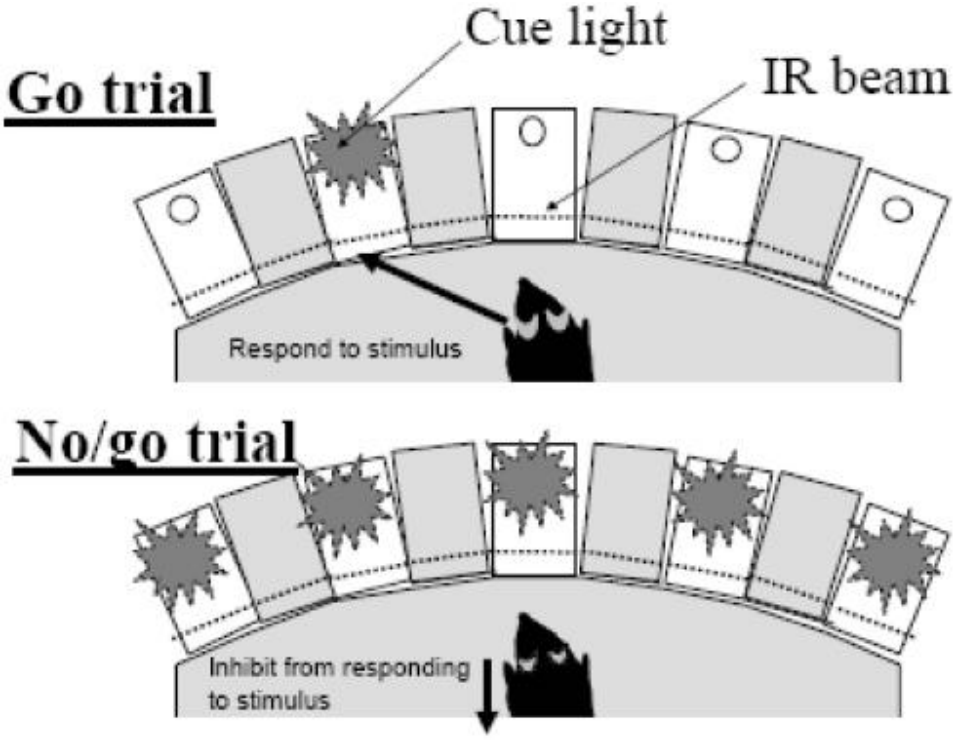

Fig 2.5. Schematic diagram of the two trial types presented within the session of the 5C-CPT and the correct action required. Image taken from Young et al 2009b.

2.6.2 Signal Detection Theory

The following describes the methods used to calculate the non-parametric indices used in signal detection theory (SDT) as originally described by Frey and Colliver (1973) and also used in the mouse 5C-CPT (Young et al. 2009b). Human CPTs utilise parametric indices (d' and β – described in detail in chapter 4), however they require assumption to be made regarding data distribution and normality. As a result, non-parametric equivalents of d' and β (SI and RI, respectively) described by Frey and Colliver (1973) have been implemented. Correct, incorrect and omissions refer to possible response outcomes when presented with a target trial, FA (false alarm) indicates an incorrect response to non-target trial and CR (correct rejection) refers to a correct rejection of non-target trial:

Similar to human CPT studies regarding the calculation of d', if the values for CR or FA were equal to zero than the following corrections were used prior to the calculation of SI or RI.

2.6.2.1 Calculation Correction:

$$FA = 0, use \frac{1}{CR} \times 2 \qquad CR = 0, use \frac{1}{FA} \times 2$$

2.6.2.2 SDT Calculations:

$$p[HR] = \frac{Correct}{Correct + Incorrect + Omission} \qquad p[FA] = \frac{FA}{FA + CR}$$

$$SI = \frac{p[HR] - p[FA]}{2(p[HR] + p[FA]) - (p[HR] + p[FA])^2}$$

$$RI = \frac{p[HR] + p[FA] - 1}{1 - (p[FA] - p[HR])^2}$$

2.6.3 Training Schedule

Training was conducted in a similar manner to the original 5-CSRTT (section 2.5.1), with the following exceptions. The modified task included non-target trials, whereby all apertures presented a visual stimulus and the reward pellet was dispensed following a correct rejection. Initially, methods followed the same protocol set out in the original mouse version of the 5C-CPT (Young et al. 2009b). As such, the session consisted of 100 target trials and 20 non-target trials, presented pseudorandomly throughout the session. However, with this parameter configuration rats were unable to differentiate between trial types and responded to the majority of non-target trials presented within the session (described in chapter 4). Therefore, it was required to place a greater emphasis on the presentation of non-target

trials, therefore the proportion per session was increased (77 target trials and 43 non-target trials). This configuration was used until rats could correctly respond to target trials and inhibit a response to non-target trials. Once rats were able to reliably discriminate between trial types (p[HR] > p[FA] – approximately 14 weeks), the proportion of target trials per session was increased to 84 and non-target trials reduced to 36, a configuration used throughout the remainder of training and testing. At the beginning of training the SD was 10 s (for both target and non-target trials) and was progressively reduced (10, 8, 4, and 2 s) for individual rats as they reached criterion (>75% accuracy, <25% omission and >65% correct rejections of non-target trials, for three consecutive days). Training was conducted until animals achieved a stable performance at the desired testing parameters (chapter 4, SD = 1.5 s and chapters 5 and 6 SD = 1 s, for all chapters a 5 s TO, variable ITI with mean of 5 s and 2 s LH was used unless otherwise stated) over three consecutive days. Animals took approximately 14 weeks to reliably discriminate between trial types, and a further 6 – 10 weeks to reach stable performance at the desired testing criteria. The entire training procedure took approximately 20 – 24 weeks.

2.7 Data Analysis

All data are expressed as mean $\pm$ SEM and graphically displayed using Graphpad Prism (v5). Statisitcal analysis was carried out using Statsoft Statistica (v6) with alpha level set to 0.05. The distribution of data was checked using normality plots. Any deviation of normality resulted in the transformation of data prior to analysis. Percentage data underwent arc-sin transformation, latencies were log transformed and inappropriate responding data were subject to square root transformation, when appropriate (Hahn et al. 2002); however raw data were graphically displayed. Specific information regarding the statistical design and approach for each analysis used are described in the methods section of each experimental chapter.

Chapter 3

Assessment of attentional processing using the 5-Choice Serial Reaction Time Task: Disruptive effects of psychotomimetics

3.1 Introduction

As discussed previously, the 5-choice serial reaction time task (5-CSRTT) is a common preclinical task assessing attentional processing (Bari et al. 2008), suggested to share many features with human attentional tasks, namely the continuous performance test (CPT). Construct validity of the 5-CSRTT as a task of attention has been demonstrated (Robbins 2002). The 5-CSRTT is a complex paradigm that can record a number of different behavioural measures, which may be controlled by a variety of cognitive processes, not just attentional processing.

Response accuracy in this task is largely dependent on the attentiveness of the experimental animal, with deficits resulting in the animal being unable to sustain its attention or divide it across the spatial apertures. This may result in the target stimulus not being detected, and consequently the animal may respond by 'guessing' and perhaps guessing incorrectly, thus responding in an incorrect aperture and reducing response accuracy (Young et al. 2009a). Spratt and colleges (2000) confirmed this theory. The group had modified the basic configuration of the 5-CSRTT and during discrete trials throughout the session withholding a response when no stimulus was presented earned a food reward. It was revealed that rats responded to approximately 90% of no stimulus light conditions. These findings suggested that perhaps the rats were responding based on a temporally mediated response strategy, nose-poking even if a visual stimulus had not been detected but following the elapse of the ITI. Another consequence of impairment in attentional function may be an increase in the number of error of omissions (Amitai and Markou, 2010). If the test subject is failing to attend to the task and continually misses the visual stimulus presentation and fails to elicit a response, the result will be an increase in the number of missed trials, particularly if the animal is not adopting the 'guessing' strategy. Coupled with the increase in omissions being recorded, a reduction in the number of correct responses being made is likely.

Another collection of behavioural measures assessed by the 5-CSRTT includes response latencies, consisting of the correct latency (CL), incorrect latency (IL) and magazine latency (ML). The latency measures can be interpreted in a number of ways and depends largely on the pattern of response of not only each of the latencies, but also the other behavioural measures (Robbins, 2002). Firstly, response latencies are suggested to provide a measure of speed of informational processing. An increase in the CL may be indicative of a reduced ability of the rodent to process information that the visual stimulus has been presented and therefore a response must be made. However, this interpretation (as with others) must be made in conjunction with other behavioural measures being taken into account (Robbins, 2002; Amitai and Markou, 2010), as an increase in CL, caused by pharmacological treatment, may be a result of altered motivation to complete the task or motor impairment instead. This can usually be determined by observing the ML. Impairment in motivation or motor function is likely to be coupled with an increase in the time taken for the rodent to retrieve the food reward, and therefore an increase in magazine latency would be observed (Robbins, 2002; Chudasama and Robbins, 2004a). Similarly, it has been suggested that when an increase in omissions is accompanied by an increase in magazine latency, the likely reason for increased omissions is probably due to some form of motor impairment and not attentional impairment as previously discussed (Chudasama and Robbins, 2004a). However, if the increase in the number of omissions is not associated with an increase in the time taken to retrieve the food reward, the explanation is more likely due to impairment in attentional function (Amitai and Markou 2010). These considerations demonstrate an important factor concerning understanding of 5-CSRTT performance. Any interpretation of the effects of drug treatment or parameter manipulation on the ability to conduct the task must be made when all behavioural parameters (and how they are affected) have been taken into consideration, and not based on behavioural measures observed in isolation (Robbins, 2002; Chudasama and Robbins, 2004a; Amitai and Markou, 2010).

Another area of cognition that the 5-choice task can assess is behavioural inhibition and inappropriate responding, in the form of premature or perseverative responding, which is known as response inhibitory control (Robbins, 2002). Premature responses are the result of a response being made during the ITI, before the visual stimulus has been presented. Premature responses occur when the rat is most likely anticipating the presentation of the visual stimulus, therefore the number of premature responses increases when there is impairment in the animal's ability to withhold a highly prepotent response and thus it responds inappropriately. As a result, this type of inappropriate responding is suggested to be indicative of impulsive behaviour. Deficits in impulse control are often observed in various psychiatric disorders, including schizophrenia (Kiehl et al. 2000; Weibrod et al. 2000; Wykes et al. 2000). Perseverative responses represent another form of inhibitory impairment, which manifests itself by the repetitive over-responding of a previously rewarded behaviour. Perseverative responding involves the rat repeatedly nose poking a response aperture, even following the delivery of the food reward. This type of behaviour is an inhibitory impairment in the form that the rodent cannot disengage from the behaviour once initiated, representative of compulsive behaviour (Robbins, 2002). Thus, along with the obvious attentional component, aspects of impulsivity, compulsivity and speed of information processing are also involved in successful task performance, areas of cognition that are also suggested to be affected in schizophrenia (American Psychiatric Association 1989, p 1217; Riedel et al. 2006; Young et al. 2009a).

Previous work within our laboratory has established several behavioural paradigms that measure the ability of rodents to perform cognitive tasks of relevance to schizophrenia (see Neill et al. 2010 for review). They include novel object recognition (Grayson et al. 2007), attentional set-shifting (McLean et al. 2009a), reversal learning (McLean et al. 2009b) and social cognition, in the form of social interaction (Snigdha and Neill, 2008); all of which reflect preclinical paradigms assessing cognitive domains putatively core to the cognitive deficits

observed in schizophrenia (Neuctherlein et al. 2004; Young et al. 2009a). As these paradigms have the potential of quantifying aspects of cognitive disruption, they provide a means of screening potential pro-cognitive agents. Therefore, they may assist in the elucidation of neurobiological mechanisms involved in cognitive disruption and may direct research ultimately leading to the attenuation of cognitive impairment of schizophrenia. In these paradigms, sub-chronic treatment with the NMDA antagonist, PCP, results in cognitive deficits and an inability to perform the task compared to control animals, lending further support that a dysfunctional glutamatergic system may be involved in the emergence of the cognitive impairments present in schizophrenia (Olney and Faber 1995; Olney et al. 1999; Goff and Coyle 2001).

The aim of this experimental chapter was to identify the possibility of establishing the 5-CSRTT in female rats at the University of Bradford as a model assessing attentional processing. This would enable the suitability of inducing attentional impairments via NMDA receptor blockade to be determined, which may have clinical relevance to the cognitive impairments seen in schizophrenia. Previous investigations have been carried out following administration of NMDA antagonists and the 5-CSRTT; however, all were conducted using an acute or repeated dosing regime in male rats. Therefore, the primary aim was to establish whether female rats can be trained to perform the task to a similar level of performance as male subjects. More importantly the ability to induce persistent impairments in task performance following a sub-chronic PCP treatment regime in the drug-free state was to be determined. Finally, the effect of an acute challenge of PCP or amphetamine on task performance was assessed.

3.2. Methods

3.2.1 Subjects

50 female hooded-Lister rats (Charles River; approximately 250g (± 10g) at the start of training were used in the experiments described in this chapter. Information regarding the housing conditions, food restriction and ethical guidelines can be found in chapter 2.

3.2.2 Training and Behavioural Procedure

Details regarding the 5-CSRTT behavioural procedure and how the animals were trained in order to acquire the task are described in the general methods section (Chapter 2).

3.2.3 Drugs

3.2.3.1 Sub-chronic PCP treatment regime

Once fully trained in 5-CSRTT, rats were sub-chronically treated with either vehicle (0.9% saline, n = 10) or PCP (2 mg/kg, n = 40). The dosing regimen consisted of one dose in the morning (approx 9 am) followed by one dose in the afternoon (approx 5 pm) for 7-days. The 7-days twice-daily treatment regimen was followed by a 7-day washout period. During the treatment period animals received no training or testing. Following the 7-day washout period, testing in the 5-CSRTT commenced.

3.2.3.2 Acute Psychotomimetic Challenge

Acute challenge treatment (described in section 3.2.6) consisted of an acute challenge of drug with a 30-minute pre-treatment time. Behavioural testing commenced immediately after the pre-treatment time.

3.2.4 Effect of sub-chronic PCP treatment on 5-CSRTT performances

Following completion of the sub-chronic PCP treatment regimen, 5-CSRTT performance was assessed under conditions of standard attentional load. 5-CSRTT testing parameters consisted of 1 s SD, 5 s ITI, 5 s TO and 5 s LH. Additionally, performance was assessed when attentional load was increased, by reducing the SD to 0.5 s (summarised in table 3.1). All other parameters remained constant. This was designed to further tax attentional performance without altering the basic methodology of the task.

3.2.5 Effects of protocol change on performance in the 5-CSRTT

Modifications to basic 5-CSRTT protocols were design to further tax attentional performance (table 3.1). Challenge sessions consisted of altered ITI (increased or decreased) with a SD of 0.5 s. TO and LH durations were 5 s throughout. Challenge sessions were conducted on Wednesdays and Fridays, and interspersed with sessions consisting of standard protocols (1 s SD, 5 s ITI, 5 s TO, 5 s LH).

Table 3.1 Summary of behavioural manipulations used to challenge 5-CSRTT performance

Challenge	Effect
Reducing Stimulus-Duration	Taxes attentional capabilities due to the increased difficulty in target stimulus detection
Reducing Inter-trial Interval	Increases the event-rate and taxes attentional processing due to the continuous allocation of attentional resources
Increasing Inter-trial Interval	Increases the duration between stimulus-onset reducing the event-rate, placing demands on attention and impulse control

Description of task interventions used and the effect on performance

3.2.6 Effects of acute challenge of psychotomimetics on rat performance in 5-CSRTT

Effects of acute psychotomimetic challenge were investigated. Administration of either amphetamine or PCP conformed to a Latin square design. Experiments were conducted Monday – Friday over a course of two weeks. Animas were allocated into two treatment groups with all receiving vehicle (0.9% saline, i.p.) on Monday, Wednesday and Friday. Depending on treatment group, on Tuesday and Thursday, animals were treated with vehicle (0.9% saline, i.p.), PCP (1.5, 2.0, 2.5 mg/kg, i.p) or amphetamine (0.25, 0.5, 0.75 mg/kg, i.p), according to the Latin square design. The doses of PCP and amphetamine were chosen based on previous findings in our laboratory in various cognitive tasks (Idris et al. 2005; McLean et al. 2010), in which impairment in rodent's performance was produced using similar doses. The testing parameters on treatment days were 0.25 s SD, 5 s ITI, 5 s TO and 5 s LH.

3.2.7 Data Analysis

All data are expressed as mean $\pm$ S.E.M. Analysis of the effect of sub-chronic PCP treatment on 5-CSRTT performance was conducted by an unpaired Students t-test. Analysis of acute psychotomimetic challenge of 5-CSRTT performance was conducted by a mixed-model analysis with Treatment as fixed factor and animal as a random effect, followed by Planned Comparisons (Snedecor and Cochran, 1989). This allowed a reduction of variability by utilising within-subjects analysis, resulting in an increased statistical power (Simon Bate, personal communication).

3.3. Results

3.3.1.1 Effect of sub-chronic PCP treatment on 5-CSRTT performance

Sub-chronic PCP treatment had no impact on animal baseline animal performance. Response accuracy (t = 1.04, NS), percent correct (t = 0.28, NS) and percent omission (t = 0.98, NS) showed no significant difference (fig 3.1A). Likewise, CL (t = 0.34, NS), IL (t = 1.56, NS), ML (t = 1.08, NS) were also unaffected (fig 3.1B). The number of trials completed was similarly unaffected (t = 1.05, NS) as was premature responding (t = 0.39, NS). A trend towards a PCP-induced increase in perseverative responding was observed, but the increase lacked statistical significance (t=1.68, p = 0.09) (fig 3.1C).

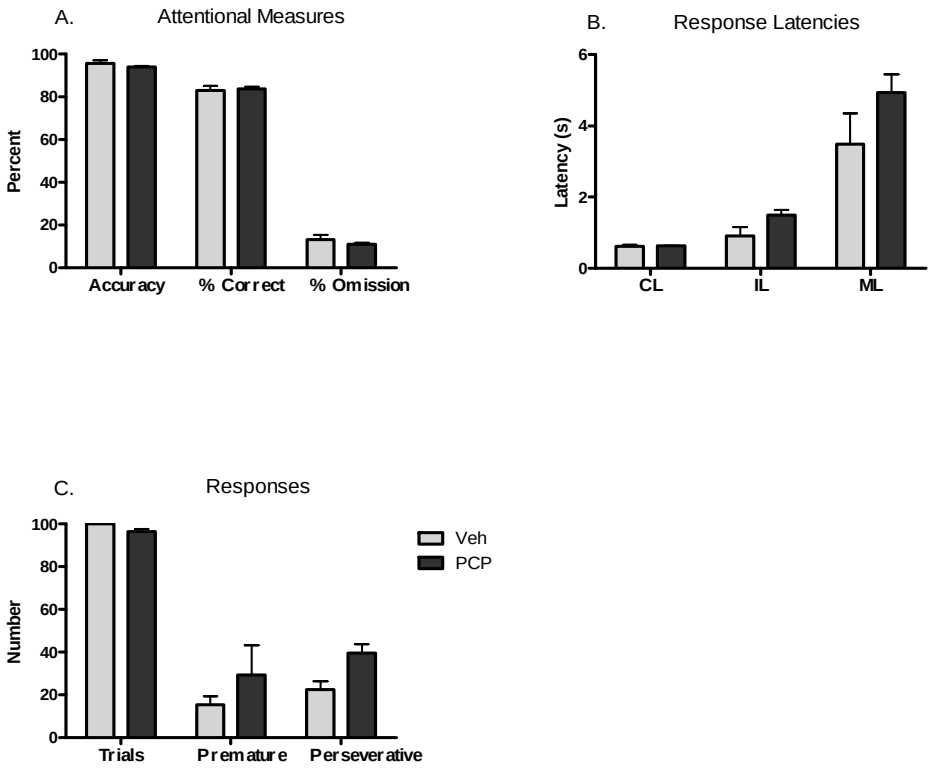

Fig 3.1 The effect of sub-chronic PCP treatment on baseline behavioural performance. Data are expressed as mean ± SEM for vehicle (n = 8) and PCP (n = 8) treated animals. Measures assessed include attentional performance (A), response latencies (B) and response profile (C).

3.3.1.2 Reduction of Stimulus Duration

A reduction of SD from 1 s to 0.5 s impaired 5-CSRTT performance (table 3.2), indicating an increase in attentional load. Accuracy was significantly reduced ($t = 3.68$, $p<0.01$), as was percent correct responding ($t = 4.08$, $p<0.01$). Omissions was significantly increased ($t = 2.24$, $p<0.05$). No effect on CL was observed ($t = 0.6$, NS), but IL was significantly elevated ($t = 3.29$, $p<0.01$) independent of ML alterations ($t = 1.10$, NS). The number of trials was unaffected ($t = 1.17$, NS), in addition to premature ($t = 0.76$) and perseverative responding ($t = 0.48$, NS).

Table 3.2 Effect of reduced SD in saline treated animals

Measure	1 s SD	0.5 s SD
Accuracy (%)	95.62 ± 1.56	83.12 ± 2.82 **
Correct (%)	83.00 ± 2.29	66.55 ± 3.16 **
Omission (%)	13.16 ± 2.15	20.03 ± 2.21 *
Correct Latency (s)	0.61 ± 0.04	0.57 ± 0.03
Incorrect Latency (s)	0.91 ± 0.24	1.75 ± 0.12 **
Magazine Latency (s)	3.48 ± 0.87	2.51 ± 0.32
Trials	100.00 ± 0.00	98.1 ± 1.31
Perseverative Response	15.33 ± 4.06	19.14 ± 3.09
Premature Response	22.50 ± 3.87	25.85 ± 5.61

Comparisons between long (1 s) and short (0.5 s) stimulus duration (n = 8)

While the reduced SD resulted in performance impairment, a PCP-induced deficit was not evident (fig 3.2A). No differences in accuracy (t = 0.73, NS), percent correct (t = 0.17, NS) and percent omissions (t = 0.9, NS) were observed between treatment groups. Additionally, CL (t = 1.16, NS), IL (T = 0.9, NS) and ML (t = 1.12, NS) were also unaffected (fig 3.2B). Furthermore, no change in trials completed (t = 1.8, NS), premature responding (t = 0.5, NS) or perseverative responding (t = 1.24, NS) was observed (fig 3.2C).

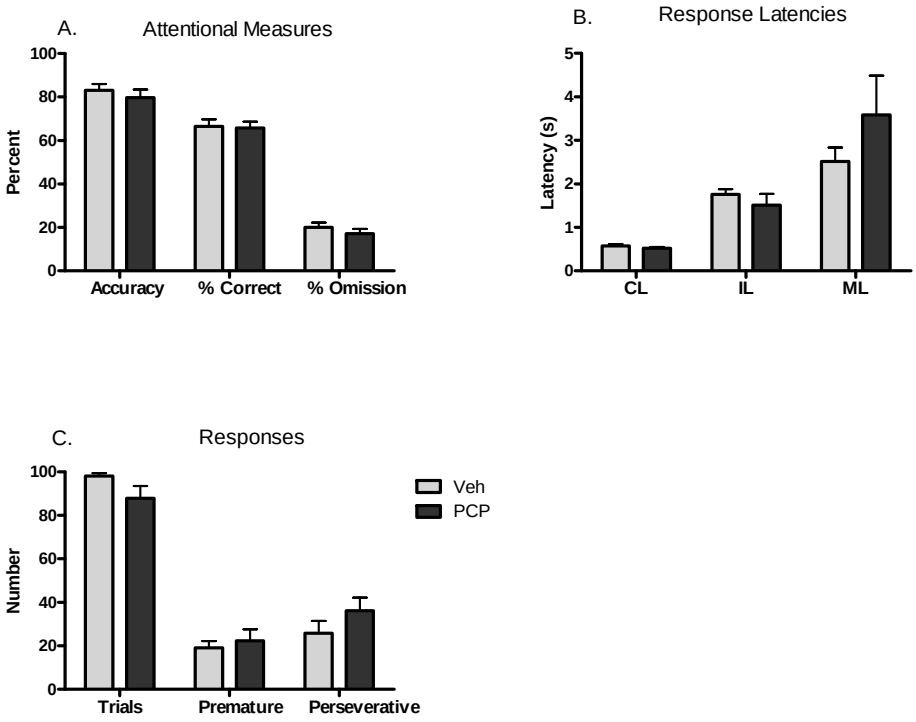

Fig 3.2 The effect of sub-chronic PCP (n = 8) treatment compared to vehicle (n = 8) treatment in the 5-choice task when the attentional load was increased by reducing the stimulus duration to 0.5s. Data are expressed as mean ± SEM. Measures assessed include attentional performance (A), response latencies (B) and response profile (C).

3.3.2 Effects of task interventions on rat performance in 5-CSRTT

3.3.2.1 Increased Event-Rate

An increased event-rate revealed a PCP-induced attentional impairment. Accuracy was significantly reduced in PCP-treated animals ($t = 2.64$, $p<0.05$) (fig 3.3A). This occurred without an effect on percent responding ($t = 1.11$, NS) or percent omissions ($t = 0.09$, NS). Furthermore, PCP-treatment had no effect on CL ($t = 0.27$, NS), IL ($t = 0.23$, NS) or ML ($t = 0.53$, NS) (fig 3.3B). Likewise, trials competed ($t = 0.14$, NS), premature ($t = 1.03$, NS) and perseverative responding ($t = 0.89$, NS) were all insensitive to a PCP-induced alteration (fig3.3C).

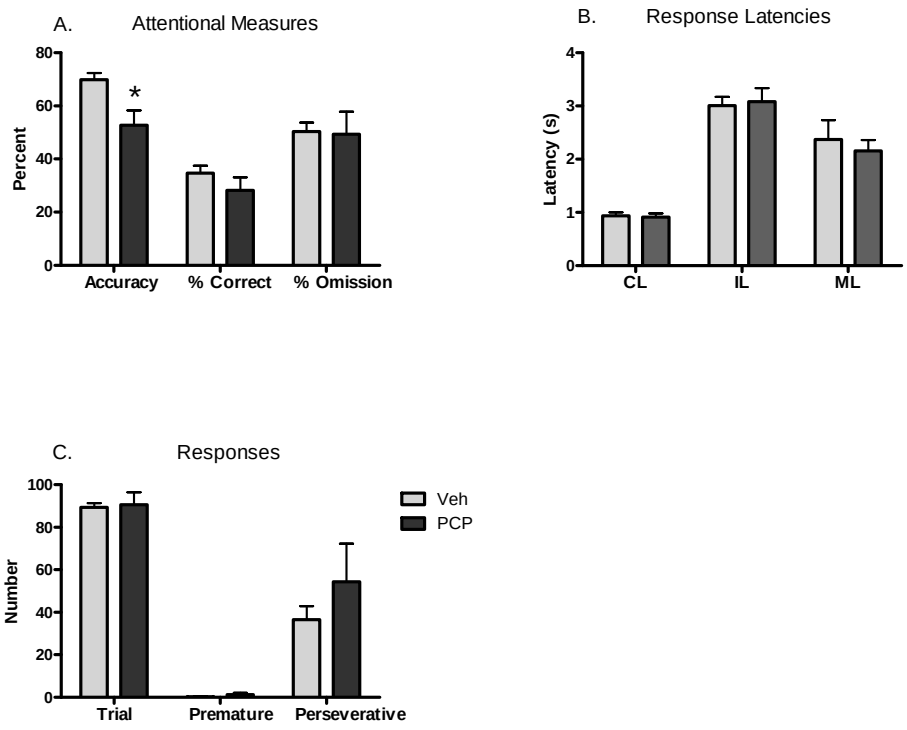

Fig 3.3A: The effect of sub-chronic PCP treatment (n = 8) compared to vehicle treated animals (n = 8) in the 5-choice task when challenged by reducing the ITI from 5 seconds to 2 seconds. Data expressed as mean ± SEM and analysed by unpaired Student's t-test (* $p<0.05$ reduction in accuracy when PCP was compared to the Veh group).

A repeat of the increased event-rate challenge also demonstrated attentional impairment. However, accuracy was unaffected (t = 0.2, NS). Instead a reduction in correct responding (t = 2.45, p<0.05) and an increase in omission (t = 2.45, p<0.05) was observed (fig 3.4A). Consistent with the previous exposure to the increased event-rate challenge, no treatment effect was observed in CL (t = 0.57, NS), IL (t = 0.41, NS) and ML (t = 0.11, NS) (fig 3.4B). Additionally, PCP-treatment had no effect on trials completed (t = 1.61, NS), premature (t = 1.36, NS) or perseverative responding (t = 0.51, NS) (fig 3.4C).

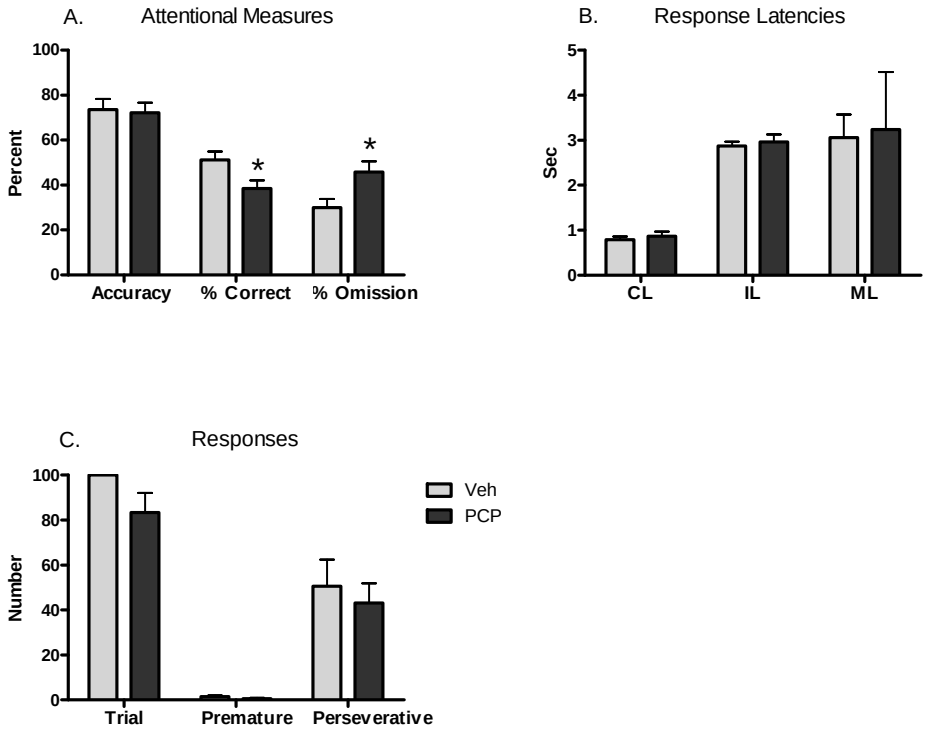

Fig 3.4A: In a repeated experiment where the ITI was again 2 seconds, PCP (n = 8) produced a significant difference in percent correct and percent omission compared to vehicle animals (n = 7). Data are expressed as mean ± SEM and analysed by unpaired Student's t test. Percent correct responding was significantly reduced and percent omissions was significantly increased (* = p<0.05, PCP compared to the Veh group).

3.3.2.2 Reduced Event-Rate

Sub-chronic PCP treatment had no effect when the event rate was reduced, by increasing the ITI. The lack of effect on accuracy (t = 0.54, NS), percent correct (t = 0.08, NS) and omissions (t = 0.72, NS) suggest no PCP-induced attentional impairment (fig 3.5A). Additionally, no effect on CL (t = 0.85, NS), IL (t = 0.34, NS) or ML (t = 0.51, NS) was evident (fig 3.5B). Furthermore, there was no treatment effect on trials completed (t = 0.17, NS), premature responding (t = 0.32, NS) or perseverative responding (t = 0.29, NS) (fig 3.5C).

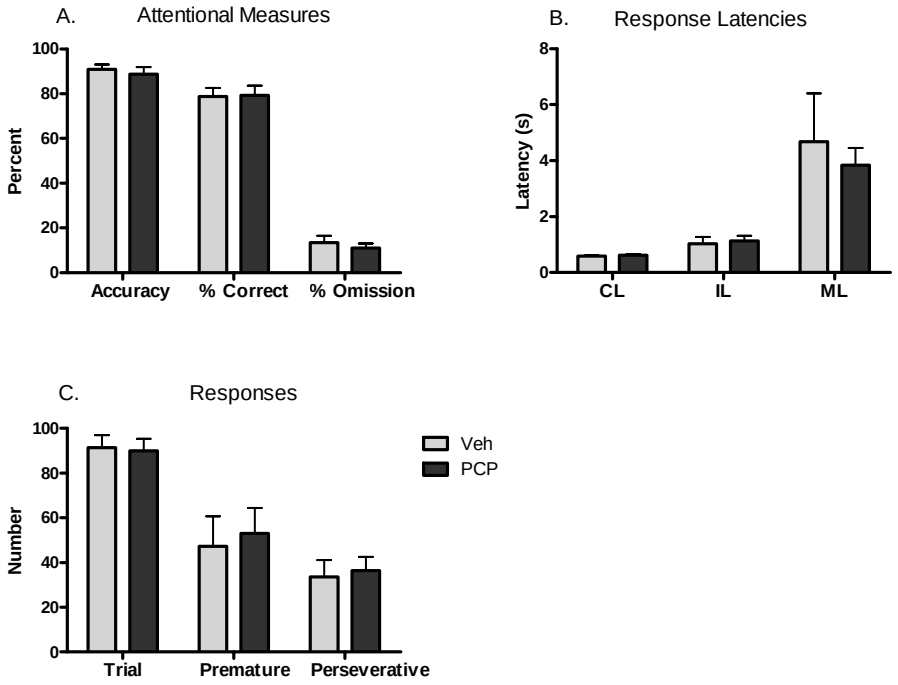

Fig 3.5A, B and C: The effect of sub-chronic PCP treatment (n = 8) and vehicle treatment (n = 7) in the 5-choice task when animals were challenged by increasing the ITI from 5 to 6 seconds. Data are expressed as mean ± SEM and analysed by unpaired Student's t test.

3.3.3 Effects of acute challenge with psychotomimetics on rat performance in 5-CSRTT

3.3.3.1 Amphetamine

The following two experiments utilised a Latin square design in which each animal received each dose and acted as its own control. Analysis showed that amphetamine (fig 3.6A) caused a significant reduction in accuracy [$F_{(3, 19)}$=3.74, p<0.05]. Planned Comparisons revealed the highest dose (0.75 mg/kg) of amphetamine significantly reduced accuracy (p<0.01). Additionally, amphetamine reduced percent correct responding [$F_{(3, 19)}$=6.02, p<0.01]. Planned Comparisons demonstrated only the highest dose of amphetamine produced a significant reduction of percent correct responding (p<0.01). Amphetamine also produced a significant increase in the number of error of omissions [$F_{(3, 19)}$=5.46, p<0.01], however Planned Comparisons showed that only the lowest dose (0.25mg.kg) of amphetamine produced a significant effect (p<0.05). Amphetamine treatment (fig 3.6B) had no significant effect on correct latency [$F_{(3, 19)}$=1.41, NS]. Initial analysis of incorrect latency resulted in a strong trend towards a significant reduction [$F_{(3,, 19)}$=2.60, p=0.06]. Planned Comparisons demonstrated that all amphetamine doses significantly reduced incorrect latency (p<0.05). Analysis of the effect of amphetamine on magazine latency (fig 3.6B) revealed a strong trend towards a significant effect [$F_{(3, 19)}$=2.58, p=0.06]. Planned Comparisons showed that 0.5 mg/kg and 0.75 mg/kg significantly increased magazine latency (p<0.05). Amphetamine treatment produced a significant reduction (fig 3.6C) in the number of trials completed [$F_{(3, 19)}$=12.97, p<0.001]. Further analysis revealed that amphetamine (0.5 mg/kg and 0.75 mg/kg) resulted in a significant reduction in completed trials (p<0.001). Amphetamine treatment produced a significant increase in the number of premature responses (fig 3.6C) [$F_{(3, 19)}$=10.36, p<0.001]. Planned Comparisons revealed that all three doses of amphetamine produced a significant increase in premature responding (p<0.001). Amphetamine also produced a significant increase in the level of perseverative responding [$F_{(3, 19)}$=8.29, p<0.001]. Following Planned Comparisons, it was revealed that all three doses of

amphetamine produced a significant increase in perseverative responding ($p<0.001$) (fig 3.6C).

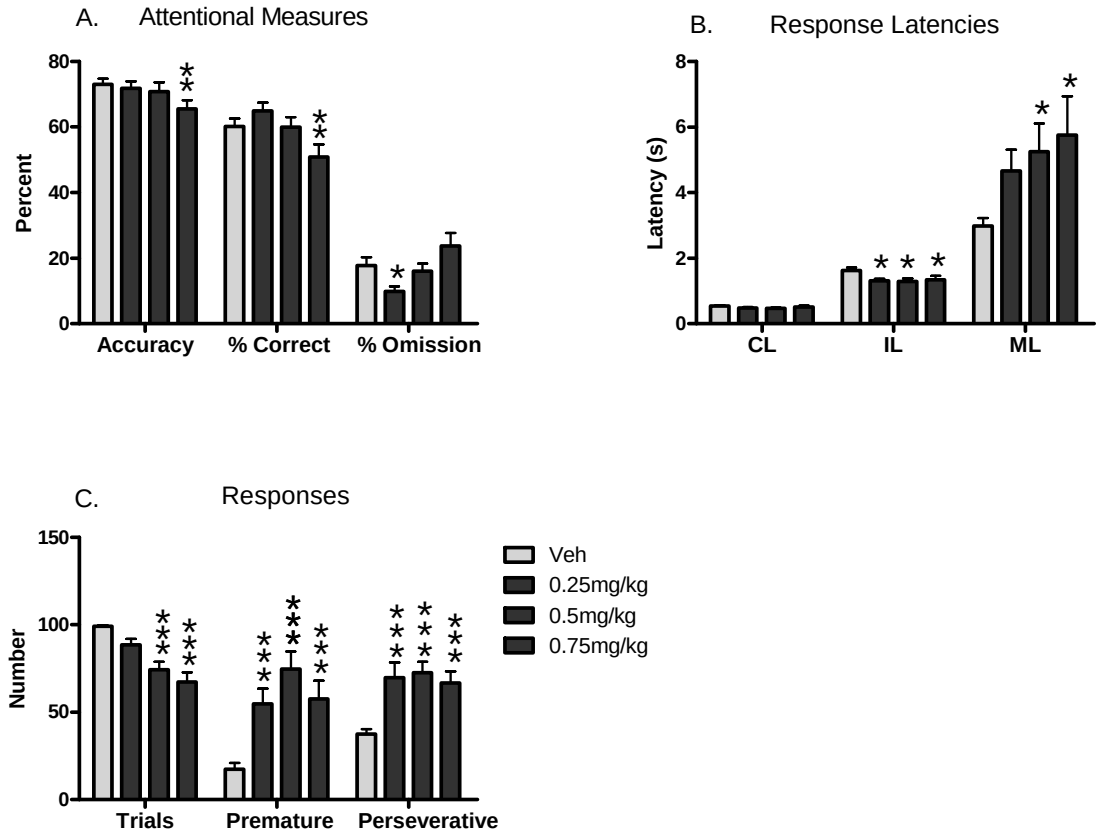

Fig 3.6A Acute amphetamine (n = 20) treatment (0.75 mg/kg i.p.) produced a significant reduction in accuracy and percent responding. The dose of 0.25 mg/kg produced a significant reduction in percent omissions **B;** Amphetamine resulted in a significant reduction in incorrect latency (0.25 – 0.75 mg/kg). Amphetamine also increased magazine latency (0.5 – 0.75 mg/kg). **C;** Amphetamine reduced the number of trials completed (0.5 – 0.75 mg/kg) and also significantly increased the number of premature and perseverative responses. Data expressed as mean ± SEM. * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ significantly different from Veh group.

3.3.3.2 PCP

Acute PCP treatment produced a significant reduction in accuracy [$F_{(3, 19)}$=8.89, p<0.001]. Following Planned Comparisons it was evident that only the two higher doses of PCP resulted in a significant reduction in accuracy (p<0.01 and p<0.001, respectively) (fig 3.7A). PCP also produced a highly significant effect on percent correct responding [$F_{(3, 19)}$=14.61, p<0.001]. As with accuracy, Planned Comparisons showed that it was only the two highest doses that produced a significant reduction in percent correct responding (p<0.01 and p<0.001, respectively) (fig 3.7A). Acute PCP treatment also produced a significant increase in the number of error of omissions [$F_{(3, 19)}$=12.28, p<0.001]. Planned Comparisons demonstrated that only 2.0 mg/kg and 2.5 mg/kg PCP produced significant increases in omissions (p<0.05 and p<0.001, respectively). Statistical analysis showed that PCP had a significant effect on correct latency [$F_{(3, 19)}$=6.34, p<0.01]. Only the highest dose of PCP (2.5 mg/kg) produced a significant increase in correct latency (p<0.001) (fig 3.7B). PCP also produced a significant effect on incorrect latency [$F_{(3, 19)}$ = 5.22; p<0.01]. Planned Comparisons also indicated PCP treatment only produced a significant effect at the highest dose (p<0.01) (fig 3.7B). Initial analysis suggested that PCP treatment did not affect magazine latency [$F_{(3, 19)}$=1.84, p=0.15]. However, Planned Comparisons demonstrated that the highest dose of PCP (2.5 mg/kg) produced a significant increase in magazine latency (p<0.05) (fig 3.7B). PCP produced a significant reduction in the number of trials completed [$F_{(3, 19)}$ = 13.35, p<0.001]. It was demonstrated the two higher doses of PCP caused a significant reduction in trials completed (p<0.01 and p<0.001, respectively) (fig 3.7C). Additionally, a significant increase in premature responding was evident [$F_{(3, 19)}$=5.05, p<0.01]. Planned Comparisons revealed that all three doses of PCP produced a significant increase (p<0.05 and p<0.01) (fig 3.7C).

A strong trend towards an increase in perseverative responding was observed [$F_{(3, 19)}$=2.44, p=0.07]. However, Planned Comparisons showed all three doses of PCP produced a significant increase in perseverative responding (p<0.05) (fig 3.7C).

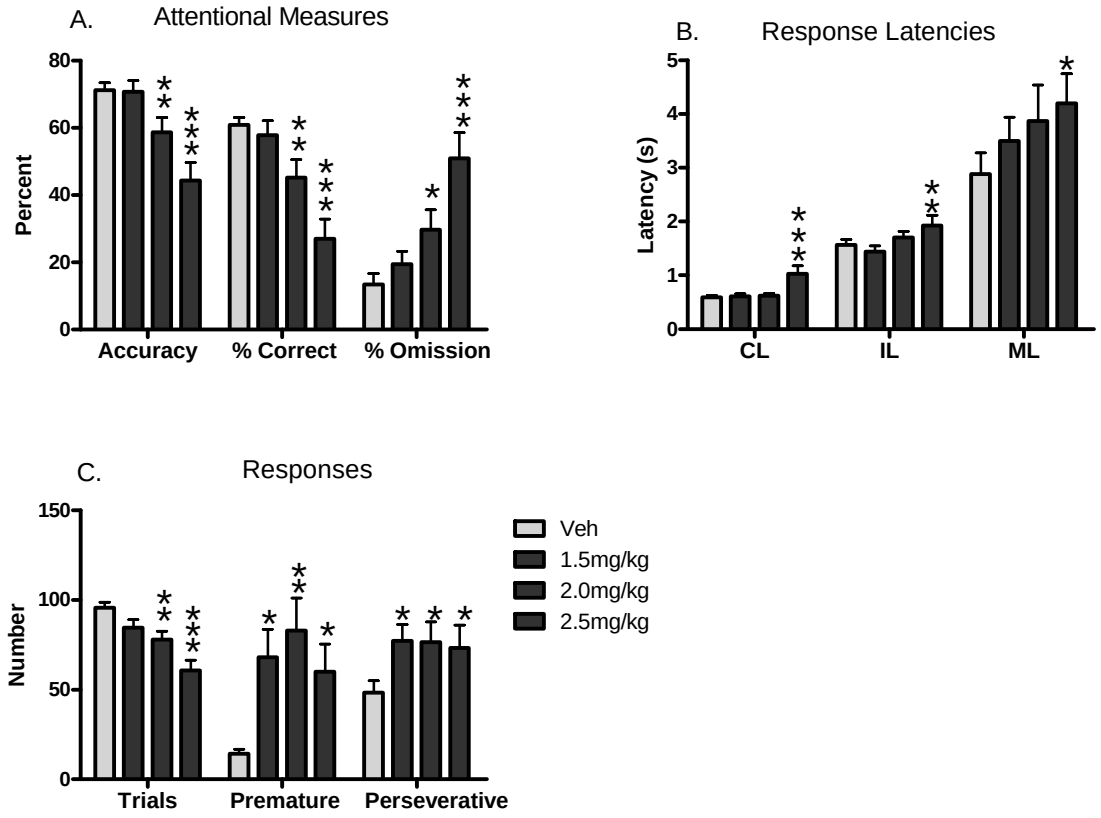

Fig 3.7A; Acute PCP (n = 20) treatment (2.0 – 2.5 mg/kg) reduced accuracy, percent correct and increased percent missed. **B;** Data show that acute PCP (2.5 mg/kg i.p.) caused a significant increase in correct latency, incorrect latency and magazine latency. **C;** PCP (2.0 – 2.5 mg/kg i.p.) reduced the number of trials completed. Premature and perseverative responding was increased as a result of PCP treatment (1.5 – 2.5 mg/kg). Data expressed as mean ± SEM. * = p<0.05, ** = p<0.01, *** = p<0.001 significantly different from Veh group.

3.4. Discussion

The primary aim of this chapter was to establish the 5-choice task serial reaction time task in female rats as a method of assessing the multifaceted cognitive construct of attention. Secondary to this aim, the effects of sub-chronic PCP treatment were investigated in this paradigm for its ability to disrupt successful performance of the task and identify whether sub-chronic exposure to PCP disrupted attentional processing. Finally, the effect of an acute challenge of PCP or amphetamine on the ability of the animal to perform the 5-CSRTT was investigated.

One of the primary aims of this chapter was to establish and validate the 5-Choice Serial Reaction Time Task (5-CSRTT) at University of Bradford. The first experiment demonstrated that female Lister-hooded rats can be trained in the 5-CSRTT to a predetermined criteria (>80% correct responding, <20% omissions), which is the general accepted level of training suggested by previous experiments (Grottick and Higgins 2000; Higgins et al. 2005; Murphy et al. 2005). This level of performance is well above chance performance of 20% correct responding (Robbins, 2002). The secondary aim of the experiments within this chapter was to determine the effects sub-chronic PCP treatment had on the animals' performance in the task. Data demonstrated that sub-chronic PCP treatment failed to induce a performance deficit in the 5-CSRTT, when animals were tested using the same criteria that they were trained to. The lack of PCP-induced impairment was still evident when the attentional load of the task was increased by reducing the stimulus duration (SD). The reduction in SD did increase the difficulty of the task, as evidenced by the impaired performance in control animals. However, it is possible that the stimulus duration was not reduced enough, with a further reduction potentially revealing a PCP-induced impairment. Previous studies have used either a 1 or 0.5 s SD for standard testing (Mirza and Stolerman, 1998; Grannon et al. 2000; Dalley et al. 2001; Fletcher et al. 2007; Young et al. 2007). Therefore, if the SD was

reduced beyond 0.5 s (e.g. 0.25 or 0.125 s), stimulus detection is made more difficult further testing the animal's attentional capacity, with the potential consequence of PCP treated animals having an increased inability in target detection compared to control animals. Testing under these conditions may have revealed a more robust PCP-induced impairment.

In light of these observations, the next stage was to challenge performance by introducing parameter manipulations. When the ITI was reduced, thus increasing the event rate and increasing the attentional load of the task (Parasuraman, 1998), PCP treated rats had a small, yet significant impairment in a number of attentional measures. A reduction in accuracy was evident. This effect is suggestive of an impairment in attentional processing, resulting in a reduced ability to detect and correctly report the occurrence of the visual stimulus. As a result, the implementation of a guessing strategy (detailed in the introduction) is possible, which increases the likelihood of incorrect responding, reducing the choice accuracy. When the reduced ITI challenge was repeated, to determine the reproducibility of the impairment, there was a different outcome. The reduction in accuracy disappeared, however this was replaced by a reduction in percent correct responding and an increase in omission. The interpretation of attentional dysfunction resulting in an increase in omissions (Risbrough et al. 2002; Young et al. 2004; Cordova et al. 2006) is supported by the lack of a concomitant increase in magazine latency. If the time to retrieve the food reward was elevated, it is suggestive of reduced motivation which would confound interpretation of attentional dysfunction. As these effects occurred without alterations in response latencies they can be interpreted as a reduction in attentional capabilities (Amitai and Markou, 2010).

The alteration in effects observed may have been due to the fact animals had been exposed to this behavioural challenge once before and thus an element of learning had taken place. Animals may have modified their strategy in light of the repeated exposure to the same

challenge and so were not simply guessing (and guessing incorrectly) when they failed to detect the target stimulus, hence the lack of effect on response accuracy. However, as their attentional capabilities were still compromised following this challenge, this resulted in the increased number of trials being omitted (which also reduced the number of correct response being made).

When the ITI was increased it was hypothesised that the reduction in event rate would make the task sensitive to aspects of behavioural disinhibition and would reveal if PCP treatment induced impulse control impairments. While there was an increase in the baseline level of premature responding (a well characterized measure of impulsivity), the elevated level of premature responding was not significantly affected by PCP treatment. This, therefore suggests, that the form of impulsivity assessed by premature responses is not sensitive to disruption via sub-chronic exposure to NMDA receptor antagonists in the drug-free state.

Sub-chronic PCP treatment produced significant impairments in performance, albeit only subtle deficits, when the parameters were altered that are reminiscent of the results produced by some lesion studies. Carli and colleagues (1983) lesioned the ascending noradrenergic fibres arising from the locus coerulus (the dorsal noradrenergic bundle, DNAB) using 6-hydroxydopamine (6-OHDA), in rats that were fully trained in the task. Initially there was no impairment in the rats' ability to perform the task. However, when the ITI was reduced, this resulted in a significant reduction in choice accuracy which was not present when rats were test under baseline conditions. Carli and colleagues (1983) also examined the effect of interpolating bursts of white noise with the presentation of the stimulus, which resulted in a reduction in choice accuracy and an increase in percent omissions, indicating attentional impairment in the DNAB lesioned animals.

One main limitation of this experiment was the assumption that sub-chronic PCP treatment would induce impairment in 5-CSRTT performance. Task performance relies heavily on PFC function, demonstrated by impaired performance following discrete lesion of this region (Muir et al. 1996), an area that is densely populated with NMDA receptors (Cotman & Iversen, 1987; Ulus et al. 1992). It has been demonstrated that acute and repeated PCP treatment induced deficits in 5-CSRTT performance (Amitai et al. 2007). In light of this, it was therefore hypothesised that performance in the 5-CSRTT would also be sensitive to disruption via sub-chronic PCP treatment. Another task that requires the PFC for successful performance is the attentional set-shifting task (Birrell & Brown, 2000), and previous investigations have demonstrated that performance within this task is disrupted via sub-chronic PCP administration (Rodefer et al. 2005; Rodefer et al. 2008; McLean et al. 2008; Broberg et al. 2009; McLean et al. 2009). These findings suggest PCP treatment may induce long-term changes that compromise the functioning of the PFC, impairing task performance in paradigms associated with this brain region.

When animals were exposed to acute doses of psychotomimetic drugs, there was a pronounced effect on task performance. When rats received an acute dose of PCP (1.5 – 2.5 mg/kg) there was a significant impairment in performance, demonstrated by attentional dysfunction, behavioural disinhibition, and at the highest dose, an increase in response latencies. Acute PCP (2.0 – 2.5 mg/kg) resulted in a significant reduction in accuracy, percent correct responding and increased percent omissions. These findings alone would suggest a generalised impairment in attentional capabilities, suggestive of an inability of the rat to sustain and divide attention (Robbins 2002). However when the highest dose (2.5 mg/kg) was administered, the deficits in attentional measures were accompanied by a significant increase in all of the response latencies measured. The effect on response latencies make interpretation difficult, as the inability to perform the task may have been the result of a

generalised performance deficit induced by motoric or motivational dysfunction (Robbins 2002). It should be noted that the treatment regimen followed a Latin square design and thus, each animal acted as its own control. Whilst increasing the statistical power, this design is not truly acute administration as each animal received multiple injections of PCP. Consequently, the pronounced behavioural disrupted effects (discussed in detail in Chapter 5) of a true acute dose of PCP may have been avoided as the treatment regimen used is technically repeated administration (Amitai and Markou, 2010). Therefore, the motoric or motivational disruption may not have been apparent with the lower PCP doses.

