EXPLORATION OF COGNITIVE AND NEUROCHEMICAL DEFICITS IN AN ANIMAL MODEL OF SCHIZOPHRENIA

Investigation into sub-chronic PCP-induced cognitive deficits using behavioural, neurochemical and electrophysiological techniques; and use of receptor-selective agents to study the pharmacology of antipsychotics in female rats

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ABSTRACT

Keywords: Rat; Phencyclidine; Antipsychotics; Cognition; Dopamine; 5-HT; GABA

Cognitive dysfunction is a core characteristic of schizophrenia, which can often persist when other symptoms, particularly positive symptoms, may be improved with drug treatment. The non-competitive NMDA receptor antagonist, phencyclidine (PCP), is a psychomotor stimulant drug that has been shown to induce symptoms characteristic of schizophrenia in humans and animals.

The aim of these studies was to use the sub-chronic PCP model in rats to investigate cognitive dysfunction in behavioural tests which have been highlighted as relevance by the MATRICS initiative (MATRICS.ucla.edu). The main tests used were attentional set-shifting, operant reversal learning, and novel object recognition tasks. The pharmacology of antipsychotics was studied in the reversal learning task using receptor selective compounds. Following this, experiments were carried out using *in vitro* electrophysiology and *in vivo* microdialysis in an attempt to investigate the mechanisms underpinning the PCP-induced cognitive deficits.

The attentional set-shifting task is a test of executive function, the extra-dimensional shift (EDS) phase relates to the ability to shift attention to a different stimulus dimension; this is impaired in patients with schizophrenia. The studies presented in chapter 2 showed that sub-chronic PCP administration impaired attentional set-shifting performance selectively in the EDS phase, a deficit which was significantly attenuated by sub-chronic administration of clozapine and risperidone, but not haloperidol. The effect of PCP was also shown to be more robust in female rats compared to males. A deficit in set-shifting ability was also observed in isolation reared rats. However, the deficits produced by PCP were more robust than the deficit produced by isolation rearing.

The reversal learning task is another test of executive function. Chapter 3 reported that sub-chronic PCP administration impairs reversal learning ability in an operant task, as demonstrated by reduced percent correct responding in the reversal phase of the reversal learning task. It was found that a D_1 agonist (SKF-38398), a 5-HT_{1A} partial agonist (buspirone), a 5-HT_{2C} antagonist (SB-243213A) and an agonist and positive allosteric modulator of the alpha 7 nACh receptor (PNU-282987 and PheTQS respectively) are able to reverse the sub-chronic PCP-induced deficit in reversal learning. Although many antipsychotics have affinity for muscarinic M_1 and histamine H_1 receptors, selective agents at these receptors were not able to improve the PCP-induced deficit.

In chapter 4, the atypical antipsychotics, clozapine and risperidone, when given alone to naïve rats had no effect on reversal learning. Haloperidol when given to naïve rats impaired performance at the highest dose. Sub-chronic PCP was again found to impair reversal learning performance. Investigative experiments revealed that the 2 min time-out could be important as a cue. Following a double reversal, olanzapine-treated rats lost the ability to switch between the rules, whereas clozapine and risperidone-treated rats could perform the double reversal. Experiments with the extended (15 min) reversal phase could allow the investigation of the time-course effects of antipsychotics or selective compounds.

The studies presented in chapter 5 found a reduction in gamma oscillations in the CA3 region of the hippocampus, following sub-chronic PCP treatment (2-5 weeks post treatment) that was paralleled by a deficit in parvalbumin immunoreactive (IR) cell density, at a similar time point (2 weeks post treatment). In contrast, a time-dependent increase in gamma oscillations was observed (6-8 weeks post treatment), at which point parvalbumin IR cell density was unchanged (8 weeks post treatment). Gamma oscillations were unchanged in the prefrontal cortex (PFC) following the PCP treatment regime. Locomotor activity tests were also carried out to ensure that the sub-chronic PCP treatment was successful.

In-vivo microdialysis revealed that vehicle-treated rats show an increase in dopamine in the PFC which is selective for the retention trial of the novel object recognition task. PCP-treated rats were unable to distinguish between the novel and familiar objects and the increase in dopamine observed in vehicle rats was absent. As a control experiment it was also shown that sub-chronic PCP did not induce anxiety-like symptoms in the elevated plus maze and open field tests.

These studies suggest that sub-chronic PCP induces cognitive deficits in behavioural tasks, and these deficits may be due to GABAergic mediated processes in the hippocampus and dopaminergic dysfunction in the PFC. These behavioural and neurochemical results are concurrent to findings observed in schizophrenia.

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2. McLean SL, Woolley ML, Neill JC (2009) Behavioural analysis of the effects of psychotomimetics and antipsychotics in an operant reversal learning task. *Submitted*.

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Chapter 1

General Introduction

1.1 Schizophrenia

Schizophrenia affects approximately 1% of the population. Schizophrenia is a thought disorder, and is characterised by both disturbed form and content of thought (Feldman *et al.,* 1997). It is an accumulation of a wide range of symptoms which are characteristically grouped together to simplify diagnosis. Symptoms were divided into positive and negative and stemming from the early work of Kraepelin (1913) and Bleuler (1950), a two-syndrome hypothesis was established by Crow (1980). The disorder has now been categorised into more distinct types including catatonic, paranoid and disorganised (Stahl, 2002).

There is strong evidence that there is a genetic predisposition to schizophrenia from many twin and adoption studies (Gottesman, 1991). Current molecular genetic research is attempting to uncover a link between schizophrenia and specific genes (see Harrison and Owen, 2003). Chromosome 22 has been associated with the disorder. Structural brain abnormalities have been found using CT scans and MRI in schizophrenic patients such as diffuse cerebral atrophy and ventricular enlargement (Weinberger, 1995; DeLisi, 1999). These results have been confirmed by post-mortem studies (Brown *et al.,* 1986). There is also strong evidence for neurochemical changes to be implicated in schizophrenia (Glantz and Lewis, 2000), with several neurotransmitter systems involved, in particular dopamine and glutamate.

1.2 Symptoms of Schizophrenia

The onset of schizophrenia tends to be 18-25 in males and 25-30 in females, with episodes reoccurring throughout life (Buchanan and Carpenter, 2005). Schizophrenia is considered a neurodevelopmental disorder (McGrath *et al.*, 2003). Many patients are unable to work due to the severity of their symptoms; therefore schizophrenia poses a

great social problem and is responsible for filling a majority of psychiatric hospital beds (Feldman *et al.,* 1997). Between 25 and 50% of patients attempt suicide, with 10% eventually succeeding. Patients often require chronic treatment as the disorder is in many cases progressive. Currently there is not a known cure for schizophrenia with drugs only treating some of the symptoms.

1.2.1 Positive symptoms of Schizophrenia

The positive symptoms (also named type I symptoms) are characterised by the more florid symptoms of the disorder and reflect an excess of normal function. These typically include delusions, auditory hallucinations, distortions or exaggerations in language and communication, disorganised speech and behaviour and agitation (Stahl, 2002). Patients who suffer from predominantly positive symptoms tend to have had acute onset of the disorder. These patients respond well to antipsychotic treatment, and when symptoms are in remission patients social functioning tends to be relatively good (Feldman *et al.,* 1997).

1.2.2 Negative symptoms of Schizophrenia

The negative symptoms (also named type II symptoms) of schizophrenia represent a loss or reduction of normal function. These symptoms are most resistant to antipsychotic treatment and symptoms may actually be exacerbated by treatment. Unlike patients with positive symptoms, patients with predominantly negative symptoms tend to show a long course of progressive deterioration, possibly reflecting long-term brain deterioration changes such as ventricular enlargement (Andreasen, 1987; Meltzer, 1987). This symptom cluster has been difficult to model, but there is currently

a social withdrawal test which may model some of these deficits (Snigdha and Neill, 2008a,b).

1.2.3 Cognitive dysfunction in Schizophrenia

Cognitive dysfunction is a core characteristic of schizophrenia (Sullivan *et al.,* 1994; Elvevag and Goldberg, 2000; Kuperberg and Heckers, 2000) and is becoming increasingly important as impaired cognition has been implicated in poor long-term morbidity (Marder and Fenton, 2004) which can often persist when other symptoms may be improved with treatment (Gold *et al.,* 1991). One reason for the residual disability in schizophrenia appears to be the long-standing cognitive deficits of the disorder (Green and Nuechterlein, 2004). Deficits often manifest in a generalised decrease in IQ (Elvevag and Goldberg, 2000). Cognitive impairments appear to be stable from first episode through to late middle age (Heaton *et al.,* 2001).

The National Institute of Mental Health (NIMH) launched the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative to address the fact that, although there are many antipsychotic drugs that alleviate psychotic symptoms, these do not reduce cognitive deficits. Thus the MATRICS initiative was established to facilitate the development of novel pharmacological approaches for the treatment of the cognitive deficits in schizophrenia (www.matrics.ucla.edu). One of the major accomplishments of this initiative was the identification of 7 primary cognitive domains that are affected in schizophrenia – attention/vigilance, speed of processing, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, and social cognition (Green *et al.,* 2004).

Whilst impairments have been reported across all of the cognitive domains identified by the MATRICS initiative above, impairments in executive function have long been considered to be a core feature of schizophrenia (Sullivan *et al.,* 1994). One aspect of executive function is the ability to modify behaviour in response to the changing relevance of a stimulus; this is commonly assessed in patients using the Wisconsin Card Sorting Test (WCST) (Berg, 1948). In this test, subjects are presented with a set of cards with 3 different features (shape, colour, and number) and instructed to sort the cards without being told the relevant feature. They must learn this based upon feedback from the tester. Once they have established which is the correct feature, the rule is switched unannounced, and subjects must learn the new rule based upon feedback. Patients with schizophrenia perform poorly on such tests as they lack the ability to inhibit a previously learned rule and are unable to shift their attention to the relevant stimulus (Goldberg *et al.,* 1987; Morice and Delahunty, 1996).

Deficits in working memory are also predominant in schizophrenia. Working memory can be defined as a limited capacity memory system for the temporary storage and manipulation of information; this can include verbal material and visuospatial images (Keefe, 2000). Functions of working memory include selective attention, maintenance of these selected events and integration of these with the experiences stored in long-term memory; these processes are essential for the operation of other types of memory, such as episodic or spatial, language, comprehension and reasoning (Baddeley, 1986).

1.3 Behavioural tests of cognition

Behavioural tests or measures are a very useful tool when developing an animal model. A major problem in developing an animal model of cognition is that involves several

domains, as highlighted by the MATRICS initiative (Green *et al.*, 2004). As a consequence, any one test cannot accurately describe these multiple dimensions of cognition. It is therefore more conceivable to use several behavioural tests to characterise different aspects of cognition. The novel object recognition, reversal learning and attentional set-shifting tasks have been highlighted by the MATRICS initiative as being relevant translational models for studying visual learning and memory and problem solving ability respectively pre-clinically (www.matrics.ucla.edu, see table 1.1). Other cognitive tests highlighted by the MATRICS initiative include tests of working memory, for example delayed non-match to sample task, and tests of sensorimotor gating, such as pre-pulse inhibition (PPI).

Table 1.1: Mapping animal tests onto cognitive domains affected in schizophrenia.

Cognitive Domain	**Animal Models/Tests**	**MATRICS Clinical Battery**
Working memory	Operant or T-maze	BACS
	DNMTP/ DMTP	WMS-III Spatial Span
	Radial arm maze	WAIS-III Letter-Number sequence
		UoM Letter-Number Span
		Spatial Delayed Response Task
Attention/vigilance	5-Choice Serial Reaction	3-7 CPT
(pre-attentive	Time Task	Identical pairs CPT
processing)	PPI, auditory gating	PPI, auditory gating
Verbal learning and		NAB- Daily Living Memory
memory		HVLT-Revised
Visual learning &	Novel Object Recognition	NAB – Shape Learning
memory		BVMT-Revised
Speed of processing	5-Choice Serial Reaction	Category fluency
	Simple Reaction time tasks	Trail making A
		WAIS-III Digit Symbol-Coding
		BACS – Symbol Coding
Reasoning & problem	Attentional Set-shifting	WAIS-III Block design
solving	Maze tasks	BACS- Tower of London
	Serial Reversal Learning	NAB – Mazes
Social cognition	Social interaction/Social	MSCEIT – Managing emotions
	recognition?	MSCEIT – Perceiving emotions

Table 1.1: Tests of cognition highlighted by the MATRICS inititative. Tests highlighted in yellow will be used in this thesis.

1.3.1 Novel object recognition

The novel object recognition task (Ennaceur and Delacour, 1988) is a non-spatial test of recognition memory which is ethologically relevant as it relies on the animal's natural tendency to explore novel environments/objects. This model is relevant to the disease since visual recognition memory is impaired in schizophrenic patients (Calkins *et al.,* 2005). The task involves the rat being placed in an arena with 2 novel objects and exploration times of each object are scored, this is know as the acquisition trial. This is followed by an inter-trial-interval, which can vary in length from 1 minute to days. The rat is then placed back into the arena with a third copy of the familiar object and a novel object, again exploration of each object is timed. The brain areas involved in object recognition depend upon the length of inter-trial interval used. Following an interval of minutes it is believed that the PFC and/or perirhinal cortex are important for recognition (this will be further discussed in chapter 6), whereas following an interval in the region of hours it is thought that the hippocampus is involved in the recognition of the objects (Hammond *et al.,* 2004).

1.3.2 Reversal learning

In the reversal learning paradigm, animals are required to perform two tasks. The first task requires the animal to inhibit a previously learned rewarded strategy; in the second task, the animal must acquire the new strategy (Mackintosh and Little, 1969). Effective performance requires intact cognitive ability; thus animals are required to demonstrate flexibility, attention, motivation, and ability to suppress a previously learned response and implement a new one (Jones *et al.,* 1991). In rats this reversal learning ability is assessed in operant chambers with two levers, an active lever which when pressed produces food from the magazine, and an inactive lever which has no consequence but

to end the trial. The task involves an initial phase for 5 minutes in which one lever is active; this is followed by a 2 minute time-out period. Then in the 5 minute reversal phase the opposite lever is now active (Abdul-Monim *et al.*, 2003; 2006; Idris *et al.*, 2005a). It has been shown that lesions of the orbital prefrontal cortex impair reversal learning ability (McAlonan and Brown, 2003; Tait and Brown, 2007; this will be further discussed in chapter 3).

1.3.3 Attentional set-shifting

The perceptual attentional set-shifting task (Birrell and Brown, 2000) investigates the ability of a rat to learn a rule and form an attentional set within the same sorting category (intra-dimensional shift - IDS), as well as the ability to shift attentional set between different sorting categories (extra-dimensional shift - EDS). Rats must carry out a series of 7 discriminations namely, simple discrimination (SD), compound discrimination (CD), reversal 1 (R1), intra-dimensional shift (IDS), reversal 2 (R2), extra-dimensional shift (EDS) and reversal 3 (R3). The attentional set-shifting task represents a rat analogue of the human Wisconsin Card Sorting Task (WCST, Berg, 1948) and CANTAB ID/ED task (Downes *et al.,* 1989) in which schizophrenic patients exhibit impaired set-shifting (Kolb and Wishaw, 1983; Haut *et al.,* 1996; Pantelis *et al.*, 1999; Tyson *et al.,* 2004). It has been reported that lesions of the medial prefrontal cortex (mPFC) produce a selective deficit in the EDS phase in attentional set-shifting (Birrell and Brown, 2000; this will be further discussed in chapter 2).

1.3.4 Pre-pulse Inhibition (PPI)

In pre-pulse inhibition of the startle response (PPI) the animals are exposed to a weak stimulus prior to a startling stimulus. The pre-stimulus causes no startle response but it

allows the animal to become aware of the startle stimulus thus reducing the magnitude of the startle response (Hert and Ellenbroek, 2000). Normal individuals are able to filter out or inhibit responding to most of the sensory stimuli they receive, thus becoming quickly accustomed to the pre-stimulus. However, schizophrenic patients have an abnormality of information processing and more specifically in filtering external stimuli (Braff and Geyer, 1990), a deficit of sensorimotor gating. The deficits in PPI observed in patients with schizophrenia can be mimicked in rats via administration of dopamine agonists (Swerdlow *et al.*, 1994), serotonin agonists and NMDA receptor antagonists (Swerdlow and Geyer, 1998; Geyer *et al.*, 2001). Furthermore, rats reared in isolation also show reduced PPI of the startle response (Geyer *et al.*, 1993; Cilia *et al.*, 2001). It is important to note that deficits in PPI are neither unique to schizophrenia nor diagnostic of the disorder (see Geyer *et al.*, 2001).

1.4 Aetiology of schizophrenia

Population, family and twin studies indicate that schizophrenia is a heritable disorder, but no single gene has a strong effect. Schizophrenia is known to affect approximately 1% of the population; however this is increased to 10% in first-degree relatives of patients with schizophrenia. In dizygotic twins, one of whom has schizophrenia the probability of the other having the illness is 20%; furthermore in monozygotic twins this chance is further increased to 50% (Gottesman, 1991, see figure 1.4). The disorder is more likely due to the synergistic interaction of multiple genes and environmental factors (Harrison and Weinberger, 2005; genetic models will be discussed in section 1.6.4).

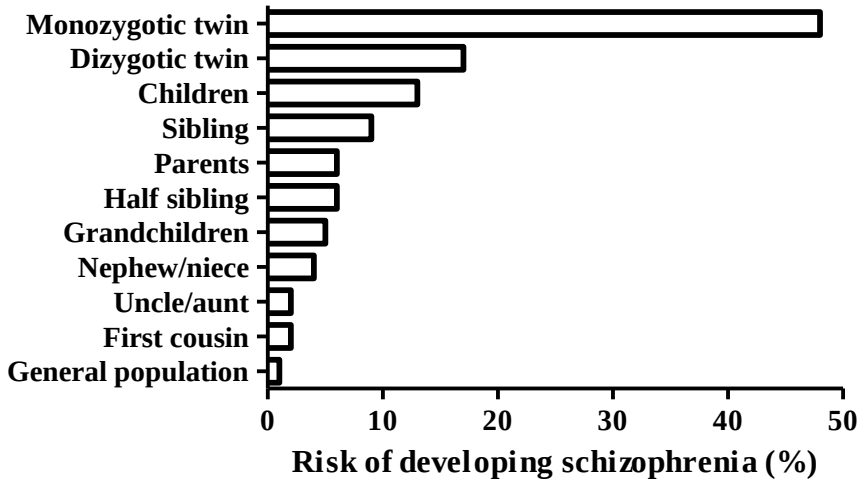


Figure 1.4: Risk of developing schizophrenia (adapted from Gottesman and Erlenmeyer-Kimling, 2001).

1.5 Neurochemical hypothesis of schizophrenia

There is strong evidence to suggest that disturbances of several neurotransmitter systems are implicated in schizophrenia. Significant attention has been directed towards the monoamines, such as dopamine and 5-hydroxytryptamine (5-HT). More recently, evidence points towards amino acid neurotransmitters such as gamma-aminobutyric acid (GABA) and glutamate (Owen and Simpson, 1995).

1.5.1 Dopamine

There are four dopamine pathways in the brain, all of which mediate different functions. The mesolimbic dopamine system projects form the ventral tegmental area to the nucleus accumbens, it is thought to have an important role in producing the positive symptoms of schizophrenia. The cell bodies of the mesocorticol dopamine pathway also arise in the ventral tegmental area and project to the limbic cortex; this pathway is believed to be involved in mediating the cognitive and negative symptoms of

schizophrenia (Weinberger *et al.,* 1988). It has been suggested that the negative symptoms of schizophrenia may arise from a dopaminergic deficit in the prefrontal cortex (hypofrontality), whereas the positive symptoms may be related to hyperdopaminergic activity in mesolimbic dopaminergic neurones (Davis *et al.,* 1991; Laruelle *et al.,* 2003). The nigrostriatal dopamine pathway projects from the substantia nigra to the striatum. This pathway is directly involved in the control of movement, therefore disordered function of this pathway whether natural or due to pharmacological intervention causes movement disorders (extrapyramidal symptoms). The tuberoinfundibular pathway connects the hypothalamus to the anterior pituitary. During treatment with antipsychotics the dopamine receptors in this pathway can be blocked, thus releasing prolactin secretion from inhibition, this can cause symptoms such as galactorrhea.

Spano *et al.* (1978) proposed the existence of two populations of dopamine receptors after it was shown that dopamine both stimulated and inhibited adenylate cyclase (AC) activity (Brown and Makman, 1972; Kebabian *et al.,* 1972). D_1 and D_5 receptors belong to the D_1-like family in that they stimulate adenylate cyclase (AC), whereas D_2, D_3 and D_4 receptors inhibit AC. D_1-like receptors are predominantly found in the PFC, while D_2-like receptors are expressed in sub-cortical regions (see Guillin *et al.,* 2007), although D_4 receptors are present in the PFC and hippocampus (Lahti *et al.,* 1998). In keeping with the dopaminergic hypothesis of schizophrenia current antipsychotics attenuate positive symptoms by blocking sub-cortical D_2 receptors (Seeman *et al.,* 1975; Creese *et al.,* 1976) but these drugs have, at best, only limited efficacy at treating cognitive deficits. As most current antipsychotics are equipotent for D_2 and D_3 receptors, it is unclear which pharmacological action accounts for their clinical profile. D_3 receptors are distributed in the mesocortical but not nigrostriatal

regions; therefore, blocking D_3 receptors may improve cognitive and negative symptoms without producing extrapyramidal adverse effects (Joyce and Millan, 2005).

1.5.2 Serotonin (5-HT)

5-HT distribution is widespread, the cells occur in several large clusters in the pons and upper medulla, which lie close to the midline and are commonly referred to as the raphe nuclei. The rostrally situated nuclei project to the many parts of the cortex, hippocampus, basal ganglia, limbic system and hypothalamus. The caudally situated nuclei project to the cerebellum, medulla and spinal cord (Rang *et al.*, 1995). Involvement of the serotonergic pathways in the pathophysiology of schizophrenia was suggested based on the observation that 5-HT agonists such as lysergic acid diethylamide (LSD) can induce or exacerabate schizophrenia-like symptoms in humans (see Roth and Meltzer, 1995). To date all of the 5-HT receptors identified are G-protein coupled receptors, with the exception of the 5-HT_3 receptor which is a ligand-gated ion channel (Barnes and Sharp, 1999; Nichols and Nichols, 2008). Each receptor is subdivided into the following subtypes; $5\text{-HT}_{1A\text{-}F}$, $5\text{-HT}_{2A\text{-}C}$, $5\text{-HT}_{3A/B}$, $5\text{-HT}_{4A\text{-}D}$, $5\text{-HT}_{5A/B}$, 5-HT_6 and $5\text{-HT}_{7A\text{-}D}$ (see Meneses, 1999). The ability of atypical antipsychotics such as clozapine to reduce extra-pyramidal adverse effects led to the interest in 5-HT_{2A} antagonists as potential targets for schizophrenia (Schmidt *et al.*, 1995). The location and pharmacology of the 5-HT receptors will be discussed in detail in section 1.7.3.

1.5.3 Glutamate

The major excitatory neurotransmitter in the brain, glutamate was first implicated in the cause of schizophrenia when it was found that phencyclidine, a glutamate NMDA receptor antagonist, induced schizophrenia-like symptoms in man (Luby *et al.*, 1959).

Glutamate was also found in decreased concentrations in the cerebrospinal fluid of schizophrenic patients together with increased density of glutamate NMDA receptors in the corpus striatum (Kim *et al.,* 1980). Sub-anaesthetic doses of ketamine, another NMDA antagonist, in healthy volunteers have also been shown to induce both positive and negative-like symptoms of schizophrenia as well as cognitive impairments assessed by the WCST (Krystal *et al.,* 1994; Malhotra *et al.,* 1996).

Selective agonists and antagonists led to the identification of three main subtypes of ligand-gated ion channels namely N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA), and kainate receptors. In 1985, Sladeczek and co-workers reported that a novel glutamate receptor stimulated phospholipase C, this gave rise to the concept of metabotropic glutamate receptors. Genetic studies indicate the existence of 7 different metabotropic receptors namely mGluR1-7 (Schoepp *et al.,* 1994). These receptors can be subdivided (see table 1.2) based on the similarity of their amino acid sequence, pharmacology and second messenger coupling (Schoepp *et al.,* 1994).

Table 1.2: Subtypes of metabotropic glutamate receptors

Receptor	**Pharmacological sensitivity**	**Second messenger system**
mGluR1 mGluR5	trans-ACPD, quisqualate	Positively coupled to phosphor-inositide second messenger system
mGluR2 mGluR3	trans-ACPD	Negatively coupled to adenylate cyclase
mGluR4 mGluR6 mGluR7	L-AP4, L-SOP	Negatively coupled to adenylate cyclise

Table 1.2: Subtypes of metabotropic glutamate receptors (Schoepp *et al.,* 1994). Trans-ACPD (trans-1-aminocyclopentane-1,3-dicarboxylic acid) is a rigid analogue of glutamate; L-AP4 (L-2-amino-4-phosphonobutyrate) and L-SOP (L-serine-O-phosphate) are phosphorylated amino acids.

1.5.3.1 NMDA receptors

NMDA receptors show significant permeability to Ca^{2+}, Na^{+} and K^{+} ions and are also subject to a voltage-dependent blockade by relatively low concentrations of Mg^{2+}, i.e. when the cell membrane is depolarised the Mg^{2+} block is removed (Feldman et al., 1997). The NMDA receptor also requires glycine as a co-agonist; both glycine and glutamate have separate binding sites and both must be present for the channel to open (see figure 1.4.3). The NMDA receptor is a heteromeric complex consisting of a combination of subunits from 3 subtypes: NR1, NR2, and NR3. There are 8 different NR1 subunits generated from alternative spicing from a single gene, 4 different NR2 subunits (A-D) and 3 NR3 subunits (A-C); the NR2 and NR3 subunits are encoded by 6 different genes (Moriyoshi *et al.*, 1991). Expression of functional recombinant NMDA receptors in mammalian cells requires the co-expression of at least 1 NR1 and 1 NR2 subunit. Most commonly NMDA receptors consist of 2 NR1 and 2 NR2 subunits of the same subtype (Dingledine *et al.*, 1999).

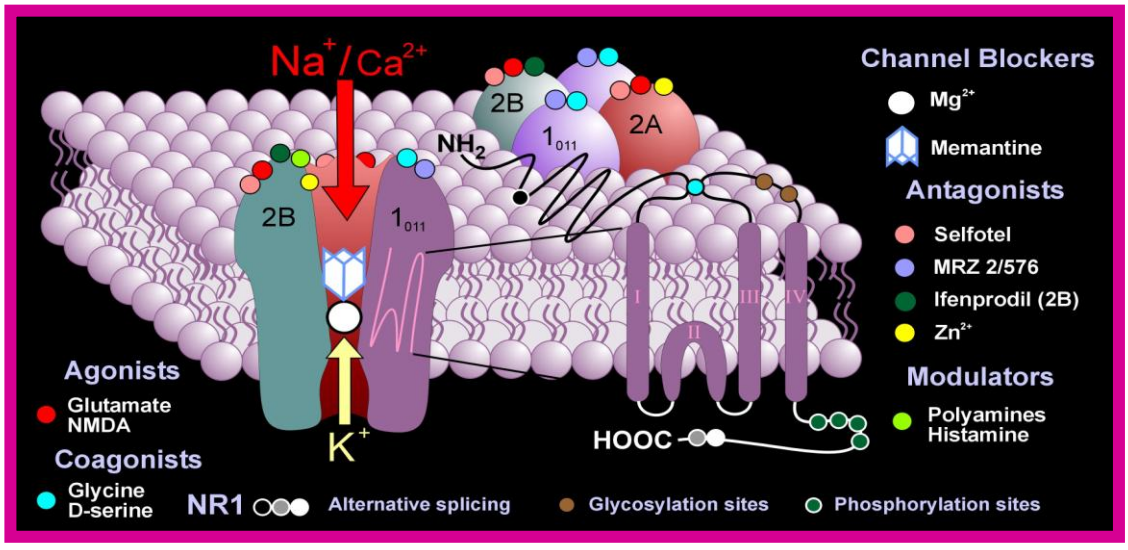


Figure 1.4.3: NMDA Receptor Model (taken from http://abdellab.sunderland.ac.uk)

The dominant hypothesis of the pathophysiology of schizophrenia until recently was the dopamine hypothesis as dopamine-releasing drugs such as amphetamine were

seen to cause psychosis. However, a purely dopamine hypothesis is limited as negative and cognitive symptoms appear to be untreated by typical antipsychotics. The glutamate hypothesis based on NMDA antagonists producing cognitive impairments may be more clinically relevant. The main glutamate hypothesis arises from the NMDA receptor hypofunction hypothesis of schizophrenia of Olney and Farber (1995). They postulated that NMDA receptor hypofunction produces a reduction in inhibitory firing mediated by GABAergic neurotransmission. In support of this hypothesis it has also been shown that glycine administration to enhance NMDA receptor function can improve schizophrenia aetiology when used in conjunction with other antipsychotic treatment (Rosse *et al.*, 1989).

1.5.4 GABA

GABA is an inhibitory neurotransmitter. GABAergic interneurons can be identified by their expression of neurochemical markers such as parvalbumin, calbindin, and calretinin, or by assessment of glutamic acid decarboxylase (GAD), the enzyme responsible for the breakdown of GABA (Benes and Berretta, 2001). Post mortem studies have found reductions in the concentration of cortical GABA (Perry *et al.*, 1979) and the activity of GAD (Bird, 1985), the enzyme that synthesises GABA. The GABA deficit does not affect all cortical GABAergic interneurons equally (Benes *et al.*, 1991) but is restricted to the basket and chandelier type of interneurons (Woo *et al.*, 1998; Zhang and Reynolds, 2002). These two types of interneurons contain the Ca^{2+} binding protein parvalbumin, and synapse onto pyramidal cells (Hendry *et al.*, 1989). Although there might be a modest reduction in the number of interneurons, the major changes are in the concentrations of particular proteins notably GAD_{67} and parvalbumin (Woo *et al.*, 1997; Lewis *et al.*, 2005). There are two isoforms of GAD; GAD_{65} is primarily

localised in axon terminals, while GAD_{67} is found in the somata and dendrites of GABAergic cells (Kauffman *et al.,* 1991).

GABA increases Cl^- conductance across the membrane, leading to a rapid hyperpolarisation of the postsynaptic cell, which accounts for the inhibitory action of GABA (Harris and Allan, 1985). GABA acts on 3 subtypes of receptors, namely $GABA_{A-C}$. The $GABA_A$ and $GABA_C$ receptors are ligand-gated ion channels and the $GABA_B$ receptor is a G-protein coupled receptor. The Cl^--dependent effects of GABA are generally mediated by the $GABA_A$ receptors as this contains the chloride channel. The $GABA_A$ receptors have different binding sites, namely the GABA (agonist/antagonist) site, benzodiazepine site, steroid site, barbiturate site and the picrotoxin site (Olsen *et al.,* 1991). $GABA_B$ receptors are located both post- and pre-synaptically; those located on the nerve terminal inhibit transmitter release. $GABA_B$ receptors are known to utilise at least 4 different effector mechanisms 1) inhibition of adenylyl cyclase, 2) stimulation of phospholipase A2, 3) increased K^+ membrane conductance, and 4) inhibition of a voltage-dependent membrane Ca^{2+} conductance (Wojcik and Holopainen, 1992).

1.5.5 Involvement of other neurotransmitters in schizophrenia

Acetylcholine (ACh) is known to play an important role in various domains of cognition, particularly attention, learning, and memory (Friedman, 2004). Indeed, cholinergic dysfunction has been shown to be central to the pathophysiology of Alzheimer's disease and has also been postulated to contribute to the cognitive deficits of various neuropsychiatric disorders, including schizophrenia (Friedman, 2004). The basal cholinergic complex sends widely diffuse afferents through 2 projections: the septohippocampal and the nucleus basalis of Meynart cortical pathways (Woolf and

Butcher, 1989). The septohippocampal pathway is important in working memory processes through hippocampal storage of intermediate-term memory (Brito *et al.,* 1983; Fadda *et al.,* 1996) whereas the nucleus basalis of Meynart cortical pathway is involved in reference memory through long-term information storage in the neocortex (Dunnett, 1985; Meck *et al.,* 1987). Pharmacologically, muscarinic receptor antagonists, such as scopolamine, produce learning impairments in healthy subjects (Sitaram *et al.,* 1978). The pharmacology of relevant ACh receptors to cognition will be discussed further in section 1.6.3 and in chapter 3.

The central noradrenergic (NA) system projects from the locus coeruleus to the prefrontal cortex where α2-adrenoceptors appear to play an important role in cognitive functioning (Arnsten, 2004). Indeed, treatment with the α2-adrenoceptor agonists, clonidine and guanfacine, has been shown to improve cognitive performance without exacerbating positive symptoms in small trials of patients with schizophrenia (Fields *et al.,* 1988; Friedman *et al.,* 2001).

1.6 Animal Models of Schizophrenia

Animal models are critical in understanding the mechanisms underlying many human diseases, and in particular psychiatric disorders. However, an animal model of schizophrenia has proved to be very difficult to develop as it is mainly a thought disorder involving complex cortical processes which may not be expected to be found in lower mammals. Consequently, the aetiology of the disease also remains poorly understood.

1.6.1 Use of pharmacological agents to mimic schizophrenia

There are many animal models of some of the symptoms of schizophrenia based on clinical observations that psychostimulant drugs such as amphetamine and phencyclidine (PCP) can produce schizophrenia-like symptoms. Amphetamine can produce the more florid positive symptoms of schizophrenia such as paranoia, hallucinations and delusions; this is modelled in animals as hyperactivity (Jentsch *et al.*, 1998). In contrast, PCP can produce both positive and negative symptoms as well as cognitive deficits (Jentsch and Roth, 1999). The apparent relationship between amphetamine and phencyclidine (PCP) induced psychosis and schizophrenia has made these compounds the primary psychotomimetic agents for modelling schizophrenia in the laboratory.