Acute PCP treatment, at all doses, produced an increase in the number of premature and perseverative responses suggestive of behavioural disinhibition. This manifested as the inability of the rat to withhold from responding before the stimulus onset (premature response) or the inability of the rat to disengage from responding to a previously rewarded behaviour (increased perseverative responding). Acute PCP treatment is reported to impair frontal cortical brain function (Verma and Moghaddam 1996; Jentsch and Roth 1999). Acute exposure to ketamine has been shown to increase regional blood flow to the frontal areas of the brain, particularly the anterior cingulate and frontomedial cortical brain regions of normal humans and schizophrenia patients (Lahti et al. 1995; Breier et al. 1997; Vollenweider et al. 1997). Therefore, it is possible that PCP, another NMDA receptor antagonist, also causes increased frontal cerebral blood flow that may result in over-activation of brain regions involved in behavioural inhibition, impairing inhibitory control following a non-monotonic dose-response curve of activation necessary for optimal performance, commonly referred to as an inverted 'U' shaped response. The inverted 'U' hypothesis, known as the Yerkes-Dodson principle (Yerkes and Dodson, 1908; Diamond et al. 2007), states that under-activation of a brain region, or a reduced release of neurotransmitter, will result in reduced performance, as the specific brain region has not been sufficiently activated. The same

applies for over-stimulation, whereby it is possible that receptors within the brain region are desensitised by the over-activation and cease to function properly, resulting in reduced activation of the brain region, and therefore impairment in behaviour.

Acute treatment with amphetamine produced a much less pronounced effect on the attentional measures of the 5-choice task, only producing significant reduction in choice accuracy and percent correct at the highest dose (0.75 mg/kg). The reduction in attentional measures was also accompanied by an increase in ML. Therefore, it is reasonable to assume that the reduction in choice accuracy and percent correct responding was potentially mediated by a disruption in locomotor activity (e.g. hyperlocomotion) or reduced motivation of the animal to perform the task, as opposed to impairment in attentional functioning. Acute amphetamine administration had a more pronounced effect on impulse control or the ability to disengage from a response, compared to the effect on these measures from an acute challenge of PCP. All three doses (0.25 – 0.75 mg/kg) increased in the number of premature and perseverative responding. Response disinhibition following amphetamine administration has previously been described in the 5-CSRTT (van Gaalen et al. 2006). These findings are possibly due to the fact that amphetamine mainly acts on the dopaminergic system, resulting in increased release of dopamine in cortical and limbic brain regions (Balla et al. 2002) possibly influencing regions of the brain that are involved with behavioural inhibition and impulse control such as the sub-regions of the PFC or the striatum (Dalley et al. 2007b). The increased release of dopamine within regions of the brain is likely to follow the same principle of the inverted 'U' hypothesis (described above) (Yerkes and Dodson 1908; Diamond et al. 2007). Therefore the behavioural effects from the increased dopaminergic activity that results from a non-selective increase in dopamine release due to an acute challenge of amphetamine, is likely to result in impaired response inhibition, that manifests as increased premature and perseverative responding. Amphetamine treatment also

significantly reduced the latency to make an incorrect response, without affecting the time taken to make a correct response. The effect amphetamine had on behavioural inhibition and the increased number of premature responses likely causes this. The reduction in IL is likely the result of the effect on impulse control, as some of the incorrect responses would have been made on the threshold of stimulus presentation. The response would have been on the verge of being a premature response, but the animal made its 'premature response' just as the ITI elapsed, and the stimulus was presented. It is more likely that the animal would make an incorrect response (4:5 chance) than a correct response (1:5 chance) and thus, the latency of this incorrect response would be a few milliseconds, significantly reducing the overall average of IL. Therefore, a reduction in IL may provide additional information regarding impulse control in addition to premature responding.

The vast majority of previous investigations into rodent performance of the 5-choice task use male rats, from Long-Evans or Hooded-Lister strains (Stolerman et al. 2000; Bari et al. 2008; McNamara et al. 2010; Thomson et al. 2010) as both these strains have pigmented retinas and are suggested to have superior vision compared to non-pigmented species. However, some groups do use albino strains, such as Wistar or Sprague-Dawley (Le Pen et al. 2003). Auclair and colleagues (2009) compared a pigmented species (Long-Evans) with an albino species (Sprague-Dawley) in order to determine species-dependent variations in task acquisition, coupled with PCP sensitivity. While Auclair and colleagues (2009) found little difference in task performance once rats were trained to criterion, they did report a difference in the effects of acute PCP administration. The group reported that Sprague-Dawley rats had a greater reduction of premature responding, whilst latency to make a correct response and levels of trials omitted were increased in response to PCP. Administration of PCP resulted in a reduction in premature responding in this study, in contrast to previous findings in the literature, which demonstrated that PCP treatment impaired behavioural inhibition (Amitai et al. 2007; Le Pen et al. 2003). However, in the case

of Auclair et al (2009), in the paradigm used, premature responses were not punished, thus removing the incentive to withhold responding until the stimulus is presented. The consequence of this is that basal levels of premature responses were elevated and were far higher than previous investigations reported. This highlights the fact that subtle variations in task parameters between laboratories may result varying intra-laboratory results and therefore drawing meaningful conclusions does require careful consideration.

As the vast majority of behavioural experimentation is carried out using male rats (Grottick and Higgins, 2000; Stolerman et al. 2000; Amitai et al. 2007; Bari et al. 2008) and the experiment conducted by Auclair and colleagues (2009) demonstrated differences in performance in the 5-CSRTT between strains of rat, it is therefore important to also consider gender differences and the impact they may have on task performance. This consideration is of particular importance as all behavioural paradigms at University of Bradford use female hooded-Lister rats; with the current study no exception. However, on the face of the present findings, there is no sex difference in general 5-CSRTT performance or the ability to acquire the task and so it appears that the ability to perform the basic task is not affected by gender. Moreover, previous investigations within our laboratory have demonstrated that cognitive performance is largely unaffected by different stages of the oestrous cycle (Sutcliffe et al. 2007; McLean et al. 2009), further supporting the absence of a gender effect.

These data have demonstrated that female hooded-Lister rats can be trained to perform in the 5-CSRTT, to predetermined criteria that is deemed sufficient by previous experiments and have a performance level well above the chance performance of 20% correct responding. It has also been shown that sub-chronic PCP treatment does not impair task performance when animals are tested using the standard training protocols. However, when the animals' performance was challenged, by manipulating task parameters sub-chronic PCP

treatment produced subtle deficits in task performance. When psychotomimetic agents were acutely administered, there was a profound and pronounced impairment in task performance. Acute PCP resulted in both attentional and inhibitory control impairments, whilst acute amphetamine primarily resulted in deficits in impulse control. However, the potential for generalised response disruption cannot be ruled out. This collection of experiments demonstrates that the 5-choice serial reaction time task is a valid behavioural paradigm for measuring various cognitive elements that are clinically affected in schizophrenia, and shows there is potential in pharmacologically inducing deficits that may model the cognitive dysfunction seen in the disorder. Future experiments will further probe the possibility that the cognitive construct of attention is sensitive to pharmacological disruption using NMDA antagonists. In order to do this, a modified version of the basic 5-CSRTT will be utilised, which has recently been validated in mice (Young et al. 2009b), which may more accurately assess attention, specifically enabling quantification of vigilance in a manner consistent with human vigilance testing. The following chapter will investigate the use of the 5-Choice Continuous Performance Test and the applicability of using rats in this paradigm.

Chapter 4

Rat Performance within the 5-Choice Continuous Performance Test:

Effects of D_1 partial agonism

4.1 Introduction

As previously discussed, the 5-CSRTT is considered to share many of the features of the human CPT (Bari et al. 2008). Despite this analogy with human CPTs, sub-chronic PCP treatment failed to induce convincing deficits (experimental chapter 3) in 5-CSRTT performance, perhaps limiting the applicability of this pharmacological model to replicate *all* of the cognitive impairment present in the disorder; or at least with the current preclinical model assessing the cognitive construct under investigation. Schizophrenia patients perform poorly in multiple versions of the CPT, including the CPT-IP, chosen by the MATRICS initiative to assess attention in schizophrenia patients (Neuchterlein et al. 2008; Kern et al. 2008), indicating that impaired attention/vigilance is inherent to the disorder (Rosvold et al. 1956; Kornetsky and Mirsky, 1966; Wohlberg and Kornetsky, 1973; Cornblatt and Keilip, 1994; Kumar et al. 2010). Impaired attention/vigilance, along with other cognitive deficits, deleteriously impact the patient's quality of life (Cornblatt and Keilip, 1994; Marder and Fenton, 2004), relating to social problem solving and skill acquisition (Green et al. 1996). Despite the myriad versions of the CPT, (CPT) (Rosvold et al. 1956), CPT-Identical Pairs (CPT-IP) (Cornblatt et al. 1988), AX-CPT (van den Bosch et al. 1996) or Conners CPT (Conner et al. 2003) (described in chapter 1), each task has some consistency in that they include both target trials requiring a response and non-target trials requiring inhibition of a response, the key to successful task performance relies on successful discrimination between trial types.

Depending on the trial presented, various response outcomes are possible in CPTs (hits, misses, correct rejections and false alarms – see table 4.1) and due to the generation of false alarms, signal detection theory (SDT) to be employed to further assess performance (See et al. 1995; Marston 1996; Steckler 2001). SDT provides researchers additional insight into the response profile of the subject and can separate attentional or vigilance impairment/improvement from alterations in the response strategy or bias of the subject (Marston 1996; Robbins 1998; Young et al. 2009b). Impairment or improvement in

sensitivity may be due to a reduced or increased ability to detect the signal, or alterations in the decision criteria of the subject; SDT enables the elucidation of the process behind task performance (Marston 1996). Thus, the inclusion of SDT measures may provide an enhanced method of revealing or quantifying impairments in CPT performance that might not be evident using omission or commission errors alone (Lam and Beale 1991; Mass et al. 2000; Riccio et al. 2002) and as such, SDT is routinely utilised in the interpretation of CPT performance (Nestor et al. 1990).

The premise behind SDT is that an individual is likely to develop a strategy to determine when it is appropriate to respond to a signal, depending on whether the salience of the signal corresponds to the individual's internal criteria of what constitutes a signal that should elicit a response (Steckler 2001; Harvey 2003). When SDT was in its infancy, researchers investigating the ability of individuals to correctly detect and report brief stimuli associated with radar scanning used statistical theory in order to aid the quantification of determining the probability of detection of brief, pulse-like signals in individuals using radar system technology (Marcum 1947). The outcome of this technique led to development of the psychological theory behind SDT and formed the basis of the analytical theory by Wilson and Swets (1954) in which they evaluated the ability of an individual to detect a visual light stimulus from a background of uniformed light 'noise'. The ultimate aim of SDT is to utilise the available indices produced by the possible response outcomes (e.g. hit, miss, false alarm, correct rejection) in order to provide an enhanced level of interpretation of the performance ability of the individual in detecting a signal from noise, irrespective of other external parameters that may be influencing performance (Marston 1996).

	Response	Non - Response
Signal Present (go trial)	***Hit***	Omission
Signal Absent (no-go trial)	False Alarm	***Correct Rejection***

Table 4.1. A description of the possible response outcomes when animals are presented with signal or non-signal trials. Correct responses are in ***italics.***

Using the types of responses described in Table 4.1, along with the frequency that they are presented throughout the task session, SDT enables researchers to generate additional indices that may aid the interpretation of task performance. CPT performance generates measures contained in table 4.1. Thus, proficient performance requires the discrimination between signal (target trials) and noise (non-target trials), SDT can quantify this level of discrimination by producing the parametric index, D prime (d'). The d' index represents a measure of signal sensitivity or the ability to discriminate between target and non-target stimuli. Therefore, a higher level of sensitivity results in a greater ability to correctly detect the signal stimuli and correctly ignore the non-target stimuli (Baerwald et al. 2005), and as a result the means of distribution between signal and noise events will be further apart (fig 4.1). Reduced sensitivity creates a greater overlap in the means of distribution, resulting in an area in which discrimination between signal and noise is impossible. Therefore, the measure d' can be interpreted as a measure of vigilance, as higher levels of vigilance in tasks such as the CPT, will generally result in a higher number of signals being detected, whilst keeping the number of false alarms to a minimum, increasing the discrimination index. This measure of discrimination has been shown to be reduced in schizophrenia patients (Mussgay and Hertwig 1990), supporting the notion that impaired vigilance is associated with the disorder.

Furthermore, also based on the measures summarised in table 4.1, SDT can generate a responsivity index known as β, which indicates the response strategy being utilised. Bias in an individual may influence performance driven by other factors such as motivation, willingness to respond, or alterations in response strategy, factors that are not necessarily driven by vigilance or cognitively mediated measures (Marston 1996; Steckler 2001). If the

value for β is high, it indicates that the subject is responding in a conservative fashion and will only respond if they are absolutely sure a signal has been presented. This will ultimately result in a low false alarm rate, as incorrect responses to non-signals are less likely. However, there is also the chance that actual signals will be missed, thus increasing the omission rate (number of misses). Conversely, if the subject is responding in a liberal fashion, reporting the detection of anything that possibly may be construed as a signal, the omission level will be reduced but at the consequence of increasing the false alarm rate. In this instance the value for β will be reduced. The advantage of SDT is that it allows researchers to determine if pharmacological intervention is increasing performance by increasing the ability of the individual at discriminating the signal from background noise (increasing d') or altering the response strategy of the individual (reflected by changes in β). By using these additional measures it can be determined exactly how increases in task performance are achieved (Dudchenko et al. 1992).

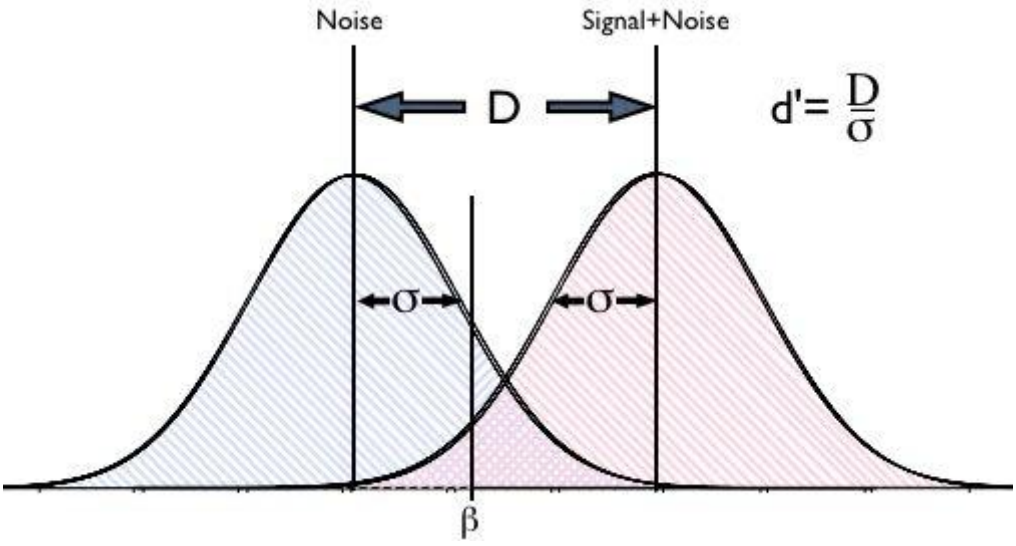

Fig 4.1. Depiction of the measures used in SDT, illustrating the means of distribution of signal and noise events and D demonstrates the discrimination of the two events. Additionally β is identified and provides information to the response strategy being used. D prime (d') is equal to the mean signal minus mean noise divided by delta, which constitutes the standard deviation of distribution. Image was taken from http://sabometrics.com/?p=242

As mentioned, the indices d' and β are parametric measures and as such, must conform to assumptions of normality, absence of skew and homogeneity of variance. If these assumptions cannot be confirmed, it is appropriate to revert to a non-parametric approach to generate these indices. Being non-parametric, fewer assumptions regarding the internal perceptions of the signal need to be made (Young et al. 2009b). In respect to d', the non-parametric alternative utilised in the current chapter is the sensitivity index (SI), first described by Frey and Colliver (1973). Moreover, the responsivity index (RI) also described by Frey and Colliver (1973) was used to represent the strategic bias used throughout the session.

Although it has been suggested that the 5-CSRTT is analogous to the human CPT (Bari et al. 2008), the 5-CSRTT only presents target trials of which there are only one of two possible response outcomes; a hit (correct or incorrect) or a miss. That is to say, the animal responds to the stimulus or it fails to report the detection of the signal. As the 5-CSRTT only presents target trials, it therefore can be implied that this task is not completely analogous to human CPTs (Young et al. 2009b) as the task lacks non-target stimuli that are present in all variants of human CPTs. The absence of trials that enable the generation of false alarms in the 5-CSRTT prevents the use of signal detection analysis being accurately employed in the interpretation of behavioural performance.

Furthermore, although the terms sustained attention and vigilance are often used interchangeably, there is a subtle difference between the two constructs (Robbins 1998). Vigilance reflects a cognitive construct described as the ability to remain alert towards incoming stimulus information (Collings 2003; Egeland et al. 2009), facilitating the state of readiness to detect and respond to unpredictable and rare events. Thus, vigilance enables the discrimination between signal and noise (Mackworth 1957; Broadbent 1971; Robbins 1998), whereas sustained attention enables an individual to focus attention over a prolonged

period enabling attentional goals to be maintained over time. As such, it has been suggested that the current configuration of the 5-CSRTT restricts the ability to accurately evaluate vigilance (Robbins 1998) in a manner consistent with human CPTs, as in most vigilance experiments the critical signals for detection fall within a backdrop of non-signal events (Warm and Dember 1987), events that are absent in the original 5-CSRTT.

A lever-based operant task developed by McGaughy and Sarter (1995) may accurately assess vigilance in preclinical testing, requiring animals to determine whether a visual cue appeared or not, prior to the presentation of the two levers (described in detail in chapter 1). The SAT task records hits, misses, correct rejections and false alarms depending on the lever press during signal or non-signal trials. However, in go/no-go paradigms (i.e. CPTs), false alarms typically constitute a failure to withhold from responding, resulting in an inappropriate response in contrast to a correct rejection that usually requires an inhibition of a response (Eagle et al. 2008). As a result it could be maintained that the non-signal trial presented within the McGaughy and Sarter (1995) paradigm, of which a correct rejection requires an active response, may have limited analogy to the non-signal trials presented in human CPTs which in contrast, a correct rejection is comprised of an inhibition of an inappropriate response (Young et al. 2009b). This discrepancy may limit accurate parallels to be drawn between the clinical and preclinical tests of vigilance.

Consequently, the 5-CSRTT has recently been modified to include not only target trials (light stimulus in one of the apertures) present in the original task, but also non-target trials (light stimulus in all the apertures) to generate a behavioural paradigm with enhanced analogy with human CPTs, called the 5-Choice Continuous Performance Test (5C-CPT) (Young et al. 2009b). Presentation of non-target trials in this task requires the animal to correctly inhibit a response, whereas a failure in action restraint, resulting in incorrect responding to the non-target trial generates a false alarm. Thus, this adaptation may improve the capability to assess vigilance in a preclinical model in a manner similar to human CPTs. Additionally, as the

5C-CPT includes non-target trials generating correct rejections and false alarms, SDT can also be utilised to provide insight into the animals' responsivity and give additional information on attentional performance in light of task or pharmacological manipulations (Dudchenko et al. 1992).

Previously discussed in chapter 1, various manipulations to basic 5-CSRTT protocols have been used to challenge performance by further taxing attentional or strategic performance (Carli et al. 1983; Stolerman et al. 2000; Grottick and Higgins, 2002; Hahn et al. 2002; Chudasama et al. 2003; Hahn et al. 2003; Winstanley et al. 2003), manipulations which include extending the duration between stimulus onset. As such, similar parameter manipulations are likely to be an appropriate method to further tax performance in animals trained in the 5C-CPT. However, caution must be exercised when employing such manipulations when the ultimate aim is pharmacologically attenuating deleterious performance. Because months of training to a specific protocol is required for animals to perform operant tasks such as 5-CSRTT or the 5C-CPT, there is the potential confound, which requires consideration; drug treatment may facilitate an increased speed of learning or improved adaptation to the modified protocol. This may weaken the interpretation of drug-induced improvement in attentional function (Hahn et al. 2003; Young et al. 2009a).

It has long been established that dopamine (DA) transmission is disrupted in the pathophysiology of schizophrenia; however, despite many years of research it is still not entirely clear how DA dysregulation and cognitive impairments in schizophrenia are connected (Cohen and Servan-Schreiber 1993). Despite this uncertainty, it is evident that dopaminergic transmission in sub-cortical (Tomasi et al. 2010) and cortical brain regions (Sawaguchi and Goldman-Rakic 1991; Goldman-Rakic et al. 2004) is linked to various cognitive processes, including attentional functioning (Nieoullon 2002). The DA D_1 receptor has been identified as one of the most promising targets for developing pro-cognitive

therapeutics for schizophrenia (Hagen and Jones 2005; Tammaninga 2006), and there is some evidence that D_1 agonists can improve sustained attention, shown by improved accuracy of responding in the 5-CSRTT in rats with poor baseline performance (Granon et al. 2000). However, enhancement of attentional performance via augmentation of the D_1 dopaminergic system in the 5C-CPT has yet to be determined. Validation of the 5C-CPT has previously only been carried out in mice and therefore, the aims of the present chapter were to firstly identify whether rats could be trained to perform the 5C-CPT. Secondly, the ability of a D_1 agonist to improve performance at stable baseline and following behavioural challenge was investigated.

4.2. Methods

4.2.1 Subjects

Female hooded-Lister rats (n=35; Charles River; approx 250 $\pm$ 10 g at the start of the experiment) were housed in groups of five on a 12 hour reversed light cycle (lights on at 7:00pm) in a temperature (21 $\pm$ 2^{o}C) and humidity (55 $\pm$ 5%) controlled environment. For details regarding animal housing conditions, food restriction and eithical guidelines, please refer to the general methods section.

4.2.2 Experimental Design

4.2.2.1 Stable Baseline Task Performance

The initial experiment investigated the effect of SKF 38393 on performance in the 5C-CPT under standard training conditions, which consisted of 1.5 s SD, 5 s TO period, 2 s LH, a variable ITI (4.0, 4.5, 5.5, and 6.0 s) which averaged 5 s, in a session consisting of 84 target trials and 36 non-target trials. Following attainment of baseline stability (for details regarding the training, please see chapter 2 section 2.6.3), animals were randomly assigned to four treatment groups, whilst also ensuring no variability between groups based on behavioural performance existed prior to drug treatment. Animals then received vehicle (distilled H_2O), 2.0, 4.0, or 6.0 mg/kg (n = 8 – 9) SKF 38393 30 minutes before behavioural testing.

4.2.2.2 Reduced Event Rate Task Challenge

The second experiment followed the same design in which animals were randomly assigned to four separate treatment groups, ensuring no variability between treatment groups and treated with either vehicle, 2.0, 4.0, or 6.0 mg/kg SKF 38393 (n = 8 – 9). The rats were then

tested in the 5C-CPT. The testing parameters were identical to the previous experiment, with the exception that the variable ITI was extended from the 5 s average used throughout training to an average of 10 s (8, 9, 11, and 12 s), thus reducing the event-rate of the task (Parasuraman, 1998). One rat was excluded from analysis following the reduced event-rate challenge (6 mg/kg treatment group) due to malfunction of an operant chamber during testing.

Baseline and challenge session were separated by two days, during which all animals received normal training sessions to ensure performance was maintained at a stable baseline, while also identifying possible lasting effects resulting from acute SKF 38393 exposure. A between-subjects design was used rather than a within-subjects Latin square design (as used in chapter 3) in order to avoid the potential learning confound that may result from repeated exposure to the extended ITI challenge session.

4.2.3 Drugs

SKF 38393 (Sigma, UK) was dissolved in distilled H_2O to give the appropriate doses (2.0, 4.0 and 6.0 mg/kg) and was injected via the intraperitoneal (i.p.) route with a 30 minute pre-treatment time at a dose volume of 1 ml/kg. All drug doses were calculated as base equivalent weight. The doses used in this study were chosen from previous behavioural experimentation conduced within our laboratory and others, which showed efficacy of SKF 38393 within this dose range in behavioural tasks of cognition of relevance to schizophrenia (Hersi et al. 1995; McLean et al. 2009). However, as previous pro-cognitive demonstrations following SKF 38393 administrations were not conducted in an attentional task, a wider dose-response was used in the current study.

4.2.4 Data Analysis

Performance for each behavioural measure in the 5C-CPT was displayed as mean ± SEM. Data were analysed by a one-way between subjects ANOVA with drug as the between subjects factor, followed by Planned Comparisons on the predicted means back to control animals. Data presented over the duration of the session was displayed as mean ± SEM and analysed using a repeated measures ANOVA (Treatment as fixed factor, Trial as repeated measure) followed by Planned Comparisons (Snedecor and Cochran, 1989) on the predicted means using Statistica v8.0 (Statsoft). Planned Comparisons were utilised to test the hypothesis that drug treatment would, depending on test parameters, improve or impair performance. Comparison of means in order to determine whether rats were able to discriminate between trial types and the determination of the effect reducing the event-rate had on task performance were displayed as mean ± SEM and analysed using a Student's t test.

4.3 Results

4.3.1 Discrimination between Trial Types

In the initial part of training (approximately 1 month in), measures relating to the 'go' component suggest animals had acquired the ability to respond to the presentation of the target stimuli (accuracy, 94%; percent correct, 91%; percent omissions, 5% - data not shown), however it was evident that animals were not discriminating between the two trial types presented within the task. This was exemplified by the high p[FA], coupled with the lack of difference between p[HR] and p[FA] (fig 4.2). Additionally SDT measures further suggested that animals could not differentiate between trial type (SI = 0.04) and have adopted an extremely liberal response strategy (RI = 0.80) (data not shown).

Fig 4.2. Comparison of p[HR] and p[FA] during the initial phase of training indicating absence of discrimination between trial types. Data expressed as mean ± SEM.

Following continued training (with a greater emphasis placed on the no-go component, described in chapter 2 section 2.6.3), animals could successfully discriminate between the two trial types presented within the 5C-CPT. There was a significant difference ($t=7.31$, $p<0.001$) between hit rate and false alarm rate (Fig 4.3A). In addition, as a correct rejection and an omission both resulted from the same type of response, it was important to determine if rats were responding appropriately and treated the two trial types differently.

Analysis showed that there was a significant difference (t=9.97, p<0.001) when percent correct rejections was compared with percent omissions (Fig 4.3B).

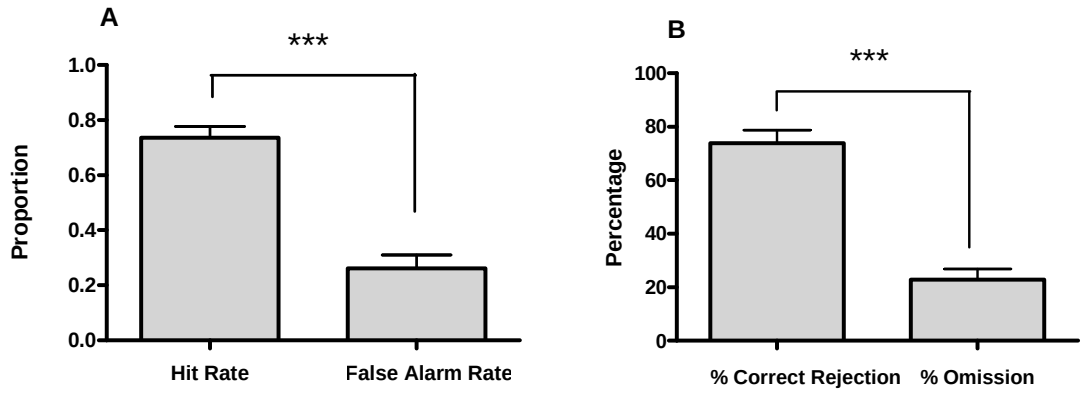

Fig 4.3 Performance of vehicle treated animals under standard training conditions highlighting the ability to perform the 5C-CPT. There was a significant difference between hit rate and false alarm rate (A) in addition to a significant difference between percent correct rejections and percent omissions (B). Data are expressed as mean ± SEM, analysed by Students t-test. *** denotes p<0.001.

4.3.2 SKF 38393 and Stable Baseline Task Performance

Effects of the partial D1 receptor agonist on stable baseline performance of rats in the 5C-CPT were examined. Table 4.2 demonstrates there was no significant effect of drug treatment on accuracy, and SKF 38393 initially resulted in no effect on percent correct responding [$F_{(3,31)}$=2.08, p=0.12], percent incorrect responding [$F_{(3,31)}$=0.98, NS] or percent omissions [$F_{(3,31)}$=1.95, p=0.14]. However, Planned Comparisons revealed a significant effect at the highest dose of SKF 38393 compared to control animals on both percent correct responding (decrease) (p<0.05) and percentage omissions (increase) (p<0.05). SKF 38393 treatment produced a significant increase in correct latency (CL) [$F_{(3,31)}$=5.25, p<0.01]. Planned Comparisons revealed that doses of 4 mg/kg and 6 mg/kg SKF 38393 resulted in a significant increase in correct latency time (p<0.05). Statistical analysis showed no significant

change in incorrect latency (IL) [F>1, ns] or magazine latency (ML) [$F_{(3,31)}$=1.47, p=0.24]. When tested under standard conditions, SKF 38393 had no effect on impulsive or compulsive responding, demonstrated by no significant change in the number of premature [F>1, ns], although a slight trend towards a significant increase in perseverative [$F_{(3,31)}$=2.04, p=0.12] responses was observed.

Table 4.2 Effect of SKF 38393 on Stable Baseline Performance

Measures	Vehicle	2.0 mg/kg	4.0 mg/kg	6.0 mg/kg
Accuracy (%)	94.35 ± 1.58	96.40 ± 0.94	95.33 ± 1.54	91.41 ± 1.75
Percent Correct	76.81 ± 4.55	78.17 ± 6.88	71.28 ± 4.70	58.49 ± 8.16 *
Percent Incorrect	4.28 ± 1.03	2.64 ± 0.71	3.26 ± 1.00	4.57 ± 0.74
Percent Omission	18.90 ± 3.99	19.19 ± 6.71	25.46 ± 4.34	36.94 ± 8.29 *
Correct Latency	0.71 ± 0.06	0.65 ± 0.03	0.93 ± 0.08 *	0.90 ± 0.05 *
Incorrect Latency	0.55 ± 0.13	0.80 ± 0.21	1.03 ± 0.27	1.14 ± 0.18
Magazine Latency	1.26 ± 0.06	1.29 ± 0.07	1.33 ± 0.07	1.46 ± 0.10
Premature	6.00 ± 2.17	6.50 ± 1.24	5.56 ± 2.24	2.67 ± 0.99
Perseverative	10.67 ± 3.36	10.75 ± 2.25	22.22 ± 4.94	20.89 ± 5.76

Measures are shown as mean ± SEM. Asterisks indicate statistically significant differences (*p<0.05) compared to the vehicle group (n = 8 – 9). Stable baseline parameters consisted of 1.5 s SD, 5 s TO period, 2 s LH, a variable ITI (4.0, 4.5, 5.5, and 6.0 s).

Analysis of SKF 38393 treatment on p[HR] showed that there was no significant impairment in the proportion of hit rate, p[HR] [$F_{(3,31)}$=2.08, p=0.12]. However Planned Comparisons of the means showed that the highest dose of SKF 38393 (6 mg/kg) resulted in a significant reduction in the proportion of target trials correctly reported (p<0.05, fig 4.4A). Treatment with SKF 38393 had no significant effect on the remaining signal detection measures (p[FA], sensitivity index [SI] and responsivity index [RI]) (Fig 4.4B, C and D.)

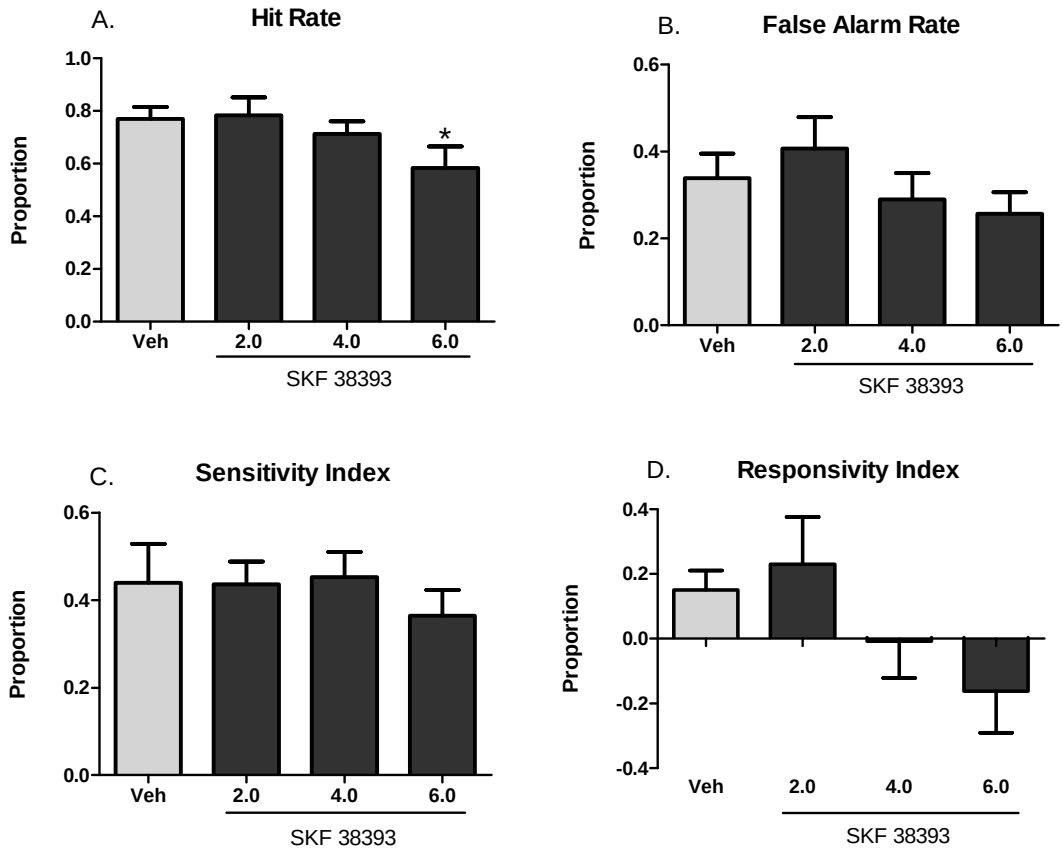

Fig 4.4 Performance of animals in the 5C-CPT tested (n = 8 – 9) using standard conditions following SKF 38393 treatment and analysed using signal detection theory. Drug treatment produced a significant reduction in p[HR] (A). There was no effect of SKF 38393 on p[FA] (B), Sensitivity Index (C) or Responsivity Index (D). Data are expressed as mean ± SEM and analysed by one-way ANOVA followed by Planned Comparisions on the predicted means. * denotes p<0.05 relative to the vehicle (Veh) group.

4.3.3 Effect of a Reduced Event-Rate on Task Performance

When the variable ITI was increased from an average of 5 s to 10 s, there was an impaired ability to correctly perform the 5C-CPT in a number of measures, displayed in table 4.3. There was no effect on accuracy ($t=1.23$, ns), but a significant reduction in percent correct ($t=2.90$, $p<0.05$) and increase in percent omission were observed ($t=2.67$, $p<0.05$). Incorrect responding was unaffected, however ($t=0.91$, ns). Correct latency and incorrect latency were

unaffected (t=0.93, ns and t=0.36, ns, respectively) but parameter manipulation resulted in a significant reduction in magazine latency (t=2.47, p<0.05). Extending the ITI dramatically increased the number of premature responses made during a session (t=4.87, p<0.0001), whereas perseverative responding was unaffected (t=0.36, ns). Reducing the event-rate had no effect on the ability to withhold a response when presented with a non-target trial (t=1.54, ns). The p[HR] was significantly reduced when the ITI was extended (t=2.95, p<0.01), but the p[FA] was unchanged (t=1.16, ns). As a result, there was a significant reduction in the sensitivity between target and non-target stimuli, characterised by a reduction in SI (t=2.82, p<0.05), which occurred without alterations in the responsivity index (t=1.05, ns).

Table 4.3 Effect of a reduced event rate on 5C-CPT performance

Measures	Stable Baseline	Reduced Event-rate
Accuracy (%)	94.35 ± 1.58	88.92 ± 3.93
Percent Correct	76.81 ± 4.55	56.41 ± 5.27 *
Percent Incorrect	4.28 ± 1.07	7.03 ± 2.68
Percent Omission	18.90 ± 3.99	37.69 ± 5.60 *
Correct Latency	0.71 ± 0.06	0.79 ± 0.06
Incorrect Latency	0.55 ± 0.13	0.69 ± 0.14
Magazine Latency	1.26 ± 0.06	1.06 ± 0.06 *
Premature	6.00 ± 2.17	36.50 ± 5.59 ***
Perseverative	10.67 ± 3.36	9.40 ± 1.51
Correct Rejections (%)	66.11 ± 5.56	55.43 ± 7.21
Hit Rate, p[HR]	0.77 ± 0.05	0.56 ± 0.05 **
False Alarm Rate p[FA]	0.34 ± 0.06	0.45 ± 0.07
Sensitivity Index	0.44 ± 0.09	0.13 ± 0.07 *
Responsivity Index	0.15 ± 0.06	0.01 ± 0.11

Effect of reducing the event-rate had on 5C-CPT performance in control animals (n = 9). Measures are shown as mean ± SEM. Asterisks indicate statistically significant differences (*p<0.05, **p<0.01, ***p<0.001) between reduced event-rate and stable baseline performance. Stable baseline parameters consisted of 1.5 s SD, 5 s TO period, 2 s LH, a variable ITI (4.0, 4.5, 5.5, and 6.0 s), whereas the reduced event-rate had an extended variable ITI (8, 9, 11, 12 s)

4.3.4 SKF 38393 and Reduced Event Rate Task Performance

SKF 38393 impaired performance when assessed during baseline level of performance. This effect could have been as a result of ceiling effects (Granon et al. 2000) as animals were at their optimal level of performance. One method used to challenge vigilance is to reduce the event rate where the period in which the animal must wait for a stimulus to appear is lengthened (Parasuraman, 1998). Rats were challenged in the 5C-CPT using an extended variable ITI challenge (Table 4.4). Analysis showed that SKF 38393 treatment had no effect on accuracy [$F_{3,30)}$=1.24, ns]. One-way ANOVA showed no significant difference in percent correct responding [$F_{(3,30)}$=2.19, p=0.11] or percentage omissions [$F_{(3,30)}$=1.72, p=0.18]. However, Planned Comparison of the means revealed that 6 mg/kg of SKF 38393 resulted in a significant increase in the percentage of correct trials per session ($p<0.05$) and a significant reduction in the percentage of trials missed ($p<0.05$). There was no treatment effect on the number of incorrect responses made throughout the reduced event-rate challenge [$F_{(3,30)}$=1.01, ns]. Statistical analysis revealed no significant effect of SKF 38393 on correct latency [$F_{(3,30)}$=0.2, ns], incorrect latency [$F_{(3,30)}$=0.27, ns] or magazine latency [$F_{(3,30)}$=1.41, ns]. Although the baseline of premature responding was increased, compared to standard conditions, SKF 38393 had no significant effect on either premature [$F_{(3,30)}$=0.59, ns] or perseverative responding [$F_{(3,30)}$=1.37, ns] when the variable ITI was extended.

Table 4.4 Effect of SKF 38393 on Reduced Event Rate Performance

Measures	Vehicle	2.0 mg/kg	4.0 mg/kg	6.0 mg/kg
Accuracy	88.92 ± 3.93	90.00 ± 2.83	94.74 ± 1.55	94.58 ± 1.13
Percent Correct	56.41 ± 5.27	63.04 ± 8.88	67.87 ± 4.65	76.14 ± 4.11 *
Percent Incorrect	7.03 ± 2.68	5.43 ± 0.65	3.46 ± 0.84	4.27 ± 0.93
Percent Omission	37.69 ± 5.60	30.90 ± 7.39	28.66 ± 4.24	19.59 ± 3.95 *
Correct Latency	0.79 ± 0.06	0.78 ± 0.05	0.78 ± 0.06	0.74 ± 0.02
Incorrect Latency	0.69 ± 0.14	0.52 ± 0.10	0.74 ± 0.24	0.73 ± 0.19
Magazine Latency	1.06 ± 0.06	1.29 ± 0.07	1.34 ± 0.22	1.41 ± 0.10
Premature	36.50 ± 5.59	33.14 ± 6.05	29.67 ± 4.05	27.38 ± 5.62
Perseverative	9.40 ± 1.51	7.71 ± 2.10	6.44 ± 1.41	5.50 ± 1.16

Measures are shown as mean ± SEM. Asterisks indicate statistically significant differences (*p<0.05) compared to vehicle group (n = 8 – 9).

Analysis showed that there was no significant effect on p[HR] as a result of SKF 38393 treatment [$F_{(3,30)}$=2.19, p=0.11], however Planned Comparisons showed a significant increase in p[HR] at the highest dose (6 mg/kg, p<0.05, fig 4.5A). There was no significant effect of drug on the p[FA] (fig 4.5B) as a result of SKF 38393 treatment [$F_{(3,30)}$=0.80, ns], however analysis revealed drug treatment produced a significant increase in the sensitivity index [$F_{(3,30)}$=4.38, p<0.05] (fig 4.5C). Planned Comparisons on the predicted means showed that both 4 mg/kg (p<0.05) and 6 mg/kg (p<0.01) SKF 38393 resulted in a significant increase in SI, while there was no significant effect of treatment compared to the control group for responsivity index [$F_{(3,30)}$=0.42, ns] (fig 4.5D).

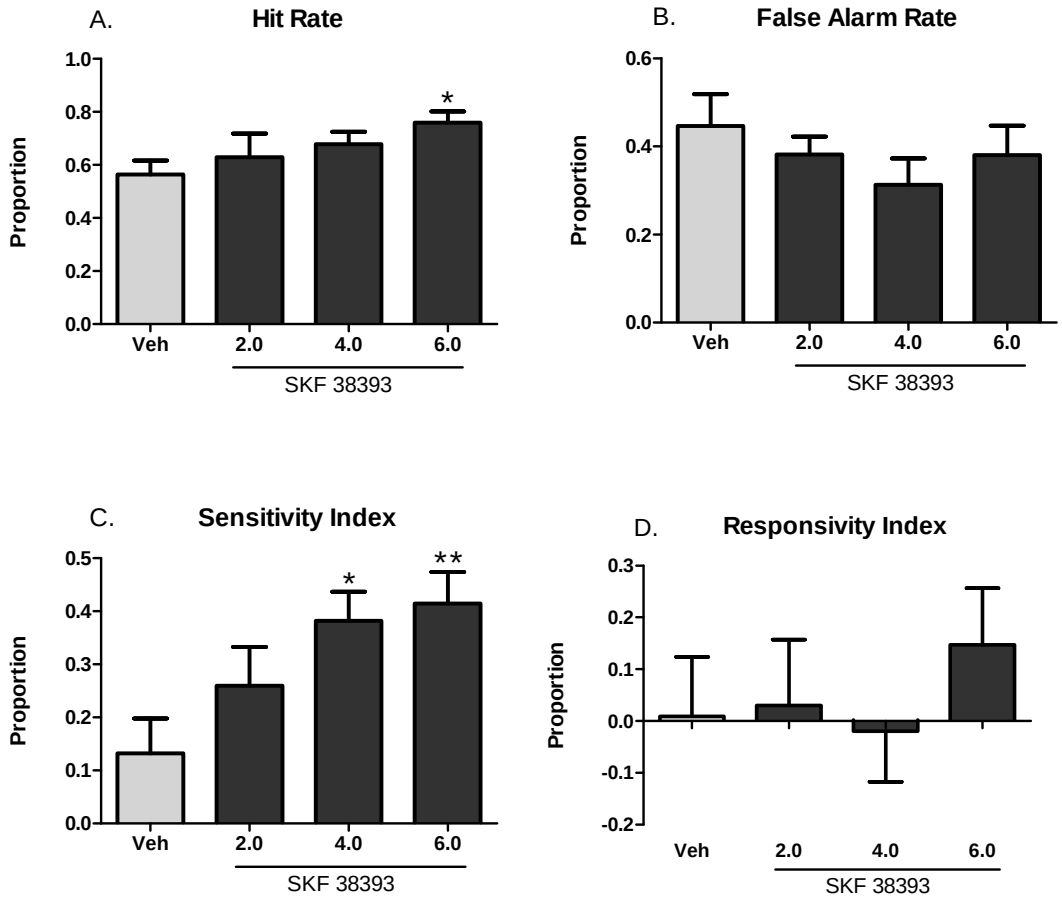

Fig 4.5 Effect of SKF 38393 on signal detection theory measures of performance in the 5C-CPT during a reduced event rate challenge (n = 8 – 9). Drug treatment resulted in a significant increase in p[HR] (A). No effect of drug was observed on p[FA] (B). SKF 38393 treatment resulted in a significant increase in the sensitivity index (C) without affecting the responsivity index of the animals (D). Data are expressed as mean $\pm$ SEM and analysed by one-way ANOVA followed by Planned Comparisions on the predicted means. * denotes $p<0.05$ and ** denotes $p<0.01$ relative to vehicle (Veh) group.

4.3.5 Reduced Event Rate Challenge – Performance across Session

Altering the protocol of a task during testing requires the animal to adjust to that protocol. Thus drug effects on performance could affect vigilance or be confounded by affecting learning of the new protocol. To assess for this learning confound, the effects of SKF 38393 on animals within the session was examined in comparison to vehicle control animals. Analysis showed that SKF 38393 resulted in a significant increase in performance across the duration of the session as measured by the sensitivity index (SI). Analysis of drug effects indicated that there was a strong treand towards a significant difference in performance ($F_{(3,30)}$=2.79, p=0.05). Following Planned Comparisons it was revealed that both 4.0 mg/kg ($p<0.05$) and 6.0 mg/kg ($p<0.01$) of SKF 38393 resulted in a significant increase in SI in the first trial bin and 6.0 mg/kg ($p<0.01$) SKF 38393 resulted in a significant increase in the second trial bin (Fig 4.6). There was no significant interaction between dose and trial number ($F_{(3,30)}$=0.17, p=0.91).

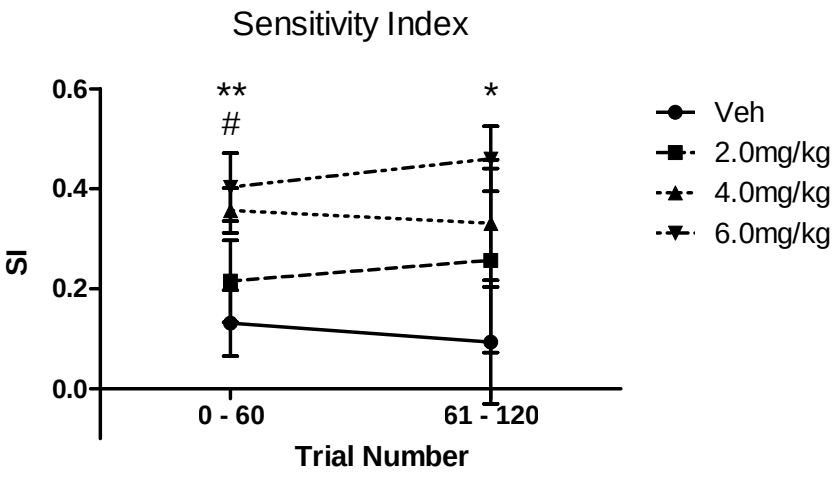

Fig 4.6 Effect of SKF 38393 treatment on performance in 5C-CPT across time in response to the reduced event rate challenge (n = 8 – 9). Drug treatment (4.0 and 6.0 mg/kg i.p.) resulted in a significant increase in sensitivity index (SI) during trials 0 – 60 and trials 61 – 120 (6.0 mg/kg i.p.). Data are expressed as mean $\pm$ SEM and analysed by one-way repeated measures ANOVA with treatment as fixed factorand trial as repeated measure, followed by Planned Comparisons on the predicted means. # denotes $p<0.05$ (4.0 mg/kg) and * denotes $p<0.05$, ** denotes $p<0.01$ (6.0 mg/kg) compared to the vehicle group.

4.4. Discussion

Firstly, these data demonstrate for the first time that rats can be successfully trained to respond to the target trials, whilst appropriately inhibiting a response when presented with the non-target trials that are presented in the 5C-CPT. Previously, it has been shown that mice can be trained to discriminate between target and non-signal trials (Young et al. 2009b), however, this is the first time it has been demonstrated rats can perform the 5C-CPT. Secondly, these data confirm that activation of dopamine (DA) D_1 receptors can improve or impair attention/vigilance in a baseline dependent manner, and thirdly supports the use of signal detection analysis as a means to quantify attentional performance and strategic bias.

Rats use a temporally mediating strategy when performing the 5-CSRTT with constant ITIs and possibly respond to the cue in a semi-automatic manner. This strategy was quantified following observations that rats responded 90% of the time following the ITI when acutely challenged with no presentation of stimuli (Spratt et al. 2000). The current study has demonstrated however, that by utilising a variable ITI and a non-target stimulus during training, the development of a temporally mediated response strategy was minimised and rats could withhold from responding to non-target trials. The start of training required a greater emphasis being placed on the proportion of non-target trials presented as initially animals responded to the majority of non-target trials, increasing the p[FA], suggesting they were treating both trial types identically. However, following continued training with the higher proportion of non-target trials, animals began to learn that a response to the non-target trial did not result in a reward and thus modified their response to the non-target trial accordingly, demonstrated by the reduction in p[FA]. Once trained to withhold from responding, the discrimination of the two trial types was quantified by comparing p[HR] and p[FA], with the former being significantly greater than the latter. Additionally, percent correct rejections and percent omissions were compared. In this instance, as both types of response essentially required the same action for target and non-target trials (i.e. a non

response); it was important to establish that performance is dependent on the type of trial presented to the animal (Young et al. 2009b). Upon analysis, it was evident that there was a clear difference in non-responses dependent upon trial type (correct rejections > omissions), suggesting the rats could make a distinction between the two trial types and responded accordingly. Thus, these data suggest that rats can be trained to differentiate and respond appropriately to two different stimulus types within the same task.

The main finding that emerged from this chapter was that D_1 receptor activation via systemic administration of the D_1 partial agonist SKF 38393 improved attention/vigilance during a demanding task challenge. These data support findings from previous studies that demonstrated D_1 receptor activation enhanced sustained attentional performance, as assessed within the 5-CSRTT, a finding that was only evident in animals that had a lower baseline performance compared to the rest of the cohort (Granon et al. 2000). The present findings demonstrate that, when the baseline performance is high (i.e. using standard task protocol), SKF 38393 did not improve attentional performance. Activation of dopaminergic D_1 receptors actually impaired performance under these conditions. This finding was demonstrated by the reduction in percent correct trials and increased trials omitted during the session, along with an increase in the latency to make a correct response. The slowed correct latency (CL) was unlikely to reflect general motoric impairment as incorrect latency and magazine latency were unaffected. Thus SKF 38393-induced slowing of CL was likely to be the result of deficits in the speed of informational processing (Robbins 2002; Young et al. 2009a). In contrast, when performance was challenged with an extended variable ITI, thus reducing the event rate (Parasuraman et al. 1998), baseline performance was reduced, exemplified by the reduced ability to respond to the target stimulus, an increase in premature responding and reduction in the sensitivity to discriminate between signal and noise. This reduction in performance, however, was attenuated by drug treatment. Augmentation of the dopaminergic D_1 system, via systemic administration of SKF 38393,

increased the percentage of correct trials and reduced the number of trials missed. As a result, the increase in vigilance, indicated by the increase in sensitivity index (SI), was likely driven by an increased p[HR] highlighting an increased capacity to detect and respond to the target stimulus. Sarter and colleagues (2001) described signal sensitivity or detectability as a method of quantifying changes in vigilance levels. Therefore, the increased SI produced by drug treatment, following the impaired performance following a reduced event rate, suggests that SKF 38393 administration increased the rats' capability to detect and report the occurrence of signals, thus indicating an improved discriminability between signal (target trial) and noise (non-target trials), which is a hallmark of enhanced vigilance.

No effect of SKF 38393 was observed on responsivity index (RI), therefore it is unlikely that performance in the task was altered due to a change in response bias or strategy, which implies that the improved SI was cognitive in nature (Marston 1996). The ability of SDT to dissociate the SI and RI and reveal no change of RI in response to drug treatment indicates that improvements in performance may be specifically due to increases in attentional ability (Dudchenko et al. 1992) and not alterations in the animals willingness to respond (Steckler, 2001). Thus, this indicates that stimulation of the D_1 dopaminergic system via systemic administration of SKF 38393 enhanced the attentional/vigilance performance of the animals only when task demands were increased.

SKF 38393 treatment most likely elicited beneficial effects via activation of D_1 receptors located in the medial prefrontal cortex (mPFC). Previous work has demonstrated that lesions in this brain region impairs attentional function resulting in reduced choice accuracy in the 5-CSRTT (Muir et al. 1996), thus highlighting the importance of the mPFC in this type of task performance. In addition, previous studies have demonstrated that SKF 38393 improved attentional processing in the 5-CSRTT in animals with a reduced performance (Granon et al. 2000; Fletcher et al. 2007), when administered by microinfusions directly into the mPFC. Furthermore, there is evidence to suggest that the DA D_1 system is involved in other cognitive

processes besides attention and vigilance, including delayed working memory (Arnsten et al. 1994; Sawaguchi and Goldman-Rakic 1994), spatial learning and memory (Hersi et al. 1995), visual learning and memory (Mclean et al. 2009) and reasoning and problem solving (Mclean et al. 2009) all of which have been shown to be dysfunctional in patients with schizophrenia (Young et al. 2009a), thus demonstrating the importance of this receptor system within frontal cortical brain regions in cognitive processes. The D_1 receptor has also been linked to motivation for reward (Young and Geyer 2010), which could have confounded the effects observed here. The lack of effect on ML makes this interpretation unlikely, however (Robbins 2002). These data therefore lend support to the hypothesis that activation of the D_1 system may be a viable target for treating cognitive disruption in schizophrenia (Sawaguchi and Goldman-Rakic 1991; Sawaguchi and Goldman-Rakic 1994; Goldman-Rakic et al. 2004; Tamminga 2006)

The reduced event-rate challenge used in the present study challenged the temporal strategy of animals, shown by the increased number of premature responses compared to baseline. Therefore, D_1 agonist treatment-induced improvement in performance may have been facilitated by increased learning; enhancing the speed at which animals adjusted to the extended ITI and not by improving attention or vigilance *per se* (Young et al 2009a). However, there was no significant reduction in the number of premature responses made in the extended ITI challenge session following drug treatment, which would have occurred had the animals adapted to the increased duration between stimuli on-set. These data therefore suggest that the improvement in performance was as a result of improved attention/vigilance and not due to improved temporal reintegration i.e. adjusting to the increase in ITI duration. As a result, it is possible that augmentation of the dopaminergic D_1 system improved performance by heightening the rats' ability to detect the signal, without enhancing within-session learning that has been demonstrated in previous studies using a reduced event-rate challenge (McGaughy and Sarter 1995; Hahn et al. 2002). The absence of

within-session learning in the present study, unlike previous studies that reduced the event-rate using a fixed ITI, was likely due to the fact that a variable ITI was utilised, thus minimising the development of a temporally-mediated strategy being implemented. Further support for this interpretation can be seen when the performance is displayed over the course of the session. An overall 'trial-independent' increase in SI following SKF 38393 treatment was observed throughout the duration of the session, whereas within-session learning would be exemplified by a performance increase as the session progressed. Moreover, this is also supported by an improvement in performance that was seen immediately at the beginning of the session, further suggesting increased performance was the result of improved ability to detect target stimuli. These data therefore suggest that activation of the D_1 system improved vigilance throughout the session and did not simply increase the speed at which the animals learned to adapt to the reduced event-rate challenge.

The impaired performance in the standard conditions in response to SKF 38393 treatment may have been as a result of an 'inverted – U' shaped dose response, whereby the level of DA stimulation is required to be at optimal levels for optimal performance. Too much or too little DA stimulation will result in less than optimal performance as has been observed in spatial short-term memory in not only rats (Zahrt et al. 1997), but a variety of species e.g. primates and humans (Vijayraghavan et al. 2007). This effect variation follows the concept of the Yerkes-Dodson principle and the link between the level of stress and arousal the relationship to behavioural performance (Yerkes and Dodson, 1908; Diamond et al. 2007). Thus, the effect SKF 38393 on task performance may depend on basal levels of DA and the optimal levels required depending on the nature of the task. In the case of the standard baseline conditions, it is likely that DA utilisation is already at a maximum and therefore any further stimulation of the dopaminergic system will have a detrimental effect on task performance. Lyon and Robbins (1975) suggested that in this case, excessive dopaminergic transmission impairs performance by progressively increasing the frequency of multiple,

concurrent behaviours and may lead to over-focused behaviour. This suggestion may hold true as there was a trend towards an increase in perseverative responding, indicating the inability to disengage from a previously rewarded response; however this increase failed to achieve statistical significance in the present study. In response to the increased ITI session, it is possible that the conditions of this task would demand an increase in catecholaminergic input, which resulted in the reduced baseline performance of rats treated with vehicle and, therefore, stimulation of the D_1 system by SKF 38393 treatment exerted a beneficial effect on task performance. As SKF 38393 is a partial agonist of the D_1 DA receptor (Tirelli and Terry 1993), future studies could investigate the effects of full DA D_1 receptor agonists, such as (+)-doxanthrine (Przybyla et al. 2009), and their effects on attention and vigilance. Furthermore, the specificity of SKF 38393 should be noted as it has been shown to show affinity for the 5-HT_{2c} receptor (Briggs et al. 1991). While involvement of the serotonergic system cannot be ruled out in the current study, previous demonstrations have indicated direct D_1 involvement in cognitive processing (Granon et al. 2000; McLean et al. 2009), future investigation should investigate the precise role of SKF 38393 and attention/vigilance. This would generate a more complete understanding of the involvement of DA D_1 receptors in this particular cognitive domain. Moreover, future studies could also investigate whether beneficial effects are mediated via D_1 or D_5 DA receptors given the fact that SKF 38393 acts equally on D_5 receptors also (Neumeyer et al. 2003; Qandil et al. 2003).

These data presented within the current chapter fail to demonstrate a vigilance decrement, identified by absence of a reduction in SI as the session progressed in control animals. In Mackworth's (1950) investigation into vigilance, testing was conducted over relatively long periods, suggesting prolonged duration is necessary to induce vigilance decrements. A vigilance decrement was observed in mice in the 5C-CPT, exemplified by a reduced SI when the session was extended from 120 to 250 trials (Young et al. 2009b). A vigilance decrement was not observed in the current study, most likely due to the short session lengths used in

testing. Future work could utilise additional task manipulations, such as reduced stimulus duration or extended session lengths, to additionally challenge attentional demands and further elucidate the mechanisms by which D_1 augmentation improves task performance. Additionally, as this study was conducted in 'normal' animals, future studies could investigate the effect augmentation of the D_1 system has on 5C-CPT performance when used in conjunction with an animal model of cognitive dysfunction present in schizophrenia patients (i.e. NMDA receptor antagonism) (Neill et al. 2010). In addition to the potentially beneficial effects of D_1 agonism, this would also investigate the potential to pharmacologically impair task performance in a manner consistent with CPT performance in a clinical setting.

In conclusion, the findings of the present study demonstrate that rats can be trained to perform the 5C-CPT, a rodent analogue of the human CPT. In addition, these findings support suggestions that activation of DA D_1 receptors can improve attentional performance in animals that are not encumbered by ceiling effects and display a reduction in performance. Importantly, the present data suggest that these effects may be observed following systemic administration of the D_1 agonists. Thus D_1 agonists may prove therapeutically beneficial in attenuating the attentional dysfunction that is evident in patients with schizophrenia. Based on the findings that rats can be trained to perform the modified version of the 5-choice task, the following chapter (5) will investigate the effects PCP-treatment has on 5C-CPT performance. However, due to the paucity of impairments following a sub-chronic treatment regimen had on the original 5-CSRTT (chapter 3), a repeated PCP treatment schedule will be utilised instead of a sub-chronic treatment regimen, and animals will be tested while the drug is still in the system in the following experimental chapter.

Chapter 5

Effects of repeated PCP treatment and dopamine D_1 partial agonism in the 5-Choice Continuous Performance Test

5.1 Introduction

As demonstrated in chapter 4, rats can be trained to respond to target trials requiring a response, and correctly withhold a response when presented with non-target trials. Therefore, it was demonstrated that rats can be trained to perform in the 5C-CPT, which is a task with enhanced analogy to the human CPT. Experiments designed to assess the disruptive effects of PCP treatment in the 5C-CPT are reported in this chapter. The aim of this work is to determine the potential of pharmacologically modelling the attentional disruption exhibited in schizophrenia patients, as demonstrated by impaired CPT performance.

It has been widely demonstrated that acute administration of NMDA antagonists produces schizophrenia-like cognitive deficits in both rats and mice. NMDA antagonism impairs the ability to successfully perform in the 5-CSRTT (Grottick and Higgins 2000; Higgins et al. 2003; Le Pen et al. 2003; Greco et al. 2005; Higgins et al. 2005; Amitai et al. 2007; Auclair et al. 2009; Paine and Carlezon 2009; Chapter 3). NMDA receptor antagonism results in performance impairments that, amongst others, include a reduction in choice accuracy and percent correct responding. These measures have been suggested to be mediated by attentional processing (Robbins 2002; Amitai and Markou 2010), therefore suggesting that blockade of the glutamatergic NMDA receptor impairs the attentional abilities of the animals, impeding their ability to correctly detect and report the occurrence of the visual stimulus within the task. This impaired ability in stimulus detection will invariably lead to the reduced accuracy and/or percent correct responding observed and previously reported.

Coupled with the attentional deficits, acute administration of NMDA antagonists (PCP, MK-801) increase the number of premature responses during a 5-CSRTT session (Grottick and Higgins 2000; Le Pen et al. 2003; Greco et al. 2005). Elevations in premature responding during the 5-CSRTT may represent higher levels of impulsivity or impaired impulse control

(Muir et al. 1996; Harrison et al. 1997; Dalley et al. 2008). However, a single dose of PCP has also been demonstrated to produce a reduction in premature responding (Auclair et al. 2009). Consequently, Amitai and colleagues (2007) suggested that the reduction in premature responding may be attributed to the generalised response disruption induced by acute PCP treatment, rather than an effect on response inhibition *per se*. Accordingly, Amitai et al. (2007) hypothesised that some of the performance impairments observed following acute exposure may be the result of a nonspecific response-suppressive effect, suggesting that deficits were the result of a generalised inability of the rat to perform in the task. This observation is in contrast to the effects observed in chapter 3, where following an acute PCP challenge there was an increase in premature responding. However, as discussed in chapter 3, the treatment regimen utilised consisted of a Latin Square design with each animal acting as its own control, therefore receiving multiple PCP exposures. As a result, the increase in premature responding observed in chapter 3 may not be the result of a true single acute exposure, and may be more akin to a repeated PCP treatment regimen (Amitai and Markou 2010), perhaps accounting for the discrepancy in premature responding observed between studies. Single acute administration of PCP (and other NMDA receptor antagonists) can result in profound behavioural deficits, including stereotypy, ataxia, sedation, paralysis of hind-limbs, locomototor impairment and/or motivational disruption (Castellani and Adams 1981; Melnick et al. 2002; Daenen et al. 2003; Amitai et al. 2007). These non-cognitive, non-specific behavioural disturbances may impair performance of operant tasks. Hence, non-specific effects arising from single acute administration may confound the observation of cognitive disruption, obscuring the final interpretation and potentially mask the cognitive-specific impairments.

In an attempt to circumvent the generalised response disruption associated with acute PCP administration, a repeated PCP treatment regimen has been developed (Amitai et al. 2007; Amitai and Markou 2009; Amitai and Markou 2010). Like acute treatment, animal

performance is assessed following a pre-treatment period. However, in contrast to acute treatment, the drug is administered multiple times, over consecutive days. The rationale for this pattern of drug exposure is that the initial behavioural disruptive effects arising from acute exposure dissipate following repeated administration, revealing a more selective cognitive-disruptive effect. The effects of ataxia, stereotypy and locomotor impairment often observed with acute PCP treatment (Castellani and Adams 1981; Melnick et al. 2002; Daenen et al. 2003) are dramatically reduced or completely ameliorated following repeated PCP exposure (Amitai and Markou 2010). Therefore, some of the more profound behavioural disturbances evident at the start of treatment, impairing the animals' ability to perform complex tasks are reduced towards the end of the treatment regimen. Hence a repeated administration facilitates the quantification of cognitive-specific impairments in behavioural paradigms, reducing the potential of interpretations being confounded by the non-specific behavioural effects of the drug. Furthermore, the profound behavioural disturbance arising from acute PCP administration demonstrates considerable inter-animal variability, with some rats exhibiting a marked sensitivity to the stereotypy or ataxic effects, whilst others are considerably less affected (Amitai et al. 2007; personal communication; personal observations). The initial exposure to PCP permits animals to be group-matched based on performance before and after the initial exposure to PCP. The group-matching procedure enables an even distribution of animals highly sensitive to the PCP-induced generalised behavioural disruption to be made throughout the treatment groups, avoiding the potential confound of unintentionally or randomly assigning all of the highly PCP-sensitive animals to the same treatment group. This is a particularly important consideration in studies involving pharmacological attenuation of the PCP-induced performance impairment.

A repeated PCP treatment regimen has been shown to impair choice accuracy and induce a reduction in the percent of correct responses in animals trained within the 5-CSRTT (Amitai et al. 2007; Amitai and Markou 2009; Amitai and Markou 2010), indicating impaired

attentional function (Robbins 2002). Systemic PCP treatment may be disrupting the functioning of the frontal and prefrontal cortical regions, as these areas are densely populated with NMDA receptors (Cotman and Iversen 1987; Ulus et al. 1992) and suggested to be involved in attentional processing (Asplund et al. 2010; Bush 2010; Lesh et al. 2010). Furthermore, local infusion of NMDA antagonists within the medial PFC (mPFC) has been demonstrated to impair choice accuracy in the 5-CSRTT (Baviera et al. 2008). Additionally, the initial response suppression which resulted in reduced premature responding (Amitai et al. 2007) was overcome following subsequent PCP exposure, resulting in impaired impulse control increasing inappropriate responding during the ITI (Amitai et al. 2007; Amitai and Markou 2009). Premature responding has also been shown to be increased following local NMDA receptor blockade within the mPFC (Mirjana et al. 2004; Baviera et al. 2008), implicating this region's involvement not only in attentional functioning, but also inhibitory control. Additionally, the Markou group have also demonstrated that repeated exposure to PCP resulted in an increase in the latency to make a correct response, which was not accompanied by an increase in magazine latency. As previously suggested (Robbins 2002; chapter 3), the increased time taken to respond to the target stimulus may be the result of PCP impairing the animal's ability to processes incoming sensory information, consistent with previous studies (Grottick & Higgins, 2000; Le Pen et al. 2003; Auclair et al. 2009). The elevation in time taken to respond to the stimulus cue is potentially mediated by a cognitive-specific process, rather than motor or motivational impairment, as locomotor or motivational effects would also result in a concomitant increase in the time taken for the animals to retrieve the food reward, an effect which was not observed.

Performance in another preclinical attentional task, the sustained attention task (SAT – described in detail in chapters 1 and 4), has been shown to be sensitive to disruption following NMDA antagonist administration (Rezvani and Levin 2003; Rezvani et al. 2008a, b; Rezvani et al. 2009). Briefly, the SAT session is composed of two trial types and requires the

animal to report the detection of a visual signal (during the signal trial) or report the absence of the signal (during the non-signal trial) by pressing one of two response levers. During the signal trial, lever presses result in either a hit (correct response) or a miss (incorrect response), thus generating a measure of response accuracy. Conversely, in the non-signal trial, lever responses result in either correct rejections or false alarms, depending on correct or incorrect detection of the non-signal event prior to lever presentation (McGaughy and Sarter 1995). The study conducted by Rezvani and colleagues (2003; 2008a, b) consisted of a session comprised of 300 trials, split into three blocks of 100 trials. Following administration of MK-801, animals showed impaired ability to correctly report the detection of the signal trials (reduction in hit rate) in the latter two blocks of trials. Furthermore, an increase in non-signal trials incorrectly reported as signal trials (increase in false alarms) was evident across the entire session, following NMDA antagonism. Taken together, the effect of MK-801 on signal and non-signal trials demonstrated by Rezvani and colleagues (2003; 2008a, b) provide supporting evidence to previous findings of attentional dysfunction following glutamatergic disruption.

As previously discussed in chapters 1 and 4, cortical dopaminergic transmission is intrinsically linked to many of the cognitive domains playing a regulatory role (Goldman-Rakic et al. 2004), and is suggested to be involved in modulating attentional functioning in humans (Nieoullon 2002) and schizophrenia patients (Cohen and Servan-Schreiber 1993). As previously described (chapter 4), the effects of dopamine on cognition follow an 'inverted-U' shaped dose response curve i.e. the Yerkes-Dodson principle (Yerkes and Dodson 1908; Diamond et al. 2007; Vijayraghavan et al. 2009). Therefore, attentional performance may be sensitive to impairment following alterations in dopamine efflux. The dopaminergic system is closely linked with glutamatergic transmission. Hirsch and colleagues (1996) have described that dendritic spines of glutamatergic pyramidal cells (located in the cortical layers) receive afferent innervations from dopaminergic neurons (Smiley et al. 1992). Glutamate

receptors are located in the synapses of fibres arising from the nigrostriatal pathway (Roberts and Anderson 1979) and dopamine receptors are located in the synapses of glutamatergic projections arising from the striatum (Schwartz et al. 1978). Furthermore, activation of NMDA receptors has been observed to alter the distribution of dopamine D_1 receptors and D_1 activation increases the expression of NR1, NR2A and NR2B subunits of the NMDA receptor (Dunah et al. 2001; Cepeda et al. 2009). These observations strongly suggest that a reciprocal relationship exists between dopaminergic and glutamatergic neurotransmitter systems (Carlsson 1995; Hirsch et al. 1996) and alterations in glutamatergic functioning may impact dopaminergic transmission, as a result influencing the cognitive processes mediated by the dopaminergic system.

NMDA receptor antagonism has been shown to affect dopaminergic transmission; however it is highly dependent on the treatment regimen used. Acute administration of PCP (and other NMDA receptor antagonists) is associated with a marked increase in dopaminergic transmission (Deutch et al. 1987; Hertel et al. 1996; Jentch et al. 1997c; Verma and Moghaddam 1997; Jentsch and Roth 1999; Jentsch et al. 2008) in both cortical (PFC) and sub-cortical (ventral striatum i.e. nucleus accumbens) regions. In contrast to acute administration, following sub-chronic PCP treatment, a reduction of dopamine turnover in the prefrontal cortex has been observed in both rats and monkeys (Jentsch et al. 1997a, b; Jentsch and Roth 1999; Noda et al. 2000). After sub-chronic PCP exposure (in the drug-free state), a persistent reduction (at least 3 weeks) of around 75% of the basal dopamine levels were observed in prefrontal cortical regions, but not ventral or dorsal striatum (Jentsch et al. 1997b; Jentsch et al. 1998). The reduction in frontal cortical dopaminergic turnover was quantified by assessing the levels of dopaminergic metabolites (dihydroxy-O-phenyl-acetic acid [DOPAC]) in relation to dopamine. Jentsch and Roth (1999) commented that the reduction in metabolite ratio (and thus utilisation levels) were not mediated by excessive neurotoxicity arising from sub-chronic PCP exposure, suggesting that the dopaminergic

abnormalities are specifically the result of abnormal levels of dopamine available. Furthermore, using autoradiography, it has been shown that sub-chronic PCP treatment reduces binding of a radioligand to dopamine D_1 receptors in the basal ganglia (medial and lateral caudate-putamen) in rats (Choi et al. 2009). These findings reveal that in contrast to acute administration, sub-chronic exposure to NMDA antagonists alters dopaminergic function, through a reduction in the ability of effective transmission; highlighting the dissociation of dopaminergic effects and various PCP treatment regimens. However, the precise effects of dopaminergic transmission following *repeated* PCP treatment are still not entirely clear. Dopamine levels may be increased, substantially or slightly, or reduced compared to basal levels. However, the potential alterations in dopamine D_1 function induced by repeated PCP treatment may be linked with the cognitive disruption observed. As a result, augmentation of the dopaminergic D_1 system may prove fruitful in mediating cognitive enhancement in experimental animals or the compromised clinical populations (Goldman-Rakic et al. 2004; Hagen and Jones 2005; Tamminga 2006; Ibrahim and Tamminga 2010).

As the preceding chapter demonstrated that rats can be successfully trained to perform the modified version of the 5-CSRTT, the aim of the current chapter was to assess the disruptive effects of PCP within the 5C-CPT. However, due to the paucity of effects observed using a sub-chronic treatment regimen in the 5-CSRTT (chapter 3); a repeated PCP treatment regimen (Amitai et al. 2007) was implemented in the current chapter. Furthermore, in light of the reciprocal interaction between the glutamatergic and dopaminergic neurotransmitter systems, the putative abilities of systemic administration of the D_1 partial agonist SKF 38393 in attenuating a PCP-induced performance disruption was investigated. The rational for this came from the observation that D_1 augmentation improved 5C-CPT performance in a baseline-dependent manner (chapter 4).

5.2 Methods

5.2.1 Subjects

Female hooded-Lister rats (n=23; Charles River; approx 250 ± 10 g at the start of the experiment) were housed on a 12 hour reversed light cycle (lights on at 7:00 pm) in a temperature (21 ± 2°C) and humidity (55 ± 5%) controlled environment. For details regarding food restriction please refer to chapter 2.

5.2.2 Treatment Regimen

The repeated PCP treatment regimen was adapted from that used by Amitai et al. (2007) (summarised in fig 5.1) and consisted of all animals being injected with vehicle (0.9% saline) for three days (days 1 – 3) followed by two days (days 4 and 5) where all animals received PCP (2.5 mg/kg i.p.). Animals were then treated with vehicle for 5 consecutive days followed by 5 consecutive days of PCP treatment. Animals were tested in the 5C-CPT on each day, 30 minutes following drug administration. During the second PCP treatment period (days 11 – 15), animals were pre-treated 60 minutes before testing (30 minutes before PCP treatment) with either vehicle (0.9% saline, n = 11) or SKF 38393 (6.0 mg/kg i.p.; the dose established as the most efficacious in chapter 4; n = 12). This dosing regimen facilitated the investigation into whether pre-treatment with a D_1 partial agonist can attenuate any disruptive effects in task performance that may arise from repeated PCP treatment. Following the 15-day treatment regimen, performance was assessed for a further 3 days (day 16 – 18) following saline administration, assessing the potential long-term impact of repeated drug treatment on 5C-CPT performance, although this is not displayed on the schematic.

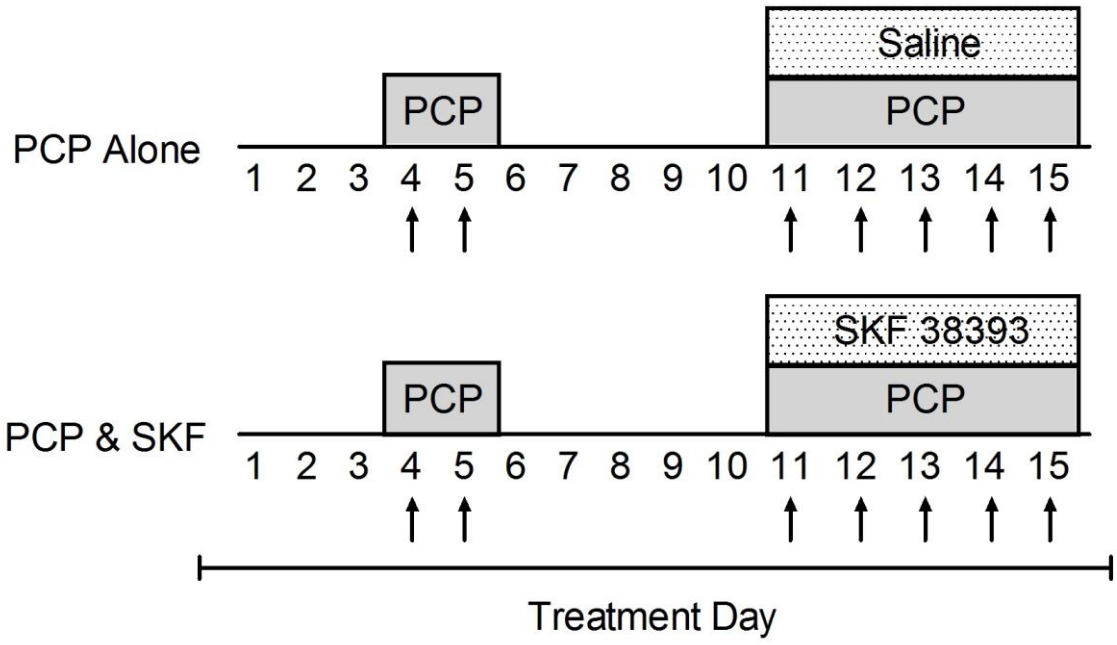

Fig 5.1 Diagram summarising the repeated PCP treatment regimen used. Arrows indicate PCP injection. On days 1-3 and 6 – 10, all animals received vehicle (0.9% saline) injections. During the second period of PCP administration (days 11 – 15), animals were pre-treated with either vehicle or SKF 38393 prior to PCP administration.

5.2.3 Group-Matching

There is a great level of individual variability regarding sensitivity to PCP, resulting in some animals having a more profound reaction to PCP treatment than others (Amitai et al. 2007). This treatment regimen enabled animals to be group-matched depending on individual responsivity to both vehicle and PCP treatment. On day 6, after all animals had been exposed to both saline (days 1 – 3) and PCP (days 4 and 5), animals were grouped into two treatment groups, based on their performance (based on several parameters) over the initial days of the treatment regimen. Animals were balanced according to the following parameters, listed in the order of importance: accuracy, sensitivity index, premature responding, percent correct responding, correct rejections, percent omissions, responsivity index, correct latency and magazine latency. The group-matching procedure ensured no

baseline differences in the initial response to PCP treatment existed between treatment groups, enabling a meaningful comparison of pharmacological attenuation of PCP-induced impairment to be made, without rats' individual sensitivity to PCP confounding interpretation.

5.2.4 Data Analysis

5.2.4.1 Effect of PCP in 5C-CPT

For each animal, the three measures from days 1 - 3 were used to produce an average baseline response per animal. An initial analysis was carried out, in the PCP treatment group that also received saline, facilitating a specific analysis investigating the effect of PCP treatment on 5C-CPT performance. This was conducted using a one-way repeated measures analysis of variance (ANOVA) approach with Day of administration as the repeated factor. All days of PCP administration (days 4 and 5 and days 11 – 15), along with the average baseline, were included in the analysis. Individual performance following each dose of PCP was compared to baseline performance using within-animal Planned Comparisons. The above analysis was repeated using animals pre-treated with SKF 38393 (days 11 – 15 only), with a repeated measures ANOVA comparing performance to the averaged baseline performance of days 1 – 3. Planned Comparisons were again utilised to assess the within-animal alteration of baseline performance.

5.2.4.2 Effect of SKF 38393 Treatment on PCP-induced Disruption

To assess the effect of treatment, a second analysis was performed on days 11 to 15 data only. Data were analysed using a two-way repeated measures analysis of covariance (RM-ANCOVA) approach with Treatment as the fixed factor, Day of administration as the repeated

factor and average baseline performance of each animal as a covariate, compensating for between-animal variability which may have existed between the animals' responses at baseline, therefore enhancing the statistical power of the approach. Between-animal Planned Comparisons were then used to assess the effect of the SKF 38393 treated group compared to the saline group on days 11 – 15.

5.2.4.3 Overall Performance

To assess the overall effect of treatment across days 11-15, performance for each animal, on each day, was averaged. Overall performance was displayed using an observed means with SEMs plot. Note: Saline and SKF 38393 are labelled on the figure to indicate future treatment as animals were only treated with saline during baseline testing. Overall performance was assessed using unpaired Students t-test.

5.3 Results

5.3.1 Effect of repeated PCP treatment on 5C-CPT performance

Initially, the effect of PCP alone was analysed in order to specifically demonstrate the effect of repeated administration on task performance. Analysis revealed that repeated PCP treatment resulted in a significant reduction in accuracy [$F_{(7, 70)} = 3.11$, $p<0.01$] (fig 5.2A) and Planned Comparisons demonstrated a significant PCP-induced reduction in accuracy on day 4 ($p<0.05$), day 5 ($p<0.01$), day 11 ($p<0.05$), day 12 ($p<0.01$), day 13 ($p<0.05$), day 14 ($p<0.01$), and day 15 ($p<0.001$). Analysis also demonstrated an overall significant increase in the percentage of incorrect responses made during the 5C-CPT session, following PCP treatment [$F_{(7,70)}=2.72$, $p<0.05$] (fig 5.2B). A significant increase was evident on day 4 ($p<0.05$), day 5 ($p<0.05$), day 11 (0.01), day 12 ($p<0.01$), day 13 ($p<0.01$), day 14 ($p<0.05$), and day 15 ($p<0.01$).

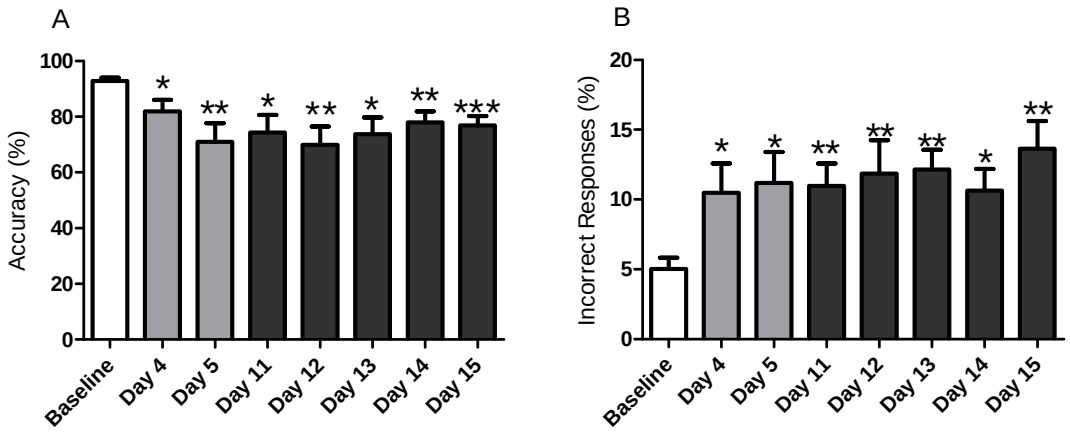

Fig 5.2 Repeated PCP administration impaired performance, indicated by a significant reduction in response accuracy (A) and an increase in percent incorrect responding (B). Data are expressed as observed mean ± SEM, n = 11. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ significant difference compared to baseline performance.

Additionally, repeated PCP administration resulted in a significant reduction in the percentage of correct responses made throughout the session [$F_{(7, 70)}$ = 4.87 p<0.001] (fig 5.3A). This reduction was evident following PCP exposure on day 4 (p<0.05), day 5 (p<0.01), day 11 (p<0.01), day 12 (p<0.001), day 13 (p<0.05), day 14 (p<0.05) and day 15 (p<0.001). This effect was coupled with a significant increase in the percentage of omissions made during the session [$F_{(7, 70)}$ = 3.85, p<0.01] (fig 5.3B). Planned Comparisons demonstrated the increase in omissions was only evident on treatment day 5, 11, 12, and 15 (p<0.01).

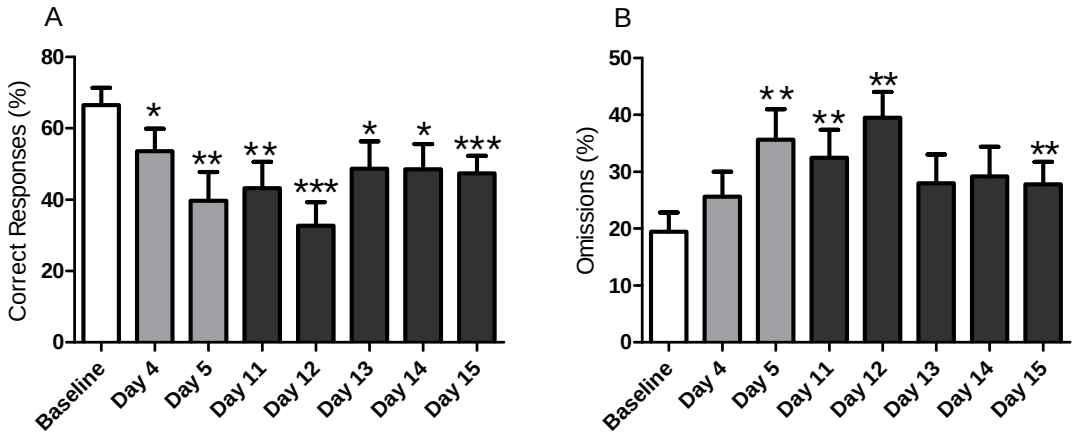

Fig 5.3 Repeated PCP administration significantly reduced percent correct responding (A) and increased percent omissions. Data are expressed as observed mean ± SEM, n = 11. * p<0.05, ** p<0.01 and *** p<0.001 significant difference compared to baseline performance.

PCP treatment induced an increase in the latency to make a correct response [$F_{(7, 70)}$ = 2.55, p < 0.05] (fig 5.4A), which was evident on day 4 (p<0.01), day 5 (p<0.01), day 11 (p<0.05), day 12 (p<0.01), and day 15 (p<0.01). One-way repeated measures ANOVA demonstrated there was no overall significant effect of PCP treatment on magazine latency [$F_{(7,70)}$=1.26, NS] (fig 5.4B), however Planned Comparisons demonstrated a significant increase in ML on day 4, 5 and 11 (p<0.05).

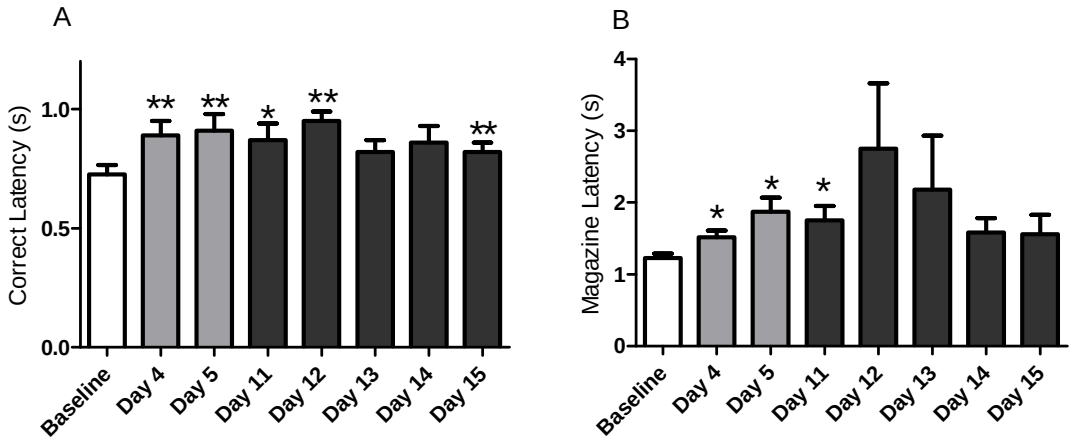

Fig 5.4 Repeated PCP administration impaired performance and significantly increased CL (A). While PCP treatment initially increased ML, any increase was not statistically significant after the third exposure to PCP (B). Data are expressed as observed mean ± SEM, n = 11. * p<0.05 and ** p<0.01 significant difference compared to baseline performance.

Repeated PCP treatment resulted in an increase in perseverative responses [$F_{(7, 70)}$ = 4.31, p<0.001] (fig 5.5A) with Planned Comparisons demonstrating a significant increase in perseveration on day 11 (p<0.05), day 12 (p<0.01), day 13 (p<0.05), day 14 (p<0.01) and day 15 (p<0.01). Similarly, a significant increase in time out responding was observed following one-way repeated measures ANOVA [$F_{(7,70)}$=5.14, p<0.001] (fig 5.5B), with Planned Comparisons demonstrating a significant increase on day 4 (p<0.01), day 5 (p<0.05), day 11 (p<0.01), day 12 (p<0.05), day 13 (p<0.01), day 14 (p<0.01) and day 15 (p<0.01). Furthermore, there was a significant increase in inappropriate premature responding [$F_{(7, 70)}$ = 4.48, p<0.001] (fig 5.5C), Planned Comparisons showed that the significant increase in premature responding was evident on day 11 (p<0.05), day 12 - 15 (p<0.01).

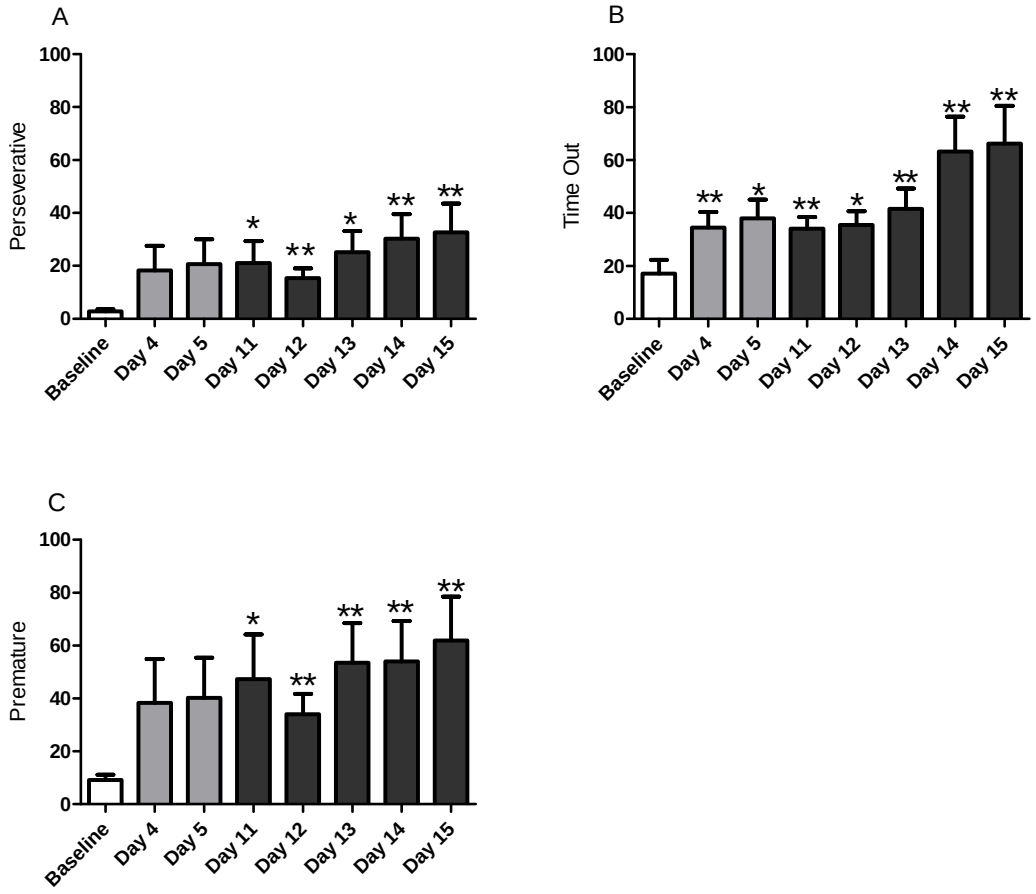

Fig 5.5. Repeated PCP administration impaired performance shown as a significant increased in perseverative responding (A), time out responding (B) and premature responding (C). Data are expressed as observed mean ± SEM, n = 11. * $p<0.05$ and ** $p<0.01$ significant difference compared to baseline performance.

Additionally, repeated PCP administration induced an inability to withhold responding in the non-signal trials, exemplified by an overall significant reduction in correct rejections [$F_{(7, 70)} = 3.22$, $p<0.01$] (fig 5.6). The reduction in percent correct rejections was evident on day 5 ($p<0.01$), day 11 ($p<0.05$), day 13 ($p<0.05$), day 14 ($p<0.05$) and day 15 ($p<0.001$).

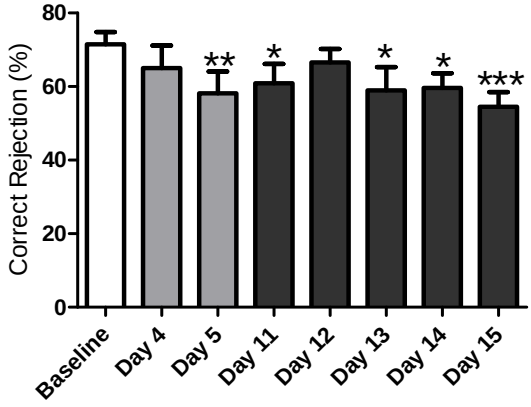

Fig 5.6 Repeated PCP administration impaired performance as significantly reduced correct rejections of non-target trials. Data are expressed as observed mean ± SEM, n = 11. * p<0.05, ** p<0.01 and *** p<0.001 significant difference compared to baseline performance.

PCP treatment also resulted in a significant reduction the hit rate when presented with target trials [$F_{(7, 70)}$ = 4.87, p<0.001] (fig 5.7A). A significant reduction in HR was demonstrated on day 4 (p<0.05), day 5 (p<0.01), day 11 (p<0.01), day 12 (p<0.001), day 13 (p<0.05), day 14 (p<0.05) and day 15 (p<0.001). A significant increase in the false alarm rate was demonstrated following PCP treatment [$F_{(7, 70)}$ = 3.22, p<0.01] (fig 5.7B), with a significant increase in incorrect responding to non-target trials evident on day 5 (p<0.01), day 11 (p<0.05), day 13 (p<0.05), day 14 (p<0.05), and day 15 (p<0.001).

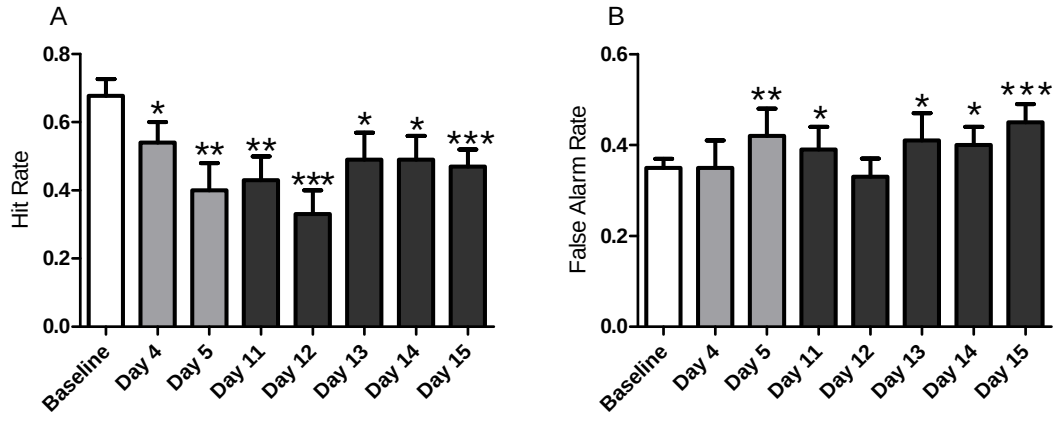

Fig 5.7 Repeated PCP administration impaired performance as significantly reduced hit rate of target trials (A) and increased the false alarm rate of non-target trials (B). Data are are expressed as mean ± SEM, n = 11. * p<0.05, ** p<0.01 and *** p<0.001 significant difference compared to baseline performance.

Due to the significant reduction and increase in HR and FAR respectively, it was apparent that repeated PCP treatment induced a significant reduction in the sensitivity index [$F_{(7, 70)}$ = 7.65, p<0.001] (fig 5.8A). This effect was demonstrated on day 4 (p<0.05), day 5 (p<0.001), day 11 (p<0.01), day 12 (p<0.001), day 13 (p<0.01), day 14 (p<0.01) and day 15 (p<0.001). The reduction in SI was independent of an overall alteration in the responsivity index, as this analysis showed no significant effect [$F_{(7, 70)}$ = 1.87, NS] (fig 5.8B). Although there was no evidence of an overall alteration in RI, Planned Comparisons indicated a significant reduction on day 11 (p<0.05) and 12 (p<0.01), following PCP treatment.

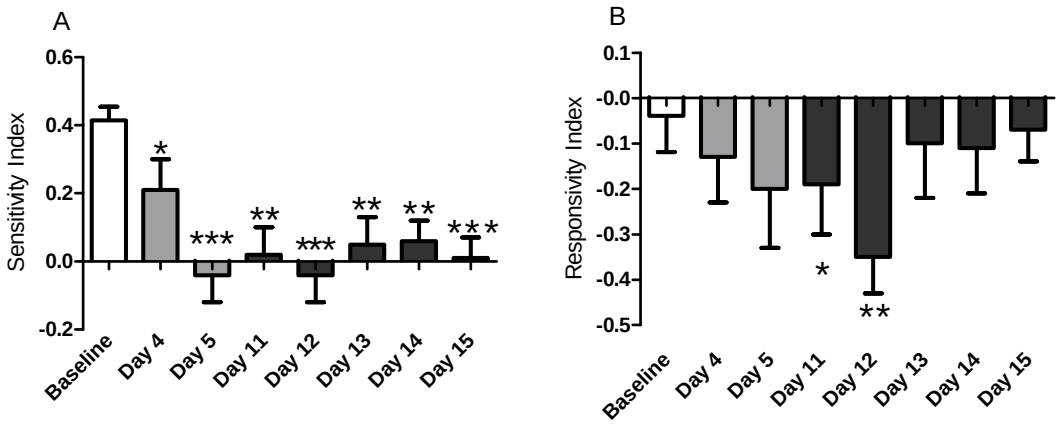

Fig 5.8 Repeated PCP treatment significantly reduced the sensitivity index (A). Repeated PCP treatment reduced RI on treatment days 11 and 12 but following subsequent administration, alterations in RI were not significantly different to that of baseline (B). Data are expressed as observed mean ± SEM, n = 11. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ significant difference compared to baseline performance.

Animals were tested on days 16 – 18 following saline administration, and performance analysed following one-way repeated measures ANOVA. Performance for each behavioural measure returned to baseline, with no statistically significant differences between days 1-3 and days 16 – 18 (data not shown).

5.3.2 Effects of SKF 38393 pre-treatment on the PCP-induced 5C-CPT disruption

The following section demonstrates the effect of pre-treatment of either SKF 38393 or saline on the PCP-induced impairment in 5C-CPT performance. The focus of the figures was to describe the between-subjects difference (two-way RM ANCOVA) during treatment days 11 – 15 (greyed area). As such, only these results are shown graphically. The within-subjects differences (one-way ANOVA) of SKF 38393-treated animals following treatment compared to baseline performance, while conducted and reported within the text, were not graphically displayed in order to maintain clairity within the figure. Furthermore, although the main focus of this section was to demonstrate the longitudinal effect of repeated drug treatment, the overall performance for both groups at baseline (days 1 – 3) and drug treatment (days 11 – 15) are displayed as column charts.

Investigation of the PCP animals pre-treated with SKF 38393 following one-way repeated measures ANOVA (Day as repeated factor) showed an overall significant effect [$F_{(5,50)}$=10.89, p<0.001]. Within-subjects Planned Comparisons demonstrated that accuracy was reduced on days 11 (p<0.01), 12 (p<0.01), 14 (p<0.01), and 15 (p<0.05), compared to baseline accuracy (data not shown). An overall significant effect was observed when percent incorrect responding was analysed by one-way repeated measures ANOVA [$F_{(5,50)}$=2.66, p<0.05], with Planned Comparisons indicated a significant increase in incorrect responding was observed on day 11 only (p<0.05) (data not shown). Two-way repeated measures analysis of covariance (ANCOVA) of the effect on accuracy demonstrated a strong trend towards an interaction between Treatment and Day [$F_{(4,80)}$=2.22, p=0.07] (fig 5.9A), but Planned Comparisons between the two treatment groups indicated a significant increase in response accuracy in SKF 38393 treated animals on days 13 – 15 (p<0.05). Although there was no significant Treatment*Day interaction for percent incorrect responding [$F_{(4,80)}$=1.33, NS] (fig 5.9C), ANCOVA did indicate an overall SKF 38393 treatment effect [$F_{(1,20)}$=14.47,

p<0.01]. Planned Comparisons between the two treatment groups showed SKF 38393 treatment significantly reduced incorrect responses made during the session on days 13 – 15 (p<0.01). Students t-test indicated that SKF 38393 treatment significantly increased (t = 2.39, df = 110, p<0.05) the accuracy of PCP treated animals (fig 5.9B). Furthermore, incorrect responding was also significantly (t = 4.86, df = 110, p<0.001) reduced in SKF 38393 treated animals (fig 5.9D).

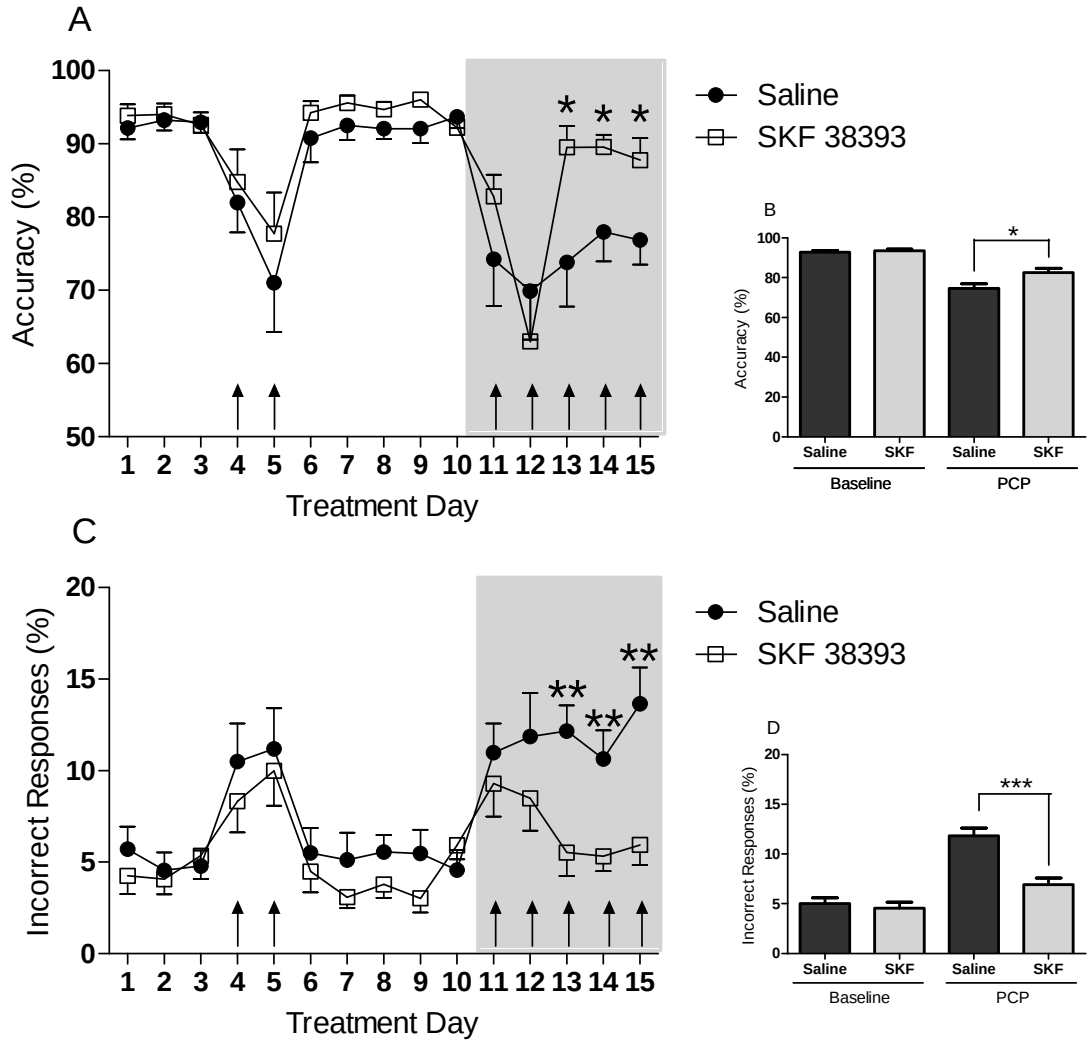

Fig 5.9. Pre-treatment with SKF 38393 significantly attenuated the PCP-induced reduction in response accuracy (A) and percent incorrect responses (C) on days 13 – 15. Overall response accuracy (B) and percent incorrect responding (D) was attenuated following SKF 38393 treatment. Data are expressed as observed mean ± SEM. Animals were pre-treated with ether saline (n=11) or SKF 38393 (n=12) prior to PCP administration. * denotes

p<0.05, ** denotes p<0.01 and *** denotes p<0.001 significant difference between treatment group. Arrows represent PCP administration.