1.6.1.1 Amphetamine

D-amphetamine causes increased dopamine by different mechanisms such as blocking reuptake via the dopamine transporter, and via disruption of dopamine's vesicular stores; this can increase locomotor activity and produce stereotypy. Stereotypy is a syndrome which includes increased sniffing, grooming, and licking (Randrup and Munkurad, 1967). These seemingly meaningless behaviours are also observed in humans with compulsive repetition in response to high doses of amphetamine. In humans, psychosis following frequent high doses of amphetamine consists of paranoid delusions, various forms of stereotyped behaviour, compulsive behaviour and predominantly visual hallucinations (Curran *et al.*, 2004; Lieberman *et al.*, 1987). In animals, treatment with amphetamine and apomorphine (a dopamine receptor agonist) increases locomotor activity, catalepsy, and stereotyped behaviour (Gambill and Kornetsky, 1976); effects

which are successfully antagonised by dopamine antagonists including antipsychotics (Ellison and Eison, 1983).

1.6.1.2 Phencyclidine (PCP)

The non-competitive NMDA receptor antagonist, phencyclidine (PCP), is a psychomotor stimulant drug that has been shown to induce symptoms characteristic of schizophrenia (Javitt and Zukin, 1991; Jentsch *et al.,* 2007). PCP and its structural analogue ketamine produce inactivation of inhibitory control by decreasing GABA release (Yonezawa *et al.*, 1998). This disinhibition of excitatory neurotransmission is referred to as NMDA receptor hypofunction (Jentsch and Roth, 1999; Olney and Farber, 1995; Olney *et al.,* 1999). PCP has been shown to disturb dopamine levels in the prefrontal cortex and nucleus accumbens. The reduction of dopamine levels in the PFC is associated with negative and cognitive symptoms of schizophrenia (Jentsch and Roth, 1999), while an increase in dopamine in the nucleus accumbens is associated with hyperlocomotion often seen in PCP-treated animals (Jentsch *et al.,* 1998). Acute administration of PCP in rats has been shown to produce deficits in the novel object recognition (NOR) task (Grayson and Neill, 2004), an operant reversal learning paradigm (Abdul-Monim *et al.,* 2003; Idris *et al.,* 2005a) and attentional set-shifting (Egerton *et al.,* 2005). Acute PCP disrupts PPI of the startle reflex (Geyer *et al.,* 1984), a test of relevance to schizophrenia as such deficits are also exhibited by patients with schizophrenia (Braff *et al.,* 1978; 1992).

Acute dosing with PCP has limitations in modelling the chronic psychotic illness or the persistent cognitive deficits of schizophrenic patients. Alternatively, repeated sub-chronic exposure to psychomotor stimulant drugs such as PCP, and d-amphetamine, produce enduring behavioural, molecular, structural and cellular changes, which are

believed to mimic schizophrenia more accurately than acute dosing (Fletcher *et al.*, 2005; Jentsch and Roth, 1999; Tenn *et al.*, 2003). Examples of these changes include reduced frontal blood flow and glucose utilisation (Hertzman *et al.*, 1990; Wu *et al.*, 1991) and reduced frontal dopamine transmission (Jentsch *et al.*, 1997). Sub-chronic administration of PCP also produces deficits in social withdrawal (Snigdha and Neill, 2008a,b). A table comparing amphetamine, PCP and isolation rearing models is shown in table 1.3.

1.6.2 Non-pharmacological models of schizophrenia

Environmental factors may alter normal processing of information during adulthood resulting in a psychotic state (Weinberger and Lipska, 1995). Abnormal development of the brain during gestation or soon after may contribute to the manifestation of schizophrenia in later life (Akil, 2000). There are several non-pharmacological models which aim to produce deficits that are caused in development.

1.6.2.1 Isolation rearing model

Early observations of behaviour in rats reared in isolation led to the description of the "isolation syndrome" (Hatch *et al.*, 1965; Sahakian *et al.*, 1977). Rats reared in isolation from weaning until adulthood show several behavioural changes, consistently including increased locomotor activity, anxiogenesis, (Puglisi-Allegra and Oliverio, 1983), enhanced sensitivity to psychoactive drugs such as amphetamine and cocaine (Jones *et al.*, 1990; Smith *et al.*, 1997) and sensorimotor gating deficits as measured by reduced pre-pulse inhibition (PPI) of the startle response (Geyer *et al.*, 1993; Cilia *et al.*, 2001). In addition, administration of sub-chronic PCP was found to enhance the locomotor activity observed in isolates (Lapiz *et al.*, 2003). Rats reared in isolation

have been shown to exhibit higher dopamine levels in the nucleus accumbens (NAC) and striatum, as well as a reduction in 5-HT levels within the NAC (Fulford and Marsden, 1998; Jones *et al.*, 1991; 1992). Studies have also demonstrated that the isolation rearing induced changes in presynaptic dopamine receptor function were also accompanied by postsynaptic dopamine changes including a down-regulation of D_2 receptors in the NAC (Hall *et al.*, 1998). Effects which may be associated with decreased presynaptic 5-HT function in the frontal cortex and hippocampus have also been demonstrated (Lapiz *et al.*, 2003). It has also been found that isolation-reared rats show decreased dopamine turnover in the mPFC (Heidbreder *et al.*, 2000), a region important for cognition (Goldman-Rakic *et al.*, 2000), particularly attentional set-shifting (Birrell and Brown, 2000). However, relatively few reports have investigated the effect of isolation rearing on cognition of relevance to schizophrenia (Weiss *et al.*, 2004, Li *et al.*, 2007, Schrijver and Wurbel, 2001; Dalley *et al.*, 2002). Although, more recent studies have shown that rats reared in isolation demonstrate deficits in novel object recognition (Bianchi *et al.*, 2006). Furthermore, treatment with the ampakine, aniracetam, or the 5-HT_6 antagonist, PRX-07037, both restored novel object recognition levels to those seen in socially housed controls (Porkess *et al.*, 2006; King *et al.*, 2007). This is due to the theories that AMPA and 5-HT_6 receptors are important for cognition in schizophrenia. AMPA receptors work closely with NMDA receptors to maintain glutamatergic function (Javitt, 2004). However, AMPA receptors are prone to rapid densenitisation; therefore ampakines have been developed as allosteric modulators of AMPA receptors and do not cause desensitisation of the receptor (Yamada *et al.*, 1998). Ampakines have been shown to enhance glutamatergic transmission, and to facilitate LTP (Hampsen *et al.*, 1998a, b). 5-HT_6 receptors have been implicated as having a role in learning and memory processes in healthy and disease states (Fone, 2008; Mitchell

and Neumaier, 2005; Woolley *et al.,* 2004; see section 1.7.3). A table comparing amphetamine, PCP and isolation rearing models is shown in table 1.3.

Table 1.3: Comparison of amphetamine, PCP and isolation rearing animal models.

	Animal model		
Impaired phenotype	**Amphetamine**	**PCP**	**Isolation rearing**
Locomotor activity	+++	++	+++
Gating	+	+	+
Cognitive behaviour	+/-	+++	++
Social behaviour	-	++	+
Schizophrenia pathology	+	++	++

Table 1.3: Comparison of amphetamine, PCP and isolation rearing animal models. The success of each model in producing deficits in the phenotype is indicated by +, ++, +++. +/- indicates some deficits depending on dosage regimen. Data taken from Schizophrenia Research Forum (www.schizophreniaforum.org).

1.6.2.2 Neurodevelopmental and lesion models of schizophrenia

Early postnatal treatment of rats with PCP on postnatal days (PNDs) 7, 9 and 11 has been proposed as a neurodevelopmental model of schizophrenia (Wang *et al.,* 2001). This treatment regimen has been reported to produce widespread neurodegeneration in brain areas relevant to the cognitive deficits observed in schizophrenic patients, such as the hippocampus and frontal cortex (Ikonomidou *et al.,* 1999; Wang and Johnson, 2005). Broberg *et al.* (2008; 2009) showed that this neonatal PCP regimen could impair performance in the attentional set-shifting task. Harich *et al.,* (2007) also showed that neonatal PCP produced impairments in social discrimination which was reversed by clozapine. In addition neonatal PCP treatment produces increased locomotor activity following an acute PCP challenge and induces deficits in PPI of the startle stimulus and delayed spatial alternation task (Wang *et al.*, 2001).

Disruption of neurogenesis by prenatal treatment with methylazoxymethanol (MAM), an anti-mitotic agent, has also been explored as a possible neurodevelopmental

model of schizophrenia. Recent studies have reported that MAM administration at gestational day 17 produced cognitive deficits as well as behavioural alterations in adult rats believed to correspond to certain aspects of the positive and negative symptoms of schizophrenia (Flagstad *et al.,* 2004, 2005; Gourevitch *et al.,* 2004; Penschuck *et al.,* 2006; Moore *et al.,* 2006; Le Pen *et al.,* 2006). Moreover, consistent with the data found in schizophrenic subjects, morphological and/or functional abnormalities such as disrupted neurogenesis and decreases in parvalbumin expressing neurons have been observed in the hippocampus, medial prefrontal cortex (mPFC) and ventral striatum of rats prenatally exposed to MAM (Flagstad *et al.,* 2004; Gourevitch *et al.,* 2004; Lavin *et al.,* 2005; Penschuck *et al.,* 2006; Moore *et al.,* 2006).

Lesions of the ventral hippocampus in neonates, but not in adults, induce the same deficits in working memory as medial PFC (mPFC) lesions in adult rats performing continuous delayed alternation and variable-delay alternation tasks (Lipska *et al.,* 2002). Neonatal ventral hippocampal-lesioned (NVH) rats also show a similar deficit prior to puberty in the radial arm maze (Chambers *et al.,* 1996). Following lesions of the NVH, rats are also impaired in the Morris water maze (Le Pen et *al.,* 2000). Multiple measures of working memory in rodents provide concordant evidence that interference with normal development in the functional circuitry of ventral hippocampus and PFC induces a postpubertal emergence of cognitive deficits consistent with findings in schizophrenia. Evidence for a role for mPFC in this process stems from studies that have shown alterations in gene expression, neuronal morphology and neurochemistry in mPFC following lesions of the NVH, consistent with the neuroanatomical connections between ventral hippocampus and mPFC in the rat (Lipska and Weinberger 2000). NVH lesions also disprupted the extra-dimensional shift phase of the attentional set-shifting task, a task known to require intact mPFC

function (Birrell and Brown, 2000) in juvenile rats (Marquis *et al.*, 2008), and deficits are also observed in the five-choice serial reaction time task (LePen *et al.,* 2003). Notably, NVH lesions in rodents also induce a postpubertal onset of deficits in sensorimotor gating or pre-pulse inhibition (PPI; Chambers *et al.*, 1996; Le Pen *et al.,* 2000; Daenen *et al.,* 2003), a consistent feature in schizophrenia spectrum disorders (Swerdlow *et al.,* 1994; Braff *et al.,* 2001; Geyer *et al.,* 2001). However, pharmacological activation/disruption of either ventral hippocampus (Bast *et al.,* 2001) or mPFC (Japha and Koch 1999) also produces PPI deficits in adult rats, suggesting that a progressive disruption of functional circuitry is not obligatory for induction of this deficit.

1.6.3 Pathophysiological changes observed in schizophrenia and similarities with PCP and isolation rearing models

In patients with schizophrenia CT and MRI longitudinal studies have suggested cerebral ventricular enlargement and hemisphere volumetric reductions (see DeLisi, 1999). Furthermore, positron emission tomography (PET) and fMRI functional measurements are often reported to be abnormal in the PFC in schizophrenia (reviewed by Wible *et al.,* 1995). Post-mortem analysis of brain tissue from schizophrenic patients has shown a reduction in GABAergic interneurons in the frontal cortex (Benes *et al.,* 1991) and hippocampus (Benes and Berretta, 2001). Calcium binding proteins (CBP), namely parvalbumin (PV), calbindin (CB) and calretinin (CR) have been used as markers of specific subpopulations of non-overlapping GABAergic interneurons in the brain. The GABAergic deficits found in schizophrenia are largely restricted to the PV-containing neurons (Lewis *et al.,* 2005). Reduced density of parvalbumin immuno reactive neurons in the hippocampus and M1 (motor area 1) region of the frontal cortex

following sub-chronic PCP treatment in the rat was recently shown (Abdul-Monim *et al.,* 2007). It has also been shown that chronic intermittent administration of PCP decreases parvalbumin mRNA expression in the prefrontal cortex of rats, and that this reduction can be reversed by clozapine but not haloperidol (Cochran *et al.,* 2003). These findings provide further support for the theory that sub-chronic PCP treatment in the rat provides a good correlate of the clinical condition.

Schizophrenic patients exhibit decreased dendritic spine density in both the hippocampus and medial prefrontal cortex (Glantz and Lewis, 2000). These structural alterations are also seen in rats reared in isolation (Varty *et al*., 1999; Silva-Gomez *et al.,* 2003; Day-Wilson *et al.,* 2006). Reduced density of parvalbumin immuno-reactive neurons in the hippocampus were also found following isolation rearing (Harte *et al.,* 2006). Levels of N-acetyl aspartic acid (NAA), a marker of neuronal integrity, were shown to be decreased in the temporal cortex of isolation-reared rats, while these changes were not observed in the hippocampus, striatum or frontal cortex (Harte *et al.,* 2004); deficits were also found in the temporal cortex only in rats that were chronically treated with PCP (Reynolds *et al.,* 2005). These decreases resemble the pattern observed in patients with schizophrenia (Fukuzako *et al.,* 1995). However, some studies have reported reductions in NAA in the frontal cortex in schizophrenia (Bertoline *et al.,* 1998; Cecil *et al.,* 1999). NMDA receptor subunit 1A mRNA expression was decreased in isolation-reared rats in the striatum and prefrontal cortex (Hall *et al.,* 2002). However, these results were not similar to those found following sub-chronic PCP administration (Snigdha, PhD thesis).

1.6.4 Genetic models of schizophrenia

Construction of mice with targeted mutations via gene knockout or transgenesis has demonstrated the ability to identify the functional significance of the targeted gene and its encoded protein (Tecott and Wehner, 2001). Several schizophrenia-susceptibility genes have been identified (Harrison and Owen, 2003). These identified genes may impact on the neurodevelopmental aspect of schizophrenia and several are involved in glutamatergic transmission particularly via NMDA receptors (Tsai and Coyle, 2002). These genes include dysbindin which is involved in the formation and maintenance of synapses (Straub *et al.,* 2002), and proline dehyrogenase (PRODH) which affects glutamatergic synapses. RGS4 (regulator of G-protein signalling-4) is a negative regulator of glutamate receptors and may have neurodevelopmental roles (De Vries *et al.,* 2000). D-aminoacid oxidase (DAAO) and G72 impact directly on NMDA receptors as DAAO metabolises D-serine, an endogenous modulator of the receptor (Mothet *et al.,* 2000), and G72 is believed to be the activator of DAAO (Chumakov *et al.,* 2002). Neuregulin-1 (NRG-1) is present in glutamatergic synaptic vesicles, and affects NMDA receptors via actions on ErbB receptors and regulation of NMDA receptor expression (Buonanno and Fischbach, 2001; Stefansson *et al.,* 2002). Catechol-O-methyltransferase (COMT) acts directly on monaminergic neurotransmission (Gogos *et al.,* 1998) and can subsequently affect other systems such as the glutamatergic system via links with the dopamine system (Grace, 1991). Disrupted-in-Schizophrenia-1 (DISC-1) is one of the strongest candidate genes through genetic and clinical association studies (Ishizuka *et al.,* 2006; Porteous *et al.,* 2006). The disruption in this gene is cosegregated with schizophrenia, major depression and bipolar disorder in a large Scottish family (Millar *et al.,* 2000; Millar *et al.,* 2001). Since its discovery

reproducible linkage between regions of DISC-1 and psychiatric diseases have been observed in many populations (see Wang *et al.,* 2008).

Despite the merits of genetic models of schizophrenia, it must be emphasised that the mutation of a specific susceptibility gene is unlikely to give rise to a phenotype that encompasses all aspects of schizophrenia; such mutants should not be considered to model schizophrenia, but, rather, to model the functional roles of genes associated with risk of schizophrenia. In addition, as such genetic mutations occur upstream from the cellular and physiological mechanisms that give rise to abnormal behaviour, they are inevitably subject to environmental influences and compensatory/adaptive effects (see Desbonnet *et al.,* 2009).

1.7 Antipsychotics

Cognitive symptoms can often persist when other symptoms may be improved with treatment (Gold *et al.,* 1991). Novel antipsychotics are required to improve cognition as the Clinical Antipsychotic Trials of Invention Effectiveness (CATIE) study revealed that atypical antipsychotics, such as clozapine, show little improvement compared to typical antipsychotics, against cognitive symptoms (Lieberman, 2006; Keefe *et al.,* 2007).