An overall significant effect on percent correct responding following SKF 38393 and PCP treatment was observed [$F_{(5,50)}$=12.75, p<0.001], with Planned Comparisons demonstrating a significant reduction on day 11 (p<0.01), day 12 (p<0.001), day 13, (p<0.05), day 14 (p<0.001) and day 15 (p<0.001) (data not shown). Similarly, an overall significant effect was observed in animals treated with SKF 38393 and PCP on the number of omissions made [$F_{(5,50)}$=11.65, p<0.001] and percent omissions was increased on day 11 (p<0.01), day 12 (p<0.001), day 13 (p<0.05), day 14 (p<0.001) and day 15 (p<0.01) (data not shown). Repeated measures ANCOVA showed SKF 38393 treatment had no between-subjects effect on the PCP-induced reduction of percent correct responding [$F_{(4,80)}$=0.7, NS] (fig 5.10A), compared to the control (saline + PCP) group. Analysis also demonstrated that SKF 38393 treatment had no effect on the PCP-induced increase in the number of trials omitted during the 5C-CPT session [$F_{(4,80)}$=0.7, NS] (fig 5.10C). There was no overall difference in percent correct responding (t = 0.21, df = 110, NS) (fig 5.10B) or percent omissions (t = 1.09, df = 110, NS) (fig 5.10D) between treatment groups following Students t-test analysis.

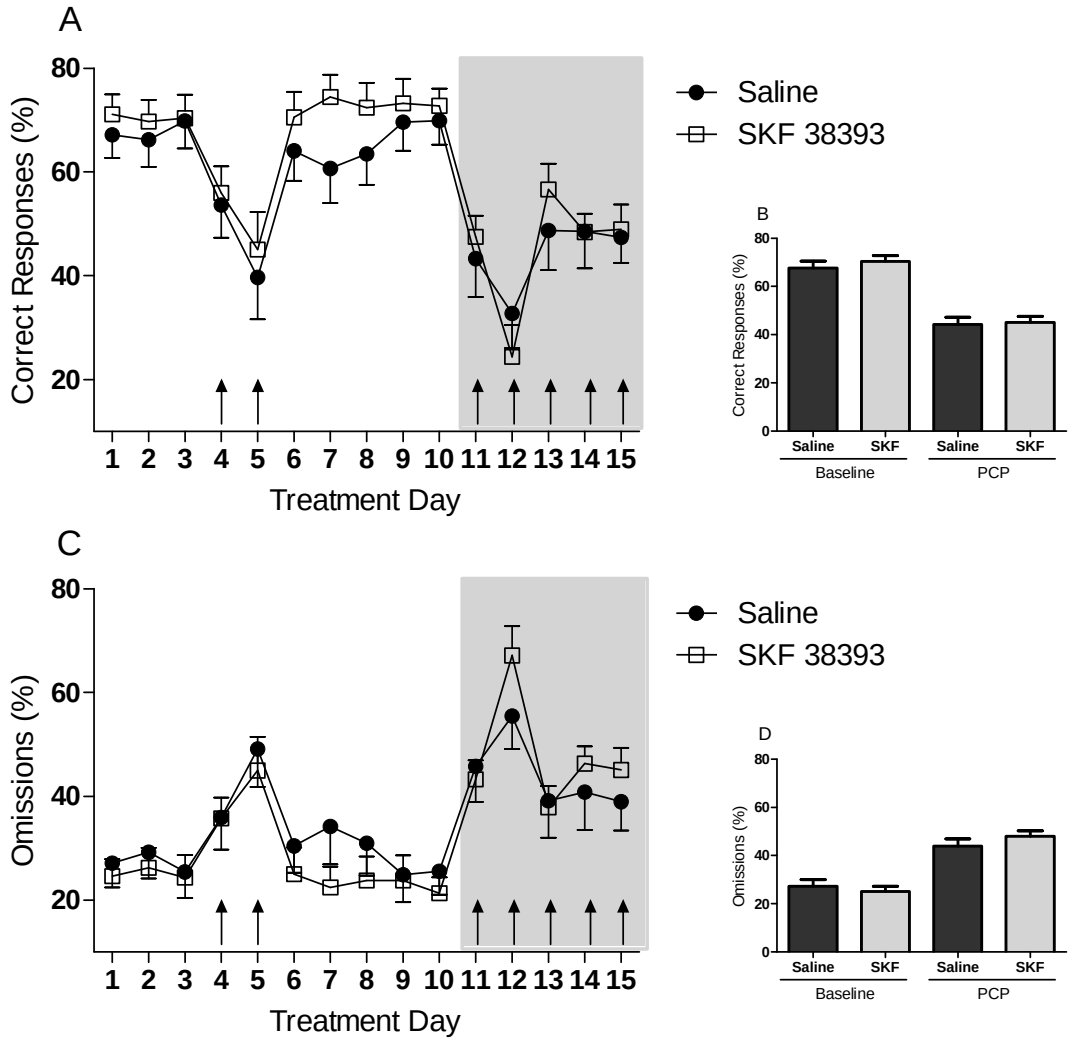

Fig 5.10 Pre-treatment with SKF 38393 had no impact on the PCP-induced reduction of percent correct responding (A, B) or the PCP-induced increase in trials omissions (C, D). Data are expressed as observed mean ± SEM. Animals were pre-treated with ether saline (n=11) or SKF 38393 (n=12) prior to PCP administration. Arrows represent PCP administration.

One-way repeated measures ANOVA of SKF 38393 treated animals showed that drug treatment significantly increased the latency to make a correct response [$F_{(5,50)}$=5.82, p<0.001], with Planned Comparisons indicating CL was increased on day 11 (p<0.01), day 12, (p<0.05), day 13 (p<0.01), day 14 (p<0.001), and day 15 (p<0.01) (data not shown). Additionally, analysis suggested there was an overall significant effect on latency to collect

the food reward [$F_{(5,50)}$=3.03, p<0.05], but Planned Comparisons demonstrated the significant increase in ML was only evident on day 11 (p<0.01), day 12 (p<0.01), day 13 (p<0.001) and day 14 (0.001) (data not shown). Any alteration of ML on day 15 was not significantly different from baseline. ANCOVA analysis demonstrated SKF 38393 treatment had no significant effect on the PCP-induced increase in correct response latency [$F_{(4,80)}$=0.5, NS] (fig 5.11A) and there was no effect between treatment groups when the effect on magazine latency was assessed [$F_{(4,80)}$=1.75, NS] (fig 5.11C). Overall analysis also indicated no difference between treatment groups for CL (t = 0.3, df = 110, NS) (fig 5.11B) or ML (t = 0.11, df = 110, NS) (fig 5.11D).

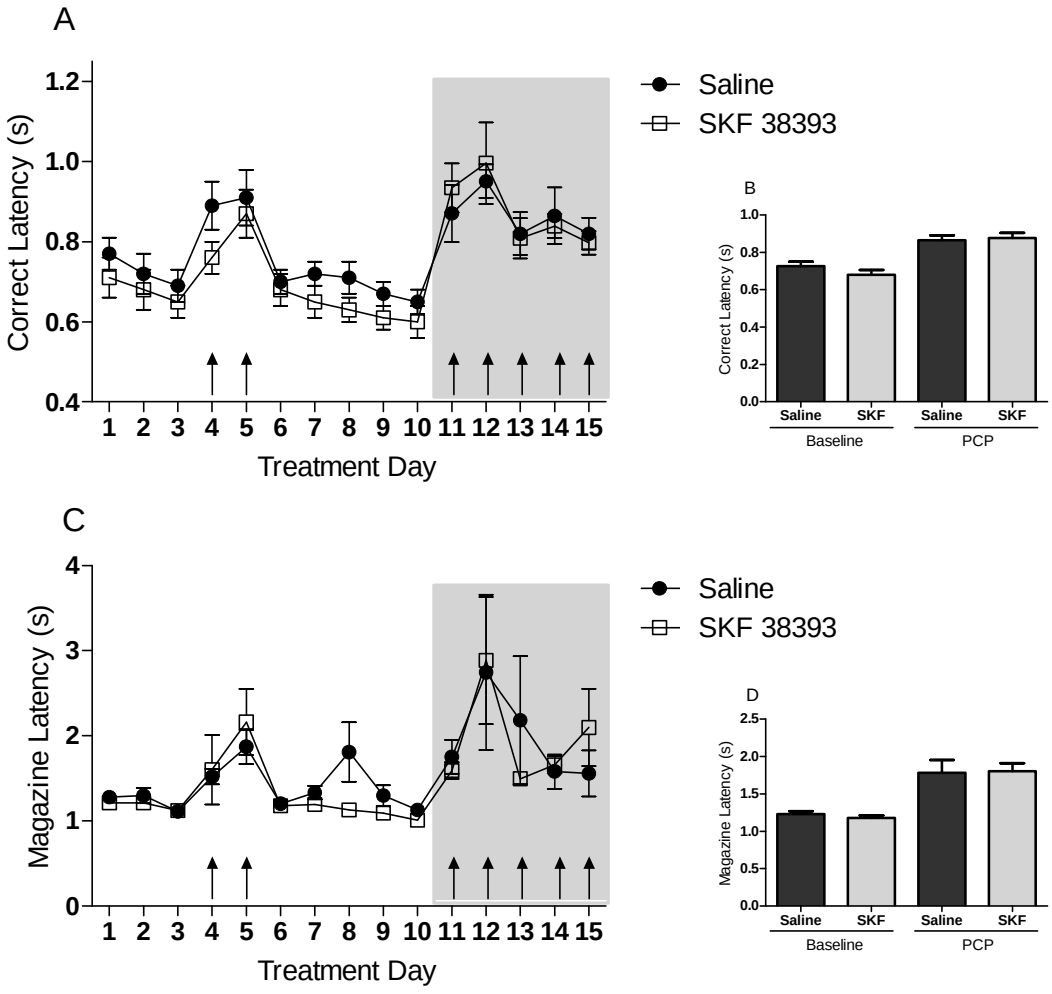

Fig 5.11. SKF 38393 treatment had no effect on the PCP-induced increase in CL (A, B). SKF 38393 treatment had no effect on the PCP-induced ML effect (C, D). Data are expressed as observed mean ± SEM. Animals were pre-

treated with ether saline (n=11) or SKF 38393 (n=12) prior to PCP administration. Arrows represent PCP administration.

Perseverative responding was also significantly increased following SKF 38393 and PCP treatment [$F_{(5,50)}$=3.99, p<0.01] and Planned Comparisons indicated perseverative responding was increased compared to baseline on day 11 (p<0.01), day 13 (p<0.01), day 14 (p<0.05) and day 15 (p<0.05) (data not shown). Additionally, there was a significant increase in inappropriate TO responding following SKF 38393 and PCP treatment [$F_{(5,50)}$=5.47, p<0.001]. Planned Comparisons demonstrated that TO responding was significantly increased on day 11 (p<0.01), day 12 (p<0.05, day 13 (p<0.001), day 14 (p<0.001) and day 15 (p<0.001) (data not shown). Likewise, there was no overall effect or treatment interaction when the effect on perseverative responding was analysed following RM ANCOVA [$F_{(4,80)}$=0.2, NS] (fig 5.12A), but Planned Comparisons of performance means between the two treatment groups demonstrated a SKF 38393-induced reduction of perseverative responding on day 12 (p<0.05). ANCOVA analysis indicated no effect of drug treatment on the number of time out responses made between treatment groups [$F_{(4,80)}$=1.56, NS] (fig 5.12C). Students t-test indicated an overall significant reduction in perseverative responding in SKF 38393 treated animals (t = 2.61, df = 110, p<0.05) (fig 5.12B). No overall difference was observed for TO responding, however a trend towards an SKF 38393-induced reduction was observed (t = 1.93, df = 110, p<0.1) (fig 5.12D).

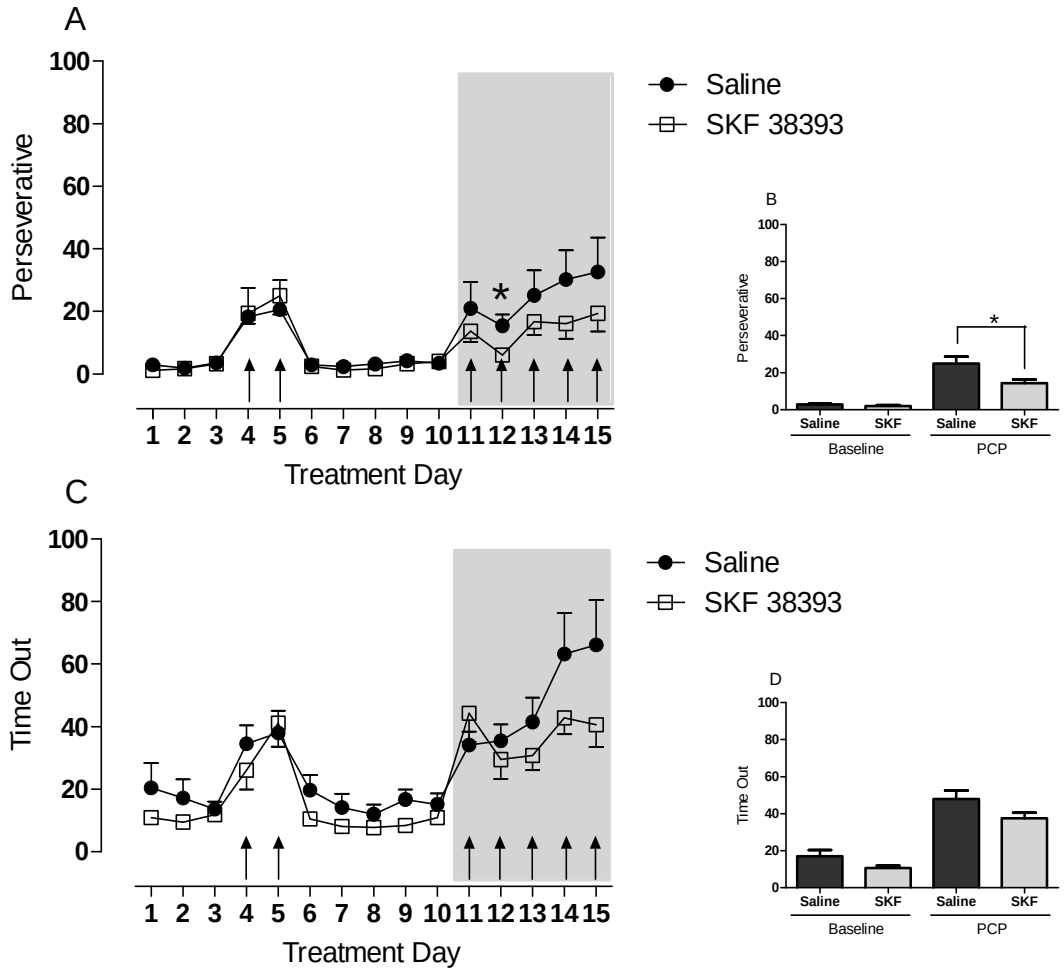

Fig 5.12. Pre-treatment with SKF 38393 significantly attenuated the PCP-induced increase in perseverative responding on day 12 (A) and resulted in an overall significant reduction (B), but had no significant effect on the PCP-induced elevation of inappropriate time out responding (C, D). Data are expressed as observed mean ± SEM. Animals were pre-treated with ether saline (n=11) or SKF 38393 (n=12) prior to PCP administration. * p<0.05 significant difference between treatment group. Arrows represent PCP administration.

Upon within-subjects analysis of SKF 38393 treated animals, there was a PCP-induced increase in premature responding [$F_{(5,50)}$=4,97, p<0.001]. Planned Comparisons indicated the number of premature responses was increased on day 11 (p<0.01), day 13 (p<0.01), day 14 (p<0.01) and day 15 (p<0.05), compared to baseline (data not shown). Between-subjects ANCOVA analysis showed no effect of Treatment on the number of premature responses

made following PCP treatment and also showed no interaction between Treatment and Day [$F_{(4,80)}$=0.12, NS] (fig 5.13A). However, Planned Comparisons indicated that SKF 38393 treatment on day 12 significantly reduced the number of PCP-induced premature responses. Overall analysis demonstrated that SKF 38393 pre-treatment significantly attenuated the PCP-induced increase in premature responding (t = 3.43, df 110, p<0.01) (fig 5.13B).

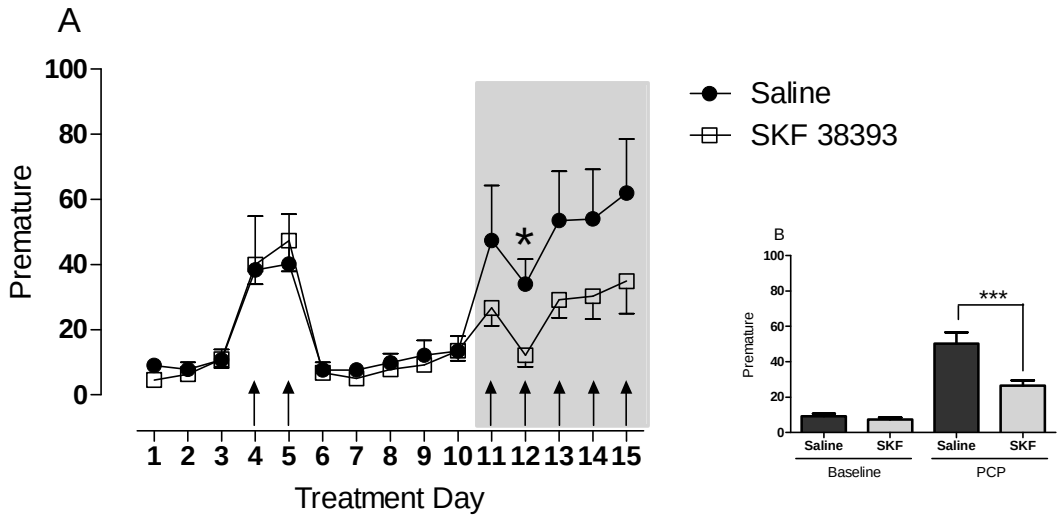

Fig 5.13 SKF 38393-treatment significantly ameliorated the PCP-induced increase in inappropriate premature responding on treatment day 12 (A). Overall PCP-induced premature responding was attenuated in SKF 38393-treated animals. Data are expressed as observed mean ± SEM. Animals were pre-treated with ether saline (n=11) or SKF 38393 (n=12) prior to PCP administration. * p<0.05 and *** p<0.01 significant difference between treatment group. Arrows represent PCP administration.

Within-subjects analysis indicated an overall significant effect on correct rejections, resulting from SKF 38393 and PCP treatment [$F_{(5,50)}$=2.70, p<0.05]. However, within-subject Planned Comparisons demonstrated that a significant reduction in correct rejections, compared to baseline performance, was only evident on day 11 (p<0.01) (data not shown). Between-subjects ANCOVA analysis indicated there was no significant interaction between Treatment and Day [$F_{(4,80)}$=1.91, NS] (fig 5.14A), but Planned Comparisons demonstrated there was a

significant SKF 38393-induced attenuation of the PCP-induced reduction of correct rejections. The SKF 38393-induced significant increase in correct rejections occurred on treatment day 15 only ($p<0.01$). However, overall Students t-test indicated a significant increase in correct rejections in PCP animals pre-treated with SKF 38393 ($t = 2.58$, $df = 110$, $p<0.05$) (fig 5.14B).

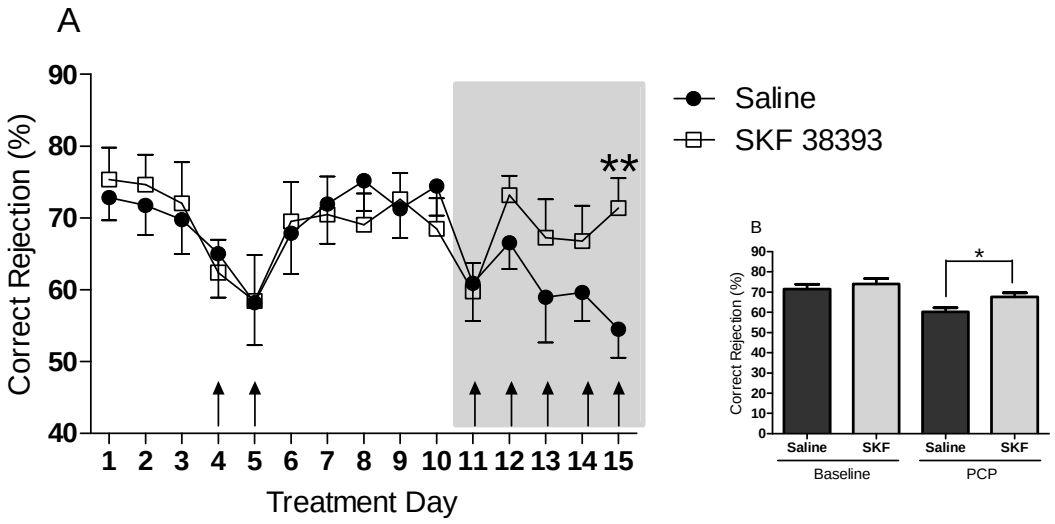

Fig 5.14 SKF 38393 treatment significantly attenuated the PCP-induced reduction in correct rejections on treatment day 15 (A). Overall correct rejections were increased in SKF 38393 treated animals (B). Data are expressed as observed mean ± SEM. Animals were pre-treated with ether saline (n=11) or SKF 38393 (n=12) prior to PCP administration. * $p<0.05$ and ** $p<0.01$ significant difference between treatment groups. Arrows represent PCP administration.

One-way repeated measures ANOVA indicated that the hit rate of SKF 38393 treated animals was significantly reduced [$F_{(5,50)}=12.75$, $p<0.001$]. Planned Comparisons indicated that HR was significantly reduced on day 11 ($p<0.01$), day 12 ($p<0.001$), day 13 ($p<0.05$), day 14 ($p<0.001$), and day 15 ($p<0.01$) (data not shown). Within-subject analysis also demonstrated a significant increase in false alarms following SKF 38393 and PCP treatment [$F_{(5,50)}=2.71$, $p<0.05$], but the only day where p[FA] was significantly elevated was day 11 ($p<0.01$) (data not shown). Between-subjects ANCOVA analysis indicated no effect of SKF 38393 treatment on the PCP-induced reduction of hit rate [$F_{(4,80)}=0.7$, NS] (fig 5.15A). In a similar manner to

the effect on correct rejections, there was no overall effect of SKF 38393 treatment on the PCP-induced increase in false alarms [$F_{(4,80)}$=191, NS] (fig 5.15C). However, Planned Comparisons demonstrated a between-subjects reduction in false alarms, showing that SKF 38393 treatment significantly reduced incorrect responding to non-target trials on day 15 ($p<0.01$). No overall effect was observed between treatment groups for hit rate ($t = 0.21$, $df = 110$, NS) (fig 5.15B). In contrast, an overall significant reduction in false alarms was observed in the SKF 38393 treatment group ($t = 2.58$, $df = 110$, $p<0.01$) (fig 5.15D).

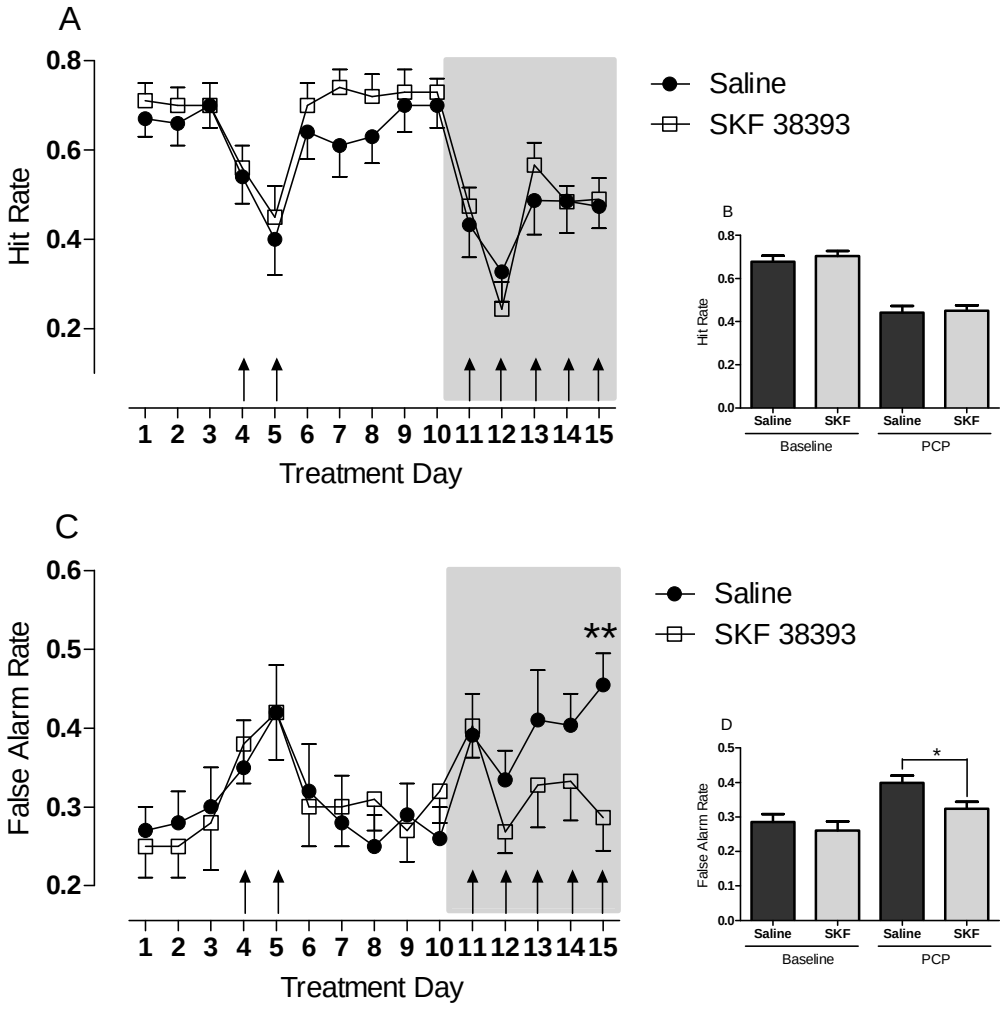

Fig 5.15. SKF 38393 treatment did not significantly attenuate the PCP-induced reduction in hit rate (A, B) but did significantly reduce the PCP-induced increase in false alarms on day 15 (C). Overall analysis also indicated a SKF 38393-induced significant reduction in false alarms (D). Data are expressed as observed mean ± SEM. Animals

were pre-treated with ether saline (n=11) or SKF 38393 (n=12) prior to PCP administration. ** p<0.01 significant difference between treatment group. Arrows represent PCP administration.

Analysis demonstrated there was a significant within-subject reduction in SI following SKF 38393 and PCP treatment [$F_{(5,50)}$=11.06, p<0.001]. Planned Comparisons indicated this reduction of SI was evident on day 11 (p<0.001), day 12 (p<0.001), day 13 (p<0.05), day 14 (p<0.001) and day 15 (p<0.01) (data not shown). This was coupled with a significant alteration in RI [$F_{(5,50)}$=7.12, p<0.001], but Planned Comparisons attributed this significant effect to a reduction on day 12 (p<0.001) and day 15 (p<0.05) (data not shown). Between-subjects ANCOVA analysis of the PCP-induced effect on SI demonstrated no overall significant Treatment*Day interaction [$F_{(4,80)}$=1.54, NS] (fig 5.16A), however Planned Comparisons demonstrated a significant between-subjects increase in SI following SKF 38393 treatment on day 15 (p<0.05). Although there was also no significant effect of drug treatment or day on the responsivity index of animals [$F_{(4,80)}$=1.09, NS] (fig 5.16C), the significant increase in SI on day 15 was also accompanied by a significant SKF 38393-induced reduction of RI on day 15 (p<0.05). Students t-test indicated a significant increase in SI in the SKF 38393 treated animals (t = 2.12, df = 110, p<0.05) (fig 5.16B), independent of alterations in RI (t = 1.28, df = 110, NS) (fig 5.16D).

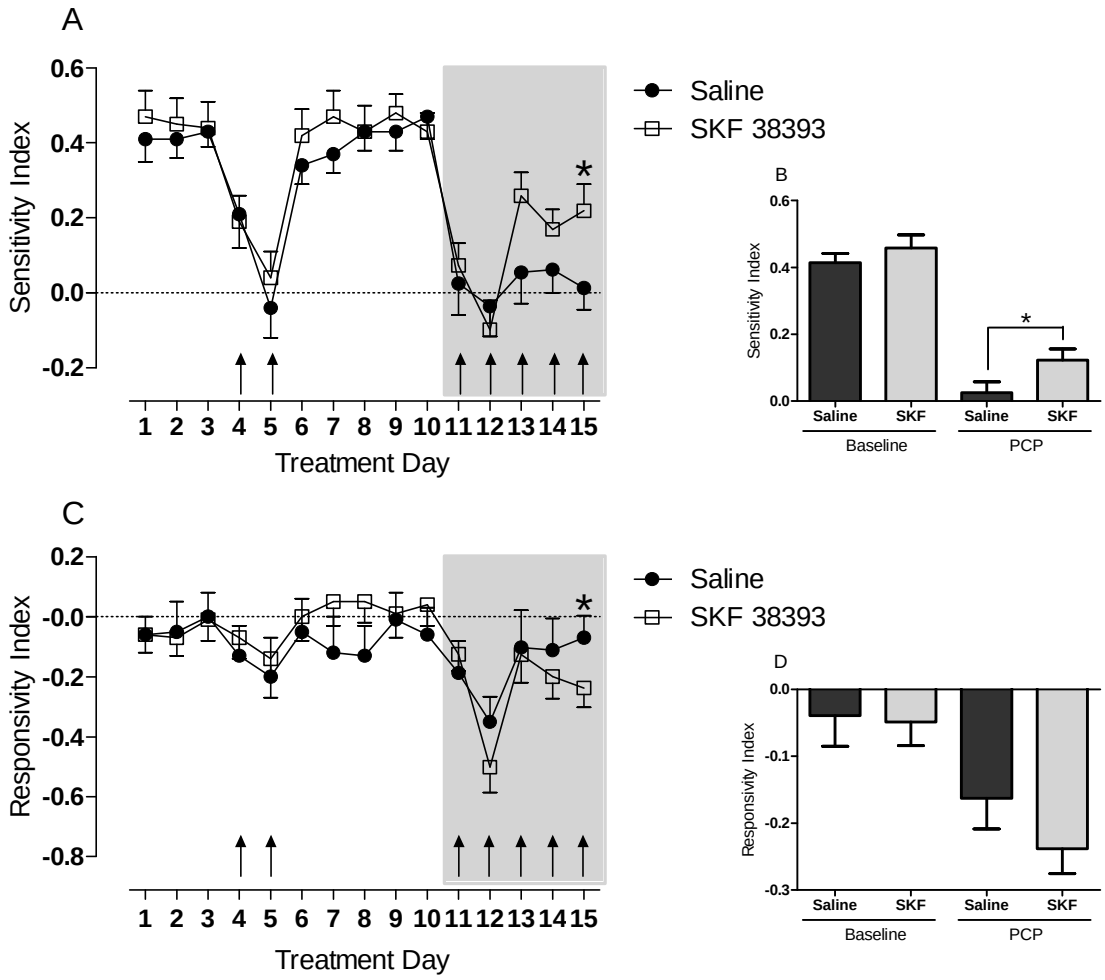

Fig 5.16 Pre-treatment with SKF 38393 significantly increased the PCP-induced reduction in sensitivity index on day 15 (A), which was coupled with a significant reduction in responsivity index on day 15 (C). However, overall SI was increased in SKF 38393-treated animals (B) independent of alterations in RI (D). Data are expressed as observed mean ± SEM. Animals were pre-treated with ether saline (n=11) or SKF 38393 (n=12) prior to PCP administration. * $p<0.05$ significant difference between treatment group. Arrows represent PCP administration.

5.4 Discussion

Firstly, these data demonstrate that repeated administration of PCP induced robust disruption of 5C-CPT performance. These findings are in agreement with previous investigations of repeated PCP treatment and impaired 5-CSRTT performance (Amitai et al. 2007; Amitai and Markou 2009; Amitai and Markou 2010). Cognitive-specific effects and not generalised behavioural disruption, which is often associated with acute PCP administration, mediated impaired performance. These findings, however, expand on those demonstrated by the Markou group as PCP treatment was shown to also impair performance during non-target trials, exemplified by the reduced ability to correctly withhold responding. Additionally, these data demonstrate that the PCP-induced impairment is susceptible to attenuation, at least partially, following augmentation of the D_1 dopaminergic system. When PCP-treatment was preceded by SKF 38393 administration there was an attenuation of the PCP-induced disruption of 5C-CPT performance on a number of behavioural parameters measured.

5.4.1 Effect of Repeated PCP treatment on 5C-CPT performance

The response to PCP was analysed separately in order to specifically assess the effect repeated administration of the NMDA receptor antagonist had on performance in the 5C-CPT. Repeated PCP administration significantly reduced choice accuracy (mediated by reduced correct responding *and* increased incorrect responding), percent correct and increased the number of omissions, indicative of impaired sustained attentional processing (Robbins 2002; Dalley et al. 2004; Amitai and Markou 2010). Exposure to PCP also increased the time taken to make a correct response (CL), suggesting PCP exposure reduced the speed of information processing. This was coupled with an initial increase of magazine latency

(ML), indicating locomotor impairment or a reduced motivation to perform the task may have induced the increased time taken to respond. However, this effect dissipated following subsequent doses of PCP. This attenuation of the elevated ML following repeated PCP exposure suggested that putative locomotor or motivational impairments were not responsible for the increased CL observed towards the latter stages of the treatment regimen. These data indicated PCP treatment induced a specific impairment in the speed of information processing, an effect observed in schizophrenia patients (Nelson et al. 1990). Additionally, the lack of a concomitant increase in ML following repeated treatment suggested that the increased number of trials omitted was likely the result of cognitive-specific attentional dysfunction.

PCP treatment produced an increase in the number of inappropriate responses made throughout the 5C-CPT session, demonstrated by an increase in perseveration and a subsequent increase in inappropriate time out responses. Increases in both of these measures suggest that PCP treatment produced a reduced ability to disengage from a behaviour once initiated, indicative of increased compulsivity or cognitive inflexibility (Amitai and Markou 2010). Increased perseveration has also been demonstrated following PCP administration in the 5-CSRTT (Auclair et al. 2009). Studies conducted by the Markou group did not find a PCP-induced increase in perseverative responding (Amitai et al. 2007; Amitai et al. 2008; Amitai and Markou 2009), however the first perseverative response initiated a time out, thus only one perseverative response could be made per trial. However, the Markou group demonstrated the number of time out responses was significantly elevated following PCP treatment, suggesting increased compulsive-like behaviour. Similarly though, the first perseverative response in the current study also initiated a time out, but in contrast to the findings demonstrated by the Markou group, PCP-treatment significantly increased not only time out responding, but also perseverative responding. This may highlight a gender-specific difference as females were used in this instance, *cf* male subjects used in all studies from the

Markou group. This may suggest females may have enhanced sensitivity to the PCP-induced failure to disengage from a response once initiated. Aspects of this type of dysfunctional behaviour have also been demonstrated in schizophrenia patients (Goldberg et al. 1988; Morice 1990). PCP-induced impairments in cognitive flexibility are not unique to the 5C-CPT (or the 5-CSRTT in previous cases), as impairments in a reversal-learning paradigm have also been demonstrated (Abdul-Monim et al. 2003; Idris et al. 2005; McLean et al. 2009). Although these studies utilised acute or sub-chronic PCP treatment regimens, they highlight the susceptibility of cognitive flexibility to NMDA antagonist disruption. Impairments in this task are only evident in the reversal phase, with performance in the initial phase being left intact, symptomatic of impaired cognitive flexibility as animals have a reduced capability to switch their response behaviour once the reward contingency has been reversed.

PCP treatment also resulted in deficits in impulse control, exemplified by the increased number of premature responses made throughout the session. Premature responses within the 5-CSRTT (or in this case, the 5C-CPT) are a well characterised measure of impulsivity (Puumala and Sitvio 1998; Evenden 1999; Robbins 2000; Amitai and Markou 2010; Besson et al. 2010) and represents a reduced ability to withhold, or a failure of the animal to inhibit, a response. Consequently, this failure of inhibitory control elevates the frequency of inappropriate responding during the ITI, before the stimulus cue has been presented. Schizophrenia patients also demonstrate increased impulsivity characterised by deficits in behavioural inhibition, resulting in an increased frequency of inappropriate responding in situations requiring the inhibition of a response (Kiehl et al. 2000; Weibrod et al. 2000; Wykes et al. 2000). PCP treatment has been shown on a number of occasions to increase the frequency of premature responding, suggesting a deficit in impulse control (Le Pen et al. 2003; Amitai et al. 2007).

Premature responding in the 5-CSRTT and 5C-CPT, however, reflect an inappropriate response *before* a stimulus has been presented. Consequently, premature responses may demonstrate limited analogy to the increased impulsivity exhibited by schizophrenia patients. As described, impulsivity is often assessed by inappropriate responding when *presented* with irrelevant stimuli that require inhibition of a response, characteristic of behavioural disinhibition demonstrated in schizophrenia patients (Green 1997). Therefore, the increased premature responding induced through repeated PCP treatment is perhaps more likely to embody a form of motoric impulsivity as opposed to behavioural disinhibition (Evenden 1999). However, the 5C-CPT also encompasses non-target trials and the correct action when presented with these trials is to inhibit a response. Coupled with the elevation in premature responding, it was demonstrated that PCP treatment also increased the number of inappropriate responses when presented with non-target trials. This resulted in a reduction in the number of correct rejections increasing the number of false alarms made throughout the session. As false alarms constitutes a failure to *inhibit* a response when the animal was *presented* with inappropriate stimuli, an increase in false alarms has enhanced analogy to behavioural disinhibition observed in schizophrenia patients (Kiehl et al. 2000; Weibrod et al. 2000; Wykes et al. 2000) over the traditional 5-CSRTT measure of impulsivity – premature responding. Taken together however, the effect of increased premature responding *and* increased false alarms suggests that PCP treatment induced a form of behavioural disinhibition, not just motoric impulsivity, of relevance to that observed in schizophrenia patients. NMDA receptor antagonism has previously been shown to reduce the number of correct rejections (ergo increased false alarms) in animals performing the sustained attention task (SAT – Rezvani and Levin 2003; Rezvani et al. 2008a, b) a task developed by McGaughy and Sarter (1995), reflecting increased inappropriate responding to non-signal stimuli. However, as discussed in chapters 1 and 4, both false alarms and correct rejections within this task require an active response. Therefore, the correct rejections

cannot be fully analogous to correct rejections in human CPTs. As the contrary measure of a correct rejection is a false alarm, the NMDA-induced increase in false alarms within the SAT (Rezvani et al. 2003; 2008a, b), may not fully encompass the behavioural disinhibition demonstrated by schizophrenia patients performing go/no-go tasks or CPTs (Cornblatt and Malhotra 2001; Neuchterlein et al. 2008). However, as previously described, the non-target trials within the 5C-CPT require the animal to withhold a response when presented with non-target trials. Therefore, the PCP-induced increase in false alarms within the 5C-CPT is representative of a failure in action restraint (Eagle et al. 2008; Young et al. 2009b), perhaps providing greater analogy than other preclinical paradigms to the clinical observations of behavioural disinhibition exhibited by schizophrenia patients.

As previously described, PCP treatment reduced target trials correctly detected (reduction in hit rate) whilst simultaneously reducing non-target trials correctly rejected (increase in false alarm rate). These effects suggested that repeated PCP administration completely abolished the animal's ability to discriminate between trial types presented within the 5C-CPT. This abolition of target discrimination was quantified by the reduction in the sensitivity index (SI). As the SI has been implemented as a means of quantifying vigilance performance in a manner consistent with human attention/vigilance assessment utilising one of the myriad CPTs (Young et al. 2009b), it can therefore be inferred that PCP treatment impaired signal discriminability, manifesting as impairment in vigilance. The 5C-CPT also generates an index of responsivity (RI), enabling the characterisation of cognitive impairment to be dissociated from alterations in strategic bias of the animal. It was evident that PCP treatment significantly reduced RI (days 11 and 12), suggesting that impaired performance may potentially be mediated by alterations in the decision criteria, shifting to a more conservative response strategy, rather than a truly cognitive mechanism (Davies and Parasuraman 1982; Parasuraman et al. 1998). However, following repeated administration of PCP, the reduction in the responsivity index returned to that of baseline, whilst reductions in target

discrimination persisted. These findings suggest that PCP treatment impaired the ability of the animal to detect and respond to target stimuli and increased the response to non-target stimuli, independently of alterations in the strategy being employed, indicating impairments being driven by cognitive mechanisms (Marston 1996; Parasuraman et al. 1998).

5.4.2 Effects of SKF 38393 on PCP-induced 5C-CPT impairment

When animals were treated with SKF 38393 prior to PCP treatment there was a partial amelioration of the PCP-induced cognitive disruption. SKF 38393 pre-treatment significantly increased the PCP-induced reduction in response accuracy. The SKF 38393-induced attenuation was not immediate, however. In the initial two days of PCP/SKF 38393 treatment there was no difference to the animals treated with PCP/saline. However, following the initial two days (days 13 – 15), there was a significant increase in response accuracy, when a between-subjects comparison was conducted. Upon inspection of the treatment effect on percent correct responding, analysis demonstrated that there was no significant effect of SKF 38393-treatment on the PCP-induced reduction in target trials correctly detected. Following analysis of percent incorrect responding it was evident that SKF 38393 pre-treatment completely attenuated the PCP-induced increase in incorrect responding after treatment day 12. These findings suggest that augmentation of the dopaminergic D_1 system attenuated the PCP-induced impairment in response accuracy, mediated by a specific amelioration of the number of incorrect responses made following PCP treatment. It is well established that acute and repeated NMDA antagonism elevates acetylcholine release in the PFC (Kim et al. 1999; Nelson et al. 2002). Additionally, cortical deafferation has been shown to impair target detection in the SAT, suggesting cholinergic transmission is involved in stimulus detection (McGaughy et al. 1996; McGaughy and Sarter 1998; McGaughy et al. 2002), and NMDA antagonism impairs target detection in the SAT

(Rezvani et al. 2003; 2008a, b). These findings potentially suggest that disrupted cholinergic transmission may be involved with the PCP-induced reduction in correct responding.

There was a significant attenuation of incorrect responding in the SKF 38393-treated animals. This suggests that aberrant dopaminergic transmission may mediate the generation of incorrect responding following PCP treatment. Augmentation of the D_1 system therefore would attenuate the PCP-induced increase in incorrect responding, mediating the partial attenuation of the response accuracy impairment. Unfortunately, a general theme within 5-CSRTT literature is to only report the effects on response accuracy or percent correct responding. It is therefore not possible to determine whether any impairment in response accuracy was driven by a reduction in correct responding, increased incorrect responding, or a combination of the two. An exception to this rule is the study published by Amitai and colleagues (2007) who demonstrated that repeated PCP treatment increased incorrect responses. Furthermore, the group demonstrated that the PCP-induced increase in incorrect responses was attenuated by chronic clozapine treatment (4.0 mg/kg/day), which may have accounted for the clozapine-induced partial attenuation of the PCP-induced reduction in response accuracy demonstrated by Amitai and colleagues (2007). It has been suggested that clozapine provides therapeutic efficacy by normalising prefrontal dopamine levels following PCP treatment (Elsworth et al. 2008). The beneficial effects in the SKF 38393 treated animals may be mediated by a similar process following clozapine treatment; normalisation of frontal cortical dopamine levels, restoring cognitive function. In addition to the lack of attenuation of the PCP-induced reduction in percent correct responding, pre-treatment with SKF 38393 had no impact on the increase in omissions following PCP treatment.

There was no difference between groups on any of the treatment days when the measures of correct latency or magazine latency (ML) were assessed. It should be noted, however, that

the effect of drug treatment (SKF 38393 + PCP) on ML followed a similar trend to PCP alone when analysed separately; while there may have been an initial significant increase in ML compared to baseline performance, any alterations in ML by the end of the treatment regimen were not statistically significant from baseline performance. This confirms that any behavioural effects observed following SKF 38393 and PCP treatment were not mediated by putative alterations in locomotor abilities or reduced motivation to perform the task. SKF 38393 treatment had a partial effect on the PCP-induced increase in perseverative responding. Analysis of performance on individual days indicated a significant SKF 38393-induced reduction in perseverative responding on day 12 only, but, comparison of the overall means between the treatment groups suggest at least a partial attenuation of the PCP-induced increase in perseverative responding. However, no effect was observed on the PCP-induced increase in time out responding. A similar effect was observed for premature responding; analysis of performance over individual treatment days indicated a significant reduction on day 12 only, whilst a comparison of the overall performance means indicated that SKF 38393 treatment induced a partial attenuation of the PCP-induced increase in premature responding.

It was revealed that SKF 38393 pre-treatment significantly attenuated the PCP-induced reduction of correct rejections. Between-subjects comparison indicated this effect was only evident on treatment day 15. As correct rejections reflect the inhibition of a response the measure is obviously sensitive to confounding effects of locomotor impairment. However, the significant SKF 38393-induced attenuation of correct rejections occurred on day 15, when there was no significant effect on ML suggesting the effect was not attributed to a generalised inability to respond thus ruling out the potential confound of locomotor impairment. The SKF 38393-induced partial amelioration of premature responding, along with the attenuation of the PCP-induced reduction in correct rejections suggests aberrant dopaminergic transmission is involved with PCP-induced impairment in behavioural

inhibition. While it has been demonstrated that chronic clozapine attenuates the increase in premature responding in the 5-CSRTT (Amitai et al. 2007), clozapine treatment had no effect on the reduction in correct rejections when MK-801 was administered to animals performing the SAT (Rezvani et al. 2008b). These findings suggest that perhaps NMDA antagonism impairs behavioural inhibition, increasing premature responding and reducing correct rejections. The amelioration of premature responding by clozapine may be due to clozapine's effect on prefrontal dopamine transmission. This could explain the effects observed in the current chapter. However, this does not account for the SKF 38393-induced attenuation of correct rejections observed (day 15) and the lack of effect demonstrated by Rezvani and colleagues (2008b). If the beneficial effects of SKF 38393 on correct rejections are to be attributed to normalization of prefrontal dopamine levels following PCP treatment, it would be expected that clozapine treatment (which also normalises cortical dopamine levels) would produce similar effects. However, a number of differences exist between the Rezvani study (2008b) and data shown in the current chapter; firstly MK-801 is used and not PCP. Whilst both are primarily NMDA antagonists, they may have subtle differences in pharmacology accounting for differing behavioural impairments. Secondly, MK-801 was administered acutely (although it can be argued that a repeated treatment regimen was used as a Latin square design was implemented). Thirdly and perhaps most importantly, clozapine was administered acutely. The efficacious effects of clozapine on PCP-induced premature responding were observed following chronic administration (Amitai et al. 2007). SKF 38393 was administered repeatedly over 5 days in the current chapter, with amelioration of correct rejections observed on the last treatment day only. Additionally, when clozapine was demonstrated to normalize prefrontal cortical dopamine levels following PCP treatment, it was also administered repeatedly (Elsworth et al. 2008). These observations could account for the SKF 38393-induced amelioration of correct rejections in the current chapter, whilst clozapine (which normalises PFC DA levels) failed to attenuate NMDA-induced reduction in

correct rejections in the SAT (Rezvani et al. 2008b). These findings attest to aberrant dopaminergic transmission involvement in behavioural disinhibition following PCP treatment.

PCP-treatment significantly reduced the hit rate, an effect that was not sensitive to SKF 38393-treatment. However, SKF 38393 pre-treatment significantly ameliorated the PCP-induced increase in false alarms on treatment day 15. Taken together, this effect led to a complete abolition of signal discrimination, an impairment that was significantly attenuated by SKF 38393 treatment. However, the PCP effect on SI was only partially attenuated by SKF 38393 treatment. This was not suprising as SKF 38393 treatment had no beneficial impact on the hit rate and so the SKF 38393-induced attenuation of SI was driven solely by the effect on the false alarm rate. As such, the SKF 38393-induced increase in SI only became apparent on day 15. While there was no overall significant effect observed between treatment groups, the SKF 38393-induced increase of the PCP-induced reduction in SI on day 15 was accompanied by a significant reduction in the responsivity index. This finding suggests that partial attenuation of SI may not have been mediated by enhanced discrimination of trial types, which would be attributed to enhanced cognitive abilities. However, the increase in SI was potentially driven by enhanced behavioural inhibition, reflected by a more conservative response strategy being utilised and therefore a reduced RI.

These data demonstrate that 5C-CPT performance is robustly disrupted following repeated PCP administration in a cognitive-specific manner, impairments that are not precluded by generalised-response disruption. The PCP-induced impairment was partially ameliorated if PCP administration is preceded by treatment with a D_1 receptor partial agonist. These findings suggest that the PCP-induced impairment observed may be mediated, at least in part, by aberrant dopaminergic transmission. However, as previously mentioned, it is still not entirely clear the effect that a repeated PCP treatment regimen has on cortical DA turnover. A single acute injection of PCP considerably increases PFC DA release (Jentsch et

al. 2008), while in contrast, during the drug-free state following sub-chronic PCP treatment there is a marked decrease in DA utilisation in the PFC (Jentsch et al. 1997a, b; Jentsch and Roth 1999; Noda et al. 2000). It has been demonstrated that glutamate is release is increased following a single acute injection of PCP (Adams and Moghaddam 2001) and evidence has recently emerged that following repeated PCP administration, glutamate is elevated above basal levels, albeit blunted compared to acute administration (Amitai et al. 2011). Although the precise effect is unclear, it is likely that the effects are similar for dopamine following repeated PCP treatment; increased above basal levels, but blunted compared to the increase elicited following a single acute dose. In this scenario, beneficial effects following dopamine D_1 partial agonism are likely mediated by SKF 38393 competing with DA for the D_1 receptor. SKF 38393 would activate the D_1 second messenger system (adenylate cyclase), but due to lower intrinsic activity of the compound, activation would be to a lesser extent than a full agonist (e.g. DA) (Stahl 2008a; Strange 2008). Thus, this would achieve the net effect of reducing the effect of elevated DA release following PCP administration, potentially attenuating cognitive function mediated by this region.

Alternatively, there is the possibility that dopamine levels are reduced following repeated PCP treatment, at least during the later exposures of the drug. This has been demonstrated following sub-chronic PCP administration, in the drug-free state (Jentsch et al. 1997a, b; Jentsch and Roth 1999; Noda et al. 2000). Reduced prefrontal DA transmission would have clinical face validity as prefrontal hypdopaminergia has been shown in schizophrenia (Abi-Dargham and Moore 2003). If there is indeed a reduction in frontal DA levels following repeated PCP treatment, cognitive improvement arising from SKF 38393 treatment would be derived from the compound acting as an agonist of the DA D_1 receptor compensating for the PCP-induced reduction in DA transmission. This could explain why an immediate SKF 38393-induced attenuation was not observed. However, the scenario of a blunted increase in DA efflux following repeated PCP treatment is more likely, as Jentsch et al. (1998) has firstly

demonstrated PFC DA levels are reduced in the drug-free state following sub-chronic PCP treatment, but there is a blunted increase in DA release when animals are re-challenged with an acute systemic dose of PCP.

As these data demonstrate, the 5C-CPT task is sensitive to disruption following PCP treatment; the aim of the next chapter was to assess the capability of a sub-chronic PCP treatment regimen to induce performance disruptions. Previous investigations within our laboratory utilise a sub-chronic pattern of exposure, assessing cognitive performance in the drug-free state. As a result, the potential of inducing persistent attentional impairments following this treatment regimen was to be explored in chapter 6.