1.7.1 Typical antipsychotics

The classical antipsychotics such as haloperidol, chlorpromazine and thioridazine, act via blockade of dopamine D_2 receptors, muscarinic cholinergic receptors, α_1-adrenergic receptors and histamine receptors (Stahl, 2002). This group of antipsychotics are effective in controlling positive symptoms of schizophrenia, but are ineffective in treating negative or cognitive symptoms. Furthermore, typical antipsychotics have

significant adverse effects such as tardive dyskinesia and extra-pyramidal symptoms due to the blockade of dopamine receptors in the nigrostriatal pathway (Casey, 1996). Hyperprolactinemia may also be a side effect when tuberoinfundibular D_2 receptors are blocked (Windgassen *et al.*, 1996). Due to these debilitating adverse effects and apparent lack of efficacy against many of the symptoms of schizophrenia, much research has been focused on developing novel antipsychotics.

1.7.2 Atypical antipsychotics

The label of "atypical" antipsychotics is usually based on the drugs' ability to reduce schizophrenic symptoms without causing extra-pyramidal adverse effects. Benefits of atypical antipsychotics include efficacy against positive, negative, and disorganised symptoms, improvements of some areas of cognition, lack of development of tardive dyskinesia and lack of hyperprolactinaemia. Some atypical antipsychotics were developed to be more selective for the D_2 receptor such as sulpiride. However, most new antipsychotics have affinity for multiple receptors, for example risperidone acts as an antagonist at 5-HT_{2A}, 5-HT_7, α_1 and α_2-adrenergic, and histamine H_1 receptors.

Atypical antipsychotics improve on the action of the typical antipsychotics by blocking 5-HT and dopamine receptors simultaneously. The significance of 5-HT is that it can control the release of dopamine to differing degrees in the four different pathways. In the nigrostriatal pathway 5-HT exhibits significant control over dopamine, therefore blocking 5-HT receptors stimulates dopamine release which consequently prevents EPS and tardive dyskinesia (Lieberman *et al.*, 1998). In the mesocortical dopamine pathway there is an abundance of 5-HT receptors, thus the atypical antipsychotics have a greater effect on 5-HT antagonism therefore increasing dopamine release. Increase in dopamine release in this area of the brain helps to reduce negative

symptoms and may also improve cognition (Lieberman *et al.,* 1998). In the tuberoinfundibular pathway dopamine inhibits prolactin release, however when D_2 receptors are blocked, hyperprolactinaemia occurs. With atypical antipsychotics, there is simultaneous inhibition of 5-HT_{2A} receptors, thus there is no longer stimulation of prolactin release, and this tends to mitigate the hyperprolactinaemia of D_2 receptor blockade. In the mesolimbic pathway, atypical antipsychotics have little impact as they fail to prevent D_2 receptor blockade.

1.7.3 Pharmacology of antipsychotics and receptor targets for cognition

Results in this laboratory have shown that atypical but not classical antipsychotics can reverse cognitive deficits induced by sub-chronic PCP administration in an operant reversal learning paradigm (Abdul-Monim *et al.,* 2006), and in a novel object recognition task (Grayson *et al.,* 2007). Molecular targets for cognitive enhancement in schizophrenia were highlighted by Gray and Roth (2007) and are shown in table 1.4.

Mounting evidence suggests that the D_1 receptor in the mPFC may be important in regulating cognitive function in schizophrenic patients. Okubo and colleagues (1997) reported a down-regulation of D_1 binding in the PFC of treatment-free/-naïve schizophrenic patients. Another study has demonstrated an association between genetic risk for schizophrenia and alterations in cortical D_1 receptor binding (Hirvonen *et al.,* 2006). It has also been shown that D_1 receptors are more abundant than D_2 receptors in the PFC of non-human primates (Lidow *et al.,* 1991), and this D_1 receptor subfamily has been implicated in working memory functions of the PFC (Arnsten *et al.,* 1994, Sawaguchi and Goldman-Rakic, 1991) one aspect of cognition impaired in schizophrenia. Thus, it is possible that stimulation of the D_1 receptor may represent a potential strategy for treating cognitive deficits associated with schizophrenia. However,

the use of D_1 receptor agonists as therapeutic agents pose difficulties for pharmacologists as dopamine function in the PFC seems to follow an "inverted-U" dose-response relationship whereby increases or decreases from an optimal level result in cognitive impairment (Goldman-Rakic *et al.*, 2000).

There is evidence to show that 5-HT_{1A} receptor density is high in the frontal cortices of patients with schizophrenia (Burnet *et al.*, 1997) and a subsequent PET study has shown an increase in cortical 5-HT_{1A} receptor binding in schizophrenia (Tauscher *et al.*, 2002). Post-mortem studies have also indicated abnormalities in 5-HT_{1A} receptor-mediated transmission in the frontal cortex of patients with schizophrenia (Sumiyoshi *et al.*, 1996). 5-HT_{1A} receptor agonism has been suggested to contribute to an atypical antipsychotic drug profile (Protais *et al.*, 1994). There are also interactions between 5-HT_{2A}, 5-HT_{2C} and 5-HT_{1A} receptors (Kapur and Remington, 1996; Meltzer, 1999). The 5-HT_{2A} antagonist, M100.907, is efficacious in improving a sub-chronic PCP-induced deficit in reversal learning (Neill *et al.*, 2008). Antagonism at 5-HT_{2C} receptors by various atypical antipsychotics (e.g. clozapine, risperidone, sertindole and ziprasidone; Roth *et al.*, 2003) may play a role in their beneficial effects and their diminished likelihood to produce to neurological adverse effects (Meltzer, 1995; 1999). Conversely, in a recent study, WAY-163909, a selective 5-HT_{2C} agonist, demonstrated anti-psychotic-like properties by reversing increased locomotor activity induced by PCP and amphetamine and reversing MK-801-induced disprution in PPI (Marquis *et al.*, 2007). Antagonism at the $5HT_{2C}$ receptor can increase food intake and body weight in rats (Bonhaus *et al.*, 1997), thus this may not be an advantageous strategy for treatment in patients as weight gain is a major adverse effect and could affect compliance (see Allison *et al.*, 1999 for meta-analysis). Furthermore, it had been found that knock-out mice lacking the 5-HT_{2C} receptors are hyperphagic and obese (Tecott *et al.*, 1995).

Antipsychotics that induce body weight gain appear to have combinations of high affinities for 5-HT_{2C}, α_1, H_1 and M_1 receptors (Baptista, 2000; Casey and Zorn, 2001; Hartfield *et al.,* 2003). In patients, olanzapine dramatically increases weight gain (Eder *et al.,* 2001). Although ziprasidone also has high affinity for 5-HT_{2C} receptors it was shown not to induce ingestive behaviour and intra-abdominal fat deposition in rats (Fell *et al.,* 2005). Indeed, it was demonstrated that ziprasidone, when co-administered with olanzapine, attenuated the olanzapine-induced hyperphagia (Kirk *et al.,* 2004). This was suggested to be due to ziprasidone's action as a 5-HT_{1A} partial agonist, a property which may protect against weight gain (Casey and Zorn, 2001).

5-HT_6 receptors have been implicated as having a role in learning and memory processes in healthy and disease states (Mitchell and Neumaier, 2005; Woolley *et al.,* 2004). High levels of 5-HT_6 receptor mRNA are found in the striatum, nucleus accumbens, hippocampus and cortex (Monsma *et al.,* 1993; Ruat *et al.,* 1993). Many atypical antipsychotics are potent antagonists at the 5-HT_6 receptor (Roth *et al.,* 2004), making it a target of interest. A selective 5-HT_6 receptor antagonist, SB 399885-T, has been shown to improve set-shifting ability (Hatcher *et al.,* 2005), this may be via facilitation of cortical and hippocampal glutamatergic activity (Dawson *et al.,* 2001). Results from this laboratory have reported that the 5-HT_6 receptor antagonist, GSK 742457, and the atypical antipsychotic sertindole a drug with high 5-HT_6 receptor affinity (Leysen, 2000), are able to reverse a sub-chronic PCP-induced deficit in reversal learning (Idris *et al.,* in preparation; Neill *et al.,* 2008). Antagonism at 5-HT_6 receptors may improve cognition by increasing dopamine (Lacroix *et al.,* 2004) and acetylcholine (Riemer *et al.,* 2003) levels in the PFC. 5-HT_7 receptors are located in the thalamus, hypothalamus, amygdala in addition to limbic and cortical regions (Thomas *et al.,* 2002; Varnas *et al.,* 2004). 5-HT_7 receptors have been implicated as having a role

in learning and memory (Hedlund and Sutcliffe, 2004; Thomas and Hagan, 2004). The 5-HT_7 receptor is a potential target of relevance to schizophrenia as many atypical antipsychotics such as risperidone and ziprasidone have affinity for this receptor (Roth *et al.,* 1994; Siegfried *et al.,* 2005; Shahid *et al.,* 2008), and some e.g. clozapine have significant inverse agonist activity (Mahe *et al.,* 2004). It has been hypothesised that 5-HT_7 receptor antagonists may reverse PCP-induced deficits via the prevention of 5-HT and/or glutamate release (Semenova *et al.,* 2008).

Nicotinic acetylcholine receptors (nAChRs) are ionotrophic receptors with a pentameric structure composed of α and β subunits, and are highly expressed in the hippocampus, cortex, striatum, and thalamus (Freedman *et al.,* 1995; Breese *et al.,* 2000). The most prevalent nAChRs are the α4β2 and α7 subtypes, both of which have been shown to have reduced numbers in schizophrenia (Freedman *et al.,* 1995; Breese *et al.,* 2000). It has been suggested that these receptor subtypes are involved in cognition (Schreiber *et al.,* 2002; Chan *et al.,* 2007; Gray and Roth, 2007; this will be further discussed in chapter 3). Muscarinic ACh receptors are G-protein coupled. Post mortem studies using the selective M_1/M_4 radioligand [^{3}H]pirenzepine have found decreased density of M_1 and/or M_4 receptors in the frontal cortex (Crook *et al.,* 2001) and hippocampus (Crook *et al.,* 2000). The M_1 receptor subtype (discussed further in chapter 3) is the most abundant muscarinic receptor in the forebrain and hippocampus (Levey *et al.,* 1991; Wei *et al.,* 1994). Furthermore, it has been shown that M_1 deficient mice demonstrate deficits in working and reference memory (Anagnostaras *et al.,* 2003). N-desmethylclozapine (NDMC), a major metabolite of clozapine, has been shown to increase ACh and DA in the frontal cortex, an effect due to the stimulation of M_1 receptors, confirming that NDMC had M_1 agonistic actions (Li *et al.,* 2005).

There are 7 known metabotropic receptors (see section 1.5.4). Targets of importance to cognition and schizophrenia are mGluR2/3 agonists and mGluR5 agonists. mGluR2 and mGluR3 receptors exhibit moderate to high expression in forebrain regions that are commonly associated with schizophrenia such as the frontal cortex, hippocampus and nucleus accumbens (Ohishi *et al.,* 1993). These receptors are localised mainly pre-synaptically on glutamate nerve terminals; the mGluR2/3 appear to act on a presynaptic site to inhibit glutamate release from the glutamate neurons (Schoepp *et al.,* 1999; Kilbride *et al.,* 1998). Agonists at the mGluR2/3 have been reported to attenuate some of the behavioural and neurochemical effects of PCP and amphetamine in animal models (Schoepp and Marek, 2002). The mGlu2/3 receptor agonists, LY3792688 and LY404039, have been found to mediate their antipsychotic-like effects in the mouse PCP and amphetamine hyperlocomotion models of psychosis through the selective activation of mGluR2 rather than mGluR3 receptors (Woolley *et al.,* 2008; Fell *et al.,* 2008). mGluR5 is abundant in the striatum, nucleus accumbens, olfactory tubercle, hippocampus and cerebral cortex (Testa *et al.,* 1994). Stimulation of mGluR5 enhances NMDA receptor function in cerebral cortex, hippocampus and striatum (Benquet *et al.,* 2002; Mannaioni *et al.,* 2001; Pisani *et al.,* 2001). In support of this theory, impairments in PPI have been found in mGluR5 knockout mice (Kinney *et al.,* 2003; Henry *et al.,* 1999).

Table 1.4: Molecular Targets for Cognitive Enhancement in Schizophrenia.

Molecular Target
D_1 agonists
D_4 antagonists
D_4 agonists
COMT inhibitors
5-HT_{2A} antagonists
5-HT_{1A} agonists
5-HT_{1A} antagonists
5-HT_4 agonists
5-HT_6 antagonists
5-HT_7 antagonists
Cholinesterase inhibitors
Nicotinic α_7 agonists
Nicotinic $\alpha_4\beta_2$ agonists
M_1 agonists
M_4 agonists
M_5 antagonists
NMDA enhancers
GlyT inhibitors
Ampakines
mGluR2/3 agonists
mGluR5 agonists
α_2-adrenergic antagonists
$GABA_A$ (α_2) agonists
$GABA_A$ (α_5) antagonists
Sigma agonists

Table 1.4: Molecular Targets for Cognitive Enhancement in Schizophrenia (adapted from Gray and Roth, 2007). Targets highlighted in yellow will be investigated and further described in this thesis.

1.8 General Aims

Phencyclidine is being extensively used to mimic behavioural deficits in different rodent tests of cognition. The principal aim of this work is to study the nature of the cognitive and neurobiological deficits in schizophrenia using the sub-chronic PCP model in female rats.

Different behavioural tests will be used to assess different aspects of cognition. This thesis will utilise the attentional set-shifting task to assess cognitive deficits in executive function using the sub-chronic PCP model and the neurodevelopmental model

of isolation rearing. An operant reversal learning task will be employed to investigate the receptor pharmacology of antipsychotics; and this test is will be altered and manipulated to elucidate if the task can be made more difficult in order to distinguish between antipsychotics.

An improved knowledge of the mechanisms by which PCP produces its effects on cognition will ultimately enhance our understanding of the pathophysiology of schizophrenia. Therefore, the effects of sub-chronic PCP on gamma oscillations in an *in vitro* electrophysiology preparation will be studied as these are believed to be disrupted in schizophrenia. In addition, immunohistochemistry will also carried out to determine whether changes in gamma oscillations in the hippocampus are linked to changes in parvalbumin immunoreactive neuron cell density. In order to further understand the neurochemical changes in cognition and to investigate the role of dopamine, *in vivo* microdialysis will be performed in behaving rats during the novel object recognition test.

Chapter 2

The effect of sub-chronic PCP, antipsychotics, and isolation rearing on attentional set-shifting performance

2.1 Introduction

Whilst impairments have been reported across all of the cognitive domains identified by the MATRICS initiative (see chapter 1), impairments in executive function have long been considered to be a core feature of schizophrenia (Elvevag and Goldberg, 2000; Nuechterlein and Dawson, 1984; Sullivan *et al.*, 1994). One aspect of executive function is the ability to modify behaviour in response to the changing relevance of a stimulus; this is commonly assessed in patients using the Wisconsin Card Sorting Test (WCST) (Berg, 1948). In more recent years a more conclusive battery of tests known as CANTAB (Cambridge Neuropsychological Test Battery) has been developed (Downes *et al.*, 1989). The intra-/extra-dimensional (ID/ED) test in CANTAB is similar to the WCST. In the ID/ED task, simple discriminations and reversals are made to ensure a rule can be learned and reversed. This is followed by the addition of a distracting variable which establishes an attentional set. Attentional shifts can then be made; firstly in the same dimension as the relevant stimuli (intra-dimensional or ID shift) and secondly the relevant dimension changes to the dimension that was previously being ignored (extra-dimensional or ED shift; Jazbec *et al.*, 2007, fig 2.1).

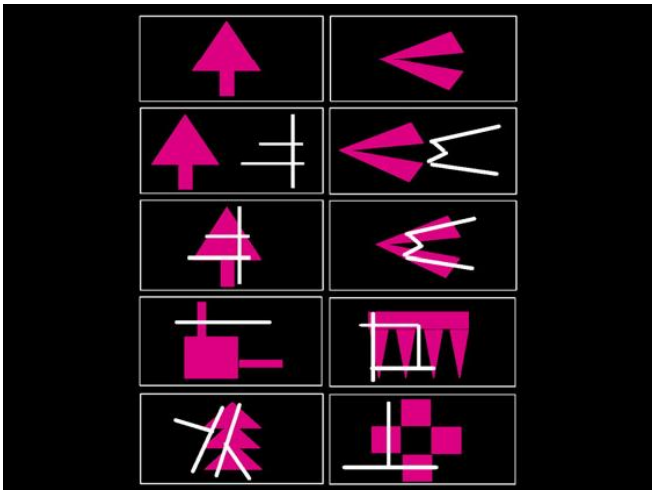


Figure 2.1: The ID/ED task without reversal stages, taken from Jazbec *et al.* (2007).

The entire CANTAB battery including the ID/ED test was successfully trialled in primates (Weed *et al.,* 1999). The images appeared on a touch screen which primates were trained to push to receive a food reward. However, the use of primates in research is extremely expensive and ethically debatable. Therefore, other animal models of the ID/ED task have been developed. In 2000 Birrell and Brown developed the perceptual attentional set-shifting procedure for the rat. This task (Birrell and Brown, 2000) investigates the ability of a rat to learn a rule and form an attentional set within the same sorting category (intra-dimensional shift - IDS), as well as the ability to shift attentional set between different sorting categories (extra-dimensional shift - EDS). Rats must carry out a series of 7 discriminations namely, simple discrimination (SD), compound discrimination (CD), reversal 1 (R1), intra-dimensional shift (IDS), reversal 2 (R2), extra-dimensional shift (EDS) and reversal 3 (R3). In the simple discrimination (SD) phase, for example if odour was selected as the relevant dimension, one odour would be baited while the other would not, both bowls would be filled with the same digging medium. Successful completion of the SD phase is followed by compound discrimination (CD) in which odour remains as the relevant dimension (using the same exemplars), along with the introduction of the new digging medium. The same odour is baited as in SD. Following CD, a reversal (R1) of the established rules in CD is carried out, so that the previously unrewarded odour was now baited, irrespective of digging media. Following this rats are presented with new exemplars to assess the intra-dimensional shift (IDS); the relevant dimension of odour continues to be the positive predictor with digging media irrelevant. The previously rewarded odour is then reversed (R2). For the extra-dimensional shift (EDS) the relevant dimension of odour is replaced by digging medium as the positive predictor. Finally, the EDS is reversed (R3)

so that the formerly negative digging medium exemplar becomes the positive predictor of the food reward.

The attentional set-shifting task represents a rat analogue of the human Wisconsin Card Sorting Task (WCST, Berg, 1948) and CANTAB ID/ED task (Downes *et al.,* 1989) in which schizophrenic patients exhibit impaired set-shifting (Kolb and Wishaw, 1983; Haut *et al.,* 1996; Pantelis *et al.,* 1999; Tyson *et al.,* 2004). The TURNS initiative has identified that this test can be used to determine the problem solving deficits described in the MATRICS cognitive battery (www.turns.ucla.edu).

It has been shown that whereas lesions of the orbital prefrontal cortex in rats selectively disrupts reversal learning (McAlonan and Brown, 2003), lesions of the medial prefrontal cortex (mPFC) produce a selective deficit in the EDS phase in attentional set-shifting (Birrell and Brown, 2000). Rats reared in isolation have been shown to exhibit structural abnormalities selectively in the mPFC (Silva-Gomez *et al.,* 2003; Day-Wilson *et al.,* 2006). Isolation-rearing also causes reduced levels of dopamine in the prefrontal cortex (Jones *et al.,* 1991), a state similar to hypofrontality observed in schizophrenia, an effect also mimicked by sub-chronic PCP treatment (Jentsch and Roth, 1999).

Acute administration of PCP in rats has been shown to produce deficits in attentional set-shifting (Egerton *et al.,* 2005). Rodefer and colleagues combined the attentional set-shifting test with a sub-chronic PCP dosing regimen (5 mg/kg twice daily for seven days followed by a ten-day drug washout period) and found a selective deficit in the EDS phase (Rodefer *et al.,* 2005), and have now investigated the pharmacology of antipsychotics in this model (Rodefer *et al.,* 2007). Performance in isolation reared rats has previously been described by Schrijver and Wurbel (2001) using an 8-arm radial maze with spatial and non-spatial visual discriminations.

Haloperidol is still a widely used antipsychotic, and it is the antipsychotic most often used to judge the efficacy of newer medications (see Irving *et al.,* 2006). Haloperidol relies heavily on its affinity for D_2-like receptors (see table 2.1). Meltzer *et al.,* (1989) defined atypical antipsychotic drugs as having higher affinity for 5-HT_{2A} receptors than for D_2 receptors. 5-HT_{2A} receptor antagonists increase dopamine release into the prefrontal cortex (PFC; Ichikawa and Meltzer, 1990). Although atypical antipsychotics such as risperidone and clozapine have high affinity for 5-HT_{2A} receptors, it is unlikely that this mechanism alone is responsible for their antipsychotic action, as both have been shown to saturate 5-HT_2 receptors at sub-therapeutic doses (Kapur *et al.,* 1999). Atypical antipsychotics have affinity for a multitude of receptors. In this attentional set-shifting chapter, clozapine, risperidone and haloperidol are to be tested against sub-chronic PCP. The *in vitro* pharmacology of these antipsychotics is shown in table 2.1 and will be further detailed in the discussion of this chapter.

Table 2.1 *In vitro* receptor binding in man (affinity constants K_i in nM) at receptors for clozapine, risperidone and haloperidol. Values taken from Siegfried *et al.,* 2005.

Receptor	Clozapine	Risperidone	Haloperidol
D_1	85	430	210
D_2	160	2	1
D_3	170	10	2
D_4	50	10	3
5-HT_{1A}	200	210	1100
5-HT_{2A}	16	0.5	45
5-HT_{2C}	10	25	>10000
5-HT_6	14	2200	9600
5-HT_7	100	2	1200
NE α_1	7	1	6
NE α_2	8	1	360
H_1	1	20	440
Muscarinic	2	>1000	5500

The first aim of this study was to investigate whether a sub-chronic PCP dosing regimen could induce a cognitive deficit in male and female rats. The second aim was to investigate whether these deficits induced by PCP in attentional set-shifting could be reversed by sub-chronic administration of clozapine, risperidone or haloperidol. The third aim was to investigate whether a non-pharmacological model could also induce a cognitive deficit in order to strengthen the validity of the paradigm. To address this, the effects of isolation rearing on cognitive performance in the attentional set-shifting task were examined as isolation rearing has been suggested to model some aspects of schizophrenia (see chapter 1).

The aim of the final experiment was to counterbalance the PCP study i.e. half of the rats tested on medium first then switching to odour and the second half on odour first then switching to medium. In this experiment the discovery trials were also scored. It would be expected that both vehicle and PCP-treated rats would make more incorrect discovery trials in the EDS phase.

2.2 Materials and Methods

2.2.1 Subjects

For experiment 1, 20 male and 20 female hooded-Lister rats (Harlan, UK), weighing 250-280 g and 200-220 g respectively at the start of the study, were used. For experiment 2, 50 female hooded-Lister rats (Harlan, UK), weighing 200-220 g at the start of the study, were used and were housed in groups of 4-5 (shown in fig 2.2.1a). For experiment 3, 20 female hooded-Lister rats (Harlan, UK) were ordered at post-natal day (PND) 14. At PND 21 rats were randomly allocated to one of two housing conditions. Half were housed in groups of five in clear plastic cages (38 x 59 x 24 cm) and half were housed in individual opaque cages (25 x 35 x 24 cm) for a minimum period of 6 weeks before testing (shown in fig 2.2.1b). Rats could hear and smell other animals but could not see (due to opaque cages) or come into physical contact with them. For experiment 4, 16 female hooded-Lister rats (Harlan, UK), weighing 200-220 g at the start of the study, were used and were housed in groups of 4-5 (shown in fig 2.2.1a).

Animals were housed in a temperature-controlled room (21 ± 2 °C) at humidity of 45-55%, and maintained on a 12:12 h light/dark cycle (lights on at 0700 h). Experimental procedures were performed in the light phase. All rats had free access to food (Special Diet Services, UK) and water until 1 week before testing began, at which point a minimal food restriction was imposed upon them (90% of free feeding bodyweight; 12 g food per rat per day) but *ad libitum* access to water continued. All experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act, UK (1986) and were approved by the University of Bradford ethical review process.

Fig. 2.2.1a Cage of 4-5 social rats.

Fig. 2.2.1b Isolation-reared housing conditions.

2.2.2 Locomotor activity

The locomotor activity (LMA) response to a novel environment was assessed using automated photocell cages (see fig 2.2.2a and 2.2.2b). The movement of each animal was monitored in 1 of 16 Plexiglas chambers (16 x 26 x 19 cm) covered with a compatible Plexiglas lid using AM1052 Activity Monitor (Linton Instrumentation). Counts were recorded by AmLogger software (supplied by GSK, Harlow, UK) by means of photo beam interruptions within the chamber. Activity was monitored every 5 min over a 60 min period and summed to give a total count. For experiment 1 10 vehicle and 10 PCP-treated rats were tested in these chambers. In experiment 2 10 social and 10 isolation-reared rats were tested. Rats were randomly allocated across the LMA boxes to ensure that all groups were evenly tested on each shelf, and an even number of rats from each group were tested at each time point, thus avoiding any horizontal placement and/or time of testing bias. For example vehicles were tested alongside PCP-treated rats and isolates were tested next to socially housed rats.

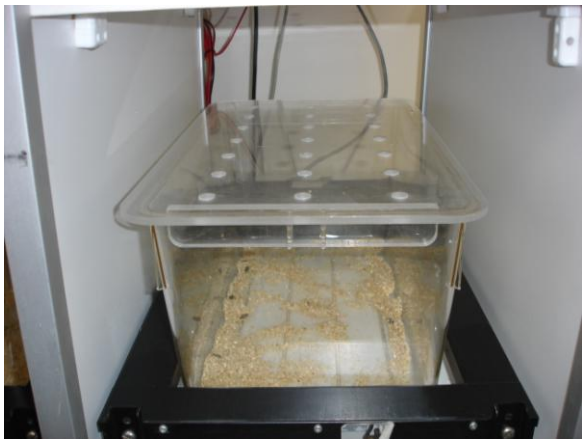


Fig. 2.2.2a An individual automated photocell cage.

Fig. 2.2.2b 16 automated photocell cages.

2.2.3 Set-shifting apparatus

The set-shifting apparatus was a modified version of that described originally by Birrell and Brown (2000) and was made in-house. The test box was essentially a modified home cage measuring 59 x 35 x 24 cm made of transparent plastic (Fig. 2.2.3a and 2.2.3b). A fixed 1 cm thick Plexiglas panel permanently divided 1/3 of the length of the box into two separate compartments (choice areas). These two choice areas could be further isolated from each other with the aid of two removable Plexiglas panels that were lowered into place along wall mounted vertical sliders. The digging bowls (glazed ceramic pots, Pets at Home, UK) were placed in each choice area, and the rat was given access by lifting the divider(s) which could also be used to deny access to the bowls, such as when an incorrect choice was made. A third removable panel divided the remaining 2/3 length of the larger compartment into two sections when in place, along the horizontal (short) axis of the test box.

Figure 2.2.3a: Photograph of the attentional set-shifting test box, plexiglas dividers were inserted into the visible slots in the lid.

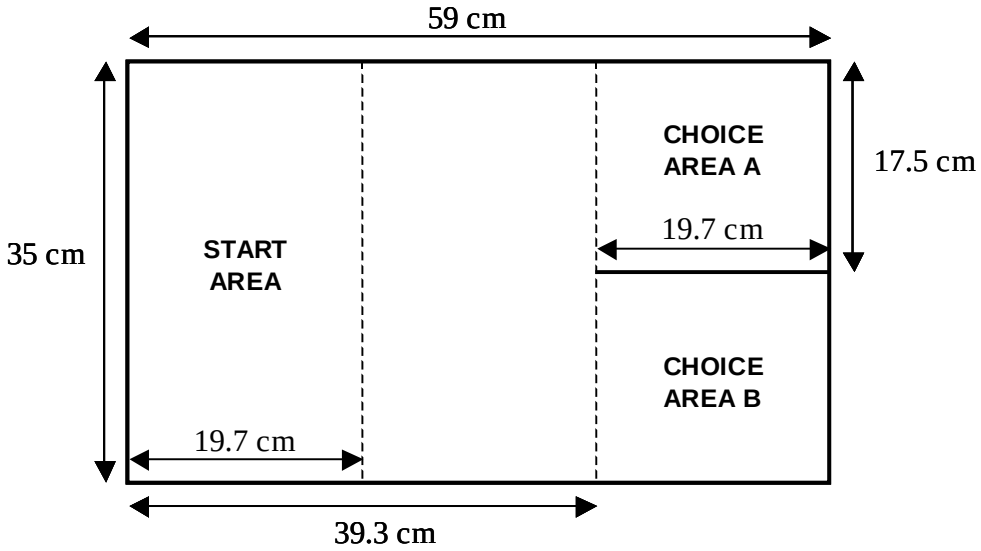


Figure 2.2.3b: Plan view of attentional set-shifting test box, showing gross dimensions, division into thirds and then into sixths to create two choice areas. Dotted lines indicate removable Plexiglas dividers and unbroken lines indicate fixed panels and walls.

2.2.4 Habituation and training

Habituation to specific aspects of the testing paradigm commenced immediately following the end of the seven-day drug washout period (see section 2.2.6). Habituation to the testing box occurred for 30 min on 3 consecutive days prior to training. Two

smooth ceramic circular digging pots (measuring 9 cm in diameter by 5 cm internal depth) identical to those intended for use during the testing phases were introduced to each home cage (figure 2.2.4a). Each bowl was filled with grade 5 sawdust (also covering the home cage floor) and baited with food reward in the form of several 1/4 honey nut loops (Kellogs, UK). Digging bowls were continuously re-baited during this habituation period and remained in the home cage for the duration of the study. Following habituation, all rats had to successfully complete the entire training regime in order to proceed to testing. Rats were first trained to dig quickly and reliably in both bowls by progressively covering a single food reward per trial with incrementally thicker layers of digging media. Once a rat had repeatedly demonstrated that it had acquired the training procedure, the dividing panels were introduced. Digging itself was defined as a vigorous movement of front paws to displace digging media and obtain food reinforcement buried 2-2.5 cm below surface level.

The second phase of training introduced the concept of simple discrimination (SD) between first medium and then, odour. Rats were presented with identical digging bowls that had been anointed (smearing a few drops of oil around the rim of the bowl using a tissue) with two aromatic oils (Strawberry and Sandlewood, Bodyshop, UK), only one of which was baited with food reward (fig 2.2.4b). Placement of bowls in either the left or right compartment was randomised with the aid of an adapted pseudorandom Gellerman schedule. Rats were permitted to explore both bowls for the first four trials irrespective of which bowl they dug in first, thereby allowing the associate of a food reward with the positive predictor. Subsequent incorrect selections ended a trial without the opportunity to explore the correct bowl. A criterion for successful learning of each discrimination was set at 6 consecutive correct trials. All

exemplars used in training were not used during testing or at any other point in the study.

Fig. 2.2.4a Ceramic bowl used for digging. **Fig. 2.2.4b** Example of aromatic oils used for the odour.

2.2.5 Testing paradigm

In all cases rats were tested in the attentional set-shifting procedure 24 hours after training, and following either sub-chronic PCP treatment or 6 weeks of isolation rearing; example timelines of these experiments are shown in figures 2.2.5. A trial was initiated by raising both dividers to give access to both digging bowls, only one of which was baited.

1. **Experiment 1 (males and females):** The first stage was the simple discrimination (SD), which was identical to the simple discrimination in the training session on the previous day, except new exemplars were used. Testing continued until the rat reached a criterion of six consecutive correct responses. In a test session, rats performed a series of discriminations (see Table 2.2.5a). For the compound discrimination (CD) a second dimension was introduced (digging medium), but the correct and incorrect exemplars remained the same (odour). For the reversals the exemplars and relevant dimensions remained the

same (odour) but the rats had to learn that the previously baited odour was now incorrect and the other odour was now correct. New exemplars were used for the ID and ED shifts. The specific exemplars used are shown in table 2.2.5b. For the ED shift, the previously irrelevant parameter (i.e. digging medium) was now relevant. It has been shown that rats find the difficulty of each discrimination change, i.e. medium to odour or odour to medium, equivalent (Birrell and Brown, 2000). Therefore in simple discrimination, odour was the relevant parameter for all rats.

2. **Experiment 2 (sub-chronic PCP and antipsychotics):** Rats were tested on digging medium in simple discrimination and odour was the parameter used in the EDS phase. For the compound discrimination (CD) a second dimension was introduced (odour), but the correct and incorrect exemplars remained the same (medium). For the reversals the exemplars and relevant dimensions remained the same (medium) but the rats had to learn that the previously baited odour was now incorrect and the other odour was now the correct one. New exemplars were used for the ID and ED shifts. The specific exemplars used are shown in Table 2.2.5b (experiment 1). For the ED shift, the previously irrelevant parameter (i.e. odour) was now relevant.
3. **Experiment 3 (isolation rearing):** Rats were tested on odour in simple discrimination and digging medium was the parameter used in the extra-dimensional shift (as in experiment 1).
4. **Experiment 4 (counterbalanced sub-chronic PCP):** Rats were trained and tested as described in detail in experiments 1 and 2. Half of each treatment group were tested on odour in simple discrimination and half were tested on digging medium in simple discrimination, therefore counterbalancing the study.

The discovery trials prior to the test trials were scored and then testing continued until the rat reached a criterion of six consecutive correct responses.