Chapter 6

Sub-chronic PCP treatment and the 5-Choice Continuous Performance Test: performance impairment following an increase of the attentional load

6.1 Introduction

As demonstrated in chapter 5, performance within the 5C-CPT is sensitive to disruption following repeated PCP administration. Disrupted performance was the result of a cognitive-specific impairment, at least following repeated administration with impairment not being confounded by generalised behavioural disruption. Although these findings demonstrate that the 5C-CPT is sensitive to cognitive disruption following PCP treatment, impaired performance was observed whilst animals were acutely intoxicated, with cognitive testing conducted 30 minutes following PCP administration.

Previous investigations within our laboratory have demonstrated that a sub-chronic PCP treatment regimen induces persistent cognitive disruption, even in the drug-free state, in several behavioural paradigms that show relevance to cognitive disruption exhibited in schizophrenia. In conjunction, sub-chronic PCP treatment also induces neuropathological disturbances that are associated with the disorder (see Neill et al. 2010 for full review). Impairment has been demonstrated in tasks that assess recognition memory, cognitive flexibility, executive functioning and social behaviour. These tasks are suggested to reflect a battery of preclinical tasks that effectively probe areas of cognition (Young et al. 2009a) that are affected in schizophrenia (Neuchterlein et al. 2004). Novel object recognition (NOR) involves a task consisting of two distinct trials; in the first the animal is presented with two identical objects (acquisition phase) which they are allowed to explore and interact with. In the second trial (retention phase) one of the objects is replaced, allowing the animal to explore and interact with one familiar object and a novel one. The NOR task is a non-rewarded, ethologically valid task that exploits the rodents' natural exploratory behaviour originally described by Ennaceur and Delacour (1988). It has been demonstrated a number of times within our laboratory that control animals spend significantly more time exploring the novel object than the familiar one in the retention phase. Conversely, animals sub-chronically treated with PCP (2 mg/kg, 7-days bi-daily followed by at least 7 day washout)

spend an equal amount of time exploring novel and familiar objects in the retention phase (Grayson et al. 2007; McLean et al. 2009; Idris et al. 2010), suggesting that recognition memory is impaired. Levels of exploration in either trial were unaffected by PCP treatment, ruling out impaired motivation impairing task performance. Thus, from these observations it is suggested PCP treatment induced impairment in recognition memory in the retention phase of the NOR task. Similarly, sub-chronic PCP treatment impairs performance in an operant reversal learning (RL) task (Abdul-Monim et al. 2006, 2007; McLean et al. 2009; Idris et al. 2010). The RL task also involves two distinct trials; the initial phase requires animals to lever press depending on a previously learned reward contingency. In the reversal phase, the reward contingency is reversed; animals are required to inhibit the previously rewarded strategy and modify their behaviour to facilitate the acquisition of the new reward strategy. PCP induced no impairment in the initial phase suggesting no overt inability to perform the task. However, when the reward contingency was switched PCP treatment resulted in a reduced ability to inhibit the previously rewarded behaviour, inducing selective impairments in the reversal phase of the task. Successful performance of the RL task requires the prefrontal cortex to be fully functioning. Select lesion studies have implicated the orbital frontal cortex (OFC) involvement in the successful inhibition of previously rewarded behaviour, with impairment observed in the reversal phase, when a previously rewarded behaviour was to be inhibited (Bohn et al. 2003; McAlonan and Brown 2003; Boulougouris et al. 2007; Tait and Brown 2007). Schizophrenia patients have shown deficits in the Wisconsin Card Sorting Task (WCST) (Haut et al. 1996), in which patients show a reduced conceptualisation of the task and demonstrate a higher incidence of perseverative errors and impaired shifting of an attentional set. Furthermore, it was described that patients with frontal tumours had comparable WCST performance deficits (Haut et al. 2006). This observation suggests frontal cortical abnormalities in schizophrenia may mediate impaired performance within this task. A rodent analogue of the WCST was developed by Birrell and

Brown (2000) and is known as the attentional set—shifting task (ASST). An attentional set is formed when the animal discriminates and responds to a stimulus based on the features of that stimulus (e.g. digging medium). The attentional set enhances the efficiency of informational processing of the stimulus features. The ASST involves 7 discriminations; simple discrimination (SD), compound discrimination (CD), reversal 1 (R1), intra-dimensional shift (IDS), reversal 2 (R2), extra-dimensional shift (EDS) and reversal 3 (R3) (Birrell and Brown 2000). The ASST assesses the ability of the animal to form an attentional set within the same sorting category (intra-dimensional shift –IDS). Additionally, the task investigates the ability to shift an attentional set between different sorting categories (extra-dimensional shift – EDS). Birrell and Brown (2000) showed that medial prefrontal cortical lesion resulted in a specific impairment in the ability of the animal to shift attentional set *between* sorting categories (EDS impairment). These findings suggest that the frontal cortical region is involved in successful task performance, facilitating the ability of the animal to switch attentional sets between stimuli. Sub-chronic PCP treatment has been shown to impair ASST performance that was also specific to impaired EDS (Rodefer et al. 2005; McLean et al. 2009). Impairment in social interaction has also been observed following sub-chronic PCP treatment (Snigdha and Neill 2008). Whist social interaction is not strictly a form of cognition, social cognitive deficits involving the evaluation and appropriate responding to cues have been observed in schizophrenia (Penn et al. 2002) and described by the MATRICS initiative as one of the cognitive domains putatively affected in schizophrenia (Nuechterlein et al. 2004). The task involves the assessment of interactive behaviours of two unfamiliar rats placed in an open-field test arena. PCP treatment has been shown to significantly reduce interaction behaviours, whilst increasing the active avoidance of social contact (Snigdha and Neill 2008) up to at least 6-weeks following PCP treatment.

These observations demonstrate that PCP treatment (2 mg/kg) administered twice a day for a period of 7-days, followed by at least one week washout, induced persistent cognitive

disruption in a number of task of relevance to the cognitive deficits observed in schizophrenia. Due to the persistence of impairments (up to 12 months in some cases – Grayson, unpublished observations), it is clear that impaired performance is not due to the transient drug effects arising from PCP treatment. Moreover, in addition to the cognitive disruption, it is evident that sub-chronic PCP treatment induces a number of long-term neuropathological changes in the brain, many of which are reminiscent of those demonstrated in schizophrenia patients.

Notably, GABAergic dysfunction has been observed in schizophrenia patients, originally proposed by Roberts in 1972, confirmed following examination of post-mortem tissue demonstrating deficits in schizophrenia patients within interneuron populations that mediate GABAergic transmission (Blum and Mann 2002). Deficits in parvalbumin (PV) containing interneurons in the PFC (Beasley and Reynolds, 1997; Beasley et al. 2002) and the hippocampus (Zhang and Reynolds, 2002) have also been identified in schizophrenia patients. Coupled with the defects in GABAergic containing interneurons in schizophrenia patients, there is also a consistent reduction in the expression of the mRNA and protein for glutamic acid decarboxylase$_{67}$ (GAD_{67}), a synthesizing enzyme for GABA in the prefrontal cortex of schizophrenia patients (Guidotti et al. 2000; Volk et al. 2000; reviewed in Neill et al. 2010). Furthermore, it has been demonstrated that mRNA expression for GAD_{67} along with GAT1 (GABA transporter) is impaired in schizophrenia and are selective for PV-immunoreactive (ir) containing neurons within the PFC (Hashimoto et al. 2003). These findings clearly implicate a dysfunctional GABAergic system is involved in the pathogenesis of schizophrenia, and due to their location, deficits that may contribute to the development of cognitive dysfunction associated with the disorder (Lewis et al. 2005), potentially mediating the fractionated thought and cognitive impairment demonstrated in schizophrenia patients. A similar dysfunction has been observed following repeated exposure to NMDA antagonists (Abdul-Monim et al. 2007; Jenkins et al. 2008), with reduction in parvalbumin containing

neurons in the hippocampus and prefrontal cortex. Additionally, reductions in the expression of GAD_{67} mRNA have also been observed in rats treated with NMDA antagonists, suggesting a reduction in the GABA synthesising enzyme (Behrens et al. 2007; Bullock et al. 2009). These data suggest that repeated glutamatergic blockade induces GABAergic dysfunction in experimental animals that is similar to the pathological observations of GABAergic dysfunction observed in schizophrenia patients, supporting the face validity of this pharmacological model for investigating schizophrenia-like behaviours.

In addition to the findings centred on GABAergic dysfunction, sub-chronic PCP treatment has also been shown to induce other pathological changes observed in schizophrenia. Studies have identified that brain-derived neurotrophic factor (BDNF) is altered in schizophrenia (Thompson et al. 2011). Reduced BDNF levels have also been observed in female rat following sub-chronic PCP treatment (Snigdha et al. 2010), which were associated with impaired cognitive performance exemplified by impaired EDS in the ASST. Additionally, sub-chronic PCP has been shown to significantly reduce the number of dendritic spine synapses in the PFC (Hajszan et al. 2006). As this region is heavily implicated in multiple domains of cognition, reduced dendritic function may contribute to the cognitive impairment observed following sub-chronic PCP exposure. Dendritic spine density of prefrontal pyramidal neurons has been shown to be reduced in schizophrenia patients (Glantz and Lewis 2000), suggestive of dysfunctional prefrontal cortex function potentially mediated by loss of spine synapses.

Furthermore, sub-chronic PCP treatment induces neurochemical alterations mirroring the clinical deficits, which are not clearly evident following acute or repeated PCP administration. In the drug-free state following sub-chronic PCP treatment, significant reductions in basal levels of glutamate and dopamine have been observed within the prefrontal cortex (Jentsch et al. 1997a, b; Murai et al. 2007). Elevations of the expression of a glutamate transporter have been observed following sub-chronic PCP treatment (Fattorini et al. 2008), potentially

accounting for the reduction in cortical glutamate levels observed in the drug-free state. While dopamine *hyperactivity* is associated with schizophrenia (Howes and Kapur 2009), hyperactivity is largely restricted to sub-cortical regions (striatum) and implicated in psychotic symptoms. Cortical dopaminergic *hypofunction*, however, is hypothesised mediate certain cognitive deficits (Davis et al. 1991; Sawaguchi and Goldman-Rakic 1991; Arnsten et al. 1994; Knable and Weinberger 1997; Goldman-Rakic et al. 2004) and is observed in schizophrenia patients (Egan et al. 2001; Guo et al. 2003). These observations provide further justification and enhance the validity of a sub-chronic treatment regimen in contrast to acute treatment, which increases prefrontal dopamine levels as a reduction in basal cortical dopamine levels are observed in the drug-free state, following sub-chronic PCP administration (Verma and Moghaddam 1997; Jentsch and Roth 1999).

The findings described above provide a considerable level of evidence to suggest that chronic exposure to NMDA antagonists replicates not only cognitive dysfunction observed in schizophrenia, but also some of the neurochemical, neuropathological and morphological findings that are also evident. Furthermore, these findings display persistence, suggesting long-term neuropathological changes are induced, mimicking those observed clinically in schizophrenia patients.

In addition to the cognitive disruption described above, NMDA receptor antagonism has been shown to disrupt attentional processing, demonstrated when animals were acutely (Chapter 3) or repeatedly administered PCP (Amitai et al. 2007; Amitai and Markou, 2009; Chapter 5). Additionally, intra-medial prefrontal cortical (mPFC) infusion of the competitive NMDA antagonist, 3-(R)-2-carboxypiperazin-4-propyl-1-phosphonic acid (CPP) also disrupts 5-CSRTT performance (Murphy et al. 2005; Baviera et al. 2008). These data further highlight the involvement of the NMDA receptor in attentional function and furthermore, implicate mPFC involvement in attentional processing. However, to date, previous studies have only

shown disrupted attentional performance (assessed using the 5-CSRTT) while animals were 'acutely' intoxicated with NMDA antagonists (Le Pen et al. 2003; Amitai et al. 2007; Amitai and Markou, 2009; Auclair et al. 2009; Chapter 3) and not following a significant washout period. Due to the paucity of information on the effect of a sub-chronic PCP regimen on 5-CSRTT performance, there is clearly a need to understand the enduring effect of long-term NMDA antagonism on attention/vigilance. Thus, the aim of the presented within this chapter was to determine the effect of sub-chronic PCP treatment, following a substantial washout period, on performance in the 5C-CPT at baseline. However, due to the lack of PCP-induced impairment observed in chapter 3 within the 5-CSRTT, a higher dose was used in the current chapter in addition to the use of behavioural challenges designed to further tax attentional processing.

6.2 Methods

6.2.1 Subjects

Female hooded-Lister rats (n=35; Charles River; approx 250 ± 10 g at the start of the experiment) were housed in groups of five on a 12 hour reversed light cycle (lights on at 7:00pm) in a temperature (21 ± 2°C) and humidity (55 ± 5%) controlled environment. Further information regarding housing conditions, food deprivation and ethical guidelines can be found in chapter 2.

6.2.2 Drug Treatment Regimen

Rats were divided into three treatment groups, matched for baseline performance (the group-matching procedure and measures that are balanced is described in chapter 5). Following group-matching, rats were sub-chronically administered PCP (2.5 mg/kg, n = 11 or 5.0 mg/kg, n = 10 i.p.) or vehicle (0.9% saline, n = 11) twice daily (9am and 5pm) for 7-days, followed by a 7-day washout period, after which behavioural testing commenced. Animals were not trained or tested during drug treatment. Rats that failed to reach a stable baseline at the desired criteria were excluded from the study (n = 3).

6.2.3 Experimental Design

6.2.3.1 Stable Baseline Performance

Rats were tested following sub-chronic PCP administration (8-days post PCP) using standard training parameters that consisted of a 1 s SD, variable ITI which had a mean of 5 s (4.0, 4.5, 5.5, and 6.0 s), a 5 s TO period and a 2 s LH. The animals were presented with 84 target trials and 36 non-target trials in a session lasting no more than 30 minutes.

6.2.3.2 Variable Stimulus Duration

Rats performance was challenged (9-days post PCP regimen) within a single session that presented a variable SD (0.25, 0.5, 0.75, and 1 s). All other parameters (TO, LH, target/non-target ratio) were as before (section 6.2.6.1) with the exception of the variable ITI. Due to a programming limitation, which would have attributed one specific SD value to a specific ITI value, the vITI was switched to a fixed ITI (5 s).

6.2.3.3 Extended Session

Rats were exposed to an extended session (11-days post PCP regimen) to further challenge vigilance performance. The session consisted of 256 trials, of which 160 were target (40 x 4 ITIs) and 96 non-target trials (24 x 4 ITIs) and lasted no longer then 60 minutes. Test parameters were the same as standard training conditions i.e. 1 s SD, variable ITI (4.0, 4.5, 5.5, and 6.0 s), 5 s TO and 2 s LH.

All animals received a normal training session on day 10, between the vSD and extended session protocol manipulations to ensure performance was maintained at a stable baseline.

6.2.4 Data Analysis

All data are displayed as observed mean $\pm$ SEM. Overall performance was analysed by one-way between-subjects ANOVA followed by Planned Comparisons. Analysis of the performance across the duration of the session was carried out by two-way repeated measures ANOVA (Treatment as a fixed factor and Trial as a repeated measures factor – session was divided into trial bins, each composed of 40 trials for the 120 trial sessions or 64 trials for the extended session). Planned Comparisons (Snedecor and Cochran, 1989) compared PCP animals to control animals in each of the separate trial bins. Assessment of

the effect of a variable SD on task performance was conducted by two-way repeated (SD as within-subjects factor and Treatment as between-subjects factor) measures ANOVA, followed by Planned Comparisons. Assessment of vSD on performance of control animals' was conducted by one-way repeated measures ANOVA (SD as within-subjects factor).

6.3Results

6.3.1 Stable Baseline Performance

A one-way ANOVA analysis revealed that sub-chronic PCP treatment had no significant effect on baseline performance for any measure, suggesting that treatment induced no impairment in the animals ability to perform the 5C-CPT when standard testing parameters were used (table 6.1).

Table 6.1 Effect of Sub-Chronic PCP on overall performance in 5C-CPT with stable baseline

Measure	Vehicle	2.5 mg/kg PCP	5.0 mg/kg PCP
Accuracy (%)	94.52 ± 1.41	93.68 ± 1.13	93.56 ± 1.49
Percent Correct	78.63 ± 3.40	74.56 ± 3.07	73.78 ± 3.56
Percent Incorrect	4.33 ± 1.03	4.88 ± 0.86	4.90 ± 1.00
Percent Omission	17.03 ± 2.84	20.56 ± 2.71	21.32 ± 3.26
Correct Latency	0.67 ± 0.04	0.64 ± 0.03	0.68 ± 0.05
Incorrect Latency	0.82 ± 0.12	0.86 ± 0.14	0.91 ± 0.10
Magazine Latency	1.22 ± 0.08	1.17 ± 0.05	1.26 ± 0.06
Perseverative Response	1.82 ± 0.85	2.00 ± 0.66	4.60 ± 2.12
Premature Response	6.55 ± 1.86	10.09 ± 1.80	11.90 ± 2.49
Time Out Response	8.18 ± 1.62	11.00 ± 3.10	14.40 ± 4.14
p[HR]	0.79 ± 0.03	0.75 ± .0.03	0.74 ± 0.04
p[FA]	0.26 ± 0.04	0.31 ± 0.05	0.34 ± 0.06
Sensitivity Index	0.54 ± 0.06	0.45 ± 0.04	0.42 ± 0.06
Responsivity Index	0.06 ± 0.07	0.06 ± 0.08	0.06 ± 0.09

Measures are shown as observed mean ± SEM. PCP treatment resulted in no significant effect under these conditions. Veh (n=11), 2.5 mg/kg PCP (n=11) and 5.0 mg/kg PCP (n=10).

A two-way repeated measures analysis of the baselines also revealed the performance did not alter with session (data not shown). No significant Dose*Trial interaction were observed for any measure.

6.3.2 Variable Stimulus Duration (vSD)

6.3.2.1 Overall Performance

A vSD was then used to challenge performance (table 6.2). Statistical analysis revealed no significant difference in accuracy between treatments [$F_{(2,29)}$=2.01, NS], however, Planned Comparisons analysis demonstrated that there was a trend towards reduced accuracy in the 5 mg/kg PCP treatment group (p=0.06) when compared to the vehicle group. A significant effect of treatment on percent correct responding [$F_{(2,29)}$=5.85, p<0.01] was observed and PCP (5 mg/kg) significantly reduced percent correct responses (p<0.01). No effect was observed when percent incorrect responding [$F_{(2,29)}$=1.02, NS] was assessed, however. A strong trend toward a significant PCP effect was observed for percent omissions [$F_{(2,29)}$=3.16, p=0.057], with Planned Comparisons showing that PCP at 5 mg/kg significantly increased percent omissions (p<0.05).

There was no significant overall PCP treatment effect on latency to respond (CL, IL, or ML) or perseverative responses although significantly increased TO responses [$F_{(2,29)}$=4.12, p<0.05] were observed at 5 mg/kg of PCP, when compared with vehicle (p<0.01). There was no significant effect on the number of premature responses [$F_{(2, 29)}$=2.36, NS], although Planned Comparisons demonstrated a trend towards a significant effect at the highest dose (p=0.07).

PCP treatment significantly reduced p[HR] [$F_{(2,29)}$=5.85, p<0.01] with Planned Comparisons revealing that 5 mg/kg PCP significantly reduced p[HR] when compared with vehicle treatment (p<0.01). There was a trend towards an increase in the p[FA] [$F_{(2,29)}$=2.78, p=0.07] with the highest dose of PCP significantly increasing p[FA] (p<0.05). Sub-chronic PCP treatment significantly decreased the SI [$F_{(2,29)}$=6.83, p<0.01] with both 2.5 mg/kg and 5

mg/kg PCP significantly reducing signal discrimination ($p<0.05$ and $p<0.001$ respectively), while there was no significant effect on the RI [$F<1$, NS].

Table 6.2 Effect of Sub-Chronic PCP on overall performance in 5C-CPT using a variable SD

Measure	Vehicle	2.5 mg/kg PCP	5.0 mg/kg PCP
Accuracy (%)	88.15 ± 1.57	85.23 ± 2.79	81.43 ± 2.55
Percent Correct	67.42 ± 2.93	62.55 ± 2.72	53.79 ± 2.79 **
Percent Incorrect	9.00 ± 1.24	10.80 ± 2.06	12.66 ± 2.01
Percent Omission	23.58 ± 2.86	26.65 ± 2.04	33.79 ± 2.79 *
Correct Latency	0.68 ± 0.03	0.58 ± 0.04	0.63 ± 0.06
Incorrect Latency	0.93 ± 0.07	0.81 ± 0.06	0.99 ± 0.11
Magazine Latency	1.18 ± 0.06	1.14 ± 0.06	1.26 ± 0.08
Perseverative Response	1.00 ± 0.54	2.27 ± 1.23	3.50 ± 1.59
Premature Response	3.82 ± 0.98	7.27 ± 1.52	9.60 ± 2.86
Time Out Response	8.09 ± 1.33	12.00 ± 2.28	16.70 ± 2.58 *
p[HR]	0.67 ± 0.03	0.63 ± 0.03	0.54 ± 0.03 **
p[FA]	0.18 ± 0.04	0.27 ± 0.03	0.32 ± 0.06 *
Sensitivity Index	0.52 ± 0.05	0.36 ± 0.04*	0.24 ± 0.07 ***
Responsivity Index	-0.21 ± 0.07	-0.12 ± 0.05	-0.17 ± 0.07

Measures are shown as observed mean ± SEM. Asterisks indicate statistically significant differences (*p<0.05, **p<0.01) compared to the vehicle group.

6.3.2.2 Within-Session Performance

Within-session analysis of performance from the vSD challenge revealed some more specific effects of sub-chronic PCP compared to overall performance. Repeated measures ANOVA revealed a Dose*Trial interaction on accuracy [$F_{(4,58)}=3.45$, $p<0.05$] (fig 6.1A). Planned

Comparisons revealed that 2.5 mg/kg PCP reduced accuracy in trial bin 1 ($p<0.05$), while 5 mg/kg PCP reduced accuracy in trial bin 3 ($p<0.05$), when compared to vehicle treated animals. PCP treatment resulted in no Trial effect [$F_{(2,58)}=2.21$, NS] and no Trial*Treatment interaction [$F_{(4,58)}=0.28$, NS] on percent omission. However, Planned Comparisons demonstrated 5 mg/kg significantly increased omissions, compared to control animals, in the first trial bin ($p<0.05$) (fig 6.1B).

PCP treatment reduced p[HR] [Treatment: $F_{(2,29)}=5.71$, $p<0.01$, Treatment*Trial: $F_{4,58}=1.75$, NS]. Planned Comparisons demonstrated that 2.5 mg/kg and 5 mg/kg significantly reduced the p[HR] during the first trial bin ($p<0.05$) (fig 6.1C). The highest dose of PCP (5 mg/kg) produced a trend towards a significant reduction in p[HR] in the trials bin 2 ($p=0.058$) but resulted in a significant reduction in p[HR] in the trial bin 3 ($p<0.05$). PCP treatment resulted in a trend toward increased p[FA] [Dose: $F_{(2,29)}=2.84$, $p=0.07$, Dose*Trial: $F_{4,58}=1.26$, NS]. Planned Comparisons showed that PCP (both 2.5 & 5 mg/kg) significantly elevated p[FA] in the last trial bin ($p<0.05$ and $p<0.01$, respectively) (fig 6.1D). In light of the effect on p[HR] and p[FA], it was clear that PCP produced a significant Trial*Dose interaction for SI [$F_{(4,58)}=2.80$, $p<0.05$], with Planned Comparisons indicating that 5 mg/kg PCP significantly reduced SI in the first two trial bins ($p<0.05$), with a more substantial reduction observed in the last group of trials when compared with vehicle treatment ($p<0.001$) (fig 6.1E). PCP treatment had no significant effect on the RI [$F_{4,58)}=1.38$, NS] (fig 6.1F).

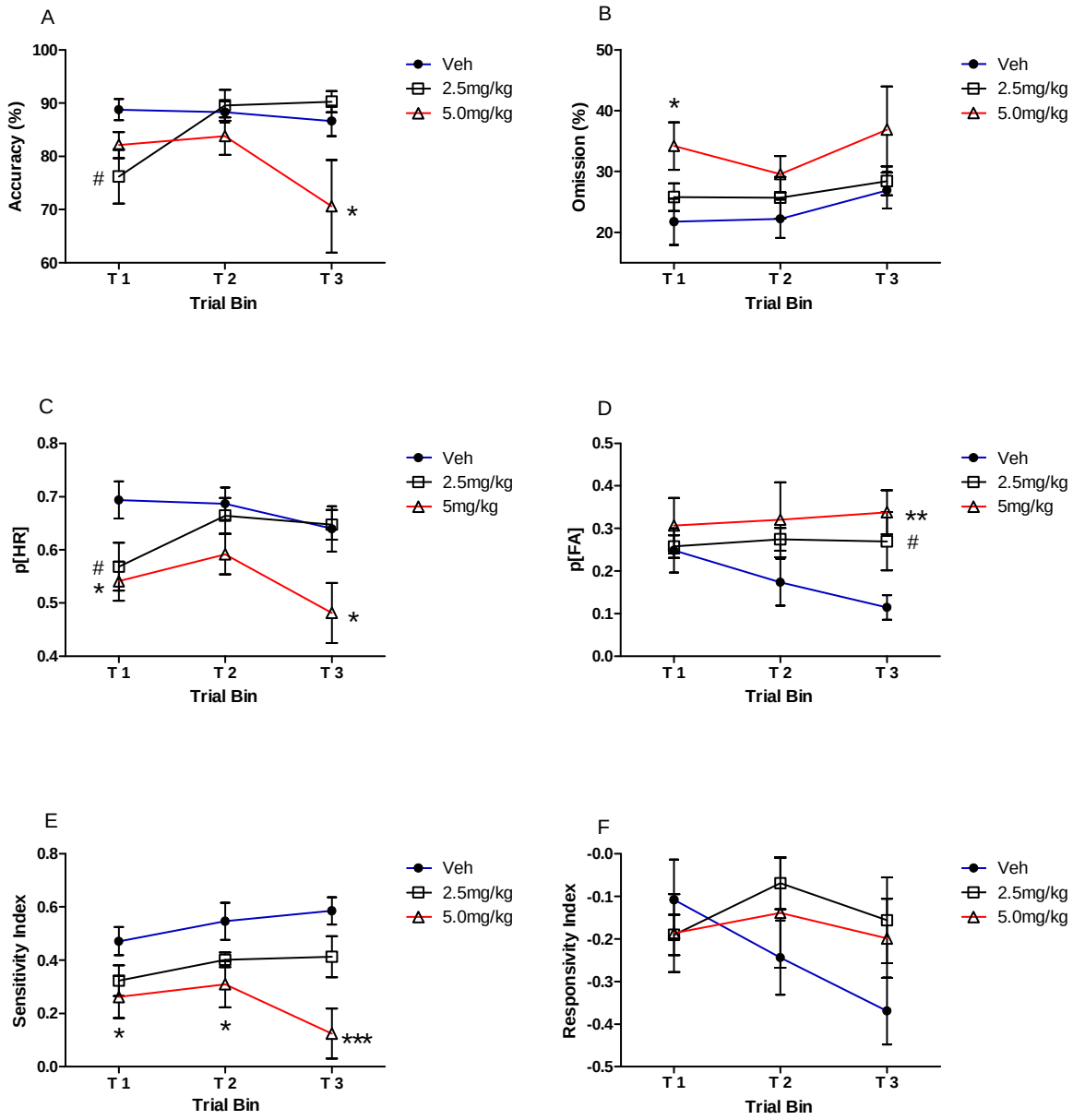

Fig 6.1 Performance displayed over the duration of the session following an acute behavioural challenge consisting of introducing a variable SD. T1 – T3 refers to the trial bin, each consisting of 40 trials. Data were expressed as observed mean ± SEM and analysed by two-way repeated measures ANOVA (Trial as within-subjects factor and Treatment as between-subjects factor) followed by Planned Comparisons on the predicted means. # $p<0.05$ significant difference in Trial bin between PCP (2.5 mg/kg, n=11) and vehicle animals (n=11). * $p<0.05$, ** $p<0.01$, and *** $p < 0.001$ significant difference in Trial bin between PCP (5.0 mg/kg, n=10) and vehicle animals.

While there was no significant difference between treatment groups in RI for each trial bin, a significant reduction of RI in vehicle treated rats was observed as the session progressed [$F_{(2,29)}$=4.08, p<0.05]. A significant reduction was observed when the RI of trials bin1 was compared to trial bin 3 (p<0.01). This progressive reduction in RI was not evident in PCP treated animals (Table 6.3).

Table 6.3 Within-session alteration of strategy in control animals following vSD challenge

Trial Bin	T1	T2	T3
Responsivity Index	-0.11 ± 0.09	-0.24 ± 0.09	-0.37 ± 0.08 **

Measures are shown as mean ± SEM. A more conservative response strategy was adopted in control animals as the session progressed, an effect that was absent in PCP treated animals. ** denotes p<0.01 when compared to trial bin 1

6.3.2.3 Effect of vSD on performance

There was a significant main effect of SD on response accuracy [$F_{(3,87)}$=56.39, p<0.001] and percent omissions [$F_{(3,87)}$=29.23, p<0.001] when a vSD was introduced, without a significant interaction between SD and Dose for accuracy [$F_{(6,87)}$=0.3, NS] or percent omissions [$F_{(6,87)}$=0.4, NS]. There was a main effect of SD on p[HR] [$F_{(3,87)}$=84.44, p<0.001], but no significant interaction between SD and Treatment [$F_{(6,87)}$=0.2, NS]. No significant effect of SD was observed on p[FA] [$F_{(3,87)}$=0.8, NS] although there was a slight trend towards a SD*Treatment interaction [$F_{(6,87)}$=1.86, p=0.09] (data not shown). There was a significant main effect of SD on SI [$F_{(3,87)}$=23.32, p<0.001], along with a significant SD*Treatment interaction [$F_{(6,87)}$=2.48, p<0.05], with Planned Comparisons demonstrating a significant PCP-induced reduction in SI following 1 s SD (5 mg/kg, p<0.05), 0.75 s SD (2.5 and 5 mg/kg, p<0.05) and 0.25 s SD (2.5 mg/kg, p<0.05; 5 mg/kg, p<0.001). No effect on SI was observed when the SD was 0.5 s. Additionally, when the effect of SD on SI was analysed specifically for

control animals, a main effect was observed [$F_{(3,30)}$=4.95, p<0.01], with Planned Comparisons showing that SI following 0.25 s SD was significantly reduced compared with SI following 1 s SD (p<0.05) (fig 6.2). There was significant main effect on the responsivity for SD [$F_{(3,87)}$=26.38, p<0.001], but no interaction of SD*Dose [$F_{(6,87)}$=0.9, ns] (data not shown).

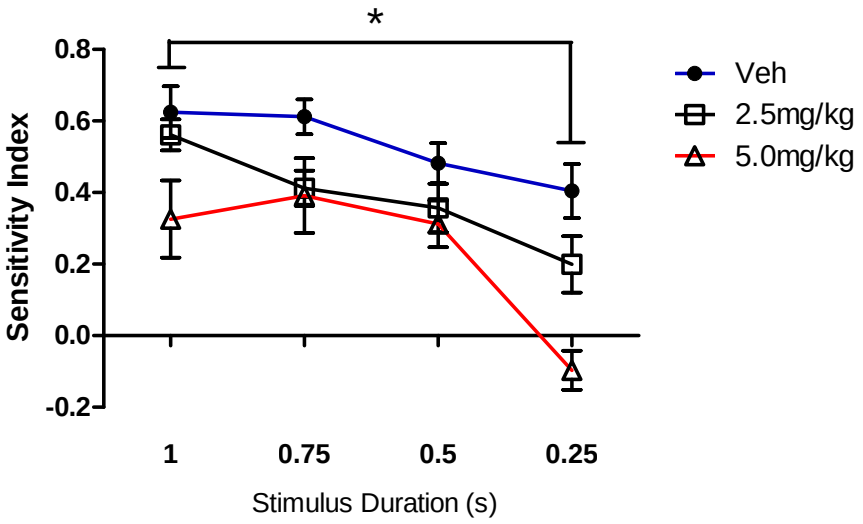

Fig 6.2 Sensitivity Index displayed for each individual stimulus duration presented throughout the session. Data are displayed as observed mean ± SEM and analysed by two-way repeated measures ANOVA (SD as within-subjects factor and Treatment as between-subjects factor) followed by Planned Comparisons on the predicted means. The effect of SD on SI was analysed specifically for control animals via one-way repeated measures ANOVA (SD as within-subjects factor) followed by Planned Comparisons. * p<0.05 reduction in SI for 0.25 s SD compared to 1 s SD in control animals.

6.3.3 Normal Baseline Performance

Following the vSD challenge, but prior to the extended session challenge (day 10), performance was assessed when protocols were returned to that of baseline. Animal performance returned to baseline with no significant differences existing between treatment groups in any of the behavioural measures assessed (data not shown).

6.3.4 Extended Session

6.3.4.1 Overall Performance

The extended session challenge (table 6.4), revealed varying effects of PCP treatment. There was no overall effect on accuracy [$F_{(2,29)}$=1.38, NS], percent correct [$F_{(2,29)}$=1.41, NS], percent incorrect [$F_{(2,29)}$=071, NS] or percent omissions [$F_{(2,29)}$=1.05, NS]. A trend toward significance was observed for CL [$F_{(2,29)}$=3.17, p=0.056], with Planned Comparisons indicating that 2.5 mg/kg PCP reduced CL ($p<0.05$). No significant effect was observed for ML [$F_{(2,29)}$=1.93, NS] or IL [$F_{(2,29)}$=1.2, NS]

There was a trend towards a significant increase in perseverative responding [$F_{(2,29)}$=2.68, p=0.08], with Planned Comparisons revealing that 5 mg/kg PCP significantly increased perseveration ($p<0.05$). Additionally, there was also a significant increase in TO responses [$F_{(2,29)}$=3.74, $p<0.05$) and Planned Comparisons indicated that 5 mg/kg PCP significantly increased TO responding ($p<0.05$). No effect on premature responses were observed [$F_{(229)}$=2.40, NS].

Sub-chronic PCP produced no significant effect on p[HR] [$F_{(2,29)}$=1.41, NS], but increased p[FA] [$F_{(2,29)}$=3.57, $p<0.05$], by both doses of PCP following Planned Comparisons analysis ($p<0.05$). There was an overall reduction in the SI [$F_{(2,29)}$=6.84, $p<0.01$] with Planned Comparisons indicating that both 2.5 and 5 mg/kg decreased discriminability ($p<0.05$ and $p<0.01$, respectively). No effect of PCP was observed for RI [$F_{(2,29)}$=1.00, NS].

Table 6.4 Effect of Sub-Chronic PCP on overall performance in 5C-CPT following an extended session

Measure	Vehicle	2.5 mg/kg PCP	5.0 mg/kg PCP
Accuracy (%)	93.85 ± 1.13	92.03 ± 1.45	90.38 ± 1.82
Percent Correct	70.17 ± 3.81	68.69 ± 3.14	61.50 ± 4.57
Percent Incorrect	4.55 ± 0.89	5.80 ± 1.02	6.33 ± 1.34
Percent Omission	25.28 ± 3.87	25.51 ± 2.86	32.18 ± 4.49
Correct Latency	0.71 ± 0.03	0.59 ± 0.03 *	0.63 ± 0.04
Incorrect Latency	1.13 ± 0.08	1.12 ± 0.12	1.27 ± 0.12
Magazine Latency	1.17 ± 0.05	1.13 ± 0.05	1.28 ± 0.07
Perseverative Response	2.00 ± 0.56	5.45 ± 1.71	7.10 ± 2.16 *
Premature Response	6.73 ± 1.44	13.55 ± 2.80	16.90 ± 5.10
Time Out Response	12.45 ± 1.29	20.55 ± 3.49	32.30 ± 8.51 *
p[HR]	0.70 ± 0.04	0.69 ± 0.03	0.61 ± 0.05
p[FA]	0.16 ± 0.04	0.28 ± 0.03 *	0.28 ± 0.04 *
Sensitivity Index	0.57 ± 0.04	0.42 ± 0.03 *	0.36 ± 0.06 **
Responsivity Index	-0.19 ± 0.10	-0.04 ± 0.06	-0.12 ± 0.07

Measures are shown as observed mean ± SEM. Asterisks indicate statistically significant differences (*p<0.05, **p<0.01) compared to the vehicle group.

6.3.4.2 Within-Session Performance

In a within-session analysis of the extended session challenge on performance repeated measures ANOVA revealed no Treatment*Trial interaction for accuracy [$F_{(6, 87)}$=1.1, NS]. A Trial effect was observed [$F_{(3,87)}$=3.09, p<0.05]. A Trial effect was observed for percent omissions [$F_{(3,87)}$=13.03, p<0.001], attributed to an increase in omissions in trial bin 4 (p<0.001). This occurred indendent of a Treatment*Trial interaction [$F_{(6,87)}$=1.13, NS] (data

not shown). A Trial effect was observed for p[HR] [$F_{(3,87)}$=14.51, p<0.001], but this occurred without a Treatment*Trial interaction [$F_{(6,87)}$=1.51, NS] (fig 6.3A). A Treatment*Trial interaction was observed for p[FA] [$F_{(6,87)}$=2.90, p<0.05], however (fig 6.3B). The significant increase in p[FA] was associated with 2.5 and 5 mg/kg PCP during the first trial bin (p<0.01 and p<0.05, respectively) and 5 mg/kg PCP in the second trial bin (p<0.01). PCP did not increase p[FA] in remaining trial bins. Although there was no Treatment*Trial interaction [$F_{(6,87)}$=1.47, NS], there was an overall Trial effect on SI [$F_{(3,87)}$=8.73, p<0.001] (fig 6.3C). ANOVA initially demonstrated no Drug effect in RI [$F_{(2,29)}$=2.40, NS]. There was a Trial [$F_{3,87)}$=10.41, p<0.001] and a Trial*Treatment interaction on RI [$F_{(6,87)}$ =2.57, p<0.05] and Planned Comparisons revealed 2.5 mg/kg and 5 mg/kg PCP increased RI in trial bin 1 only (p<0.01 and p<0.05, respectively) (fig 6.3D).

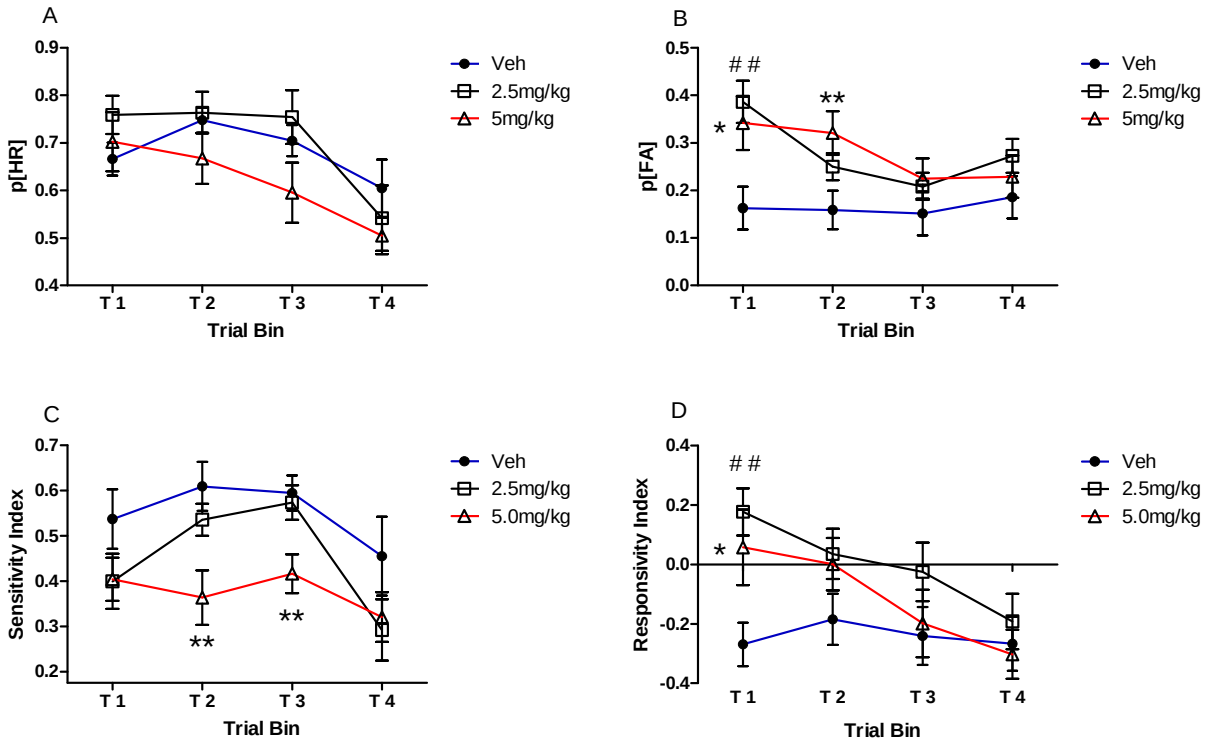

Fig 6.3 Performance displayed over the duration of the session that was extended to 256 trials. T1 – T4 refers to the trial bins each consisting of 64 trials. Effect of extended session on hit rate (A), false alarm rate (B), sensitivity index (SI) and responsivity index (D) are displayed. # p<0.05 and ## p<0.01 significant difference in Trial bin

between PCP (2.5 mg/kg, n=11) and vehicle animals (n=11). * denotes p<0.05 and ** denotes p<0.01 significant difference in Trial bin between PCP (5 mg/kg, n=10) and vehicle animals.

6.3.4.3 Vigilance Decrement

Analysis of the control animals' performance demonstrated a trend towards a main effect when SI was analysed by repeated measures (trial as within-subjects factor) ANOVA [$F_{(3,30)}$=2.58, p = 0.07], with Planned Comparisons indicating that Trial bin 4 was significantly reduced (p<0.05) when compared with Trial bin 2 (fig 6.4).

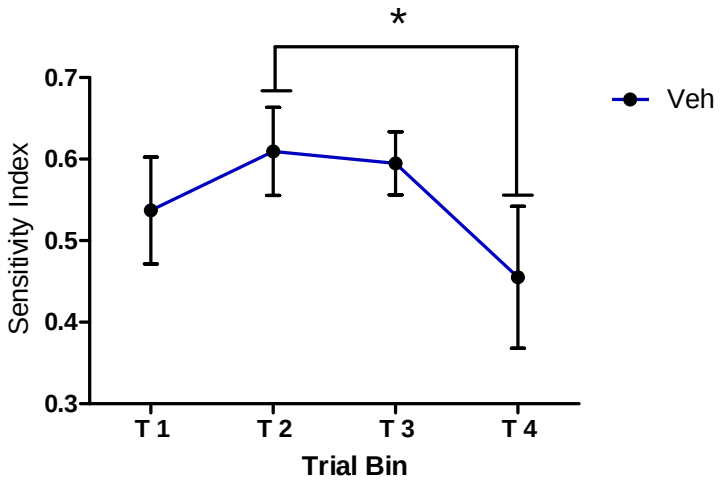

Fig 6.4 Sensitivity Index (SI) of control animals (n=11) following the extended session challenge. T1 – T4 refers to the trial bins each consisting of 64 trials. Data were expressed as observed mean ± SEM and analysed by one-way repeated measures (Trial as within-subjects factor) ANOVA followed by Planned Comparisons. * p<0.05 Trial bin 4 (trials 193 – 256) significantly reduced compared to Trial bin 2 (trials 65 – 128).

6.4 Discussion

The present study supports the hypothesis that sub-chronic PCP treatment impairs performance in rats, in a manner consistent with impaired vigilance seen in patients with schizophrenia, up to 11 days after cessation of treatment. These data suggest that PCP treatment induces persistent cognitive disruption, resulting in impaired 5C-CPT performance. However, these data also indicate that elucidation of these deficits requires animals' performance to be challenged beyond that of baseline. Hence, in some cases, sub-chronic PCP-induced deficits only became evident when the performance was displayed in discrete time bins throughout the session. To our knowledge, this is the first time that sub-chronic PCP-induced impairments in the drug-free state in the pre-clinical test of vigilance, the 5C-CPT have been demonstrated in rats.

Successful performance of the 5C-CPT requires animals to be thoroughly trained with a highly pre-potent response necessary for task completion. While acute or repeated PCP treatment resulted 5-CSRTT deficits at baseline (Amitai et al. 2007; Amitai and Markou, 2009; Chapter 5), the lack of impairment on baseline performance in the present study suggests that a sub-chronic PCP dosing regimen does not lead to task disruption, when performance was assessed at a baseline level of attentional demands. Although this is in contrast to previous investigations, a major methodological difference exists as previous studies were conducted when animals were under the influence of PCP treatment (Le Pen et al. 2003; Amitai et al. 2007; Amitai and Markou, 2009; Auclair et al. 2009) and not in the drug-free state. The complete absence of performance impairment during baseline testing is similar to the lack of effect observed in chapter 3, using the 5-CSRTT even though a higher dose of PCP was used in the current chapter. Sub-chronic PCP treatment results in a number of persistent neuropathological abnormalities (Jentsch et al. 1997a; Jentsch et al. 1997b; Cochran et al. 2003; Hajszan et al. 2006; Abdul-Monim et al. 2007; Jenkins et al. 2008; Snigdha et al. 2010). However, it could be that none of these neurochemical, morphological or metabolic

disturbances contribute to attentional disruption under normal task demands. Normal baseline performance in the 5-CSRTT following dorsal noradrenergic bundle lesions (Carli et al. 1983; Cole and Robbins, 1992) or dopamine depletion of the dorsal striatum (Baunez and Robbins, 1999), have been observed previously with deficits only apparent during increasing task demands, which included reducing stimulus intensity, stimulus duration, event-rate, or interpolating bursts of distracting white noise (see Robbins, 2002). These findings, along with the trend towards impairment in chapter 3 following parameter manipulations, prompted the use of behavioural challenges within the 5C-CPT in order to determine if performance deficits induced by sub-chronic PCP treatment could be elucidated.

By manipulating the test parameters to further tax attentional demands, it was demonstrated that PCP treated animals had a reduced ability to perform the 5C-CPT, compared to control animals. The variable SD (vSD) task challenge was chosen specifically because it does not fundamentally alter the protocol the animal is responding to. Alterations in the fundamental task parameters (i.e. extending the ITI) can lead to adaptation or modification of performance strategy. Thus changes in performance following a vSD challenge are less likely to reflect within-session learning, as has been observed elsewhere (Hahn et al. 2002; see Young et al. 2009a). When a vSD was introduced, so increasing the attentional demands of the task (McGaughy and Sarter, 1995; Jones et al. 1995; Mirza and Stolerman, 1998; Parasuraman, 1998), animals treated with PCP exhibited a reduced performance consistent with attentional dysfunction. Although there was no significant effect on response accuracy, a strong trend towards a PCP-induced reduction was observed. When the effect of PCP-treatment and accuracy was assessed in discrete trial bins, across the duration of the session, significant impairments were observed. Animals treated with the lower dose of PCP had an initial reduction in accuracy, but this effect disappeared as the session progressed. Conversely, the initial response accuracy of animals treated with 5 mg/kg PCP was no different to control animals. However, as the session progressed the

response accuracy reduced, being significantly lower in the last group of trials. As response accuracy is a well described measure of sustained attention (Muir 1996; Robbins 2002), these findings suggest that PCP-treatment is inducing deficits in attentional processing. In contrast to the effects observed following repeated PCP treatment (chapter 5), the number of incorrect responses made following sub-chronic PCP remained unaffected. The lack of an effect on incorrect responding indicated that a 'guessing strategy' was not utilised following failed target detection, demonstrating the differential attentional response profile following repeated or sub-chronic PCP treatment. Furthermore, this suggested that the reduction in accuracy was mediated solely by impaired target detection, exemplified by the reduction in correct responses. In light of this effect, an overall reduction in percent correct responding was observed. This was coupled with an increase in trials omitted, consistent with previous reports using the standard 5-CSRTT (Amitai and Markou, 2010). Like the reduction in response accuracy, it has been suggested that these findings represent impaired attentional functioning, as long as a concomitant increase in response latencies is not observed. An increase in response latencies would potentially suggest locomotor impairment or reduced motivation resulting in a reduction in responding (hence decreased % correct and increased % omissions). As there were no PCP-induced effects on the response latencies, particularly the magazine latency, these confounding effects can be ruled out, supporting the notion of a PCP-induced attentional deficit following the vSD challenge.

There was no significant increase in perseverative responding in PCP-treated animals, but there was an increase in time out (TO). TO responding is an under-reported and under-studied measure in 5-CSRTT/5C-CPT testing but, along with preservative responding, TO responding is suggested to represent compulsive-like behaviour or cognitive inflexibility (Dalley et al. 2002; Amitai and Markou 2010). Thus, an increase in TO responding may suggest that PCP-treated animals showed impaired cognitive flexibility, inappropriately responding during a TO period resulting from deficits in response inhibition. This suggestion

is supported by previous findings in our laboratory in which PCP-treatment induced deficits in reversal learning impairing performance during the reversal phase, when inhibition of a previously rewarded response was required (Abdul-Monim et al. 2006, 2007; McLean et al. 2009; Idris et al. 2010). However, deficits in response inhibition appear to be selective for inappropriate responding during the TO period; PCP-treatment did not increase premature responding. While there was a trend towards an increase in premature responding with the higher PCP dose, the increase lacked statistical significance. The lack of a significant increase in ITI responses indicates that sub-chronic PCP treatment does not result in deficits in impulse control, in contrast to acute or repeated PCP treatment which dramatically increases premature responding (Chapter 3; Chapter 5). This suggests that while NMDA antagonism induces pronounced deficits in behavioural inhibition while the drug is in the system, long-term abnormalities resulting from sub-chronic PCP treatment are not associated with motoric impulsivity, as assessed by premature responding in the 5C-CPT. This is supported by the absence of alterations in premature responding in chapter 3, when the effect of sub-chronic PCP treatment was assessed in the 5-CSRTT.

The addition of non-target stimuli in the 5C-CPT provides a method to further assess this deficit, however, by a method not available in 5-CSRTT testing. In comparison with control animals, PCP treatment reduced the rats' ability to withhold a response when presented with non-target stimuli, therefore suggesting impairment in action restraint (Eagle et al. 2008). There was an overall reduction in correct rejections (thus, increased false alarms). However, assessment of performance across the session indicates inappropriate responding to non-target trials is only elevated in the latter stages of the session. It appears as if control animals are improving their performance when presented with non-target trials (reducing p[FA]) as the session progresses, an action that is inhibited by PCP-treatment. Therefore PCP-treatment is perhaps not *increasing* inappropriate responding but impairing the *reduction* of inappropriate responding, an effect which occurs in control animals.

The differential findings regarding premature responding and false alarms perhaps suggests a fractionation of the PCP-induced deficit in impulse control; motoric impulsivity assessed by premature responding is unaffected, while behavioural disinhibition assessed by false alarms is compromised. These findings correlate with clinical observations. Schizophrenia patients show dysfunction when presented with stimuli requiring response inhibition, increasing inappropriate responding compared to control subjects (Kiehl et al. 2000; Weibrod et al. 2000; Wykes et al. 2000). However, it would be pertinent to investigate the precise nature of this deficit. Does schizophrenia increase behavioural *disinhibition,* increasing inappropriate responding or does schizophrenia impair the development of behavioural *inhibition*, preventing the reduction of inappropriate responding? So far, clinical reports only demonstrate the overall effect and not the performance throughout the course of a session. Therefore, a full and direct comparison between PCP treated animals and schizophrenia patients cannot be accurately substantiated.