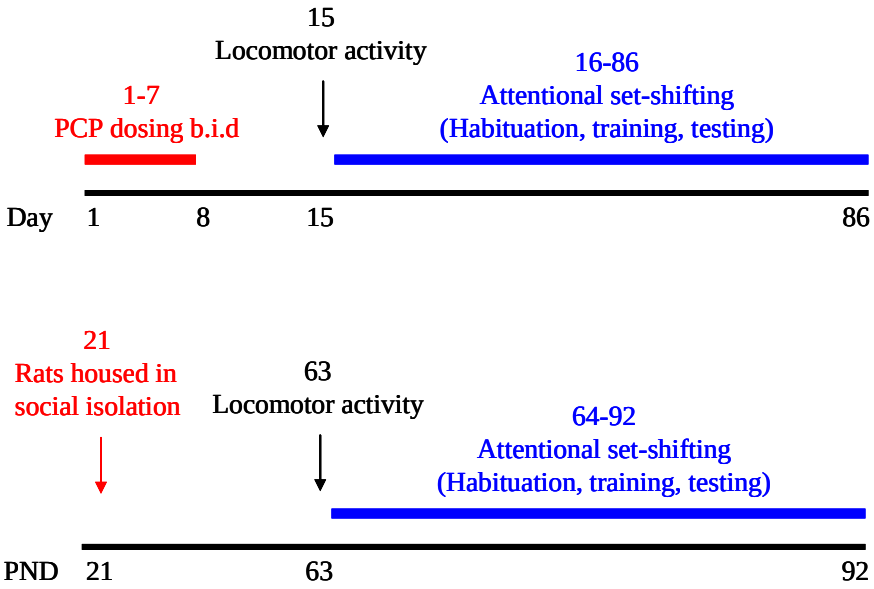


Figure 2.2.5: Example timeline of an attentional set-shifting experiment following sub-chronic PCP treatment and isolation rearing.

Table 2.2.5a Example order of discriminations presented to rats undertaking the attentional set-shifting task. Only rewarded stimuli were baited with food reward. [M1 = medium 1; O1 = odour 1]. Table adapted from Birrell and Brown (2000).

Discriminations	Dimensions		Exemplar combinations	
	Relevant	Irrelevant	Rewarded	Unrewarded
SD	Medium	Odour	M1	M2
CD	Medium	Odour	M1/O1	M2/O1
			M1/O2	M2/O2
Rev1	Medium	Odour	M2/O1	M1/O1
			M2/O2	M1/O2
IDS	Medium	Odour	M3/O3	M4/O3
			M3/O4	M4/O4
Rev2	Medium	Odour	M4/O3	M3/O3
			M4/O4	M3/O4
EDS	Odour	Medium	O5/M5	O6/M5
			O5/M6	O6/M6
Rev3	Odour	Medium	O6/M5	O5/M5
			O6/M6	O5/M6

Table 2.2.5b Specific exemplars used (presented in pairs) in each phase of the attentional set-shifting task. Odours were applied around the rims of digging bowls which were filled with various digging media depending upon the phase being tested. The significance of pairing within a test phase ensures that, for example, rose is always accompanied with white flower within a test trial [O = Odour, M = Medium].

Dimension	**Pairing 1 (CD)**	**Pairing 2 (IDS)**	**Pairing 3 (EDS)**
Odour	Rose = O1 White flower = O2	Green Meadow = O3 Coconut = O4	Orange = O5 Almond = O6
Medium	Wood shavings = M1 Cat litter = M2	Small pebbles = M3 Aspen = M4	Fine sawdust = M5 Wood blocks = M6

2.2.6 Drugs

In experiment 1 half of the males (n=10) and half of the females (n=10) were dosed with either 2 mg/kg PCP (Sigma, UK) or vehicle (0.9% saline) by the intraperitoneal (i.p.) route twice daily for seven days. For experiment 2 the study design involved five groups (n = 10 per group initially); rats were given either 2 mg/kg PCP (*n* = 40) or vehicle (*n* = 10). For experiment 4 half of the rats were given either 2 mg/kg PCP (*n* = 8) or vehicle (*n* = 8). Dosing with sub-chronic PCP or vehicle was followed by a washout period of a further seven days. In experiment 2 one week following the seven-day washout period, PCP-treated rats were sub-chronically dosed for seven days (daily, i.p. route) with vehicle (0.9% saline), clozapine (2.5 mg/kg), haloperidol (0.05 mg/kg) or risperidone (0.2 mg/kg). Rats were trained 30 minutes after dosing on the seventh day, and they were tested on day 8 30 minutes after drug administration.

The dose of haloperidol was chosen on the basis of a previous study showing that 0.05 mg/kg haloperidol significantly attenuated a d-amphetamine-induced reversal learning impairment in female hooded-Lister rats (Idris *et al.*, 2005a). Furthermore, this dose of haloperidol has been shown to occupy 50% of dopamine D_2 receptors (Kapur and Seeman, 2001; Kapur *et al.*, 2003). The doses of clozapine (2.5 mg/kg) and risperidone (0.2 mg/kg) were chosen on the basis of previous work in the laboratory showing efficacy against sub-chronic PCP in the reversal learning paradigm (Abdul-Monim *et al.*, 2006; Neill *et al.*, 2006) and NOR task (Grayson *et al.*, 2007). PCP HCl (Sigma, UK) was dissolved in 0.9% saline (Jentsch *et al.*, 1997b; 1998). Haloperidol (Serenace liquid, 2 mg/kg, Baker, UK) was diluted with distilled water. Clozapine (Tocris, UK) and risperidone (provided by GSK, UK) were dissolved in a minimum volume of acetic acid, made up to volume with distilled water and pH adjusted to 6 with 0.1M NaOH. Antipsychotics were given sub-chronically as a pilot study showed that

acute dosing with clozapine and haloperidol prevented rats from being able to attend to the task due to slight sedation.

2.2.7 Pilot antipsychotic study

A short pilot study was undertaken, during which the training and testing procedures were fine-tuned and developed. During this time 8 rats were tested, of which only 5 managed to complete the test. This may have been due to the odours from other rats remaining on the testing boxes as they were wiped clean between subjects but it became apparent that it would be more beneficial to have the boxes machine cleaned in between rats. The major change was to the dosing of the antipsychotics. It was originally planned to administer the drugs acutely 30 min prior to testing; however, it was soon clear that this would not be possible as clozapine and haloperidol were sedating or causing motor impairments in the animals too much for them to be able to complete the task. Subsequently, it was decided to give the drugs sub-chronically. Therefore, a dosing, training and testing regimen was devised, so that each rat was treated once daily, with the drug or vehicle for the 6 days prior to training; the day of training; and the day of testing. Although risperidone at this dose did not cause sedation, in order to make a true comparison between the antipsychotics this was also to be dosed sub-chronically.

2.2.8 Data and statistical analysis

In experiment 1 only total trials to reach criterion and average time per trial were scored. In experiments 2 and 3, percentage errors and frequency of both bowl exploration before digging were also scored. LMA data were analysed by Student's unpaired t-test and are expressed as total counts in a 60 min period. Data for total trials to criterion (TTC), average time per trial (ATT), percentage of errors and exploration of both bowls

are expressed as mean ± SEM. Data were analysed by a two-way ANOVA (set-shifting phase x treatment group or housing), followed by post-hoc Student's independent t-test. If the ANOVA was not significant planned comparisons were still carried out in the stage of interest i.e. the EDS phase. For experiment 4, data were analysed by three-way ANOVAs (set-shifting phase x treatment x order of testing [media or odour first]). All analysis was performed using SPSS for Windows software (SPSS Inc., Version 13).

2.3 Results

2.3.1 Experiment 1: Effects of sub-chronic PCP in female and male rats in attentional set-shifting.

Effects of sub-chronic PCP in female and male rats on total trials to criterion

A total of 30 rats were successfully trained and tested, rats (3 female vehicle, 2 female PCP, 2 male vehicle, 3 male PCP) were excluded from the study due to incompletion of the training within a single session as rats were required to complete the simple discriminations in training within 30 trials. Extra trials were not given in order to ensure all rats had the same level of training before testing. Treatment groups were female vehicle (n = 7), female PCP (n = 8), male vehicle (n = 8), and male PCP (n = 7). Treatment did not have any effect on performance during habituation or training.

In female rats a two-way ANOVA using phase as a within-subjects factor and drug treatment as a between-subjects factor revealed no significant interaction ($F_{1,13}$ = 0.48; p=0.50). A slight increase in trials to criterion in the EDS phase was observed in PCP-treated rats to (17.5 ± 2.6) from (12.6 ± 1.8) in the vehicle-treated group, however this increase was not significant (p = 0.15). Post-hoc analysis did not reveal any significant differences between the groups (fig. 2.3.1a).

In male rats a two-way ANOVA using phase as a within-subjects factor and drug treatment as a between-subjects factor revealed no significant interaction ($F_{1,13}$ = 0.17; p=0.68). Post-hoc analysis did not reveal any significant differences between the groups (fig. 2.3.1b).

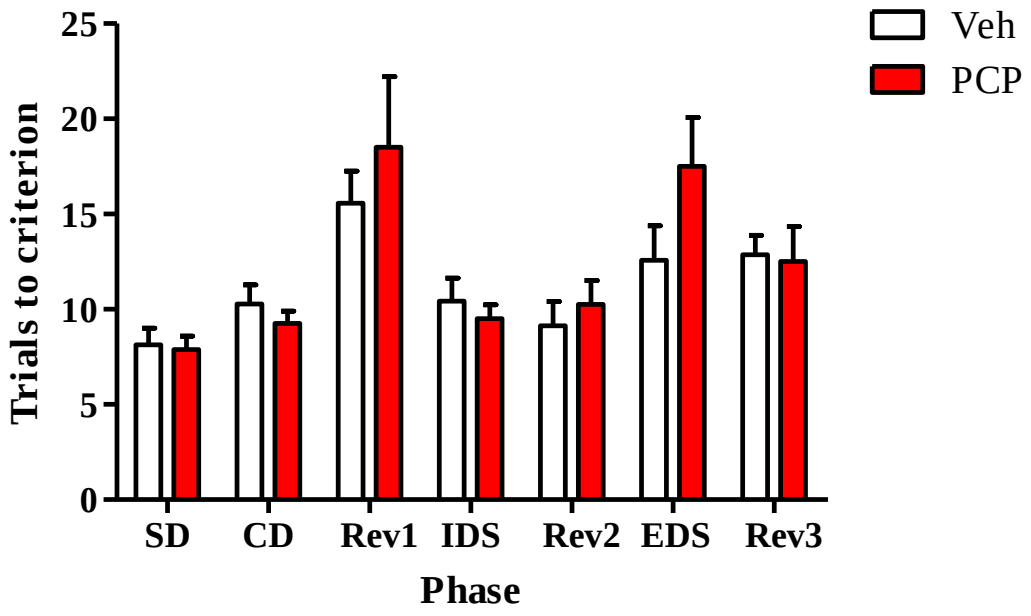


Figure 2.3.1a The total trials to criterion in the attentional set-shifting task in female rats. Data are expressed as mean ± SEM (vehicle n=7; PCP n=8). No significant differences between vehicle and PCP-treated rats at any stage of the task, analysed by Student's t-tests.

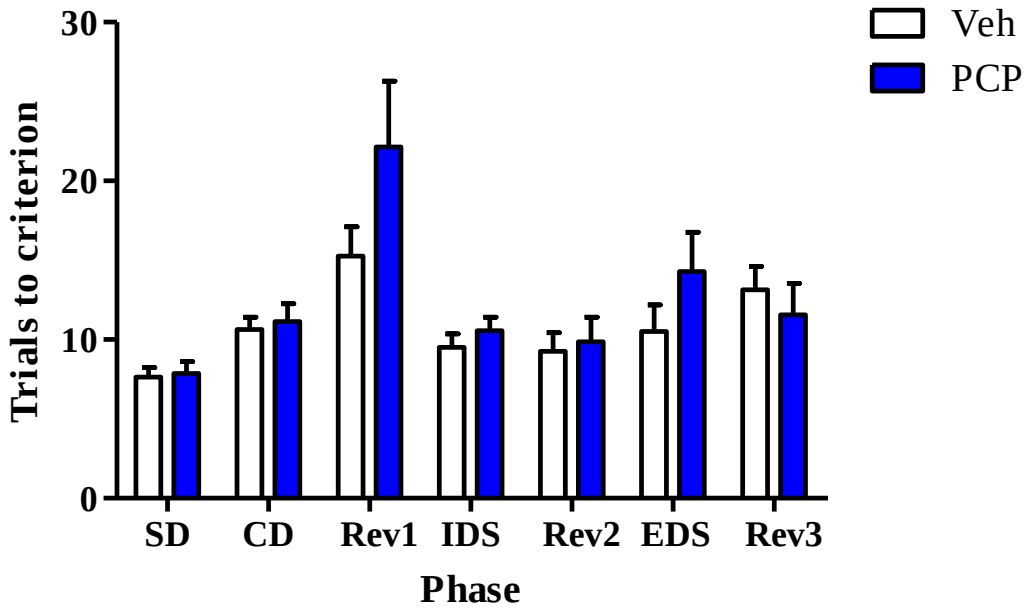


Figure 2.3.1b The total trials to criterion in the attentional set-shifting task in male rats. Data are expressed as mean ± SEM (vehicle n=8; PCP n=7). No significant differences between vehicle and PCP-treated rats at any stage of the task, analysed by Student's t-tests.

Effects of sub-chronic PCP in female and male rats on average time taken per trial

In female rats a two-way ANOVA using phase as a within-subjects factor and drug treatment as a between-subjects factor revealed no significant interaction ($F_{1,13} = 1.26$; p=0.28). However, post-hoc analysis did reveal a significant increase in the average time taken per trial in PCP-treated compared to vehicle-treated rats in the EDS phase only (p<0.05; fig. 2.3.1c).

In male rats a two-way ANOVA using phase as a within-subjects factor and drug treatment as a between-subjects factor revealed no significant interaction ($F_{1,13} = 0.21$; p=0.65). Post-hoc analysis did not reveal any significant differences between the groups (fig. 2.3.1d).

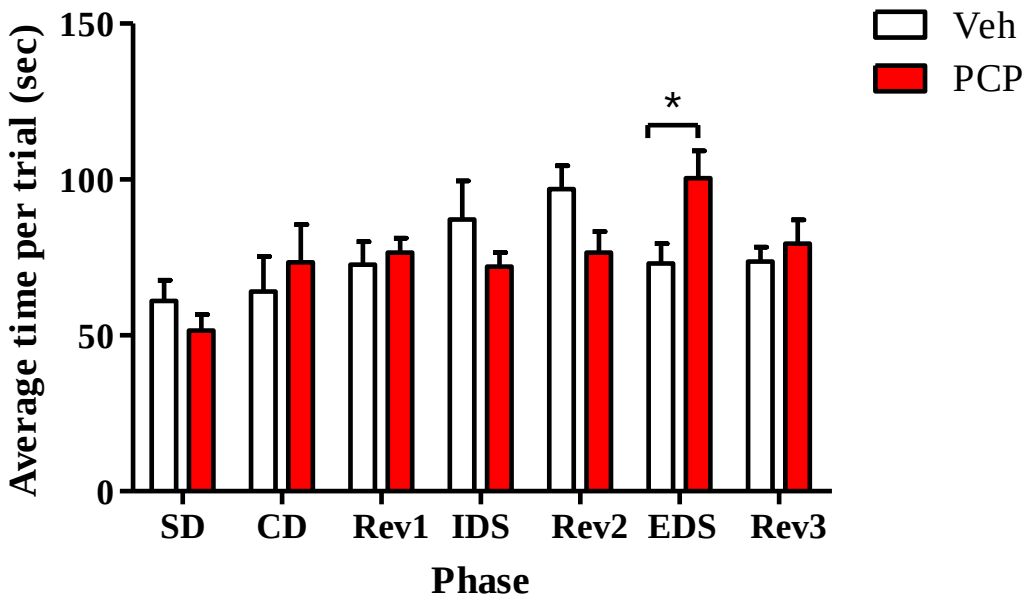


Figure 2.3.1c The average time taken per trial in the attentional set-shifting task in female rats. Data are expressed as mean ± SEM (vehicle n=7; PCP n=8). PCP significantly increased the average time taken per trial in the EDS phase compared to vehicle-treated rats, *p<0.05.