The PCP-induced reduction in correct detection of target stimuli, coupled with the increased frequency of incorrect responding to non-target stimuli ultimately results in a reduction in signal sensitivity, quantified by the PCP-induced reduction in the sensitivity index (SI). The SI has been implemented as a non-parametric method of displaying d' commonly used in human CPTs to assess performance, reflecting a measure of vigilance. D' is consistently reduced in schizophrenia patients, indicating stimuli discrimination deficits are inherent to the disorder (Nestor et al. 1990; Parasuraman, 1998; Mass et al. 2000; Baerwald et al. 2005; Wang et al. 2007; Park et al. 2010). The PCP-induced reduction of SI, following the vSD challenge, is consistent with the impaired performance of patients with schizophrenia in human CPTs. Furthermore, while PCP induced an overall significant reduction in SI, assessment of performance across the session demonstrates that the PCP-induced reduction was more substantial in the latter phase of the session compared to control animals. The reduction in SI was initially driven by the reduction in hit rate (T1 and T2); however in the last

trial bin, the SI reduction was mediated both by the reduction in hit rate and the increased false alarm rate. These data suggest that PCP treatment induced an element of cognitive fatigue, resulting in increasing deficits in signal discrimination as the session progressed, an effect that was absent in control animals. Additionally, it has been observed that healthy volunteers infused with ketamine demonstrated impairment in CPT performance, exemplified by a decreased hit rate, increased false alarm rate and impaired signal sensitivity (Krystal et al. 1994; Umbricht et al. 2000; Heekeren et al. 2008). These findings are similar not only to those observed in schizophrenia patients, but also to the PCP-induced impairment demonstrated within the current chapter. However, these studies involved acute ketamine exposure, and did not investigate the enduring effects of chronic ketamine use on CPT performance. This type of investigation is inherently difficult however, given the ethical considerations concerning the repeated administration of ketamine to healthy volunteers.

The presence of target and non-target stimuli in the 5C-CPT also provides the ability to assess response strategy (bias). Changes in SI (as described above) are less likely to reflect deficits of the construct being measured (vigilance) if the change was due to alterations in bias (Marston, 1996). The reduction in SI in the present study was not accompanied by alterations in the responsivity index measure of bias (RI) between treatment groups, however, suggesting that impairment in signal sensitivity were mediate by cognitive mechanisms (Marston, 1996; Steckler, 2001). Thus, it is likely that PCP treatment truly resulted in impaired vigilance, manifested as a reduced ability of animals to discriminate between signal and noise, consistent with reduced d' exhibited by schizophrenia patients in human CPTs (Nestor et al. 1990; Parasuraman, 1998; Mass et al. 2000; Baerwald et al. 2005; Wang et al. 2007; Park et al. 2010).

There were no significant differences between the RI and treatment groups, when assessed over the whole session or within individual trial bins. However, it was apparent that the RI of

control animals significantly reduced as the session progressed, suggesting that animals were modifying their strategy in light of the increased cognitive demands of the challenge. This modification of strategy was absent in PCP treated animals. These data, therefore, suggest that PCP treatment may have reduced the ability of animals to adapt to the increased attentional load of the task, which may account for the reduced performance when challenged with a vSD. As discussed previously, the p[FA] of animals treated with saline progressively reduced as the session progressed, possibly as a result of adaptation to the challenge of varying SDs or consistent, and therefore more predictable ITIs, which was reflective of their more conservative bias over time. Varying the SD in mice while maintaining a variable ITI does not result in improved performance over the session (J. Young Personal Communication) suggesting that, in the present studies, control animals may be honing a temporally-mediating strategy (Spratt et al. 2000) within the session, consistent with previous reports when using extended ITI challenges (Hahn et al. 2002). Noradrenergic depletion also impairs rats' ability to adapt to variable ITI challenges (when trained with a constant ITI) in the 5-CSRTT (Cole and Robbins, 1992), while noradrenaline levels as measured by microdialysis only increase during such challenges (Dalley et al. 2001). Thus PCP-induced deficits in the vSD challenge are possibly the result of the inhibition of the adaptation of the better strategy, evidenced by the absences of alterations in RI. These effects may reflect alterations in noradrenergic levels following PCP treatment. Dopaminergic transmission has also been implicated in the formation and execution of strategy (Robbins and Roberts 2007). Moreover, it is well established cortical dopaminergic levels are sensitive to sub-chronic PCP treatment, showing persistent reductions following treatment (Jentsch et al. 1997a, b; Elsworth et al. 2008). This, along with potential NAergic dysfunction, may impair the development of a more conservative response strategy being implemented during the vSD challenge, accounting for disrupted performance.

Vigilant performance was further challenged by extending the session duration. The most robust finding was a PCP-induced reduction in SI primarily mediated by an increased p[FA] indicative of response disinhibition. During the start of the session (trials 0 – 64) this was coupled with increased RI, indicating a more liberal strategy than vehicle treated animals and the PCP-treated animals may simply have been increasing their responding to all signals. The initial impairment in performance therefore may have been due to a shift in the animals' decision criteria as opposed to alterations in cognitive processing *per se*. However, the increase in RI disappeared as the session progressed while p[FA] remained high. Moreover the p[HR] was unaffected, suggesting that the animals were not simply hyper-responsive to all stimuli. Furthermore, the PCP-induced SI decrement was only evident in the middle part of the session (trials 65 – 192), independent of changes in RI, indicative of reduced performance resulting from cognitive dysfunction (Dudchenko, 1992; Marston, 1996). While the PCP-induced impairment in SI was absent in the latter part of the session (trials 193 – 256), this was primarily due to a reduction in SI in the vehicle treated animals. This is likely due to cognitive fatigue in control animals. There was a significant reduction in SI (from T2 to T4) in control animals, suggesting a reduction in signal sensitivity even in the control group. This is suggestive of a vigilance decrement, consistent with that observed in human CPTs (Parasuraman, 1998; Riccio et al. 2002) and also in mice in the extended session 5C-CPT challenge (Young et al. 2009b). Studies assessing vigilance in humans traditionally utilise relatively long-term performance sessions, typically extending from 30 minutes to several hours (Parasuraman et al. 1998). The seminal studies conducted by Mackworth (1950) involved subjects observing a clock face for periods of up to 2-hours. Subjects had to report the occurrence of rare and unpredictable double-jumps of the clock hand (signal) from the frequent and predictable single jumps (noise). Mackworth (1950) reported that correct signal detection fell dramatically as the session progressed, comparing performance in the first 30 minutes to the next 30 minutes. The decline in performance is a phenomenon

referred to as vigilance decrement (Parasuraman et al. 1998; Grottick and Higgins 2002; Young et al. 2009b). There is the danger that impaired performance towards the latter part of the session may have been induced by excessive satiety. The increased volume of reward pellets dispensed during the extended session may have resulted in reduced motivation to perform the task. However, the lack of alterations in reward latency throughout the session suggested that motivation remained consistent (Robbins, 2002). Therefore, satiation is unlikely, further supporting the hypothesis that a vigilance decrement was observed. The vigilance decrement observed in control animals' following the extended session challenge is in contrast to improved performance over time in the vSD challenge, supporting the use of an extended session challenge to further probe vigilance. Although the short session used in the vSD challenge may not have been sufficient to induce a vigilance decrement in control animals. However the reduction in SI in PCP-treated animals may suggest that drug treatment reduced the threshold for decrements in vigilance to become evident. Vigilance decrements have been observed in aged rats performing an extended version of the 5-CSRTT (Grottick and Higgins 2002), characterised by a reduction in response accuracy as the session progressed. Additionally, a vigilance decrement has been observed in mice, utilising an extended session in the 5C-CPT (Young et al. 2009b). Impaired performance in this instance was demonstrated by a progressive reduction in SI throughout the session, independent of alterations in RI. These findings reveal that experimental animals are sensitive to deterioration of performance consistent with the vigilance decrement observed in human attentional studies, enhancing the validity of such testing.

The extended session challenge also revealed that sub-chronic PCP-induced a reduction in correct latency (CL), without affecting reward or incorrect latencies. These findings suggest that sub-chronic PCP results in a specific enhancement of the speed of information processing resulting in a quicker correct response to target stimuli. This is in contrast to PCP-induced slowing of responding observed following acute administration or repeated PCP

treatment (chapter 3; chapter 5), although the effects following acute treatment were likely to be non-specific motoric effects as other latency measures were also slowed (Le Pen et al. 2003; Amitai et al. 2007). One study has demonstrated that acute MK-801 reduced CL in the 5-CSRTT (Grottick and Higgins, 2000). The MK-801-induced reduction in CL appeared to be dose-specific and only evident following low-dose administration and not following a higher dose. These effects resemble those observed in the current chapter as the sub-chronic PCP-induced reduction in CL was only evident in the group treated with the lower PCP dose. In addition to the findings in the current chapter, the MK-801-induced reduction in CL occurred without alterations in the reward latency, indicating a specific cognitive effect as it was not the result of a generalised response-hyperactivity. Thus, NMDA antagonism (either acute or sub-chronic) may reduce CL in a dose specific manner.

In summary, these data demonstrate that a sub-chronic PCP treatment regimen can induce persistent deficits that result in attentional impairment in the 5C-CPT. However, disrupted performance was only detected following increased attentional demands. These data therefore suggest that the cognitive domain of attention/vigilance, although susceptible, may be less sensitive to the disruptive effects of sub-chronic PCP in rats compared with acute or repeated PCP treatment. This conclusion is drawn from the observation that other cognitive domains (such as episodic memory, cognitive flexibility, social cognition and executive functioning), are disrupted following sub-chronic PCP treatment, without the need for further task challenge (see Neill et al. 2010 for a full review). It may be that PCP-induced deficits in these tasks are mediated via a different mechanism to those observed in the current study, although further experiments will be required to test this hypothesis. As there are, however, impairments in performance that were evident some 11 days following the cessation of drug treatment, this indicates that persistent changes are associated with this PCP-treatment regimen. As described above, GABAergic dysfunction is observed following sub-chronic PCP treatment, along with morphological abnormalities. Coupled with cognitive

impairment, neurochemical and morphological abnormalities appear to be enduring, persisting at least for several weeks. The enduring morphological effects and their relation to attentional disruption following a sub-chronic PCP treatment regimen (using a dose of 5 mg/kg) were to be investigated in the next chapter, utilising structural magnetic resonance imaging (MRI) in combination with the 5-CSRTT and PPI.

Chapter 7

Structural brain abnormalities and attentional disruption following sub-chronic PCP treatment

7.1 Introduction

Coupled with the profound psychotic, behavioural and cognitive disturbances associated with schizophrenia, there appears to be a number of structural and functional brain abnormalities associated with the disorder. Extensive work in schizophrenia patients has demonstrated a reduction in grey matter, white matter and overall brain volume, in addition to an enlargement in the volume of the ventricles (Meyer-Lindernberg, 2010). Studies have reported a widespread reduction in cortical thickness associated with the disease (Kuperberg et al. 2003; Goldman et al. 2009; Rimol et al. 2010). Furthermore, Rimol et al. (2010) also demonstrated substantial sub-cortical volume reductions and, consistent with previous findings (Shenton et al. 2001), also demonstrated that ventricular enlargement was present in patients with schizophrenia. In fact, enlargement of the ventricular system has been one of the most consistent and reproducible findings that has emerged from neuroimaging studies in schizophrenia. Moreover, magnetic resonance imaging (MRI) studies have demonstrated there is typically a 2% reduction in total brain tissue volume in schizophrenia patients (Wright et al. 2000), highlighting the gross structural abnormalities associated with the disease pathology. A recent multimodal study, utilising functional and structural imaging, has indentified the medial prefrontal cortex (mPFC) as a region of the brain with prominent abnormalities in schizophrenia, coupled with dysfunction in the dorsal lateral prefrontal cortex (DLPFC) (Pomarol-Clotet et al. 2010), characterised by volumetric reductions, white matter abnormalities and hyperactivation or a failure of deactivation. Yoon et al. (2008) conducted a study investigating the functional relationship between brain activation and performance in the CPT-AX, one of the many variants of the human attentional task, the continuous performance test (CPT). It was determined that impaired performance in this task by schizophrenia patients was related to a reduced level of activation in the dorsal lateral prefrontal cortex, thus highlighting the correlation between cognitive functioning and specific neuronal regions, and implicating the frontal cortex in attentional processing.

Antagonism of the glutamatergic NMDA receptor has been suggested to be a valid means of pharmacologically modelling aspects of schizophrenia neuropathology (Olney and Farber, 1995; Coyle, 2006). Chronic blockade of the glutamatergic NMDA receptor produces a number of cognitive (Neill et al. 2010; Chapter 6), metabolic (Cochran et al. 2003), morphological (Hajszan et al. 2006), and pathophysiological and neurochemical (Abdul-Monim et al. 2006; Jenkins et al. 2008; Snigdha et al. 2010) abnormalities that are also observed in schizophrenia patients. Therefore, the use of NMDA antagonists in neuroimaging studies provides a means of assessing the functional, structural and cognitive consequences of glutamatergic disruption in humans, and may provide a means of determining the validity of this pharmacological method of investigating schizophrenia.

Investigation of white matter using diffusion tensor imaging (DTI) in chronic ketamine users identified bilateral frontal and left temporoparietal abnormalities consisting of reduced fractional anisotropy, changes that were correlated to the level of consumption of ketamine (Liao et al. 2010). In addition, the decreased fractional anisotropy located in the medial frontal cortex seen in ketamine users showed striking consistency with that demonstrated in schizophrenia patients (Pomarol-Clotet et al. 2010). Examination of chronic ketamine users also revealed structural abnormalities, as assessed by voxel based morphometry (VBM), characterised by reductions in grey matter density (Liao et al. 2011). These findings demonstrated that chronic blockade of NMDA receptors produced a reduction in frontal grey matter density, specifically located in the left superior frontal gyrus and right middle frontal gyrus. Grey matter abnormalities within the mPFC and DLPFC regions also have been demonstrated in individuals with schizophrenia (Pomarol-Clotet et al. 2010). The findings demonstrated by Liao and colleagues (2010; 2011) revealed, for the first time, that chronic exposure to a glutamatergic antagonist induces persistent structural changes in humans that are reminiscent to the alterations in brain morphometry present within the clinical population. However, human NMDA antagonist abusers are likely to be poly-drug users.

Thus, it is difficult to determine whether these effects were specifically mediated by ketamine, another psychoactive drug or a combination of both influences. Due to ethical considerations, human imaging studies in controlled settings are not permitted to chronically administer NMDA antagonists to volunteers, only acute. Therefore, the use of preclinical imaging studies utilising experimental animals can be implemented facilitating the elucidation of the effects of NMDA antagonism within the brain.

The use of preclinical experimental model enables researchers to draw conclusions on causal mechanisms underlying neurocognitive impairment associated with chronic NMDA receptor blockade. Furthermore, preclinical studies facilitate a level of sample control that cannot be achieved in a clinical setting. Chronic intermittent administration of PCP has been identified to induce metabolic hypofunction within prefrontal cortical regions. The imaging technique 2-deoxyglucose autoradiography (2-DG) has indicated a reduction in glucose utilisation within a number of cortical regions. PCP treatment induced a regionally specific reduction in local cerebral glucose utilization, including the medial orbital cortex and throughout all layers of the prelimbic cortex. In addition to cortical metabolic hypofunction, it was demonstrated PCP treatment was associated with reduced glucose metabolism within sub-cortical structures such as the lateral lemniscus and reticular nucleus of the thalamus (Cochran et al. 2003). Prefrontal glucose utilisation has been shown to be correlated with not only the presence, but also the severity of cognitive and negative symptoms of schizophrenia (Buchsbaum and Hazlett 1998; Hazlett et al. 2000; Cochran et al. 2003). Furthermore, dysfunctional neuronal metabolism within the temporal lobes has been linked to the development of psychotic symptoms and thought disorder (Buchsbaum and Hazlett 1998). These findings demonstrate that effective neuronal metabolism is essential for normal brain activity and erroneous glucose utilisation may be intrinsically involved with schizophrenia symptomatology. Moreover, the findings reported by Cochran and colleagues (2003) indicate that chronic NMDA receptor antagonism may induce functional impairment in brain

regions that share functional homology with impairments present in human PCP abusers performing frontally controlled tasks (Wu et al. 1991) as well as schizophrenia patients (Wolkin et al. 1992; Tamminga et al. 1992), as assessed by positron emission tomography.

Impaired functioning of cortical regions demonstrated in schizophrenia patients and human PCP users and now experimental animals following PCP administration may mediate the cognitive impairments observed. Prefrontal cortical regions have been extensively associated with the involvement in higher-order cognitive processing (Volz et al. 1998; Hazlett et al. 2000; Miller and Cohen 2001; Lesh et al. 2010) and therefore abnormal neuronal activity may lead to cognitive dysfunction. Additionally, it has also been demonstrated that acute administration of PCP produced altered relative cerebral blood volume (rCBV) in distinct corticolimbic-thalamic structures, including medial prefrontal, cingulate, orbitofrontal and retrospenial cortices (Gozzi et al. 2008), in a pharmacological MRI (phMRI) study. These findings demonstrate that systemic administration of PCP results in hypofrontality of cortical brain regions implicated in cognition relative to schizophrenia. Thus, these findings further enhance the cross-species translational validity of utilising NMDA antagonists to pharmacologically induce abnormal neuronal functioning of cortical brain regions.

Schizophrenia is associated with impairments in cognition across a number of domains (Marder et al. 2004; Neuchterlein et al. 2004; Ibrahim and Tamminga, 2010), many of which can be modelled in experimental animals (Young et al. 2009a) and are susceptible to enduring disruption following sub-chronic NMDA receptor antagonism (Neill et al. 2010). Previous experiments have demonstrated that attentional processing is impaired following repeated PCP treatment (Amitai and Markou 2010; chapter 5) or in the drug-free state following sub-chronic PCP treatment (chapter 6). In addition to voluntary attentional processing (sustained, divided, selective), sensorimotor gating reflects passive or 'automatic'

attentional processing and involves the pre-processing of incoming sensory information (Graham, 1975). Sensorimotor gating is usually assessed using pre-pulse inhibition (PPI) of a startle response (Braff and Geyer 1990). When a startling stimulus is preceded by a non-startling 'prepulse', the startle reflex elicited has a diminished intensity. This phenomenon is observed in all mammalian species (Braff et al. 2001). PPI deficits are evident in schizophrenia patients (Braff et al. 1978, 1992; Swerdlow et al. 1994) and have been suggested to be associated with cognitive fragmentation and the generation of thought disorders (Perry and Braff et al. 1994). Inhibition of a startle reflex has been shown to be disrupted in rats following administration of psychostimulants, including NMDA receptor antagonists (Mansbach and Geyer 1989, 1991). These findings suggest that glutamatergic disruption results in deficits in sensorimotor gating which is consistent with that observed in schizophrenia patients.

As described above, in addition to the cognitive disruption induced by NMDA antagonism, blockade of the glutamatergic system also results in a number of functional, neurophysiological and neurochemical abnormalities that are present in schizophrenia patients. While the metabolic abnormalities and neuropathalogical commonalities are well characterised following NMDA receptor antagonism, there is a distinct lack of preclinical data characterising the structural abnormalities following sub-chronic PCP administration. This is especially true of studies that were not limited to a region of interest approach. Therefore, the aim of this experimental chapter was (1) to confirm whether sub-chronic PCP treatment induces attentional disruption, characterised by attentional impairment on the 5-CSRTT; (2) whether PCP treatment affects startle reactivity; (3) whether sub-chronic PCP treatment is associated with enduring structural brain abnormalities.

7.2 Methods

7.2.1 Subjects

Male Lister-hooded rats (n=20; Charles River; weighing approx 350 ± 10 g at the start of the experiment) were housed in groups of four on a 12 hour reversed light cycle (lights on at 7:00pm). Details regarding the environmental conditions, food restriction and ethical considerations can be found in the general methods section (chapter 2).

7.2.2 Behavioural Assessment

Behavioural assessment was conducted both before and after sub-chronic PCP treatment. This enabled the longitudinal assessment of cognitive performance, enabling within-subjects assessment of performance alterations following PCP treatment to be made.

7.2.3 5-Choice Serial Reaction Time Task

Animals were trained to perform the 5-CSRTT as described in chapter 2. Additionally, the equipment used during the experimental procedures is also described within the general methods chapter. The following 5-CSRTT testing conditions were conducted before and after sub-chronic PCP treatment. Details regarding the duration between PCP treatment and behavioural challenge is described in the experimental design section.

7.2.3.1 Stable Baseline Performance

Performance was assessed using the protocols designated in Training Stage 7, which consisted of a 1 s SD, 5 s ITI, 5 s TO period and a 5 s LH. Animals were presented with 100 trials in a session lasting no longer than 30 minutes. Pre-treatment assessment of stable baseline performance was conducted when animals performed consistently to the criteria described in the general methods section.

7.2.3.2 Variable Stimulus Duration (vSD)

Rat performance was challenged within a single session that presented a variable SD (0.25, 0.5, 0.75, and 1 s) as opposed to a fixed SD (1 s). Every SD was presented pseudo-randomly, 25 times each in a session consisting of 100 trials. All other parameters (TO, LH and ITI) were as before.

7.2.3.3 Variable Inter-trial Interval (vITI)

Rats were exposed to a variable ITI session as a further behavioural challenge. The fixed ITI of 5 s now consisted of a variable ITI (2, 4, 6, and 8 s), which was presented in a pseudo-random order throughout the session (25 times for each individual ITI). Other testing parameters were identical to standard training conditions i.e. 100 trials, 1 s SD, 5 s TO and 5 s LH.

Both challenge sessions were interspersed with standard training sessions, before and after PCP treatment ensuring stable performance was maintained throughout the experimental procedure.

7.2.4 Pre-pulse Inhibition

Following 5-CSRTT testing, (stable baseline performance and behavioural challenges) all animals were tested in startle chambers (Kinder Scientific, USA), investigating the attenuation of startle reflex by the presentation of a non-startling pre-pulse. Pre-pulse inhibition (PPI) testing was conducted both before and after sub-chronic PCP treatment.

7.2.4.1 Description of PPI Paradigm

No training was required for startle experiments. The session was configured following the protocols described within the training manual published by Geyer and Swerdlow (2001). The session began with a 5 min habituation period during which a background noise (white

noise) was presented with an intensity of 65 dB. The session consisted of 36 trials comprised of either pulse only trials (120 dB) or trials containing a pre-pulse (3, 6, or 12 dB above background noise; Swerdlow and Geyer 2001). Trials were separated by variable inter-trial intervals (ITI; 8 – 23 s) which averaged 15 s. The initial part of the session, following the habituation period, was composed of 6 consecutive pulse only trials, which was followed by 25 trials consisting of pulse only and three individual pre-pulse trials, presented in a randomised order, which was then followed by 5 consecutive pulse only trials. Testing was conducted in three startle chambers and data collection was conducted by (Startle Monitor, Kinder Scientific) software and analysed in Excel (Microsoft). Each chamber was calibrated for intra-chamber consistency, thus enabling the accelerometer used to quantify the startle response to assess a reaction up to a force of 15 Newton's (N).

7.2.4.2 Description of Startle Stimulus

The pre-pulse trials were constructed as follows; the noise level increased above background noise (3, 6, or 12 dB) for a period of 20 msec, after which the noise level was reduced back to 65 dB. After a period of 80 msec the software began recording the startle response (recording window of 250 msec) and a pulse was delivered (120 dB), which had a rise time of < 2 msec and a duration of 40 msec. After the pulse, the noise level reduced to background level and the trial ended, initiating the ITI. Pulse only trials were constructed in the same manner, only without the 20 msec pre-pulse. Each of the various pulse or pre-pulse trials were interspersed by variable ITIs and the session lasted for approximately 10 minutes.

7.2.4.3 Calculation of Pre-pulse Inhibition

The level of pre-pulse inhibition was expressed as a percentage of the full startle response. This enabled differing amplitudes of startle response to be normalised. The inhibition of the startle response was calculated for each individual animal and averaged, indicating the level

of startle inhibition for each pre-pulse trial. The following calculation was used to calculate the percentage of pre-pulse inhibition of the startle response when the stimulus was preceded by a non-startling pre-pulse:

PPI = 100 x {[pulse only units – (prepulse + pulse units)] / (pulse only units)}

7.2.5 Sub-chronic PCP Treatment Regimen

Animals were treated with saline (0.9% saline, n = 10) or PCP (5 mg/kg, n = 10) via the interperitoneal (i.p.) route. The sub-chronic PCP treatment regimen consisted of 7 twice-daily (approximately 9am and 5pm) injections followed by a 7-day washout period. During this treatment regimen and washout duration animals received no behavioural training or testing and were kept in home cages. Food restriction continued throughout the experiment.

7.2.6 Induction and Maintenance of General Anaesthesia

Animals were anaesthetised during the MRI procedure. Animals were placed into the induction chamber and anaesthesia was induced using 5% isofluorane in 0_2 (2L/min) for a period of 3 – 5 minutes. Once animals' paw-pinch reflex was absent, animals were removed from the induction chamber and transferred to the MRI bed. Anaesthesia was maintained with a flow-rate of 2 – 2.5% isofluorane in 0_2 (2L/min) throughout the duration of the scanning procedure. Animals were fixed in a stereotaxic head frame. Respiration rate, core temperature and blood oxygenation level were monitored throughout the scanning procedure. Upon completion of the scanning procedure, animals were removed and placed in a recovery chamber and monitored throughout recovery, after which they were returned to an isolation cage within the holding room until the following morning.

7.2.7 Structural Magnetic Resonance Imaging

Once the animal was fixed in the MRI bed, the cradle was inserted into the bore of the 4.7 tesla MRI magnet (Bruker BioSpec 47/40 System). Following the alignment of the scanning region, the scan sequence was initiated; firstly diffusion tensor images were acquired followed by high resolution structural images. Total scan time was approximately 1 hr 45 mins and yielded an image resolution of 160 µm. Images were analysed in SPM5 with the SPM Mouse plug-in (Wellcome Department of Clinical Neurology, London (http://www.fil.ion.ucl.ac.uk).

7.2.8 Experimental Design

Once trained, all 20 animals were tested in the 5-CSRTT using standard training conditions and then exposed to two behavioural challenges. Figure 7.1 summarises the experimental design used. Following the 5-CSRTT baseline and challenge sessions, baseline PPI performance was recorded in all 20 animals. Upon completion of baseline behavioural testing, baseline MRI scanning was conducted utilising VBM and DTI techniques. Following the completion of baseline behaviour and structural imaging, animals were then dosed with either PCP or saline following a sub-chronic dosing schedule. To determine treatment groups, animals were group-matched based on 5-CSRTT performance before and after behavioural challenges, along with PPI startle responsivity, in order to create two treatment groups with no significant variation between behavioural measures. The group-matching procedure used was identical to that that described in chapter 5 and 6. In addition to the group-matching procedure, animals were ranked depending on baseline 5-CSRTT performance and based on the rank and treatment group, the order in which they were scanned was determined. This ensured there was no variability or bias throughout the scanning procedure. Animals were then treated according to the sub-chronic PCP regimen, outlined above. The dosing schedule was staggered to ensure all animals received identical

durations between drug washout, post-dose behavioural testing and MRI scanning. Following the wash-out period, animals were re-tested in the 5-CSRTT, using stable baseline conditions (8-days post-PCP) and the acute behavioural challenges (variable SD and variable ITI; 10 – 13 days post-PCP), PPI (14 days post-PCP) and finally animals were re-scanned following the same procedure used previously (15 days post-PCP). On the day following MRI scanning (16 days post-PCP), brains were removed, snap-frozen and stored at -80^{o}C, enabling *ex-vivo* analysis to be conducted.

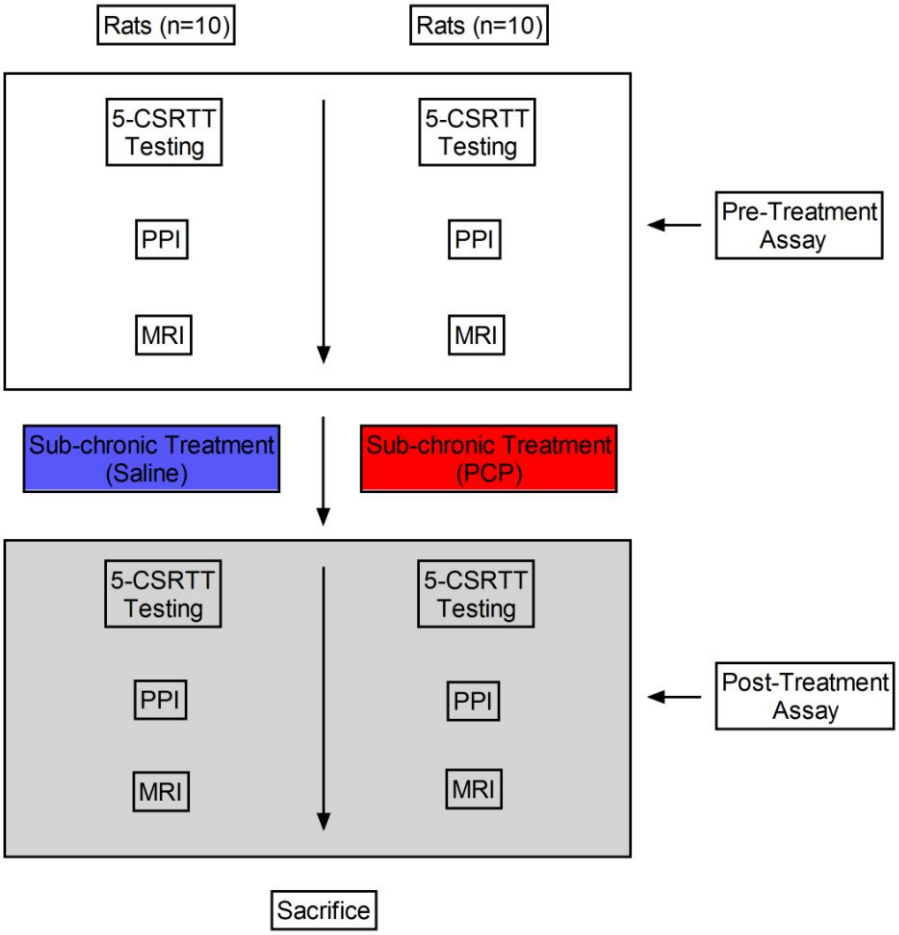

Fig 7.1 A schematic diagram showing an overview of the experimental design used. Sub-chronic treatment consisted of 7-day twice daily administration, followed by 7-day washout period. 5-CSRTT testing includes baseline attentional load and two challenge sessions.

7.2.9 Data Analysis

To analyse the longitudinal, within-subjects effect of sub-chronic PCP treatment before and after treatment, a two-way repeated measures analysis of variance (ANOVA) was utilised. 'Day' (before or after treatment) was the repeated measure and 'Treatment' (saline or PCP) was the fixed between-subjects factor. Repeated measure ANOVA was used to assess performance for standard parameters session and following the vSD and vITI challenge sessions. Planned Comparisons (Snedecor and Cochran 1989) were used to assess post-dose performance differences compared to pre-dose performance. To specifically address the post-dose between-subjects differences of the treatment groups, a one-way analysis of covariance (ANCOVA) was carried out with Treatment as the fixed factor. Pre-treatment performance was used as a covariate, compensating for between-animal variability that may have existed among the animals response prior to drug treatment, therefore enhancing the statistical power of the approach. Again, ANCOVA analysis was used to assess performance following standard parameters session, and the vSD and vITI challenge sessions. Assessment of performance when presented with individual SD (1, 0.75, 0.5, 0.25 s) or ITI (8, 6, 4, 2 s) was conducted by two-way repeated measures ANOVA. The between-subjects factor was Treatment, and repeated measure was SD or ITI. Additionally, a two-way repeated measures ANOVA was also used to determine performance across the session. The 5-CSRTT session was split into trial bins, each containing 50 trials (T1 and T2). Treatment group was the between-subjects factor and trial bin the repeated measure.

A two-way repeated measures ANOVA was used to analyse startle reactivity, before and after PCP treatment. The repeated measure was the pre-pulse amplitude and the fixed factor was the treatment group. Planned Comparisons were used to compare startle response of each pre-pulse to each other. Pre and post-treatment conditions were analysed individually and

like 5-CSRTT figure, saline and PCP labels within the pre-treatment groups indicates future treatment.

Structural MR images were processed using SPM5 following a standard VBM paradigm for animal imaging (Sawiak et al. 2009). Following manual alignment, brains were segmented into grey and white matter maps using the SPM Mouse toolbox (www.wbic.cam.ac.uk/~sjs80/spmmouse.html) and DARTEL (Ashburner and Friston 2000). Modulated GM maps were prepared for each subject scan pre- and post-administration and smoothed with an 800µm isotropic Gaussian smoothing kernel. Voxel-wise paired Student's t-tests were performed two-tailed at each voxel to identify group differences. To control for multiple comparisons, an adjusted *p*-value of $p<0.005$ in combination with an extent threshold of 100 voxels was used. Additionally, a family-wise error (FWE) correction was applied.

7.3 Results

7.3.1 Voxel-Based Morphometry

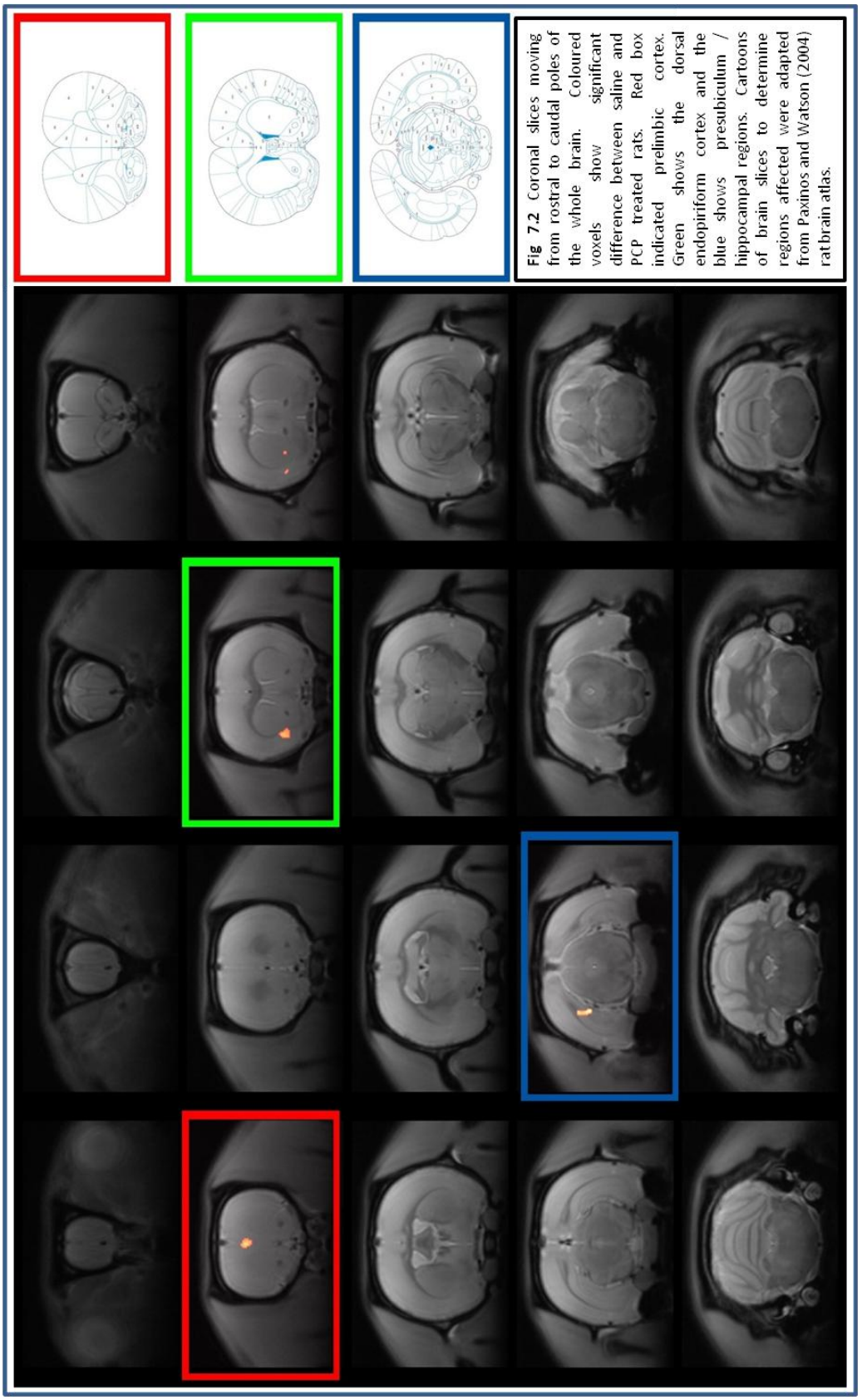

Fig 7.2 Coronal slices moving from rostral to caudal poles of the whole brain. Coloured voxels show significant difference between saline and PCP treated rats. Red box indicated prelimbic cortex. Green shows the dorsal endopiriform cortex and the blue shows presubiculum / hippocampal regions. Cartoons of brain slices to determine regions affected were adapted from Paxinos and Watson (2004) rat brain atlas.

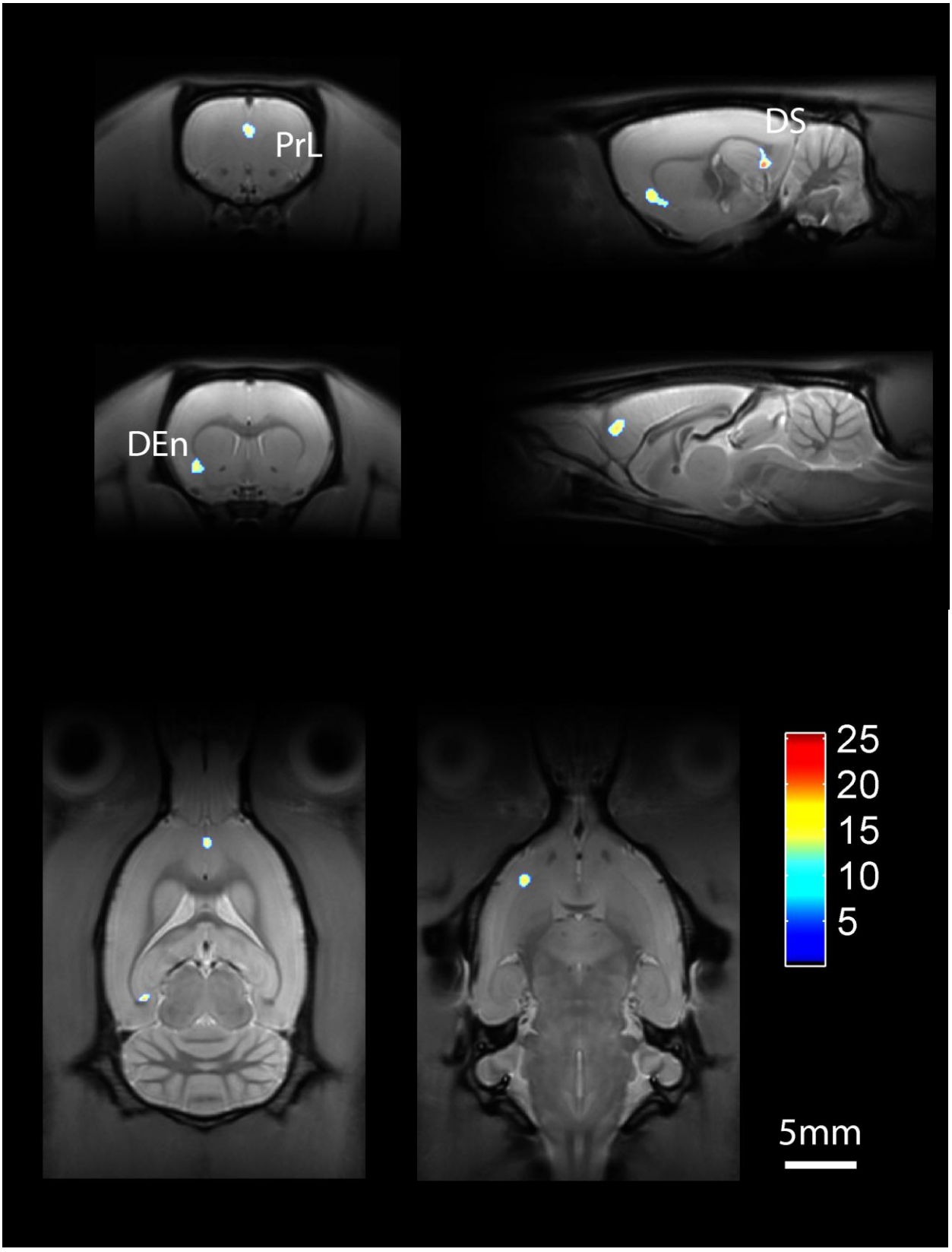

Fig 7.3 Overview of regional GM score differences in rats administered either saline (n=10) or PCP (n=10). Regions include prelimbic cortex (Prl), dorsal presubiculum (DS) and dorsal endopiriform cortex (DEn). Colour gradient indicates two-tailed t-test *F*-score with 18 degrees of freedom.

Fig 7.2 displays rostral to caudal coronal brain slices showing significant differences in GM class between saline and PCP-treated animals. A FDR and FWE threshold of *p*=0.005 for controlling the type 1 error was applied. The PCP-induced morphological abnormality within prelimbic cortex (PrL) survived correction for multiple comparisons (FWE or FDR) when small volume correction was applied to the frontal cortex ($p<0.05$). Voxel clusters within the hippocampal formation/dorsal presubiculum (DS) and dorsal endopiriform cortex (DEn) survived a weak correction for multiple comparisons. However, when more stringent controls (FDR, FWE) are applied the effect lost statistical significance. Fig 7.3 represents an overview of brain morphology, showing coronal, sagittal and horizontal slices. Colour gradient represents *F*-scores (2/18 degrees of freedom) indicating intensity of MRI signal. Table 7.1 summarises the MRI results, displaying corrected and uncorrected *p* values, *F*-scores and region.

Table 7.1 Description of sub-chronic PCP induced structural changes

Extent (voxels)	*p* value uncorrected	*p* value FWE corrected	*F*-score	Region
191	0.00035	<0.05	19.3	Prelimbic
296	0.00047	=	18.2	Dorsal Endopiriform
119	0.00008	=	25.9	Presubiculum

Summary of the PCP-induced effect on brain morphology. Cluster region determined from Paxinos and Watson (2004) rat brain atlas

7.3.2 5-CSRTT - Stable Baseline Performance

The following describes the effect of sub-chronic PCP treatment on 5-CSRTT performance. As discussed in section 7.2.9, a repeated measure ANOVA was used to analyse the longitudinal effect, assessing performance before and after sub-chronic PCP treatment. Conversely, a

one-way ANCOVA analysis was used to determine the post-treatment between-subject effect. Performance in the 5-CSRTT when animals were tested using standard training parameters was analysed. Two-way repeated measures ANOVA demonstrated no Treatment effect [$F_{(1,18)}$=0.83, NS] or Treatment*Day interaction [$F_{(1,18)}$=1.29, NS] for response accuracy under standard training parameters (fig 7.4A). Additionally, one-way ANCOVA demonstrated no significant between-subjects effect following sub-chronic PCP treatment [$F_{(1,17)}$=1.59, NS].

Analysis of the percent correct responding in PCP treated animals' indicated an overall significant effect for Day [$F_{(1,18)}$=6.11, $p<0.05$], without an effect on Treatment [$F_{(1,18)}$=0.39, NS] or a Treatment*Day interaction [$F_{(1,18)}$=2.93, NS]. Planned Comparisons revealed the significant Day effect was mediated by a significant reduction in percent correct responding following PCP treatment ($p<0.01$), an effect that was absent in control animals (fig 7.4B). While the effect lacked statistical significance, the one-way ANCOVA demonstrated a trend towards a PCP-induced reduction in percent correct responding following drug treatment [$F_{(1,17)}$= 3.12, $p<0.1$].

There was no Treatment effect [$F_{(1,18)}$=0.81, NS] or Treatment*Day interaction [$F_{(1,18)}$=0.88, NS] when the longitudinal effect on incorrect responses was analysed (fig 7.4C). Similarly, there was no between-subjects effect following ANCOVA analysis [$F_{(1,17)}$=1.21, NS].

Analysis of percent omissions demonstrated a significant Day effect [$F_{(1,18)}$=6.17, $p<0.05$], which occurred independently of a Treatment effect [$F_{(1,18)}$=0.00, NS] or Treatment*Day interaction [$F_{(1,18)}$=1.63, NS]. Planned Comparisons demonstrated that PCP treatment resulted in a significant increase in omissions following sub-chronic treatment ($p<0.05$). This effect was not evident in control animals (fig 7.4D). Between-subjects comparisons revealed no significant difference in omissions between treatment groups following treatment [$F_{(1,17)}$=1.19, NS].

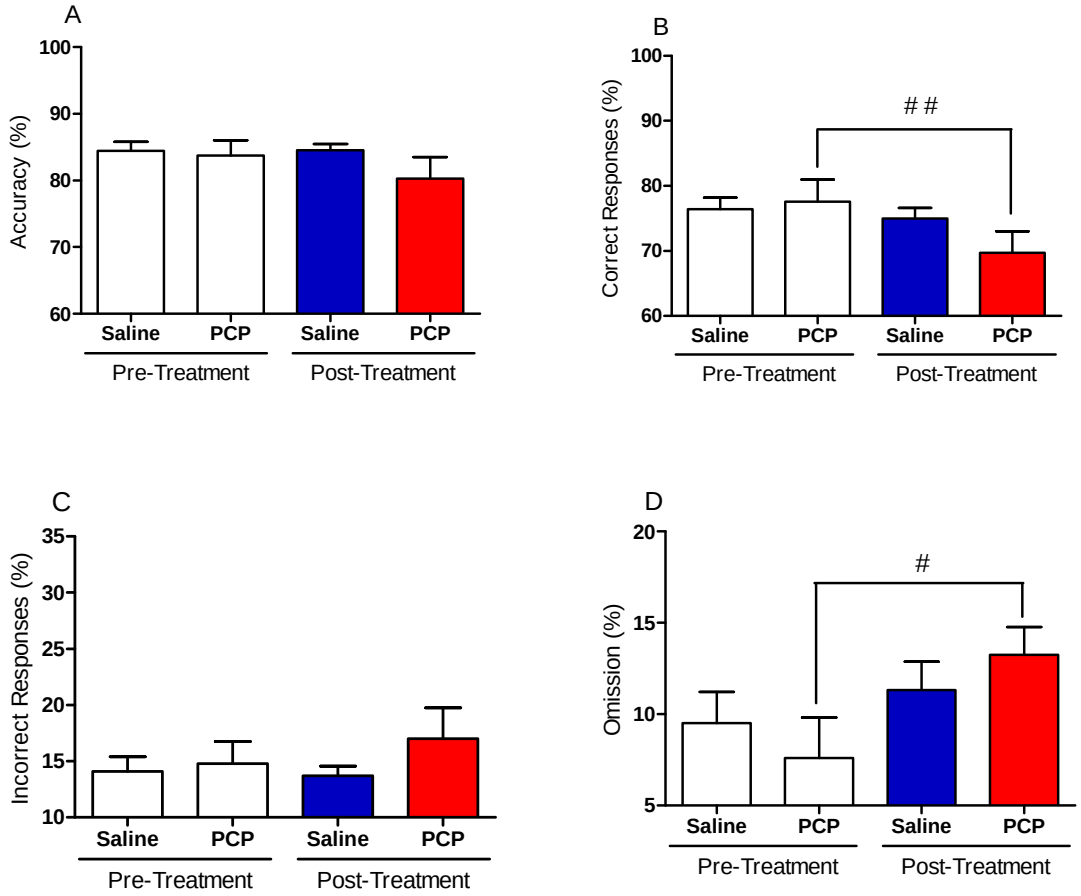

Fig 7.4. Performance before and after sub-chronic PCP treatment in the 5-CSRTT using standard parameters. Measures displayed include response accuracy (A), percent correct responding (B), percent incorrect responding (C) and percent of omissions (D). **denotes $p<0.01$ following between-subjects analysis of the saline (n=10) and PCP (n=10) treatment groups, post-dose. # denotes $p<0.05$ and ## $p<0.01$ within-subjects difference in PCP-treated animals when compared with pre-treatment performance.

PCP treatment had no significant effect on perseverative responding, indicated by the absence of a Treatment effect [$F_{(1,18)}$=2.26, NS] or Treatment * Day interaction [$F_{(1,18)}$=2.30, NS]. Furthermore, no effect between saline and PCP treated animals was observed [$F_{(1,17)}$=0.82, NS]. Likewise, sub-chronic PCP treatment had a non-significant within-subjects effect on premature responding, demonstrated by the lack of Treatment effect [$F_{(1,18)}$=0.68, NS] or Treatment*Day interaction [$F_{(1,18)}$=0.06, NS] (table 7.2). This was accompanied by no significant effect following one-way ANCOVA analysis [$F_{(1,17)}$=0.00, NS] (table 7.3).

Table 7.2 Effect of PCP treatment on baseline performance in the 5-CSRTT

Parameter	Pre-Treatment	Post-Treatment
Perseverative (n)	157.80 ± 28.01	161.80 ± 26.86
Premature (%)	5.90 ± 1.30	9.55 ± 3.31
Correct Latency (s)	0.69 ± 0.04	0.76 ± 0.05 *
Magazine Latency (s)	1.39 ± 0.06	1.29 ± 0.06 *

Within-subjects comparison of standard 5-CSRTT performance before and after PCP treatment

Investigation of the effect on correct latency (CL) revealed a significant Treatment*Day interaction [$F_{(1,18)}$=6.95, p<0.05]. Planned Comparisons attributed this interaction to a PCP-induced increase in CL (p<0.05), with no effect observed in control animals (table 7.2). Analysis of the between-subjects effect demonstrated a significant PCP-induced increase in CL [$F_{(1,17)}$=6.86, p<0.05] (table 7.3).

Analysis of the longitudinal effect on magazine latency demonstrated no effect of Treatment [$F_{(1,18)}$=0.29, NS] or a Treatment*Day interaction [$F_{(1,18)}$=0.34, NS] but did reveal a significant Day effect [$F_{(1,18)}$=7.39, p<0.05]. Planned Comparisons indicated that the ML was significantly reduced in PCP treated animals following treatment (p<0.05), without an effect in saline treated animals (table 7.2). However, there was no significant difference between treatment groups following ANCOVA analysis [$F_{(1,17)}$=0.5, NS] (table 7.3).

These analyses demonstrate that post-treatment comparisons between vehicle and PCP treated animals reveal no significant difference in the attentional measures assessed, when animals were assessed under a low attentional load. However, an increase in CL was observed. Upon assessment of the pre vs post within-subject effect of PCP treatment, subtle deficits were observed. Correct responding was reduced and the number of omissions was increased. Furthermore, CL was increased and this was coupled with a reduction in ML.

Table 7.3. Baseline 5-CSRTT performance following sub-chronic PCP treatment

Parameter	Saline	PCP
Perseverative (n)	131.20 ± 11.39	161.80 ± 26.86
Premature (%)	11.21 ± 1.79	9.55 ± 3.31
Correct Latency (s)	0.68 ± 0.05	0.76 ± 0.05 *
Magazine Latency (s)	1.36 ± 0.09	1.29 ± 0.06

Between-subjects comparison of treatment groups during standard 5-CSRTT parameters following sub-chronic treatment

7.3.3 Variable Stimulus Duration (vSD) Challenge Session

Based on previous observations (chapters 3 and 6) that baseline performance is generally unaffected following sub-chronic PCP treatment, a vSD challenge session was implemented to further tax attentional processing. Two-way repeated measures ANOVA demonstrated a significant longitudinal Treatment effect [$F_{(1,18)}$=5.04, p<0.05] and a Treatment*Day interaction [$F_{(1,18)}$=7.79, p<0.05] for response accuracy. Planned Comparisons demonstrated the significant effect was attributed to a PCP-specific reduction in accuracy following treatment (p<0.01). One-way ANOCVA analysis of the between-subject post-dose performance also demonstrated a significant difference between treatment groups [$F_{(1,17)}$=9.85, p<0.01] (fig 7.5A).

The effect on percent correct responding demonstrated a significant Day effect [$F_{(1,18)}$=4.58, p<0.05] a strong trend towards a Treatment effect [$F_{(1,18)}$=4.07, p=0.058], but no Treatment*Day interaction [$F_{(1,18)}$=2.75, NS]. Planned Comparisons demonstrated a significant within-subjects reduction in percent correct for PCP treated animals (p<0.05) but not saline treated animals. A strong trend towards a significant between-subjects effect was observed following one-way ANCOVA analysis [$F_{(1,17)}$=4.17, p=0.057] (fig 7.5B).

Repeated measures ANOVA showed a significant Treatment*Day interaction when the number of incorrect responses was analysed [$F_{(1,18)}$=8.67, p<0.01]. A trend towards a Treatment effect [$F_{(1,18)}$=3.11, p<0.1] was also observed. Planned Comparisons indicated this effect was mediated by a within-subjects PCP-induced increase in incorrect responding (p<0.01). ANCOVA analysis revealed a significant between-subject effect [$F_{(1,17)}$=9.67, p<0.01] when saline and PCP-treated animals' were compared following drug treatment (fig 7.5C). No Treatment effect [$F_{(1,18)}$=0.4, NS] or Treatment*Day interaction [$F_{(1,18)}$=0.00, NS] was observed when the number of omissions was analysed. Similarly, no between-subjects effect was observed following ANCOVA analysis [$F_{(1,17)}$=0.03, NS] (fig 7.5D).

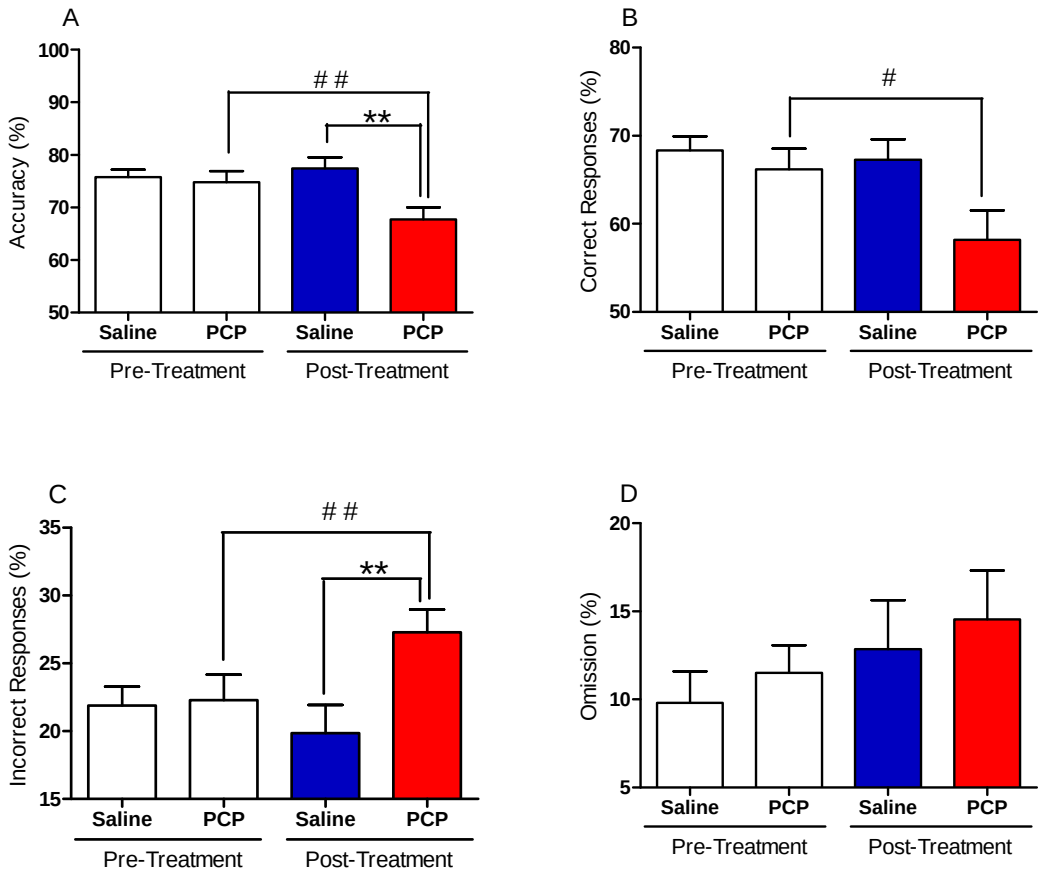

Fig 7.5 Effect of a variable stimulus duration (vSD) challenge session on performance, before and after sub-chronic PCP treatment. Measures displayed include response accuracy (A), percent correct responding (B), percent incorrect responding (C) and percent of omissions (D). **denotes p<0.01 following between-subjects analysis of

the saline (n=10) and PCP (n=10) treatment groups, post-dose. # denotes p<0.05 and ## p<0.01 within-subjects difference in PCP-treated animals when compared with pre-treatment performance.

Two-way ANOVA of perseverative responding demonstrated no Treatment [$F_{(1,18)}$=1.08, NS] or a Day effect [$F_{(1,18)}$=1.89, NS] or a Treatment*Day interaction [$F_{(1,18)}$=0.27, NS] (table 7.3). Furthermore, no difference existed between control and PCP groups following ANCOVA analysis [$F_{(1,17)}$=0.00, NS] (table 7.4). Within-subjects analysis of the PCP-induced effect on premature responding demonstrated a significant Day effect [$F_{(1,18)}$=6.04, p<0.05], independent of a Treatment effect [$F_{(1,18)}$=0.14, NS] (table 7.3). However, a trend towards a Treatment*Day interaction was observed [$F_{(1,18)}$=3.94, p=0.06]. Planned Comparisons demonstrated a significant within-subjects reduction in premature responding in saline treated animals (p<0.01, data not shown). No effect was observed in PCP-treated animals (table 7.4). Despite the significant within-subjects reduction in saline treated animals, between-subjects analysis following sub-chronic treatment demonstrated no significant difference [$F_{(1,17)}$=2.15, NS] (table 7.5).

Table 7.4 Effect of PCP treatment on 5-CSRTT performance following a vSD challenge

Parameter	Pre-Treatment	Post-Treatment
Perseverative (n)	167.40 ± 32.48	149.50 ± 30.16
Premature (%)	6.90 ± 1.39	6.33 ± 2.38
Correct Latency (s)	0.71 ± 0.03	0.76 ± 0.05
Magazine Latency (s)	1.37 ± 0.04	1.36 ± 0.07

Within-subjects comparison of performance before and after PCP treatment during vSD challenge

No significant within-subjects Treatment effect [$F_{(1,18)}$=0.41, NS] or Treatment*Day interaction [$F_{(1,18)}$=0.45, NS] were observed when correct latency was analysed (table 7.4). Likewise, no significant effect was observed following one-way ANCOVA analysis [$F_{(1,17)}$=0.64, NS] (table 7.5).

Additionally, no significant within-subjects Treatment effect [$F_{(1,18)}$=0.00, NS] or Treatment*Day interaction [$F_{(1,18)}$=0.09, NS] was observed when magazine latency was analysed (table 7.4). This lack of effect was accompanied by no significant difference between treatment groups following drug treatment [$F_{(1,17)}$=0.08, NS] (table 7.5).

Table 7.5 5-CSRTT performance during vSD challenge session following sub-chronic PCP treatment

Parameter	Saline	PCP
Perseverative (n)	120.00 ± 12.36	149.50 ± 30.16
Premature (%)	4.79 ± 1.02	6.33 ± 2.38
Correct Latency (s)	0.71 ± 0.05	0.76 ± 0.05
Magazine Latency (s)	1.36 ± 0.09	1.36 ± 0.06

Between-subjects comparison of treatment groups during vSD challenge session

In contrast to the paucity of effects during baseline conditions, when performance was challenged using a vSD session PCP treated animals exhibited substantial attentional impairment. This was exemplified by a PCP-induced reduction in response accuracy and percent correct responding, which was coupled with an increase in the number of incorrect responses made. The number of omissions throughout the session was unaffected. These effects displayed remarkable behavioural specificity as no other parameters were sensitive to impairment.

7.3.3.1 Effect of Stimulus Duration

As substantial effects were observed following the vSD challenge but not duringthe baseline session, it was important to assess the effect as function of SD. Repeated measure ANOVA demonstrated a significant Treatment effect [$F_{(1,18)}$=9.22, p<0.001] and a significant SD effect [$F_{(3,54)}$=12.47, p<0.001] for response accuracy (fig 7.6A). Analysis failed to detect a significant Treatment*SD interaction [$F_{(3,54)}$=0.69, NS]. Pair-wise comparisons demonstrated PCP treatment reduced accuracy when animals were presented with a 1 s (p<0.05) and 0.75 s SD (p<0.05). A trend towards a significant PCP-induced reduction in accuracy was observed for 0.5 s SD (p = 0.06).

Similarly, a significant Treatment effect [$F_{(1,18)}$=5.06, p<0.05] and SD effect [$F_{(3,54)}$=20.27, p<0.001] was observed when percent correct responding was assessed (fig 7.6B). While lacking statistical significance, a strong trend towards a significant Treatment*SD interaction was observed [$F_{(3,54)}$=2.74, p = 0.052]. PCP treatment significantly reduced percent correct responding during 1 s (p<0.05) and 0.75 s SD (p<0.05).

Additionally, a Treatment effect [$F_{(1,18)}$=7.75, p<0.05] and a SD effect [$F_{(,3,54)}$=10.48, p<0.001] was observed when percent incorrect responding was analysed (fig 7.6C). No significant Treatment*SD interaction was evident [$F_{(3,54)}$=0.73, NS]. A significant PCP-induced increase in incorrect responding was observed during 1 s (p<0.05) and 0.5 s SD (p<0.05), with a strong trend towards a significant increase when presented with a 0.75 s SD (p = 0.06).

When percent omissions were analysed, no Treatment effect [$F_{(1,18)}$=0.15, NS] was observed (fig 7.6D). A slight trend towards a SD effect [$F_{(3,54)}$=2.19, p<0.1] was evident, without a significant Treatment*SD interaction [$F_{(3,54)}$=0.97,NS].

The vSD challenge increased the attentional load, evidenced by the reduction in performance as the SD is shortened, even in control animals. PCP treatment reduced performance, which

resulted in significant impairment compared to control animals at the longer SDs only. A PCP-induced performance reduction was not evident at the shorter SD as even control animals displayed impaired ability to correctly perform the task. Measures affected include response accuracy, percent correct responding and incorrect responding. Omissions were not susceptible to a PCP-induced impairment.

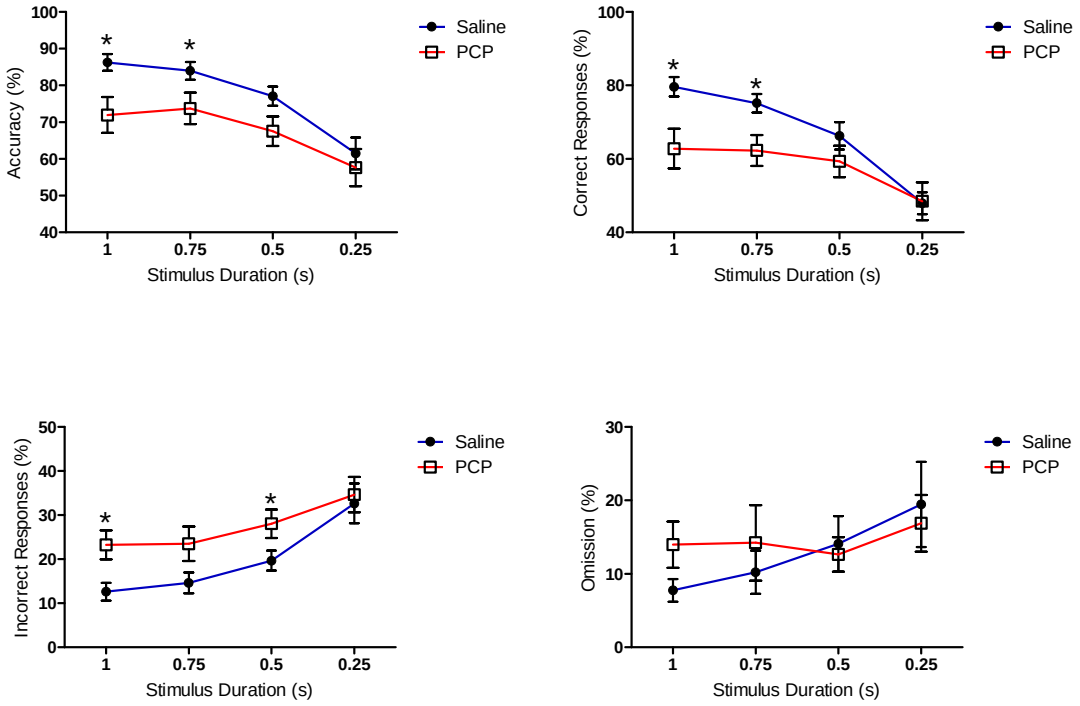

Fig 7.6 Effect of stimulus duration on 5-CSRTT performance. Measures displayed include response accuracy (A), percent correct responding (B), percent incorrect responding (C) and percent omissions (D). * denotes significant difference ($p<0.05$) between saline (n=10) and PCP (n=10) animals when presented various SDs

7.3.3.2 Performance Throughout vSD Challenge Session

To determine the potential of PCP treatment to induce a performance decrement, the ability to conduct the task throughout the session was assessed. Analysis of session performance revealed a significant Treatment effect [$F_{(1,18)}=10.21$, $p<0.01$] for response accuracy (fig 7.7A).

This occurred independently of an overall Trial effect [$F_{(1,18)}$=2.69, NS], but was coupled with a significant Treatment*Trial interaction [$F_{(1,18)}$=5.96, p<0.05]. Within-session comparisons demonstrated a significant PCP-induced reduction in T2 compared with T1 (p<0.01). Additionally, a PCP-induced reduction in accuracy was observed in T2 when compared to control animals (p<0.01).

A significant Treatment effect [$F_{(1,18)}$=4.91, p<0.05] was observed when percent correct responding was analysed (fig 7.7B). Although lacking statistical significance, a trend towards a significant Trial effect [$F_{(1,18)}$=3.06, p<0.1] and a strong trend towards a Treatment*Trial interaction [$F_{(1,18)}$=3.82, p=0.06] was observed. PCP treatment induced a within-subjects (p<0.05) and between-subjects (p<0.05) reduction in T2.

Analyses of percent incorrect responding revealed a significant Treatment effect [$F_{(1,18)}$=7.67, p<0.05] and a Treatment*Trial interaction [$F_{(1,18)}$=4.97, p<0.05] (fig 7.7C). An overall significant Trial effect was not observed, however [$F_{(1,18)}$=1.09, NS]. These effects were attributed to a significant PCP-induced within-subjects (p<0.01) and between-subjects (p<0.05) increase in incorrect responding in T2.

No Treatment [$F_{(1,18)}$=0.53, NS] or Trial effect [$F_{(1,18)}$=1.35, NS] was observed for omissions across the session (fig 7.7D). Furthermore, no Trial*Treatment interaction was evident [$F_{(1,18)}$=0.18, NS].

Again, it was evident that PCP treatment selectively impaired overall response accuracy, percent correct responding and incorrect responding. Interestingly, the performance deficit was localised to the second trial bin, indicating a PCP-induced reduction in the ability to sustain performance throughout the duration of the session.

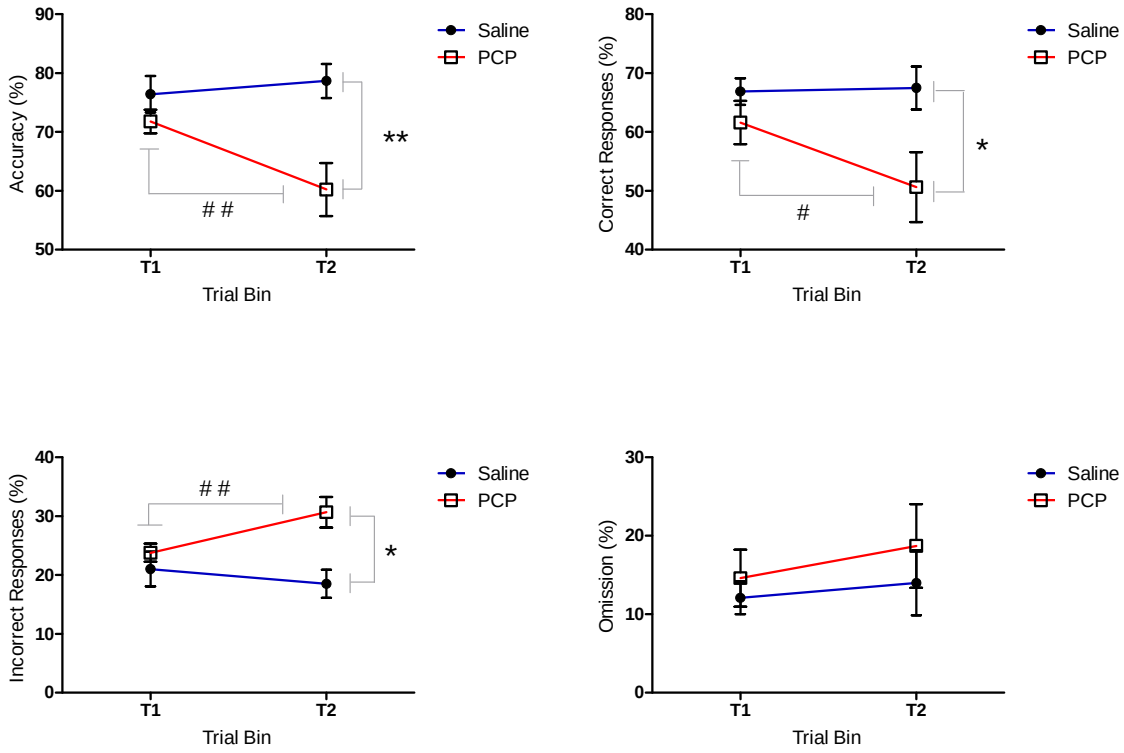

Fig 7.7 5-CSRTT session performance during the vSD challenge. Measures displayed include response accuracy (A), percent correct responding (B), percent incorrect responding (C) and percent omissions (D). T1 reflects the first 50 trials of the session and T2 the last 50 trials. # and ## denotes within-subjects difference ($p<0.05$ and $p<0.01$, respectively) in PCP treated animals from T1 and T2. * and ** denotes significant difference ($p<0.05$ and $p<0.01$, respectively) between saline (n=10) and PCP (n=10) treated animals in T2.

7.3.4 Variable ITI Challenge Session (vITI)

In a similar manner to the vSD challenge, 5-CSRTT performance was further taxed by exposing animals to a single session consisting of a vITI. Repeated measures ANOVA demonstrated a strong trend towards a significant Treatment effect [$F_{(1,18)}=3.76$, $p=0.06$] but no Treatment*Day interaction [$F_{(1,18)}=2.66$, NS] for response accuracy. Planned Comparisons however demonstrated a significant PCP-induced reduction in response accuracy ($p<0.05$), compared to pre-treatment performance. No effect was observed for saline treated animals.

A between-subjects ANCOVA reported a significant treatment effect [$F_{(1,17)}$=5.89, p<0.05], indicating a significant reduction of response accuracy in PCP-treated animal (fig 7.8A).

The effect on response accuracy was not mediated by erroneous correct responding. Percent correct responding was reduced in PCP-treated animals, but the effect was non-significant. Analysis demonstrated no significant Treatment effect [$F_{(1,18)}$=2.49, NS] and no Treatment*Day interaction [$F_{(1,18)}$=0.35, NS] (fig 7.8B). Additionally, no significant between-subjects Treatment effect [$F_{(1,17)}$=1.79, NS] was observed following drug treatment.

Thus, the PCP-induced effect on response accuracy was likely the result of increased incorrect responding. Hence, ANOVA analysis demonstrated that there was no significant within-subjects Treatment effect [$F_{(1,18)}$=2.71, NS] or a Treatment*Day interaction [$F_{(1,18)}$=2.53, NS] for the number of incorrect responses made. However, following one-way ANCOVA analysis a significant Treatment effect [$F_{(1,17)}$=6.09, p<0.05] was demonstrated, indicating a significant PCP-induced increase in the number of incorrect responses made throughout the session (fig 7.8C).

Comparable with the effect following the vSD challenge, analysis of the within-subject effect on omissions revealed no significant Treatment effect [$F_{(1,18)}$=0.55, NS] or a Treatment*Day interaction [$F_{(1,18)}$=0.16, NS]. This was coupled with no significant Treatment effect following one-way ANCOVA analysis [$F_{(1,17)}$=0.01, NS] (fig 7.8D).

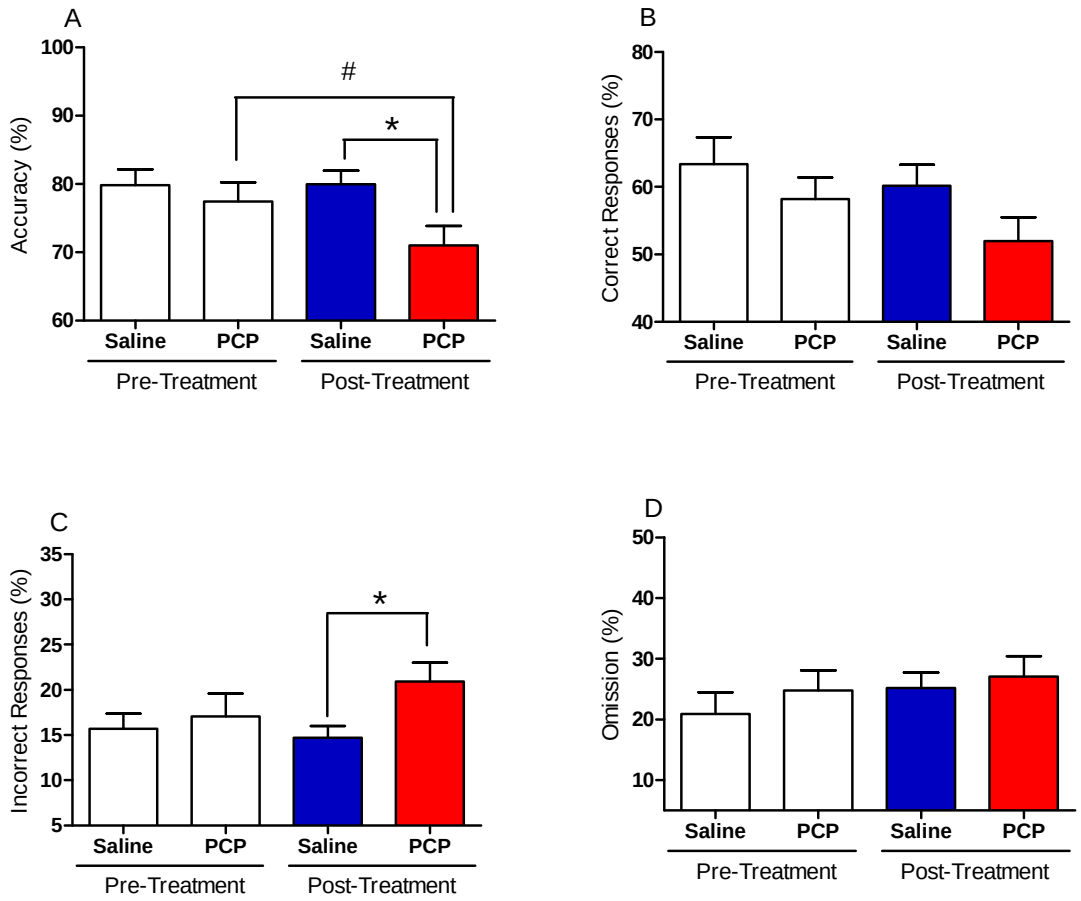

Fig 7.8 Effect of a variable ITI challenge session on performance before and after sub-chronic PCP treatment. Measures displayed include response accuracy (A), percent correct responding (B), percent incorrect responding (C) and percent of omissions (D). *denotes $p<0.05$ following between-subjects analysis of the saline (n=10) and (n=10) PCP treatment groups, post-dose. # denotes $p<0.05$ difference following within-subject analysis of PCP-treated animals when compared with pre-treatment performance.

The number of perseverative responding was unchanged following PCP treatment. No Treatment [$F_{(1,18)}$=1.14, NS] or Day effect [$F_{(1,18)}$=0.72, NS], or a Treatment*Day interaction [$F_{(1,18)}$=0.03, NS] were observed (table 7.6). Additionally, ANCOVA analysis between control and PCP animals also revealed no difference between treatment groups [$F_{(1,17)}$=0.26, NS] (table 7.7).

Table 7.6 Effect of PCP treatment on 5-CSRTT performance following a vITI challenge

Parameter	Pre-Treatment	Post-Treatment
Perseverative (n)	181.00 ± 36.39	171.70 ± 35.47
Premature (%)	21.66 ± 2.93	28.55 ± 2.68
Correct Latency (s)	0.83 ± 0.06	0.98 ± 0.09 *
Magazine Latency (s)	1.34 ± 0.04	1.29 ± 0.05

Within-subjects comparison of performance before and after PCP treatment during vITI challenge

Following repeated measures ANOVA on premature responding made during the sessions, a Treatment*Day interaction was observed [$F_{(1,18)}$=6.42, p<0.05]. Planned Comparisons demonstrated a strong PCP-induced trend towards a significant increase in premature responding (p=0.07), compared to pre-treatment performance (table 7.6). No effect was observed for control animals. Between-subjects analysis demonstrated no significant Treatment effect [$F_{(1,17)}$=2.92, NS] existed following sub-chronic PCP treatment for premature responding (table 7.7).

No within-subject Treatment effect [$F_{(1,18)}$=0.65, NS] or a Treatment*Day interaction [$F_{(1,18)}$=2.76, NS] was observed when correct latency (CL) was analysed (table 7.6). However, Planned Comparisons indicated a significant increase in CL in PCP treated animals (p<0.05), when CL was compared to pre-treatment performance. No between-subject Treatment effect [$F_{(1,17)}$=2.59, NS] was observed following ANCOVA analysis on CL (table 7.7).

No Treatment [$F_{(1,18)}$=0.08, NS] or Treatment*Day interaction [$F_{(1,18)}$= 0.9, NS] was observed when the magazine latency was analysed (table 7.6). Additionally, there was no significant between subjects effect on ML, post-treatment [$F_{(1,17)}$=1.01, NS] (table 7.7).

Table 7.7. 5-CSRTT performance during vITI challenge session following sub-chronic PCP treatment

Parameter	Saline	PCP
Perseverative (n)	130.20 ± 16.59	171.70 ± 35.47
Premature (%)	24.26 ± 2.80	28.55 ± 2.68
Correct Latency (s)	0.84 ± 0.07	0.98 ± 0.09
Magazine Latency (s)	1.34 ± 0.09	1.29 ± 0.05

Between-subjects comparison of treatment groups during vITI challenge session following sub-chronic treatment

These results show that while a PCP-induced impairment was evident following a vITI challenge, it appears as if the deficit was not as substantial as that which followed the vSD challenge. A PCP-induced reduction in response accuracy was observed, specifically mediated by an increase in incorrect responding. The attentional impairment was coupled with a between-subject slowing of CL. Although these effects are perhaps less substantial than those observed during the vSD challenge, there is consistency in that the effects again display a high level of behavioural specificity.

7.3.4.1 Effect of Inter-Trial Interval

Comparable with the vSD challenge, it was important to display the effect that each ITI duration had on task performance. Repeated measures ANOVA of response accuracy indicated a significant Treatment effect [$F_{(1,18)}$=4.90, $p<0.05$] and a significant ITI effect [$F_{(3,54)}$=8.58, $p<0.001$] (fig 7.9A). No Treatment*ITI interaction was observed [$F_{(3,54)}$=1.08, NS]. Planned Comparisons demonstrated PCP reduced response accuracy when animals were presented with a 2 s ITI only ($p<0.05$).

There was no Treatment effect [$F_{(1,18)}$=2.48, NS] when percent correct responding was assessed (fig 7.9B). A significant ITI effect [$F_{(3,54)}$=26.22, p<0.001] was observed without a significant Treatment*ITI interaction [$F_{(3,54)}$=0.47, NS].

However, a significant Treatment effect [$F_{(1,18)}$=5.09, p<0.05] was evident for percent incorrect responding (fig 7.9C). This occurred independently of an ITI effect [$F_{(3,54)}$=1.92, NS] or a Treatment*ITI interaction [$F_{(3,54)}$=1.15, NS]. Planned Comparisons demonstrated that PCP treatment significantly increased incorrect responding compared to control animals, an effect only evident during the 2 s ITI (p<0.05).

There was no Treatment effect [$F_{(1,18)}$=0.16, NS] when the effect on omissions was analysed (fig 7.9D). An ITI effect was evident [$F_{(3,54)}$=17.56, p<0.001], without a Treatment*ITI interaction [$F_{(3,54)}$=0.3, NS].

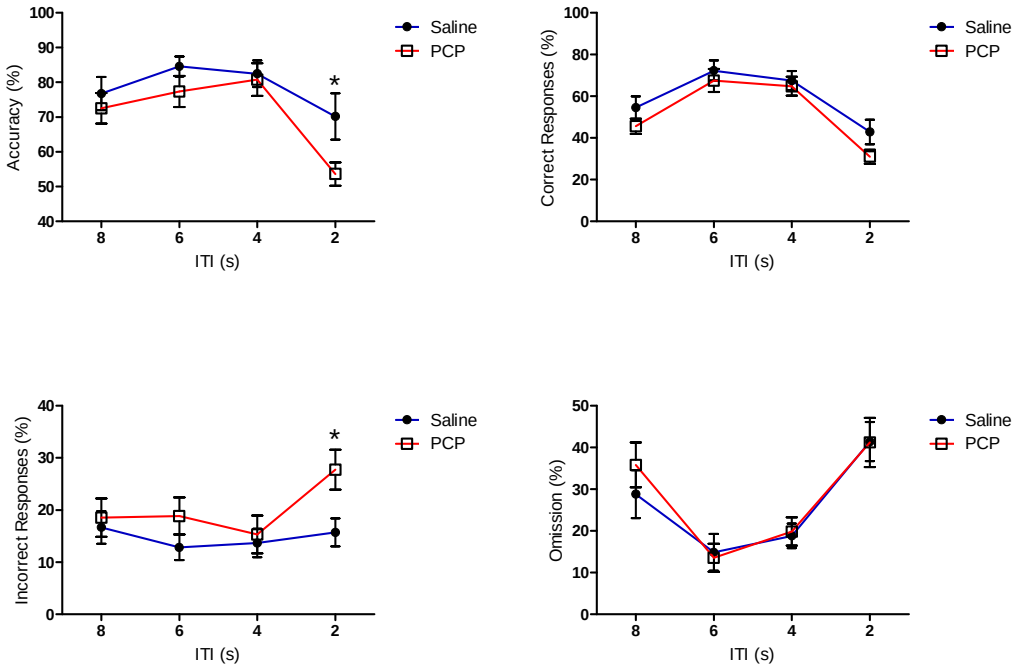

Fig 7.9. – 5-CSRTT performance displayed as function of inter-trial interval. Measures displayed include response accuracy (A), percent correct responding (B), percent incorrect responding (C) and percent omissions (D). * denotes significant difference (p<0.05) between saline (n=10) and PCP (n=10) animals when presented various ITIs

These results demonstrate that, during the vITI challenge session, PCP-induced performance impairment was evident only during the shortest ITI. This may explain why the the overall performance deficit was less substantial than that observed following this vSD challenge session. Extreme deviations from the norm (5 s ITI) resulted in impaired performance in both control and PCP animals (e.g. reduction in percent correct and increased omissions). Incorrect responding was only affected in PCP-treated animals at the shortest ITI and so a PCP-induced reduction in response accuracy was only evident following the 2 s ITI.

7.3.4.2 Performance Throughout vITI Challenge Session

Finally, the performance following the vITI challenge was assessed across the session, spilt into two trial bins. A Treatment effect [$F_{(1,16)}$=11.38, p<0.01] was observed when response accuracy was assessed across the session (fig 7.10A). This occurred independently of a Trial effect [$F_{(1,16)}$=1.05, NS] or a Treatment*Trial interaction [$F_{(1,16)}$=2.35, NS]. A trend towards a PCP-induced within-subjects reduction in accuracy in T2 was observed (p<0.1), but a significant reduction in T2 was observed between PCP and control animals (p<0.01).

A strong trend towards a Treatment effect [$F_{(1,16)}$=4.22, p = 0.056] was observed when percent correct responding was assessed. No Trial effect [$F_{(1,16)}$=0.04, NS] or Treatment*Trial interaction [$F_{(1,16)}$=2.20, NS] was observed (fig 7.10B). Comparisons demonstrated that no significant within-subjects effect was evident, but a significant effect between control and PCP animals was evident in T2 (p<0.05).

When the effect on incorrect responses was analysed, a significant Treatment effect [$F_{(1,16)}$=10.23, p<0.01] was observed (fig 7.10C). This occurred without a Trial effect [$F_{(1,16)}$=1.12, NS] or a Treatment*Trial interaction [$F_{(1,16)}$=1.13, NS]. Consistent with the response accuracy and correct responding impairment, a significant effect was only observed

in T2 between control and PCP animals ($p<0.01$). No within-subject effect was observed in either control or PCP animals, comparing T1 with T2 performance. Again, despite the PCP-induced attentional defict observed following the vITI challenge, omissions were unaffected. No significant Treatment [$F_{(1,16)}=0.33$, NS] or Trial effect [$F_{(1,16)}=0.27$, NS] were revealed when the number of omissions were analysed (fig 7.10D). Additionally, there was no significant Treatment*Trial interaction [$F_{(1,16)}=1.28$, NS].

Thus, in a similar manner to that observed following the vSD challenge, a PCP-induced deficit was only evident in the second trial bin. Along with response accuracy and incorrect responding deficits, a PCP-induced reduction in correct responding was also observed. The lack of an effect in this measure when overall performance was displayed may be the result of the subtlety of this reduction, which was masked when the overall performance was displayed.

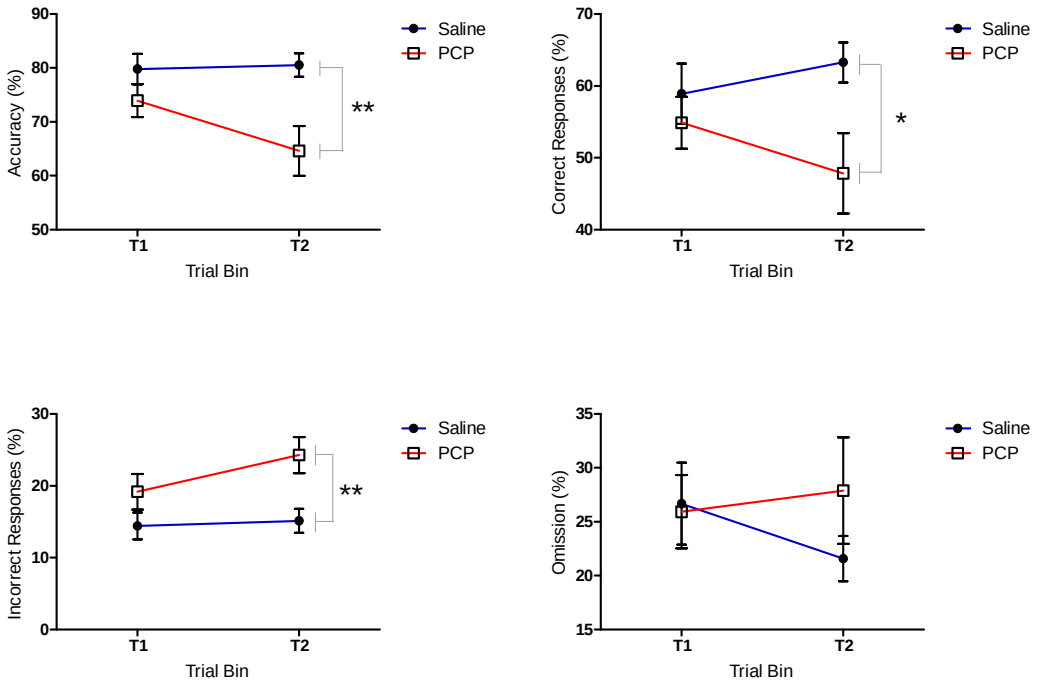

Fig 7.10. 5-CSRTT session performance following vITI challenge. Measures displayed include response accuracy (A), percent correct responding (B), percent incorrect responding (C) and percent omissions (D). T1 reflects first 50

trials of the session and T2 the last 50 trials.. * and ** denotes a significant difference (p<0.05 and p<0.01, respectively) between saline (n=10) and PCP (n=10) treated animals in T2.

7.3.5 Pre-pulse Inhibition

Analysis of startle reactivitly indicate no difference in PPI between treatment groups [$F_{(1,18)}$=2.18, NS] and no Group*PPI interaction [$F_{(2,36)}$=0.66, NS]. However, a significant PPI effect was observed [$F_{(2,26)}$=22.57, p<0.001]. Planned Comparisons indicated that a pre-pulse of 12 dB significantly inhibited the startle response (fig 7.11A). This effect was evident in both animals that were to receive saline (p<0.001) and those that were to receieve PCP (p<0.01). Following sub-chronic PCP treatment there was no significant Treatment effect [$F_{(1,18)}$=0.12, NS] or a Treatment*PPI interaction [$F_{(2,36)}$=0.58, NS]. Consistent with pre-treatment startle assesment, a significant PPI effect was observed [$F_{(2,36)}$=29.35, p<0.001]. This was attributed to a significant attenuation of the startle response following a 12 dB pre-pulse in both saline (p<0.001) and PCP (p<0.001) treated animals (fig 7.11B). A pre-pulse of a lower magnitude had no significant effect on inhibition of the startle response.

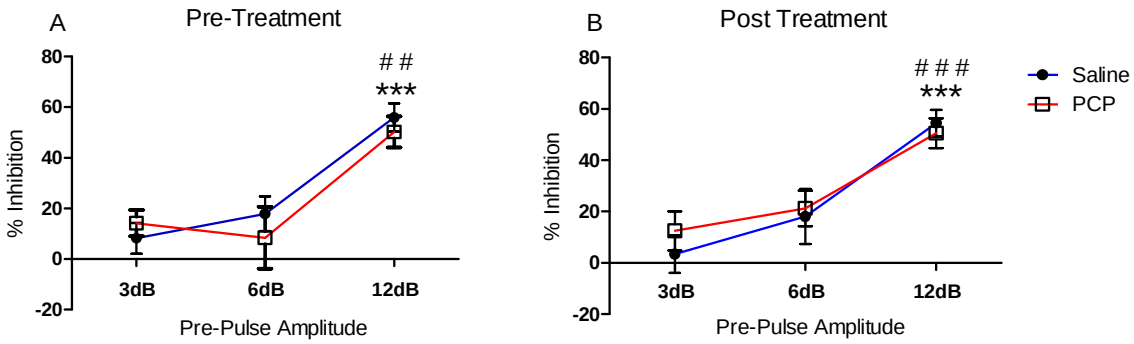

Fig 7.11. Pre-pulse inhibition of the startle response before animals underwent sub-chronic treatment (A) and following drug treatment (B). The level of PPI was demonstrated by percent inhibition. *** denotes p<0.001 in saline (n=10) animals and ## denotes p<0.01 and ### denotes p<0.001 in PCP (n=10) animals (12 dB compared with 3dB).

7.4 Discussion

These data demonstrate that morphological abnormalities are evident in rats treated sub-chronically with PCP. Structural MRI revealed an increase in MRI signal in discrete cortical and sub-cortical brain regions following sub-chronic PCP treatment. In addition to structural abnormalities, a persistent attentional disruption was observed. In the drug-free state at least 13 days after cessation of treatment, 5-CSRTT performance was impaired in a manner characteristic of disrupted sustained attention. The PCP-induced attentional deficit was exemplified by a reduced ability to sustain performance throughout the whole session, when the attentional load had been increased. In contrast, startle responsivity and the inhibition of the startle response was unaffected following PCP treatment. These findings reveal, for the first time, that sub-chronic PCP treatment produces structural abnormalities in brain regions associated with attentional performance, abnormalities that are evidently sensitive to detection with MRI imaging. These structural abnormalities may contribute to the observed sustained attentional impairment, but importantly, appear to be dissociable from deficits in passive attentional pre-processing.

Structural MRI identified regional abnormalities following systemic PCP treatment, localised to the prelimbic cortex, sub regions of the hippocampus and presubicular structures, and the dorsal endopiriform cortex. These findings suggest that PCP treatment results in regionally specific, morphological aberrations. The hippocampus has been suggested to play an important role in cognitive processes, and is intrinsically involved in aspects of memory (Kesner and Dakis 1993; Eichenbaum 2004; Gimbel and Brewer 2011). As such, structural deficits localised within the hippocampal formation, bordering with the subicular regions, may contribute to the persistent performance impairments in the novel object recognition task (NOR) observed following sub-chronic PCP treatment (Grayson et al. 2007; McLean et al. 2009; Idris et al. 2010). With respect to the attentional disruption observed, structural

abnormalities within the medial prefrontal cortex (specifically, the prelimbic cortex sub-region) were evident. The mPFC cortex has been hypothesised to be involved in the PCP-induced attentional disruption. Indeed, the mPFC has been suggested to play a pivotal role in many aspects of cognition in rats, humans and schizophrenia patients (Miller 2000; Uylings et al. 2003; Lesh et al. 2010). Therefore, PCP-induced structural abnormalities within the prelimbic cortex may be central to the persistent cognitive deficits that follow sub-chronic PCP administration (Neill et al. 2010).

Voxel-based morphometry (VBM) is an incredibly powerful tool, enabling the assessment and evaluation of neurological disease *in vivo*. It allows whole brain morphology to be assessed, without being bound by a region of interest approach (Job et al. 2002). MRI can be conducted as a recovery procedure, facilitating the visualisation of structural morphology in live animals. Alternative approaches, such as confocal microscopy, histological techniques or electron microscopy, are all terminal procedures. Animals are culled, brains harvested and treated, prepared and the procedure conducted. Consequently, MRI is the only imaging technique that enables volumetric differences, following drug treatment, to be assessed within a longitudinal design.

However, a number of potential caveats or pitfalls exist potentially limiting its application and between-study comparison (Whitwell 2009), limitations that warrant further discussion. These restrictions are particularly pertinent when comparing clinical and preclinical findings. VBM involves a number of processing steps in order to generate and analyse the images acquired, which include registering, segmenting and smoothing the images. Registering the images ensures all the voxels from each subject are aligned to the same sterotaxic position, known as spatial normalisation. This produces a template image, matching all images used in the study, accounting for regional volume variability between subjects within the sample. Voxels are then segmentated into differing tissue compartments, depending on whether the

voxel contained grey matter, white matter, or CSF. The images are then smoothed. The smoothing process ensures that data conforms to the Gaussian field model of variance. Smoothing the images increases the sensitivity of detecting a change by reducing the variance between subjects (Ashburner and Friston 2000; Whitwell 2009). While these processing steps are essential for the construction and analysis of VBM images, one or all of these steps can vary slightly between studies, introducing potential confounds that may hinder accurate comparisons (Whitwell and Jack 2005). In addition, there is no conventional threshold for significance. The final interpretation of a study may depend on the *p* value assigned to each analysis, or what method was used to correct for multiple comparisons. Due to the extraordinarily high number of comparisons made (comparison of millions of voxels), safeguards to protect against type 1 error are necessary. Two common approaches used are the family-wise error (FWE) or false discovery rate (FDR). However, Whitwell (2009) demonstrated that depending on the statistical threshold and method used to correct for multiple comparisons, even within the same subjects, differing conclusions could be drawn. The FWE is a conservative approach, whereas the FDR is more lenient and therefore depending on the approach used, different patterns of grey matter changes could be observed. The increase in MRI signal demonstrated in the current study survived a weak correction for multiple comparisons, but not when a more stringent approach was used if the whole brain was used. However, alterations in the prelimbic cortex did survive more stringent corrections ($p<0.05$ following FDR or FWE) when a small correction volume was applied to the frontal cortex. The small volume correction is appropriate, given the *priori* hypothesis that changes would be evident within the frontal cortex. However, it was not appropriate to apply a small volume correction to the sub-cortical regions given the absence of a *priori* hypothesis for alterations within these regions, and therefore they may reflect a type 1 error.

Furthermore, in addition to differences in how the data are compiled and analysed, giving rise to potential confounds in human studies, differences in the subjects selected can also contribute to a source of heterogeneity amongst studies (Whitwell 2009). Factors such as sample size, age, gender, IQ scores, and handedness in either the control or disease population can introduce subtle differences within the morphology of the sample, altering the homogeneity of the cohort under investigation. Moreover, aspects such as disease stage (chronic Vs first episode psychosis), treatment history, substance abuse, traumatic head injury or pathological comorbidity may also play a pivotal role in the final interpretation of imaging studies. While the factors regarding sample selection may provide a source of variability, they can also be carefully guarded against with a comprehensive selection criterion and matching process. Nevertheless, they may, along with methodological differences, potentially contribute to the inconsistencies demonstrated within the clinical imaging literature (Honea et al. 2005). An advantage of preclinical investigations is the high control of subject factors, enabling a level of homogeneity within the subject sample that would be difficult to rival in a clinical setting. Hence, influences of previous exposure to antipsychotic medication, poly drug abuse or disease stage can be eliminated as a source of variance within the current study. The level of homogeneity coupled with the control of confounding factors within preclinical research has exciting potential. For the first time, the ability of a drug to alter brain morphology and induce persistent cognitive deficits has been demonstrated in a longitudinal manner. This enabled the precise elucidation of regional specific structural deficits and the development of cognitive disturbances to be visualised, *in vivo*.

The prefrontal cortex (PFC) receives afferent connections from multiple areas and also projects efferent fibres to a number of cortical and sub-cortical structures (Groenewegen et al. 1990). The prelimbic cortex, a subdivision of the PFC, has been suggested to innervate a number of structures located within the limbic system, generating circuitry that is involved in

the mediation of cognitive processes (Vertes 2004). Some controversy exists regarding trans-species homology of the frontal cortex (Kolb 1990; Preuss 1995), questioning the existence of comparable primate dorsolateral prefrontal cortical structures in non-primate species. However, due to reciprocal connections and more importantly, the functionality of the rat PFC (including the prelimbic sub-region), it has been suggested that this region shares analogy with the DLPFC in primates (Ongur and Price 2000; Brown and Bowman 2002; Vertes 2004). The DLPFC is suggested play a crucial role in human cognitive control (Miller and Cohen 2001; Lesh et al. 2010). Moreover, the DLPFC has consistently been implicated in cognitive disturbances in schizophrenia (Callicott et al. 2000; Cannon et al. 2005; Yoon et al. 2008; Lesh et al. 2010), and attentional performance (MacDonald and Carter 2003). With respect to the findings in the current chapter, the prelimbic cortex in rats has previously been demonstrated to be intrinsically involved in attentional processing. Various pharmacological or lesion studies have indicated this regions' involvement in performance of visuospatial attentional tasks, indicating prelimbic mediation of sustained attention (Granon et al. 1998; Broesrsen and Uylings 1999; Granon et al. 2000).

PCP treatment may have induced regional specific structural abnormalities within the prelimbic cortex due to specific disinhibition of the GABAergic interneurons located within this region. This population of interneurons provide local inhibitory tone to the excitatory pyramidal cells (Lopez-Gil et al. 2007). Extensive GABAergic deficits have been observed following PCP treatment (Abdul-Monim et al. 2007; Jenkins et al. 2008; Amitai et al. 2011). Excitatory disinhibition may contribute to morphological abnormalities observed following NMDA antagonism (discussed in detail in chapter 8). Additionally, neuroimaging studies in human volunteers have demonstrated specific neuronal activation of the PFC following systemic ketamine infusion (Breier et al. 1997), but no significant effect on metabolic activity in other regions. These findings suggested that systemic ketamine administration results in a focalized activation of the prefrontal cortex in humans. Investigation of the effect systemic

PCP treatment has on neuronal activity in experimental animals' revealed dramatic activation of prefrontal cortical neurons. The vast majority of cortical neuronal activation, demonstrated by increased *c-fos* expression or extracellular electrophysiology recordings, was contained within the prelimbic cortex (Kargieman et al. 2008). Furthermore, neurotoxicity arising from high concentrations of NMDA antagonists was restricted to corticolimbic structures (Olney et al. 1989). This apparent regional selectivity following systemic PCP administration may provide an explanation to why cortical structural abnormalities were localised to the prelimbic cortex, despite the ubiquitous distribution of NMDA receptors (Petralia et al. 1994). It should be noted, however, it is possible that structural abnormalities were evident in additional regions, but were below the conservative statistical threshold of detection used within this study.

Sub-chronic PCP treatment resulted in enduring performance deficits within the 5-CSRTT. For the first time, a subtle impairment in 5-CSRTT functioning was described when animals were tested using baseline attentional demands. In this instance, PCP treatment resulted in a reduction in percent correct and an increase in the number of omissions. These effects were coupled with an increase in correct latency, but a reduction in magazine latency. These findings could suggest that animals had impaired attentional functioning, which manifested as target trials not being detected, and therefore omitted. The effect on response latencies suggested impaired speed of cognitive processing. As the time taken to collect the food reward was in fact reduced, impaired locomotor or reduced motivation was unlikely. As impaired locomotor abilities or sedation was ruled out, increased omissions (and consequent reduced correct responding) was likely mediated by attentional deficits. However, these effects were subtle and only detected following within-subjects analysis. Repeated measures analyses are generally more sensitive than a between-subjects approach as it eliminates intra-animal variability, therefore enhancing the statistical power of the design (Lambdin and Shaffer 2009). Previous investigations in this thesis utilising a sub-chronic PCP treatment

regimen (chapters 3 and chapter 6) assessing attentional performance were designed using a between-subjects approach. This may account for the lack of statistical significance when attentional performance was investigated previously – even when a comparable PCP dose was used (chapter 6). The only effect that was evident following a between-subjects approach when animals were tested using the standard parameters was an increased latency to make a correct response. Again, this was not evident in previous investigations, but this may have again been due to the current experimental design utilised. The design enabled an analysis of covariance to be conducted taking previous animal performance into consideration, which also enhanced the statistical power of the between-subjects comparisons (S Bate, Personal Communication). The increased CL may indicate a speed/accuracy trade off (Robbins 2002; Young et al. 2009a), whereby the animals may have been sacrificing the speed of responding to ensure a higher accuracy facilitating maximum reward. This was also supported by the lack of incorrect responding. Although, it should be noted, as these effects represent performance immediately following sub-chronic treatment (during which animals received no training or testing), they may reflect subtle alterations in the ability to resume a previously learned task. As such, although some significant effects were observed, caution should be exercised when drawing conclusions about their meaning.

Task-related interventions are an accepted technique of further taxing attentional demands, depending on the parameter manipulation utilised. When the stimulus duration was variably shortened (vSD), more robust performance impairments were revealed. PCP treatment induced a significant reduction in response accuracy, percent correct responding and significantly increased incorrect responding. As PCP treatment had no adverse effects on response latencies (particularly ML), elements of motor impairment or impaired motivation did not contribute to erroneous responding. Thus, sub-chronic PCP treatment resulted in a robust impairment in 5-CSRTT performance indicating attentional disruption. It has previously been suggested that animals trained with a constant ITI utilise a temporally

mediated strategy to respond to stimuli within the 5-CSRTT (Spratt et al. 2000). Therefore, in the event of failed target detection, the animal was likely to exploit a guessing strategy. This type of responding could explain the increased incorrect responding and the lack of PCP-induced increase in omissions. Once the ITI had elapsed (and the animal had missed the stimulus presentation), a guessed response was more likely to be incorrect than correct. Therefore responding when the stimulus was not detected increased incorrect responding and reduced correct responding and response accuracy. These effects are consistent with attentional impairment (Robbins 2002).

Performance displayed as a function of SD demonstrates performance was impaired, even in control animals, as the SD was reduced. This supports the notion of utilising the vSD challenge as a means to tax attentional processing. PCP-induced performance impairment was restricted to the longer SD only. Response accuracy and percent correct responding was significantly reduced, compared to saline animals, when the animals were presented with the 1 and 0.75 s SD. PCP-treatment increased the number of incorrect responses made during 1, (a strong trend with 0.75 s) and 0.5 s SDs. The lack of effect at the shorter SDs can be attributed to the impaired performance in control animals. Previous experiments have shown performance was reduced not only in lesioned rats, but also in control animals when the SD was reduced to 0.25 s (Muir et al. 1996). While the performance of PCP-treated animals is impaired as the SD is shortened, the effect is more substantial in control animals. It is possible that when faced with the vSD challenge, PCP-treatment resulted in a performance deficit regardless of the SD. With the longer SD, the duration was sufficient for control animals to successfully detect their presentation whilst PCP treated animals could not, and therefore a significant difference emerged. However, when the SD was reduced, control animals' performance was impaired to that of PCP-treated animals; hence no performance deficit was detected.