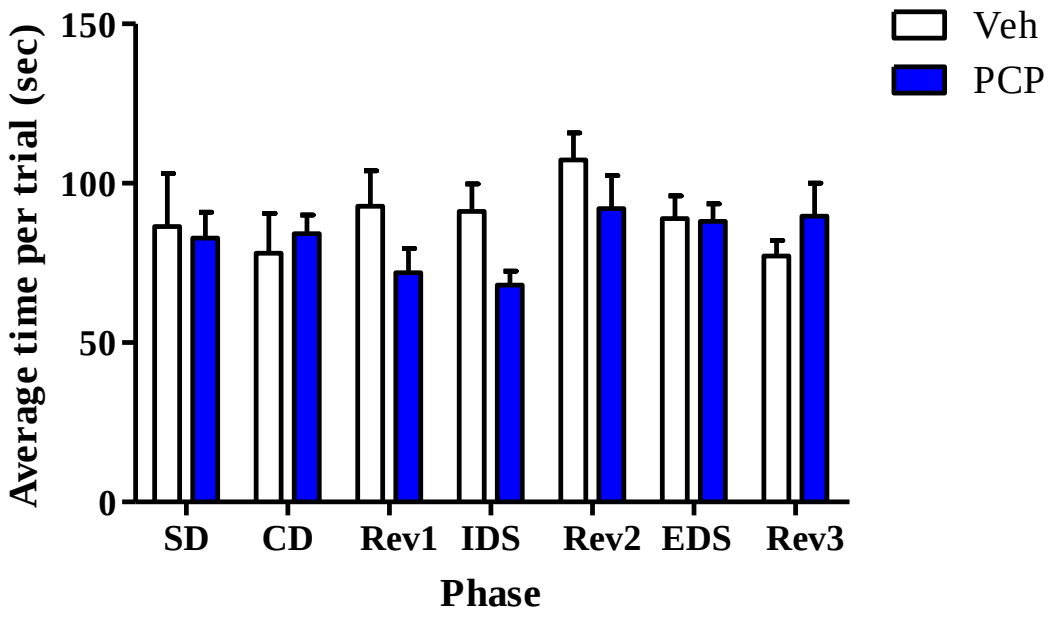


Figure 2.3.1d The average time taken per trial in the attentional set-shifting task. All data are expressed as mean ± SEM (vehicle n=8; PCP n=7). No significant difference between vehicle and PCP-treated rats at any stage of the task.

2.3.2 Experiment 2: Effects of clozapine, risperidone and haloperidol in sub-chronic PCP-treated rats in attentional set-shifting.

Effects of sub-chronic PCP on locomotor activity

Locomotor activity (LMA) was assessed following the 7-day washout period. Student's unpaired t-test showed that PCP-treated rats were not significantly different in total LMA over the 60 min test period compared to vehicle-treated rats ($p = 0.46$; fig. 2.3.2a).

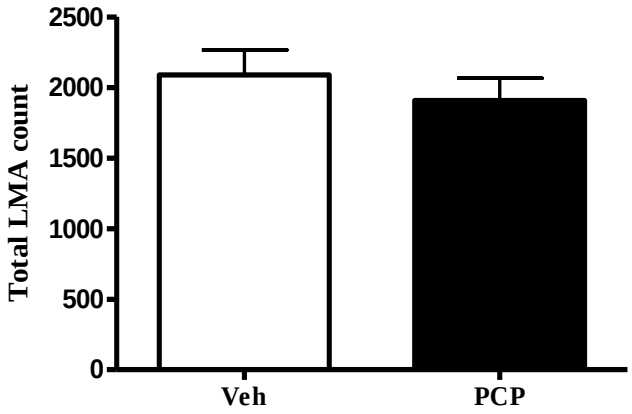


Figure 2.3.2a The effect of sub-chronic PCP (2 mg/kg, i.p. twice daily for 7 days) on locomotor activity in a novel environment. Data are expressed as mean ± SEM activity counts over a 60 min period ($n = 10$ per group). No significant difference between the groups.

Effects of antipsychotics on total trials to reach criterion (TTC)

A total of 42 rats were successfully trained and tested, 8 rats were excluded from the study due to inability to complete the training within a single session as rats were required to complete the simple discriminations in training within 30 trials. Extra trials were not given in order to ensure all rats had the same level of training before testing. Treatment groups were vehicle + vehicle ($n = 8$), PCP + vehicle ($n = 9$), PCP +

clozapine (n = 9), PCP + haloperidol (n = 7) and PCP + risperidone (n = 9). Drug treatment did not have any effect on performance during habituation or training.

A two-way ANOVA using phase as a within-subjects factor and drug treatment as between-subjects factor revealed a significant interaction ($F_{4,37}$ = 5.4; p<0.01). An increase in trials to criterion in reversal 1 was observed in all treatment groups, although these increases were not significant compared to SD. PCP+haloperidol-treated rats required the most trials in reversal 1 to reach criterion (11.4 ± 1.4) compared to vehicle-treated rats (7.3 ± 0.6), however this increase was not significant (p = 0.08). Post-hoc analysis within the EDS phase (fig. 2.3.2b) revealed that PCP significantly (p<0.01) increased the number of trials to reach criterion when compared to vehicle. This deficit was significantly (p<0.01) attenuated to the same extent by sub-chronic clozapine and risperidone, but not by sub-chronic haloperidol**.**

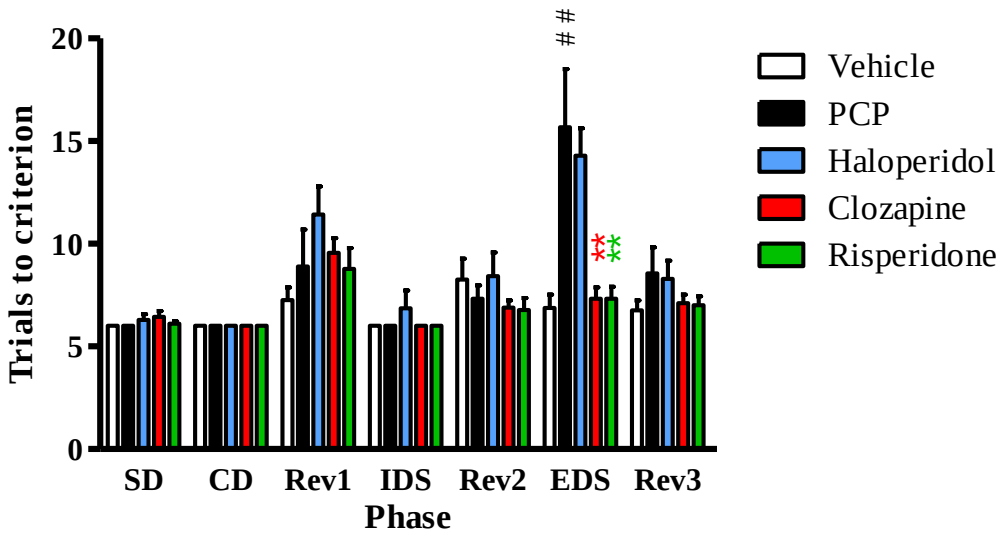


Figure 2.3.2b The effect of sub-chronic PCP (2 mg/kg; i.p. twice daily for 7 days; n = 9) against vehicle-treated (n = 8) female rats on trials to reach criterion in attentional set-shifting and effects of sub-chronic administration of clozapine (2.5 mg/kg; n = 9), haloperidol (0.05 mg/kg; n = 7), risperidone (0.2 mg/kg; n = 9) in PCP-treated rats. Data are expressed as mean ± SEM and were analysed by two-way ANOVA and post-hoc Dunnett's t-test (p<0.01$^{\#\#}$ compared to vehicle group in EDS; p<0.01** compared to PCP group in EDS).

Effects of antipsychotics on average time taken per trial (ATT)

A two-way ANOVA was applied using phase and drug treatment (as previously described) and revealed a significant interaction in the ATT ($F_{4,37} = 3.9$; $p<0.01$). Post-hoc analysis showed that PCP-haloperidol treated rats required significantly more time to complete trials compared to vehicle-treated rats in the SD ($p<0.05$), CD ($p<0.05$), Rev1 ($p<0.001$) and Rev3 ($p<0.05$) phases (fig. 2.3.2c).

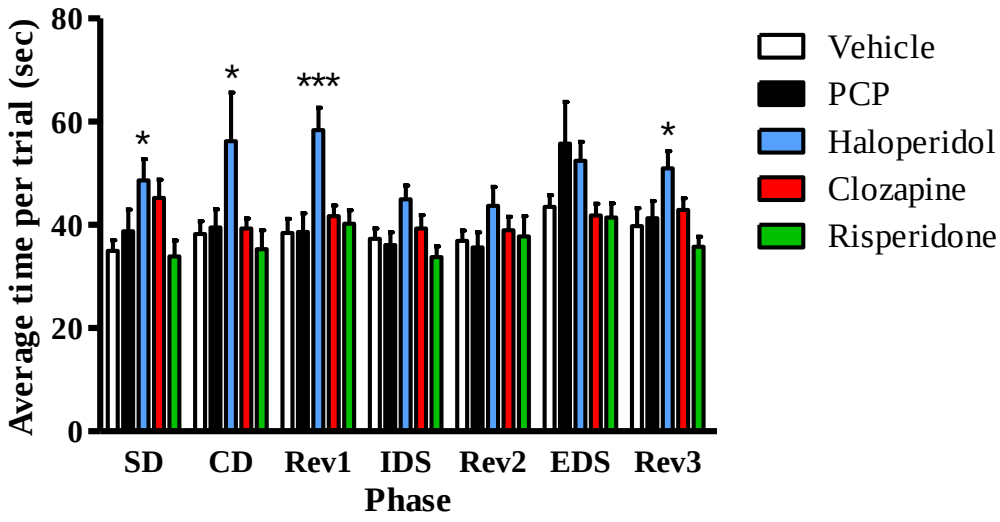


Figure 2.3.2c The effect of sub-chronic PCP (2 mg/kg i.p. twice daily for 7 days; $n = 9$) against vehicle-treated ($n = 8$) female rats on average time taken per trial (sec) in attentional set-shifting and effects of sub-chronic administration of clozapine (2.5 mg/kg; $n = 9$), haloperidol (0.05 mg/kg; $n = 7$), risperidone (0.2 mg/kg; $n = 9$) in PCP-treated rats. Data are expressed as mean ± SEM and were analysed by two-way ANOVA and post-hoc Dunnett's t-test ($p < 0.05$*; $p < 0.001$*** compared to vehicle in that phase of the task).

Effects of antipsychotics on percentage of errors

A two-way ANOVA revealed a significant interaction ($F_{4,37}$ = 4.2; p<0.01). Post-hoc analysis revealed significant (p<0.001) increases in the percentage errors in the EDS phase in PCP-vehicle and PCP-haloperidol treated rats when compared to the vehicle group (fig. 2.3.2d). The PCP-clozapine and PCP-risperidone groups committed significantly (p<0.01) less percentage errors compared to the PCP-vehicle group in EDS phase. In the first reversal the only significant difference was between the PCP-treated and PCP-clozapine groups (p<0.05), with the PCP-clozapine group making significantly (p<0.05) more percentage errors than the PCP-vehicle group.

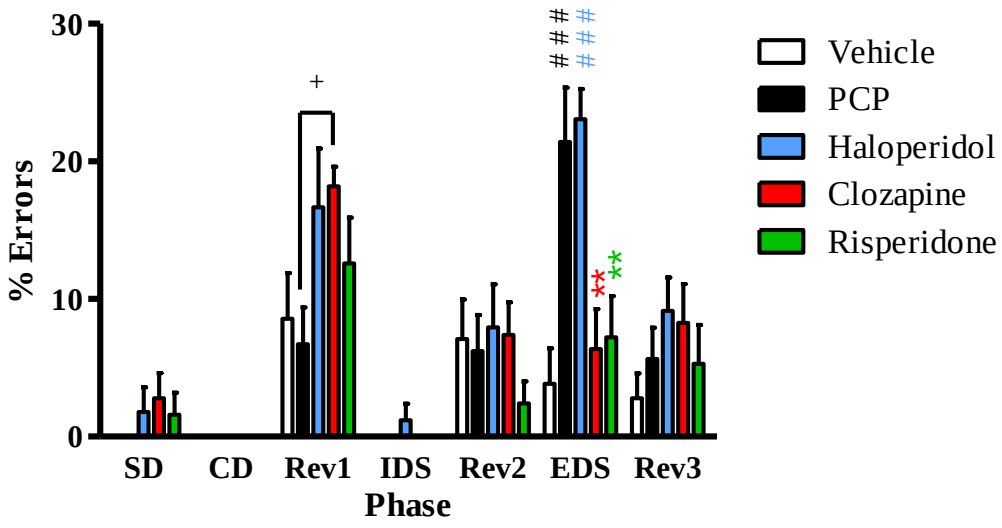


Figure 2.3.2d The effect of sub-chronic PCP (2 mg/kg, i.p. twice daily for 7 days; n = 9) against vehicle-treated (n = 8) female rats on percentage errors in attentional set-shifting and effects of sub-chronic administration of clozapine (2.5 mg/kg; n = 9), haloperidol (0.05 mg/kg; n = 7), risperidone (0.2 mg/kg; n = 9) in PCP-treated rats. Data are expressed as mean ± SEM and were analysed by two-way ANOVA and post-hoc Dunnett's t-test (p < 0.001$^{###}$ compared to vehicle group in EDS; p < 0.01** compared to PCP group in EDS; p < 0.05^{+} compared to the PCP treated group in reversal 1).

Effects of antipsychotics on exploration of both bowls before digging

A two-way ANOVA was applied; comparing the drug treatment group to the phase of the task showed that there was no overall significant difference in the exploration of both bowls ($F_{4,37}$ = 2.1; p=0.101). Frequency of exploration of both bowls was significantly greater in the PCP-treated rats compared to vehicles in the EDS phase (p<0.01; fig. 2.3.2e), this effect was significantly attenuated by clozapine [p<0.001].

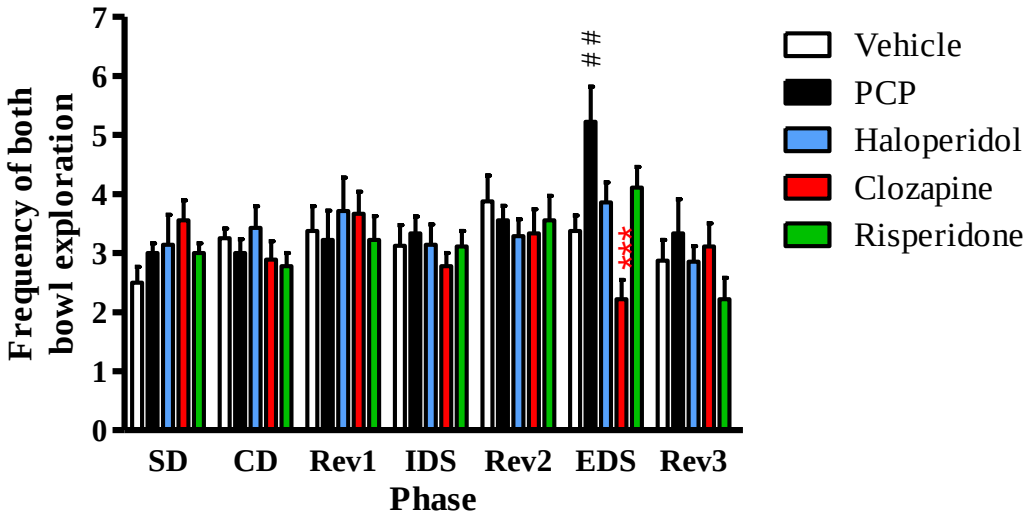


Figure 2.3.2e The effect of sub-chronic PCP (2 mg/kg, i.p. twice daily for 7 days; n = 9) against vehicle-treated (n = 8) female rats on frequency of both bowl exploration before digging in attentional set-shifting and effects of sub-chronic administration of clozapine (2.5 mg/kg; n = 9), haloperidol (0.05 mg/kg; n = 7), risperidone (0.2 mg/kg; n = 9) in PCP-treated rats. Data are expressed as mean ± SEM and were analysed by two-way ANOVA and post-hoc Dunnett's t-test (p<0.01$^{\#\#}$ compared to vehicle group in EDS; p<0.001*** compared to PCP group in EDS).

2.3.3 Experiment 3: Effects of isolation rearing on attentional set-shifting performance.

Effects of isolation rearing on locomotor activity

Locomotor activity (LMA) was assessed following 6 weeks of isolation rearing. An independent Student's t-test showed that there was a significantly larger response to a novel environment in the isolation-reared rats compared to the socially reared rats [$p<0.01$; Fig. 2.3.3a].

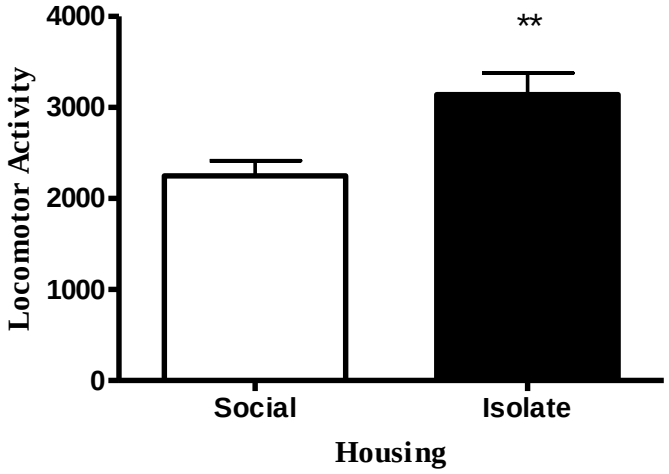


Figure 2.3.3a The response to a novel environment as measured using locomotor activity in isolation-reared and socially-reared rats. All data expressed as mean ± SEM (n=10 per group) of total locomotor activity counts over a 60 min period. An independent Student's t-test showed that total locomotor activity was significantly greater in the isolates compared to the socials (**$p<0.01$).