When performance was assessed over the duration of the session, PCP-induced impairments were restricted to the latter half of the session. Response accuracy, percent correct responding and percent incorrect responding were all impaired following PCP treatment, in trial bin two. These data suggest that PCP treatment induced a performance decrement, resulting in the inability to sustain attention for the duration of the session. This observation may explain why performance was robustly disrupted at the longer SD during this challenge session, but not during the standard parameter session (also 1 s SD). Initial performance of PCP animals during the vSD challenge session was no different to control animals. However, the vSD challenge would increase the overall attentional load of the task, as the shorter SDs would require a higher level of attention to detect (Bushnell 1998). PCP treatment may result in animals being more sensitive to cognitive fatigue when attentional demands are elevated. Thus, PCP-treated animals began to display attentional impairments as the session progressed, consistent with a reduced ability to maintain sustained attentional abilities throughout the entirety of the session. This effect was absent in control animals, suggesting that although performance was impaired following the vSD challenge, the control animals' could maintain attentional processing throughout the duration of the session.

Previous investigations utilising neurotoxic lesions of the prelimbic cortex that assessed attentional performance reveal encouraging findings (Muir et al. 1996; Broersen and Uylings 1999 Chudasama and Muir 2001). Although lesion effects on response accuracy were absent, an increase in omissions was observed without concomitant alterations in magazine latency, therefore suggesting attentional disruption (Chudasama and Muir 2001). The effect of prelimbic lesions was also assessed in the sustained attention task (SAT). The authors had implemented non-parametric signal detection analysis as a means to assess vigilance, which demonstrated reductions in signal sensitivity following prelimbic cortex lesion when the stimulus duration of the target signal was reduced (Chudasama and Muir 2001). This not only indicates that the prelimbic cortex is involved in attentional performance, but further

highlights the need in some cases of increasing attentional demands to elucidate impaired performance. While neurotoxic lesion studies implicate the prelimbic cortex in attentional performance, pharmacological investigation has demonstrated the importance of not only the prelimbic cortex, but also dopaminergic transmission and attentional performance (Granon et al. 2000). Therefore, enduring abnormalities in the prelimbic cortex following sub-chronic PCP treatment may mediate the robust disruption of performance when the attentional load was increased in the vSD challenge session. Moreover, reductions in prefrontal basal dopamine levels following sub-chronic PCP treatment (Jentsch et al. 1997b) may also contribute to the cognitive impairment observed in the current chapter.

Perseverative responding was not affected within the current study, which is in contrast to increased perseveration following neurotoxic lesion to the prelimbic cortex (Chudasama and Muir 2001). However, perseverative responses were not punished in the current study and merely recorded. Previously, studies have demonstrated that when premature responding was monitored without consequence the baseline was exceptionally high (Auclair et al. 2009). As there was no TO period following an inappropriate premature response, it removes the incentive to withhold inappropriate responses. This, therefore, changes the nature of the response making direct comparisons with studies that do punish inappropriate responding difficult (Amitai and Markou 2010). The lack of PCP-induced perseverative responding in the current study may be due to a similar effect observed by Auclair and colleagues (2009). The high baseline level of perseverative responding within the current experiment demonstrates that the incentive to withhold from inappropriate perseveration had been removed.

In support of the attentional disruption observed following a vSD challenge session, impaired performance was also demonstrated following a vITI challenge session. PCP-treated animals exhibited reduced response accuracy. Impaired accuracy was primarily mediated by an

increase in the number of incorrect responses made throughout the session. While the reduction of percent correct responding in PCP-treated animals lacked statistical significance, there was a trend towards a treatment-induced reduction which would have contributed to the reduction in response accuracy. Again, these effects occurred without any alteration in the number of omissions made throughout the session. Like the vSD challenge, the lack of an effect on omissions, coupled with the increase in incorrect responding suggests animals were utilising a guessing strategy following failed stimulus detection (Spratt et al. 2000). In addition to the disruption of attentional measures of the 5-CSRTT, sub-chronic PCP treatment also significantly increased the CL following within-subjects analysis. As this increase in time taken to initiate a response occurred independent of impairments in motivation or sedation, increased CL was likely mediated by impaired speed of informational processing (Amitai and Markou 2010).

When performance was assessed for each of the four ITI durations used, a PCP-induced impairment was only evident during the 2 s ITI. When presented with a 2 s ITI, response accuracy was reduced, a reduction mediated by increased incorrect responding. In a similar fashion to the vSD challenge, the vITI challenge impaired performance that was restricted to the second trial bin. In addition to the reduced accuracy and increased incorrect responding, a PCP-induced reduction in percent correct responding was also revealed in T2. The lack of impairment in this measure when displayed as overall session performance was possibly due to the PCP-induced effect being more subtle that that observed in response accuracy and incorrect responding. When the overall performance was displayed, the effect on correct responding was masked, something not observed with response accuracy or incorrect responding. Thus, a PCP-induced deficit in correct responding emerged during the second trial bin. These findings further suggest that PCP treatment induced a reduced ability of sustaining attention throughout the whole session, consistent with the vSD challenge session. Moreover, no effect on omissions was observed providing further evidence for the

utilisation of a temporal strategy being implemented. Furthermore, these findings support the need for assessing performance across the session in order to reveal subtle performance deficits. The observation of impaired performance during the high event-rate condition (2 s ITI) and performance decrement are in agreement with those demonstrated by Dalley and colleagues (2004b). The group revealed that mPFC cholinergic lesion (which included the ventral prelimbic cortex) resulted in 5-CSRTT performance impairment, which was particularly evident not only during conditions of an increased attentional load, but also as the session progressed, with performance deficits only apparent in the latter stage of the session. These findings further support the notion that the prefrontal cortex is involved with the mediation of sustained attentional performance throughout the whole session and the adaptation to increased attentional demands. PCP-induced abnormalities within the prelimbic cortex are likely to contribute to the inability to sustain accurate performance for the required duration during attentionally challenging sessions.

While an enduring impairment was observed in the 5-CSRTT, there was no treatment effect on inhibition of a startle response. Pre-pulse inhibition (PPI) of an acoustic startle response is a measure of sensorimotor gating and is consistently shown to be impaired in schizophrenia patients, as well as their non-symptomatic relatives (Weike et al. 2000; Braff et al. 2001). NMDA antagonism has been shown to impair the pre-pulse inhibition (Geyer et al. 2001; Scwabe et al. 2005), impairing the diminution of the startle response when the full stimulus is preceded by a non-startling pre-pulse. However, PPI disruption is absent in animals following long-term exposure to PCP (in the drug-free state), despite persistent neuropathological changes following repeated exposure (Martinez et al. 1999). While PPI impairment were observed 24 hours following chronic PCP treatment, impairment dissipated by 48 hours, despite persistent cognitive impairment being observed in the form of impaired extra-dimensional shift (EDS) in the attentional set-shifting task (Egerton et al. 2008). These findings, along with the lack of PPI impairment in the current chapter, suggest that sub-

chronic PCP treatment does not result in persistent functional, structural or neuropathological abnormalities that underpin sensorimotor gating deficits. The enduring cognitive effects observed by Egerton and colleagues (2008) however, may be the result of PCP acting on the prelimbic cortex. Lesion of the mPFC, (including the prelimbic cortex) produced impairments in EDS in animals assessed in the attentional set-shifting task (Birrell and Brown 2000). The susceptibility of prelimbic cortex to PCP-induced structural abnormalities may also contribute to the persistent impairment in attentional set-shifting observed.

The observation in the current chapter that sub-chronic PCP treatment impairs 5-CSRTT performance upon an increased attentional load (sustained divided attention), along with the previous demonstration that EDS impairment is evident (selective attentional shifts) (Egerton et al. 2008; McLean et al. 2008), but not PPI deficits suggests a dissociation in PCP-induced attentional disruption. PPI is an involuntary process describing passive attentional pre-processing, filtering incoming sensory information. In contrast, the attentional modalities susceptible to PCP-induced disruption reflect higher-order cognitive processes. As such, morphological abnormalities within the prelimbic cortex may impede the ability to conduct these voluntary processes, without affecting the ability to actively attend to or filter incoming sensory information. Moreover, sub-chronic PCP-induced deficits, when observed, are only evident during attentionally challenging aspects of a task. Deficits in attentional set-shifting occur only during the EDS phase, and not during the intra-dimensional shift (IDS) (McLean et al. 2008). Additionally, sustained attention deficits are only revealed when the attentional load of the task is increased, and are particularly evident as the session progresses. Thus, as PPI is an involuntary pre-processing measure and PCP-induced deficits are only apparent during demanding challenges, this may account for the distinction in passive and spatial sustained attentional deficits observed in the current chapter. Startle responsivity was assessed in the drug-free state, confirming this treatment regimen is not suitable for

investigating PPI deficits. In contrast, NMDA antagonism does result in robust PPI deficits, whilst animals are under the influence of the drug. This is elegantly demonstrated by Martinez and colleagues (1999) who report chronic PCP treatment produced PPI impairment only immediately following administration (10 minutes), but not following a substantial delay (6 hours). While the absence of a PPI deficit after 6 hours is in contrast to that observed by Egerton and co-workers (2008) who demonstrated impaired PPI up to 24 hours post-treatment, a different PCP treatment regimen was utilised in the two studies, which may have differentially altered dopaminergic transmission thus accounting for discrepancies in the duration of PPI deficits. It is well documented that acute exposure to NMDA antagonism induces pronounced increase in dopaminergic efflux (Moghaddam et al. 1997). Thus, excessive dopaminergic activity may be one mechanism pivotal to disrupted inhibition of startle reactivity. In support, it has previously been demonstrated that dopaminergic agonists, or drugs that promote dopaminergic transmission, (e.g. amphetamine, cocaine, and apomorphine) disrupt startle inhibition (see Geyer et al. 2001 for full review). In contrast to acute NMDA antagonism, a pronounced *reduction* in basal cortical dopamine levels following sub-chronic PCP treatment is seen (Jentsch et al. 1997a). This reduction in basal dopamine transmission may explain the lack of PPI deficit observed in the current chapter. However, decreased cortical dopaminergic activity, coupled with prelimbic cortical abnormalities, may contribute to the 5-CSRTT deficits observed following an increased attentional load.

In summary, these data demonstrate, for the first time, that sub-chronic PCP treatment induces regional-specific morphological brain abnormalities. These structural abnormalities were accompanied by selective, dissociable attentional impairments; sustained attention was disrupted following a task-related intervention yet passive attentional pre-processing was unaffected. These findings revealed that sub-chronic PCP treatment impairs cognitive processing, and more importantly, displays regional selectivity for brain areas associated with schizophrenia pathophysiology.

Chapter 8

General Discussion

8.1 Introduction

Results presented in this thesis show that sustained visual attention is susceptible to disruption by treatment with the NMDA receptor antagonist PCP. Attentional disturbances are suggested to be core symptoms of schizophrenia, to underlie higher-order cognitive impairment, and to precede episodes of psychosis and are evident in non-symptomatic relatives (Chen and Faraone 2000). Therefore, improved understanding of the biological mechanisms that underpin attentional performance is paramount. There is a major clinical unmet need with regards to cognitive deficits, resulting in a lack of effective cognitive remediation (Goldberg and Gold 1995; Marder et al. 2004). Attenuation of the attentional disruption associated with schizophrenia may play an important role in improving the long-term outcome of the patient and facilitate improved social and occupational reintegration.

While these findings demonstrate attentional impairment following PCP treatment, the profile of PCP-induced attentional impairment is highly dependent on the treatment regimen utilised; acute administration induced a performance impairment that was coupled with a generalised response disruption, potentially precluding the interpretation of cognitive impairment. Repeated treatment resulted in a selective and substantial performance deficit, without concomitant motivational deficits, and therefore was most likely mediated by specific cognitive disruption. Performance following these treatment regimens was assessed shortly after PCP administration, while animals were under the influence of PCP. Therefore, the more pronounced effects may have been attributed to the transient effects of drug treatment. In contrast, sub-chronic PCP treatment assessed performance in the drug-free state, following a substantial washout period. While attentional processing was sensitive to disruption, the impairment observed was less pronounced and required the attentional load of the task to be increased to elucidate performance deficits.

8.2 Experimental Overview

Attentional performance was initially assessed using the 5-choice serial reaction time task (5-CSRTT), a well characterised and validated task assessing several aspects of the multifaceted construct of attention; namely sustained and divided attention (Robbins 2002). The experiments in chapter 3 demonstrated that sub-chronic PCP treatment had little effect on the animals' ability to conduct the visuospatial attentional task, in the drug-free state. The treatment regimen (2 mg/kg twice daily for 7-days) was chosen because of the long-standing demonstration that pronounced and persistent impairment in a variety of cognitive paradigms are produced (see Neill et al. 2010). Following various task-related interventions, subtle PCP-induced performance impairments were observed, indicating the potential of revealing attentional impairment in the drug-free state. The demonstration of at least a subtle performance deficit prompted the full characterisation of PCP-induced attentional disruption, utilising various treatment regimens. The outcomes of this investigation into the nature of PCP-induced attentional disruption are discussed below.

Firstly, these findings demonstrated that rats can be trained to perform a modified version of the 5-CSRTT. The modification resulted in a task that consisted of target and non-target trials, known as the 5-choice continuous performance test (5C-CPT). Despite the myriad of continuous performance tests (CPTs) used in human attentional assessments, each version conforms to the basic premise of target trials requiring a response, and non-target trials requiring the inhibition of a response. Robbins (1998) suggested that the current 5-CSRTT configuration may limit its application to accurately probe vigilance, which has been described as the ability to remain alert to incoming sensory information, discriminating salient information amongst a backdrop of irrelevant stimuli (Warm and Dember 1987; Collings 2003; Egeland et al. 2009). As such, the 5C-CPT may allow the construct of not only sustained attention, but also vigilance to be more accurately assessed in a preclinical setting,

in a manner more consistent with human studies. Schizophrenia patients consistently demonstrate impairments when performing the CPT. Patients display reduced hit rates, increased false alarm rates and thus exhibit reduced signal sensitivity (Nestor et al. 1990; Chen and Faraone 2000). PCP treatment was shown to impair signal discrimination, comparable with the impaired CPT performance exhibited by schizophrenia patients. Depending on the treatment regimen utilised, PCP treatment impaired signal discrimination, mediated by either a reduction in hit rate, increase in false alarm rate, or a combination of the two.

In addition, it was demonstrated that dopamine transmission, specifically D_1 dopaminergic transmission, was involved in attentional processing. Previous investigations have implicated the D_1 dopaminergic system and cognitive processing – however this work largely focused on the domain of memory (Sawaguchi and Goldman-Rakic 1991; Goldman-Rakic et al. 2004). The findings described in chapter 4 expand on previous findings that augmentation of the D_1 system improves attentional performance, in a baseline dependent manner (Granon et al. 2000). The use of the 5C-CPT enable the demonstration that D_1 receptor activation by the D_1-like specific partial agonist, SKF 38393, improved performance as a result of enhanced signal sensitivity. Moreover, improvement was only evident in animals whose performance had been impaired following increased attentional demands. Furthermore, this experiment also demonstrated *impaired* attentional performance during baseline attentional demands. These findings emphasise the principle of an 'inverted-U' shaped dose-response of the dopaminergic system (Vijayraghavan et al. 2007). During low attentional demands, D_1 receptor activation increased omissions and impaired speed of processing; suggesting that increased dopaminergic D_1 transmission reduced attentional abilities, under baseline conditions. In contrast, when attentional demands had been increased, reducing baseline performance, D_1 activation enhanced target detection via a reduction in omissions. This led to enhanced signal sensitivity, an effect that occurred independent of alterations in

responsivity or strategic bias. Hence, when the demands of the task were increased, activation of the dopaminergic system increased the readiness to respond, improving overall performance. The baseline-dependent improvement of 5C-CPT performance is largely in agreement with findings reported by Granon and colleagues (2000), although differences do exist. The main discrepancy in findings within chapter 4 is the absence of an improvement in response accuracy. This may be the result of methodological differences; a longer SD was used in the current study, as was a variable ITI (instead of the fixed ITI used in the Granon study). These differences may have changed the response strategy of the animals, and improved attentional performance was displayed with slight variation as a result. Moreover, SKF 38393 was systemically administered in the current investigations, whereas Granon and colleagues (2000) utilised local prelimbic cortical infusions. Furthermore, Granon and co-workers (2000) demonstrated a baseline-dependent improvement in poor performing animals. In contrast, chapter 4 revealed an improvement following a behavioural challenge. Encouragingly, however, the reduction in omissions observed following SKF 38393 treatment is consistent between studies, suggesting that DA transmission within the prelimbic cortex exerts some control of attentional performance. Another aspect of consistency between the two studies is the relative specificity of the behavioural effects. Of the common parameters that are assessed in both the 5C-CPT and 5-CSRTT, only omissions were improved. While accuracy was increased in the Granon study (2000), the effect on correct responding was not reported. Given the effect on omissions, it is likely that prelimbic infusion of SKF 38393 increased correct responding (thus increasing accuracy). While in the current investigation (chapter 4) accuracy was not improved, correct responding was increased; an effect mediated by a reduction in omissions, suggesting that SKF 38393 treatment improved 5C-CPT performance with a high level of behavioural specificity, primarily enhancing the attentional measures.

Following the successful demonstration that rats can be trained to perform the 5C-CPT, the disruptive effect of PCP treatment was investigated. For the first time, it was revealed that repeated PCP treatment disrupts 5C-CPT performance (chapter 5). These findings were in agreement with those demonstrated by Amitai and colleagues (2007), who reported disrupted 5-CSRTT performance following a repeated PCP treatment regimen. PCP treatment significantly reduced response accuracy, indicating impaired attentional functionality. This was coupled with response disinhibition, elevating the number of premature responses. The use of the 5C-CPT not only supported the observations of Amitai and colleagues (2007), but also expanded on them; demonstrating the attentional disruption was characterised by a pronounced impairment in signal sensitivity. Reductions in sensitivity index (SI) were mediated by a combination of impaired target detection (reduced hit rate) and behavioural disinhibition (increased false alarm rate). This is in agreement with the consistent clinical observation that schizophrenia patients exhibit impaired CPT performance, exemplified by disrupted signal discrimination (Nestor et al. 1990; Parasuraman, 1998; Mass et al. 2000; Baerwald et al. 2005; Wang et al. 2007; Park et al. 2010). These findings suggest that PCP treatment abolished the ability of successful discrimination between target and non-target stimuli, effects that were not confounded by alterations in motivation or strategic bias. Dysregulation of the glutamatergic system, or the consequential downstream effects, may therefore be central to the impairment in attention/vigilance observed in schizophrenia patients.

Furthermore, chapter 5 revealed that the performances deficit following repeated PCP treatment was subject to a partial reversal following D_1 receptor activation. These findings suggest that augmentation of the dopaminergic D_1 system partially attenuated the PCP-induced impairment in response accuracy, mediated by a specific amelioration of the number of incorrect responses made following PCP treatment. It has been suggested that target detection, defined as “the entry of information concerning the presence of a signal in a

system that allows the subject to report the existence of the signal by an arbitrary response indicated by the experimenter" (Postner et al. 1980) is controlled, at least partially, by cholinergic transmission (McGaughy et al. 1996; McGaughy and Sarter 1998; McGaughy et al. 2002). Therefore, the PCP-induced reduction in percent correct responding observed may be attributed to excessive cortical cholinergic transmission. Acute and repeated NMDA antagonism has been shown to elevate acetylcholine release in the prefrontal cortex (Kim et al. 1999; Nelson et al. 2002). This could account for the insensitivity of the PCP-induced reduction in percent correct responding following administration of a dopaminergic D_1 partial agonist. However, aberrant dopaminergic transmission may be involved in the generation of incorrect responses in the 5C-CPT; PCP treatment has profound effects on cortical dopaminergic transmission (Jentsch and Roth 1999; Jentsch et al. 2008). This effect may account for not only the PCP-induced increase, but also the SKF 38393-induced attenuation in incorrect responding. The dissociation of effects on correct and incorrect responding highlights the importance of fully describing the behavioural consequence of drug administration on task performance. Unfortunately, a general theme within 5-CSRTT literature is to only report the effects on response accuracy. It is therefore difficult to determine whether effects on response accuracy are driven by alterations in correct responding, increased incorrect responding, or a combination of the two. An exception to this rule is the study published by Amitai and colleagues (2007), who demonstrated that repeated PCP treatment increased incorrect responses, contributing to the PCP-induced accuracy deficit. Furthermore, the group demonstrated that the increase in incorrect responses was attenuated by chronic clozapine treatment (4.0 mg/kg/day), which may have accounted for the clozapine-induced partial attenuation of the PCP-induced reduction in response accuracy (Amitai et al. 2007). It has been suggested that clozapine may provide therapeutic efficacy by normalising prefrontal dopamine levels following PCP treatment (Elsworth et al. 2008). The beneficial effects in the SKF 38393 treated animals may be

mediated by a similar process as that induced by clozapine treatment; normalisation of PFC dopamine levels, thus restoring cognitive functioning. However, clozapine has a remarkably complex pharmacology (Coward 1992), and additional mechanisms involved in the attenuation of PCP-induced cognitive impairment cannot be ruled out. In addition to the lack of effect on the PCP-induced reduction in percent correct responding, SKF 38393 treatment had no impact on the increased omissions, suggesting these measures were mediated and impaired by another mechanism. The SKF 38393-induced improvement of the PCP-induced 5C-CPT attentional disruption may in fact be mediated by enhanced behavioural inhibition. The PCP-induced deficits in accuracy and SI (both quantitative measures of attentional performance) were only partially ameliorated in the SKF 38393 treated animals. Response accuracy and SI were improved following the reduction of incorrect responding and false alarms, respectively. The inability to correctly detect and report the occurrence of the target stimulus was unaffected in SKF 38393 treated animals. Thus, D_1 receptor activation may improve the PCP-induced attentional disruption specifically through behavioural inhibition and not improved target detection, enhancing attentional performance by reducing inappropriate responding. Interestingly, there was a delayed onset of the beneficial effects of SKF 38393 pre-treatment, with attenuation emerging after the third injection. Further work is required to elucidate the precise nature of the delayed onset of action.

Investigation into the enduring disruptive effects of PCP treatment demonstrated, for the first time, that 5C-CPT performance is sensitive to impairment in the drug-free state following a sub-chronic PCP treatment regimen (chapter 6). In contrast to the attentional disruption observed in chapter 5, baseline performance was unaffected in chapter 6; a behavioural challenge that increased the attentional load was required to elucidate the attentional deficit. A variable SD was implemented, reducing the SD than the one presented during baseline. Bushnell (1998) argued that if the duration of stimulus presentation is reduced, a greater level of attention is required in order to detect its occurrence. This was

exemplified by the reduction in vigilant performance as the SD was reduced, even in control animals. However, this effect was exacerbated in animals treated with PCP, demonstrated by the reduction in SI that was more pronounced as the SD was reduced. Interestingly, when the stimulus duration was variably shortened there were no differences between PCP treated (5 mg/kg) and control animals in the initial part of the session. However, control animals could generally sustain attentional performance throughout the entirety of the session, despite the increased attentional load. This ability was abolished in PCP treated animals and the PCP-induced deleterious performance emerged as the session progressed. These observations indicate that sub-chronic PCP treatment introduced an element of cognitive fatigue that was not evident in control animals, impairing the ability to sustain accurate performance throughout the duration of a session of increased attentional load. This effect was illustrated by the reduction in response accuracy and the amplified reduction in SI within the last trial bin. The effect of sub-chronic PCP treatment displayed behavioural specificity; attentional measures were impaired, but aspects of impulsivity were dissociated. Premature responding was unaffected yet false alarms were elevated, an effect that was also restricted to the final trial bin. The increase in false alarms was attributed to the inhibition of a reduction in erroneous responding; control animals progressively improved their performance when presented with non-target trials, whereas PCP treated animals did not. Therefore, the sub-chronic PCP treatment regimen did not induce behavioural disinhibition, but rather appeared to inhibit the development of behavioural inhibition. However, the effect on behavioural disinhibition was further dissociated as time out (TO) responding was increased. This reflects an inability to withhold inappropriate repeat responses. Thus, while premature responding was unaffected and false alarms were increased due to the reduced ability to implement a better strategy, TO responses were significantly increased following PCP treatment.

Extending the 5C-CPT demonstrated a vigilance decrement in control animals, indicated by the reduction of SI as the session progressed. This enhances the validity of the 5C-CPT for assessing the sub-division of attention. This decline in attentional performance is evident not only in mice performing the 5C-CPT (Young et al. 2009b), but is also a feature of human attentional testing (Parasuraman, 1998; Riccio et al. 2002). PCP-induced performance impairments were evident, but less pronounced than those observed during the vSD challenge session. This is most likely the result of the basic task configuration being similar to that of baseline testing, and so the initial attentional load was reduced. As a result the PCP-induced reductions in accuracy were absent, but increased perseverative and TO responding were evident. Additionally, alterations in the number or frequency of non-target trials presented initially increased the number of false alarms, reducing the SI. Importantly though, these findings demonstrated that control animals are sensitive to deteriorations in performance, consistent with the vigilance decrement observed in human studies (Mackworth 1950), thus enhancing the validity of the 5C-CPT and the assessment of vigilance in rats.

Sub-chronic PCP-induced attentional impairments were not restricted to the 5C-CPT; chapter 7 also for the first time demonstrated disruptions in 5-CSRTT performance following sub-chronic PCP treatment. Consistent with previous observations, impaired attentional function was only evident following an increase in the attentional load. As a result, challenge sessions consisting of a variable SD or variable ITI were implemented, increasing the demands of the task and elucidating a PCP-induced performance deficit. Consistent with chapter 6, PCP-treatment appeared to introduce cognitive fatigue, reducing the ability to maintain performance throughout a session of increased attentional demands. As such, the PCP-induced performance impairment was restricted to the second trial bin. Yet again, the PCP-induced performance impairment displayed remarkable behavioural specificity; attentional deficits were the most pronounced effects with aspects of behavioural inhibition left intact.

In contrast to chapter 6, inappropriate repeat responses were unaffected, even during challenge sessions. However, as discussed in chapter 7, perseverative responses while recorded, did not induce a TO period. Thus, the nature of the response was altered and the incentive to withhold responding removed. As such, a PCP-induced increase was not observed. Comparable with that observed in chapter 6, the vSD challenge session increased attentional load as a higher level of attention was required to detect and respond to the target stimulus, resulting in a reduction in performance of control animals. PCP treatment impaired performance during the longer SDs, with the absence of impairment at the shortest SD, attributable to the reduced performance of the control animals. Following the vITI challenge session, a PCP-induced impairment was only detected during conditions of a high event-rate, with an ITI of 2 s. This aspect of the challenge session impairs performance due to the continuous allocation of finite attentional resources (Dalley et al. 2004a).

Although the findings in chapter 7 demonstrate a PCP-induced performance deficit was baseline dependent, the profile of impairment differed to that indicated in chapter 6. The impaired performance in chapter 7 was primarily driven by an increase in incorrect responding, in contrast to the increase in omissions observed in chapter 6. This discrepancy in effects may have been the result of fundamental methodological differences between the two experiments. Firstly, male rats were used in chapter 7 and not females. It is possible that the PCP-induced attentional disruption manifests with subtle differences depending on gender. Secondly, and perhaps more importantly, a fixed ITI was used, in contrast to the vITI in chapter 6. It has been highlighted that rats' tend to utilise a temporally-mediated strategy within the 5-CSRTT when trained with a fixed ITI (Spratt et al. 2000), responding semi-automatically. As such, upon failed target detection it is more likely that the animal implemented a guessing strategy, which in turn is likely to increase the frequency of incorrect responses. This is supported by the paucity of alterations in omissions observed within chapter 7. When animals were trained using a variable ITI, thus minimising the development

of a temporal strategy (chapter 6), sub-chronic PCP treatment tended to increase omissions rather than incorrect responses during effortful conditions. Both measures can be the consequence of inattentiveness, but emphasise the effect basic protocol configuration may have on the ultimate impairment profile observed. Interestingly, sub-chronic PCP treatment reduced correct responding irrespective of the response strategy being employed, supporting the conclusion of attentional impairment despite methodological differences.

Importantly, in addition to 5-CSRTT performance impairments, chapter 7 indicated for the first time that sub-chronic PCP treatment can induce structural brain abnormalities that are identifiable using *in-vivo* MRI. The integrity of the prelimbic cortex, a sub-region of the mPFC suggested to be functionally analogous to the DLPFC in primates (Brown and Bowman 2002), was altered following sub-chronic PCP treatment. This effect, coupled with the baseline-dependent performance deficits suggests the prelimbic cortex may be essential in optimizing and maintaining behavioural output specifically during more effortful conditions. Furthermore, the PCP-induced attentional impairment observed in chapter 7 was dissociable between voluntary and passive pre-attentional performance; 5-CSRTT deficits were evident yet PPI was intact. With focus to sustained attention/vigilance, sub-regions of the PFC may have dissociable specificity, and mediate behavioural control that is dependent on the level of attention required. As a result, elements of sustained attention under effortful conditions are susceptible to sub-chronic PCP-induced performance deficits, whereas automatic attentional pre-processing is unaffected in the drug-free state.

8.3 Underlying Mechanisms

The investigations contained within this thesis demonstrate that sustained attention/vigilance is sensitive to disruption following PCP treatment, when assessed after a

substantial washout-period. However, these findings do not fully elucidate the mechanism involved, and therefore the precise neurobiological process underpinning the dysfunctional performance observed is still unclear. What is clear, however, is that an additional challenge is necessary to reveal the performance impairment and the prelimbic cortex is sensitive to PCP-induced morphological disturbances. While further work is needed to fully understand the mechanisms involved, based on the current findings coupled with existing evidence I will propose a working hypothesis that will attempt to explain the PCP-induced deficit in sustained attention.

Evidence suggests that the prefrontal cortex is implicated in attentional function (Dalley et al. 2004; Brennan and Arnsten 2008). The rodent medial PFC can be divided into three sub-regions; anterior cingulate cortex, prelimbic cortex and the infralimbic cortex, with neurotoxic lesion studies demonstrating regional specificity regarding the mediation of attentional performance (Muir et al. 1996; Chudasama et al. 2001; Passetti et al. 2002). A robust deficit in response accuracy is evident following neurotoxic lesion of the mPFC that includes the anterior cingulate cortex, even during baseline attentional demands (Muir et al. 1996). Conversely, lesions of the infralimbic cortex tended to increase premature responding, suggesting this sub-region is involved in the mediation of impulse control. Interestingly, lesions of the prelimbic cortex have been shown to robustly increase perseverative responding, with attentional functioning remaining intact. The effects described above demonstrate a dissociation of the functional aspects of the 5-CSRTT suggesting they are, at least in part, mediated by specific sub-regions of the PFC. However, this fractionation of performance deficits was evident when animals were assessed during baseline conditions and not following an increased attentional load. The structural brain abnormality indicated in chapter 7 was regional specific, with the prelimbic cortex the only sub-division of the mPFC subject to morphological aberration. Furthermore, the attentional deficit was baseline dependent, evident only following an increase in the attentional load.

The prelimbic cortex may therefore mediate more effortful attentional performance, being recruited specifically during attentionally demanding challenges, providing 'top-down' optimization of limited attentional resources and coordinating the maintenance of attentional goals in challenging situations. If during low attentional demands, the prelimbic cortex does not mediate attentional function, but provides the necessary input during more effortful, novel conditions (e.g. shortened SD, unpredictable stimulus presentation), this may accommodate the PCP-induced performance deficit that was only revealed upon behavioural challenge. With the structural integrity of the prelimbic cortex compromised following PCP treatment, the necessary input to coordinate behaviour was lost. However, during low attentional demands, input of the prelimbic cortex is not required for attentional output and so PCP-induced performance impairment is not evident.

A neurotoxic lesion of the prelimbic cortex impairs attentional performance in the sustained attention task (SAT) (Chudasama and Muir 2001). Importantly, the deficit was baseline dependent; signal sensitivity was unaffected during a longer SD, yet impaired when the SD was shortened. This suggests that the prelimbic cortex mediates attentional functionality in an attentional load-dependent manner, with increasing attentional demands revealing lesion-induced performance impairment. Additionally, following lesion of the prelimbic cortex and assessment in the 5-CSRTT, there was no attentional impairment when animals were tested during baseline conditions (Chudasama and Muir 2001). The lack of 5-CSRTT impairment following prelimbic cortex lesion supports the hypothesis that the prelimbic cortex is not critical in the mediation of performance during low attentional demands. When Chudasama and Muir (2001) increased the attentional load, by reducing the SD, the prelimbic cortex lesion group exhibited an increase in omissions. While lesions of the prelimbic cortex had no effect on response accuracy in this instance, as discussed previously, an increase in omissions can be interpreted as attentional impairment.

Conversely, Passetti and colleagues (2002) demonstrated baseline attentional impairment (reduced accuracy) following lesion of the ventral mPFC, which included the prelimbic and infralimbic cortex in the 5-CSRTT. However, the SD used by Passetti and co-workers was considerably shorter (0.35 s) than the current investigations, and consequently may account for the discrepancy between observations as the attentional load under 'baseline' was already increased. Moreover, following a further increase of attentional load (reducing the SD by half) animals with ventral mPFC lesion were more sensitive to attentional impairment, highlighting this regions importance in performance during demanding conditions. Muir and colleagues (1996) assessed 5-CSRTT performance at baseline and following a challenge of the attentional load. Lesions of the medial frontal cortex (which included the prelimbic cortex) produced a performance deficit during baseline conditions. Similar to that observed by Passetti and colleagues (2002), the baseline deficit could be attributed to the inclusion of regions other than the prelimbic cortex in the lesion area. When the demands of the task were increased, there was a trend towards an exaggeration of the lesion-induced deficit. These findings point towards the involvement of the prelimbic cortex and the mediation of 5-CSRTT performance during situations of increased attentional load. However, non-specific lesion or differing protocol configurations make direct comparisons with existing literature difficult.

The evidence discussed above supports a role of the rat prelimbic cortex in attentional processing, demonstrating involvement in 5-CSRTT and SAT performance in a baseline-dependent manner. Further evidence to suggest the prelimbic cortex may be involved in effortful attention comes from the indication that this region may be central to performance of the attentional set-shifting task (ASST). Lesion of the mPFC increased the number of trials to criterion in the extra-dimensional shift discrimination (Birrell and Brown 2000). Other aspects of the ASST, such as discriminations based on the same perceptual stimuli (intra-dimensional shift, IDS) or reversal learning, were not affected, suggesting the medial frontal

cortex lesion (centred around the prelimbic and infralimbic cortices) impairs the ability to shift attentional-sets. Further evidence implicating the mPFC and maintenance of effortful performance arises from lesion of the ascending dorsal noradrenergic bundle (DNAB) fibres. A consequence of DNAB lesion is depletion of NA within the mPFC (including the prelimbic cortex) that resulted in selective EDS deficits (Tait et al. 2007). While DNAB lesions also result in noradrenaline (NA) depletion of other mPFC regions, it implicates not only the prelimbic cortex, but also NA in effortful processing. The effects observed by Tait and colleagues are reminiscent of those demonstrated by Carli and co-workers (1983) who also showed that DNAB lesions deplete cortical NA levels. Carli and colleagues (1983) then demonstrated that 5-CSRTT performance was initially unaffected following DNAB lesion, yet impaired following distractions or parameter manipulations. Additional work is required to fully understand the involvement of sub-regions of the mPFC and the role of NA and attentional processes, but taken together, these findings suggest cortical NA levels and effortful attentional processing may be linked. Another aspect that warrants further investigation is the role of cortical dopaminergic transmission and attentional processing. As Granon and colleagues (2000) demonstrated, DA within the prelimbic cortex is involved in sustained attention in a baseline dependent manner. Furthermore, sub-chronic PCP treatment has been shown to reduce cortical DA (Jentsch et al. 1998), and sustained attentional deficits following sub-chronic PCP treatment are also baseline dependent (chapters 6 and 7). Thus, morphological abnormalities within the prelimbic cortex, coupled with PCP-induced DA reductions may impair sustained attention during conditions of increasing attentional load. Figure 8.1 summarises the involvement of the mPFC and attentional output, during baseline and increased attentional demands. Based on my findings, coupled with previous investigations, it was hypothesised that the susceptibility of the prelimbic cortex to structural abnormalities following sub-chronic PCP treatment impairs the optimization or modification of strategy when faced with increasing attentional

demands, impairing behavioural output specifically during these conditions. During low attentional demands, attentional performance is primarily mediated through regions other than the prelimbic cortex (e.g. anterior cingulate cortex). Therefore despite the structural abnormalities following PCP treatment, attentional performance is not affected. However, during conditions of increased attentional load, the prelimbic cortex is recruited to coordinate behavioural output and maintain finite attentional resources; with task performance less reliant on other regions of the mPFC. PCP-induced structural abnormalities under these conditions result in a reduction in attentional performance.

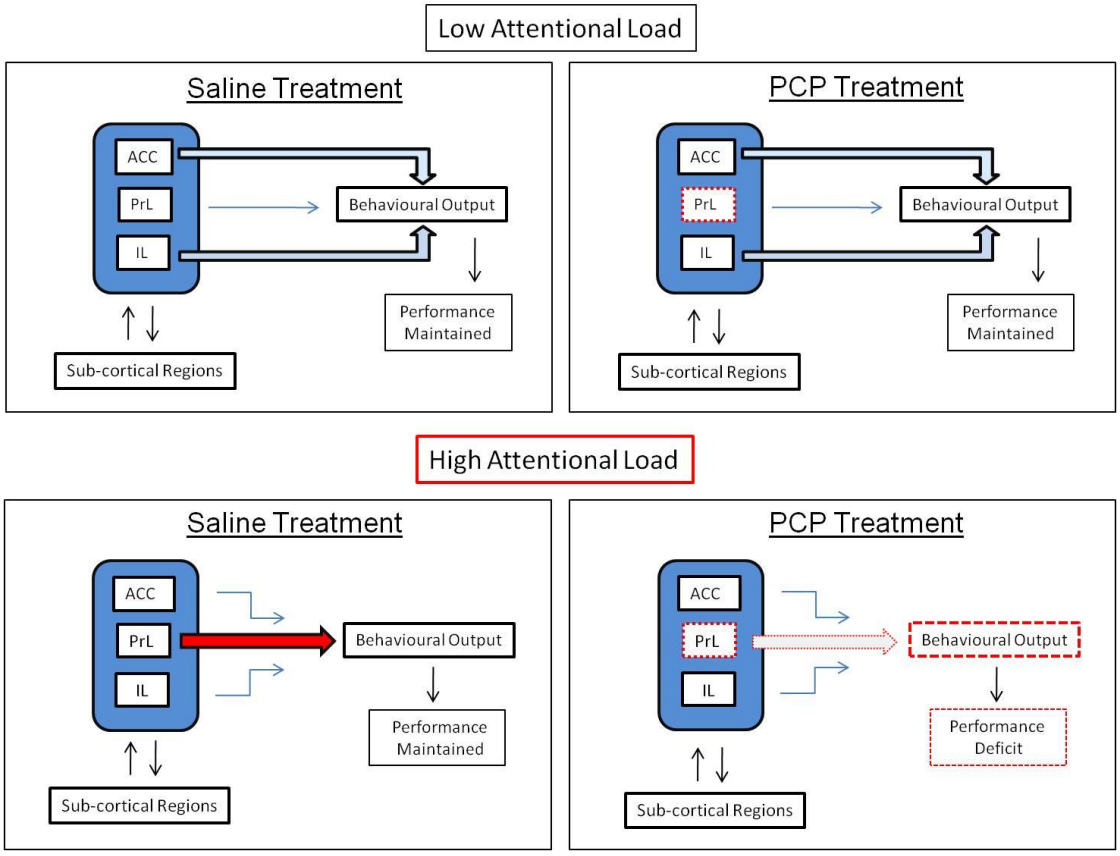

Fig 8.1 Diagram hypothesising the potential mechanism involved in the baseline-dependent PCP-induced attentional impairment. During low attentional demands attentional performance is mediated by regions other sub-regions of the mPFC along with reciprocal connections with sub-cortical regions. Despite morphological abnormalities within the prelimbic cortex following PCP treatment, differential regional recruitment maintains attentional performance. When the attentional load is increased, the prelimbic cortex is recruited to maintain finite attentional resources and sustain performance. During conditions of increased attentional load, a structural

abnormality within the prelimbic cortex impairs the ability to maintain behavioural coordination and deficits in sustained attention are observed. ACC indicates the anterior cingulate cortex. PrL represents the prelimbic cortex and IL the infralimbic cortex, which collectively form the medial prefrontal cortex (mPFC). Dashed red lines indicated compromised structural integrity leading to an attentional-load dependent deficit.

This work has established persistent attentional impairments following sub-chronic PCP treatment, in the drug-free state, which were coupled with structural abnormalities. However, translational aspects were a consistent consideration throughout this work and an obvious limitation has been highlighted; a discrepancy exists between the morphological abnormalities induced by sub-chronic PCP treatment and that observed in chronic ketamine users and schizophrenia patients. The current findings indicate an increase in MRI signal, whereas a reduction of grey and white matter density is observed in chronic ketamine users (Liao et al. 2010, 2011) and also schizophrenia patients (Pomarol-Clotet et al. 2010). It has been suggested that schizophrenia is a neurodevelopmental disorder, with the individual susceptible to abnormal neurodevelopment in their formative years leading to aberrant neuronal morphology. Because of this, Insel (2010) suggested that the onset of psychosis and the emergence of schizophrenia symptomatology may in fact be the late stages of the disorder, and not the onset. Additionally, reductions in grey and white matter densities observed in chronic ketamine users were apparent following several years of escalating abuse and level of consumption also correlated with the level of grey/white matter reduction (Liao et al. 2010, 2011). The treatment regimen used in chapter 7, while demonstrated previously to induce enduring cognitive and neuropathological disruptions (see Neill et al. 2010), did not replicate morphological changes observed in the clinic. However, while the treatment regimen utilised is known as 'sub-chronic' PCP administration, it is comparatively short; schizophrenia is a chronic disorder and the structural changes demonstrated in human ketamine abusers were observed over a prolonged period (mean usage ~2 years). These observations may account for the inconsistency in morphological findings between clinical

and preclinical studies. While sub-chronic PCP treatment provides an excellent method to model many of the cognitive, neurochemical and neuropathological features of schizophrenia, it is just a model and will never replicate the disorder in its entirety. Furthermore, as schizophrenia is very much a neurodevelopmental disorder, with clinical symptomatology emerging in early adulthood, grey matter changes are not linear (Pantelis et al. 2005; Theberge et al. 2007). However, the morphological findings do indicate NMDA receptor antagonism produces structural deficits that are detectable in experimental animals using MRI. Furthermore morphological alterations are located within a region that has important implications in schizophrenia. For future considerations, it would be interesting to determine the longevity of these structural abnormalities.

Although the findings indicate an increase in MRI signal, in contrast to that observed in schizophrenia patients and human ketamine users, the potential mechanism of this increase MRI signal may provide important insight into the neurobiological processes that mediate the effects observed following sub-chronic PCP treatment. The treatment regimen used has previously been shown to induce a 40% reduction in dendrite spine synapse in the medial prefrontal cortex, regions that included the prelimbic cortex (Hajszan et al. 2006). The PCP-induced reduction in dendrite spine synapses mimics that observed in schizophrenia patients in which prefrontal pyramidal cell dendrite spine density is reduced (Glantz and Lewis 1997; Glantz and Lewis 2000). Dendritic spine loss in schizophrenia is not restricted to frontal cortical regions as abnormal spine formation has been observed in the hippocampus and dorsal thalamus (Harrison 2004; Lewis 2004; Owen et al. 2005). As the approach used by Hajszan and colleagues (2006) was restricted by a ROI, the effect of PCP and dendritic spines in other regions was not determined. The susceptibility of additional brain regions and PCP-induced dendritic spine loss should be further explored.

It is well established that sub-chronic PCP treatment induces a robust reduction in basal cortical DA levels in both rats and monkeys in the drug-free state (Jentsch et al. 1997a, b; Jentsch et al. 1998; Jentsch and Roth 1999; Elsworth et al. 2008), in regions that include the prelimbic cortex. DA depletion of the ventral tegmental area following 6-OHDA lesion has also been shown to reduce dendritic length and spine density of layer V pyramidal cells in the prelimbic cortex (Wang and Deutch 2008). In contrast, but supporting this observation, elevations of cortical DA levels following chronic psychostimulant administration has been shown to increase PFC pyramidal dendritic length and spine density (Robinson and Kolb 1997, 1999). These findings indicate that PCP-induced reductions in cortical DA levels may mediate the PCP-induced reduction in dendritic spine synapses. It has been suggested that reductions in dendritic spine synapses may contribute to the persistent cognitive impairment observed following sub-chronic PCP administration (Hajszan et al. 2006). However, whilst dendritic reductions may play a role in the cognitive impairment observed, it is unlikely to account for the increased MRI signal demonstrated.

Crucially, Hajszan and colleagues (2006) demonstrated that the reduction of dendrite spine synapses was accompanied by a 60% increase in the density of astroglial branches, leaving the actual number of astrocytes unchanged. While the reduction in dendritic connections may contribute to the persistent cognitive impairment present in the drug-free state, a PCP-induced increase in astrocyte process formation may account for the increased MRI signal observed in chapter 7. Hajszan and colleagues (2006) initially suggested that the increased proliferation of astrocytes may have been the result of PCP-induced neurotoxicity, however signs of neurotoxicity (e.g. cellular degeneration) were not observed following electron microscopic inspection. In fact, the dose used in the current study is well below that demonstrated to induce aspects of neuronal toxicity (Olney et al. 1989; Nakki et al. 1995). Hajszan and colleagues (2006) went on to suggest that the increased astrocyte branching was simply to “occupy the cleared out space” which resulted from the reduction in dendritic

spine density. However, it is possible that astroglial density is increased in response to PCP exposure as a neuroprotective mechanism, with evidence emerging supporting this hypothesis. Astrocytes contain glutamate transporters and mediate homeostasis by absorbing and recycling excess glutamate via excitatory amino-acid transporters (Gluck et al. 2002; Katsel et al. 2011). It has been described that astrocytic processes orient towards regions where extrasynaptic glutamate concentration is elevated (Witcher et al. 2007; Eroglu and Barres 2010). Acute NMDA antagonism results in a dramatic increase in glutamate release (Moghaddam et al. 1997) in the prefrontal cortex. Excessive glutamate can result in calcium overload and induce excitotoxic cell death (Mark et al. 2001). Therefore, the proliferation of astroglial processes may occur in response to increased glutamate levels, facilitating the necessary glutamate uptake to prevent neuronal toxicity. An increase in astroglial branching arising as a protective mechanism may also account for the blunted increase (compared to single acute) in glutamate levels following repeated PCP administration (Amitai et al. 2011). In support of this, it has also been demonstrated that sub-chronic PCP treatment (albeit a different treatment regimen than the one used within this thesis) increased the expression of the cortical astrocytic glutamate transporters GLT-1/EAAT2 (Fattorini et al. 2008). Fattorini and colleagues (2008) also investigated the effect of PCP treatment on proteins that mediate vesicular packaging and release of glutamate. The absence of PCP-induced reductions in the proteins mediating glutamate release suggested that increased expression of a cortical glutamatergic transporter accounted for the decreased basal glutamate levels seen following sub-chronic PCP treatment.

Eroglu and Barres (2010) argued that it is functionally important that astroglial processes do not fully encapsulate neuronal synapses. As neuronal connections are three-dimensional, activation of neighbouring neurons may be important for functional efficiency. It has been demonstrated that in hippocampal formations, there are regions of the synapse that are free of astrocytes, enabling the escape of neurotransmitters from the synaptic cleft facilitating

trans-synaptic activation (Ventura and Harris 1999). It has also been observed that increased perisynaptic astrocytic processes can obstruct synaptic junctions and impede *trans*-synaptic neurotransmission (Witcher et al. 2010). These findings, along with the increased astroglial process density and glutamate transporter expression following sub-chronic PCP treatment (Hajzsan et al. 2006; Fattorini et al. 2008) suggest that abnormal morphology may result in disrupted cognitive processes. As it has been described that astroglial processes orient towards areas where glutamate concentration is elevated (Witcher et al. 2007; Eroglu and Barres 2010), glutamate releases is increased in the mPFC following NMDA antagonism (Katayama et al. 2007), and astroglial density is increased in the medial PFC following sub-chronic PCP treatment (Hajzsans et al. 2006), the increased prelimbic MRI signal observed in chapter 7 may be the result of increased astrocytic proliferation. This, along with the presence of neurochemical and neuropathological effects arising from sub-chronic PCP treatment, may contribute to the persistent cognitive impairment observed.

8.4 Future Experimental Direction

While the 5C-CPT represents a preclinical task that is analogous to the human CPT, it is not without its limitations. Human CPTs are generally experimenter-paced; that is the stimulus cues (akin to each trial) are presented to the subject independent of the subject's action. The 5C-CPT (along with the 5-CSRTT), however, is self-paced; the animal initiates each trial in the form of food reward collection. While efforts to limit the animal-paced aspect of the task can be implemented (e.g. stimulus onset-asynchrony), the animal still initiates each trial and so can be argued to still be self-paced. Operant task performance is mediated by the desire to earn food reinforcement. As such, it would be impossible to have a preclinical task that assesses performance over consecutive trials which is completely experimenter-paced, as the motivation to perform the task would be eliminated. However, it would be feasible to

further enhance the translational qualities of the 5C-CPT by introducing at least an element of experimenter-paced protocol into the execution of the task. Theoretically, it would be possible to train animals to perform the task with reward pellets only being dispensed following an extended number of completed or correct trials, instead of each successful trial. Obviously, validation work to determine the break point of the animal would need to be conducted. Furthermore, in the event of pharmacological manipulations (e.g. PCP administration), it would need to be ascertained that drug treatment was not altering the break point or motivation to perform the task, which could be misinterpreted as attentional disruption. This adaptation would at least remove the complete self-paced nature of the task. Although, it should be noted that in addition to schizophrenia inducing disruptions in classic experimenter-paced CPTs, impaired performance has also been observed in self-paced CPTs (J Young, Personal Communication). Furthermore, impaired vigilance has also been demonstrated in hyperactive children using a self-paced task assessing visual attention, characterised by a reduction in hits and an increase in false alarms (Goldberg and Konstantareas, 1981). Thus, the use of a self-paced paradigm for human or animal studies may not be completely invalid. Another potential issue is the obvious spatial component of the 5-CSRTT and 5C-CPT, the animals must scan a horizontal array in order to detect the presentation of the visual stimulus. In contrast, visual stimuli are presented in one location in human CPTs. A human version of the 5C-CPT has recently been developed. Consistent with traditional human CPTs, schizophrenia patients' exhibit reduced signal sensitivity, mediated by reduced hit rate that occurred independently of strategic bias alterations (J. Young, Personal Communication). While there was a trend towards an increased false alarm rate, it suggests impaired signal sensitivity was primarily mediated by reduced target detection. These observations suggest that the spatial component of the 5C-CPT may not confound the translational qualities of the task.

The experiment in chapter 7 demonstrated structural abnormalities following PCP treatment. MRI does not provide insight into the causality of the structural abnormality, only the presence of morphological alterations and the location. Furture investigations should determine the origin of the increased MRI signal. Discussed above, evidence suggests that a potential consequence of sub-chronic PCP treatment is astrocytic proliferation, which may account for the increased MRI signal observed. Firstly, it would be important to confirm an increase in astrocyte proliferation was evident following sub-chronic PCP treatment. Secondly, the impact of this proliferation could be explored. Of particular interest is an endogenous α7 nicotinic antagonist synthesised exclusively in astrocytes, known as kynurenic acid (KYNA). Fluctuations in KYNA levels can impair or enhance cognitive processing (Zmarowski 2008; Potter et al. 2010), modulate extracellular glutamate and DA levels in various brain regions, including the mPFC (Carpenedo et al. 2001; Wu et al. 2006) and modulate basal and evoked PFC Ach release (Zmarowski et al. 2009). Importantly, increased KYNA levels have been observed in schizophrenia patients (Erhardt et al. 2001; Schwarcz et al. 2001). A PCP-induced increase in KYNA levels, along with dendritic spine reductions and GABAergic deficits, may contribute to persistent impairments in cognition.

In summary, these findings highlight that attention/vigilance is sensitive to a PCP-induced disruption. Profound cognitive deficits are observed while the animal is under the influence of PCP but importantly, impairment is also demonstrated in the drug-free state following a sub-chronic treatment regimen. The performance deficit following sub-chronic PCP treatment is also dissociable between effortful and passive attentional processing. Furthermore, in addition to the apparent cognitive impairment, sub-chronic PCP treatment produces morphological abnormalities that may, along with the neurochemical and neuropathological disturbances, contribute to the cognitive impairment demonstrated.

Chapter 9

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