Effects of isolation rearing on total trials to reach criterion

A total of 17 rats were successfully trained and tested, 1 isolate and 2 social rats were excluded from the study due to non-completion of the training within a single session, as rats were required to complete the training of simple discriminations within 30 trials. A two-way ANOVA using phase as a within-subjects factor and housing as a between-subjects factor showed no significant effect ($F_{1,15}$ = 0.018; p=0.894). Isolation-reared rats showed a selective deficit at the EDS stage of the task requiring significantly more trials to reach criterion than socially-housed rats at this stage of testing only (p<0.01; fig. 2.3.3b).

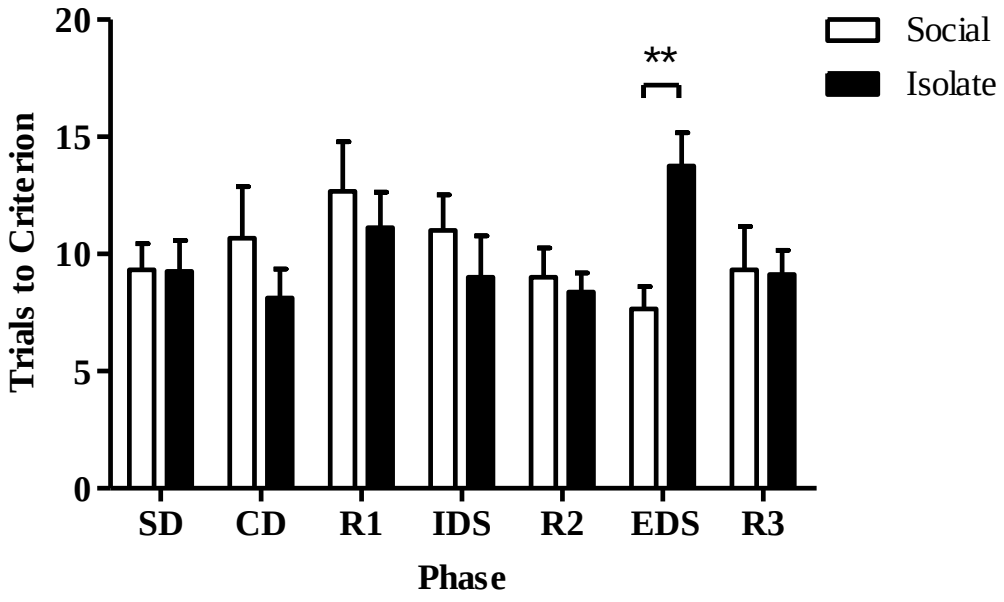


Figure 2.3.3b The total trials to criterion in the attentional set-shifting task in isolation-reared and socially reared rats. All data are expressed as mean ± SEM (isolates n=9; socials n=8) of total trials to criterion. Total trials to criterion was significantly greater in the isolates compared to the socials in the extra-dimensional shift phase (**p<0.01).

Effects of isolation rearing on average time taken per trial

A two-way ANOVA using phase as a within-subjects factor and housing as a between-subjects factor showed no significant effect ($F_{1,15}$ = 0.599; p=0.452). There was a significant reduction in the ATT in isolation-reared compared with socially reared rats in reversal 3 (p<0.05; fig. 2.3.3c).

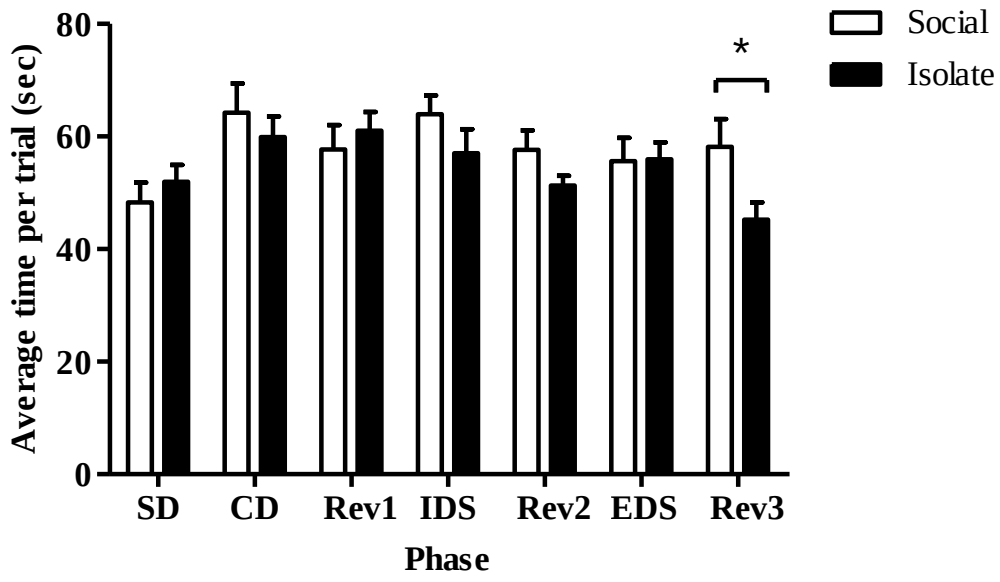


Figure 2.3.3c The average time taken per trial in the attentional set-shifting task in isolation-reared and socially reared rats. All data are expressed as mean ± SEM (isolates n=9; socials n=8). Social rats required significantly more time per trial than isolation-reared rats in the reversal 3 phase of the task (*P<0.05).

Effects of isolation rearing on percentage of errors

A two-way ANOVA with phase as a within subjects factor and housing as a between subjects factor showed no significant effect ($F_{1,15}$ = 0.044; p=0.838). There were no significant differences between socials and isolates at any stage of the task (fig. 2.3.3d).

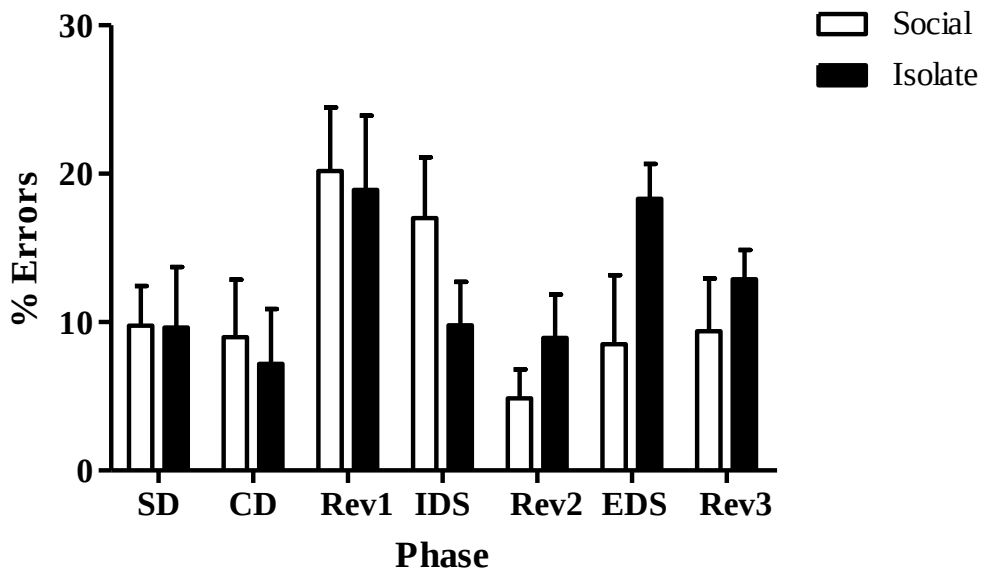


Figure 2.3.3d The percentage of errors made during the attentional set-shifting task in isolation-reared and socially reared rats. All data expressed as mean ± SEM (isolates n=9; socials n=8). No significant differences were observed.

Effects of isolation rearing on exploration of both bowls before digging

A two-way ANOVA with phase as a within subjects factor and housing as a between subjects factor showed no significance ($F_{1,15}$ = 1.979; p=0.180). Frequency of exploration of both bowls was significantly greater in the isolates compared to the socials in the extra-dimensional shift phase (p<0.01; Fig. 2.3.3e).

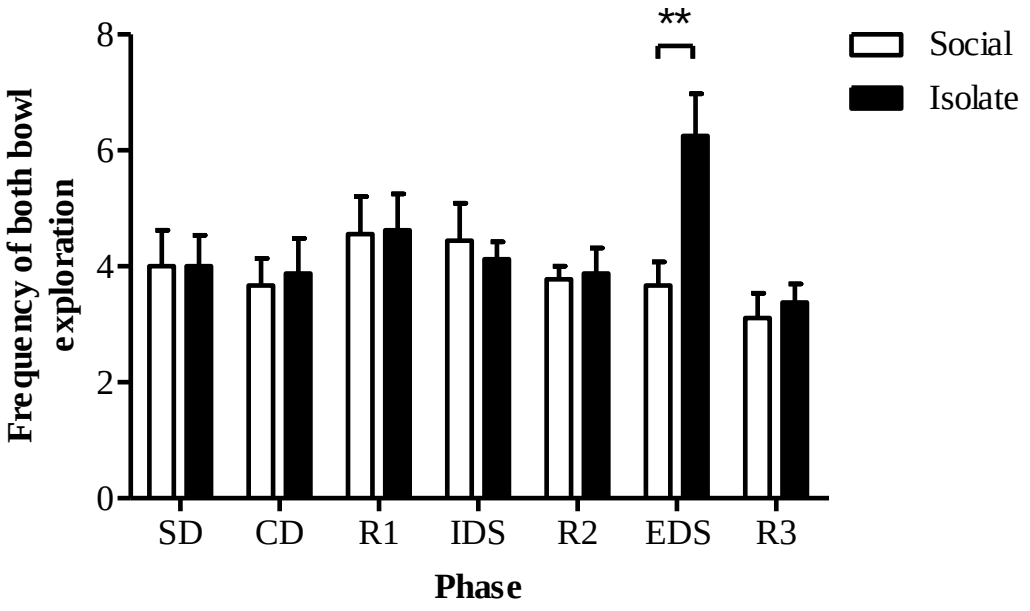


Figure 2.3.3e The total frequency of exploration of both bowls before digging in the attentional set-shifting task in isolation-reared and socially reared female rats. All data are expressed as mean ± SEM (isolates n=9; socials n=8) of frequency of exploration. Frequency of exploration of both bowls was significantly greater in the isolates compared to the socials in female rats in the extra-dimensional shift phase (**p<0.01).

2.3.4 Experiment 4: Effects of sub-chronic PCP in a counterbalanced attentional set-shifting study, and the effect of discovery trials.

Effect of sub-chronic PCP on trial to reach criterion

All 16 rats were successfully trained and tested. Drug treatment did not have any effect on performance during habituation or training. A three-way ANOVA using phase as a within-subjects factor and treatment and order of testing (media or odour first) as a between-subjects factors showed a significant interaction ($F_{1,12}$ = 3.3; p<0.01). PCP-treated rats showed deficits in the reversal 1 and reversal 3 stages (p<0.05) and a significant deficit in the EDS phase (p<0.001), requiring significantly more trials to reach criterion (fig. 2.3.4a). There was no effect of order of testing ($F_{1,12}$ = 0.02; p=0.89).

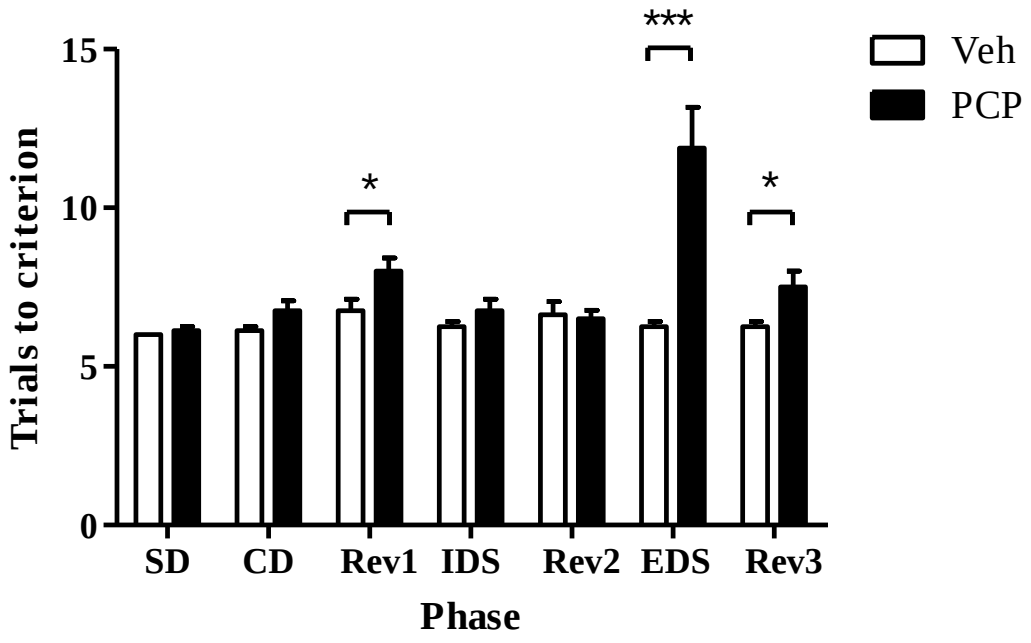


Figure 2.3.4a The total trials to criterion in the attentional set-shifting task. All data are expressed as mean ± SEM (n=8). PCP significantly increased trials to criterion in reversal 1 and 3, *p<0.05, and in the EDS phase, ***p<0.001

Analysis of the number of correct discovery trials

A two-way ANOVA using phase as a within-subjects factor and treatment and order of testing (media or odour first) as a between-subjects factors showed no significant effect ($F_{1,12} = 0.36$; $p=0.90$). There were no significant differences between vehicle and PCP-treated rats at any stage of the task (fig. 2.3.4b). Bonferroni multiple comparisons test showed that, in vehicle rats, the number of correct discovery trials was significantly decreased from IDS to EDS ($p<0.01$) and this effect was also observed in PCP-treated rats ($p<0.001$). In both vehicle and PCP-treated groups the number of correct discovery trials were significantly increased in SD when compared to the Rev1 and EDS phases of the task ($p<0.001$). There was no effect of order of testing ($F_{1,12} = 0.47$; $p=0.51$).

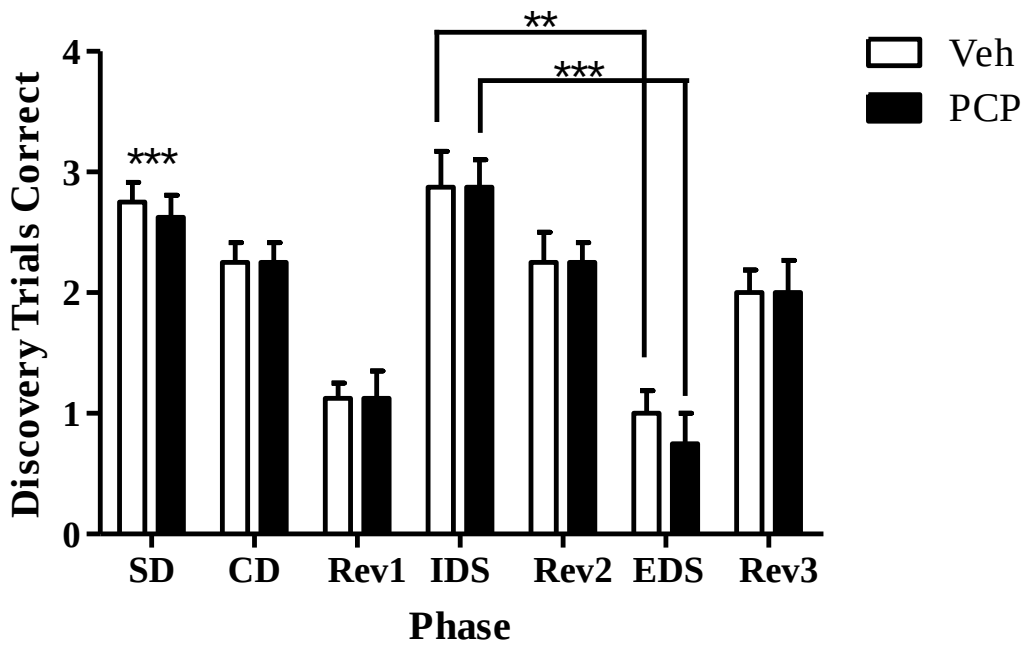


Figure 2.3.4b The number of correct discovery trials in the attentional set-shifting task. All data are expressed as mean ± SEM (n=8). No significant difference between vehicle and PCP-treated rats at any stage of the task. In both PCP and vehicle-treated rats the number of correct discovery trials was significantly lower in the EDS phase compared to IDS; **p<0.01, ***p<0.001. In both vehicle and PCP-treated groups the number of correct discovery trials was significantly increased in SD when compared to the Rev1 and EDS phases of the task ***p<0.001.

Effect of sub-chronic PCP on average time taken per trial

A two-way ANOVA using phase as a within-subjects factor and treatment and order of testing (media or odour first) as a between-subjects factors showed no significant effect ($F_{1,12} = 0.76$; p=0.61). There were no significant differences between vehicle and PCP-treated rats at any stage of the task (fig. 2.3.4c). However there was a significant effect of order of testing ($F_{1,12} = 7.54$; p<0.01).

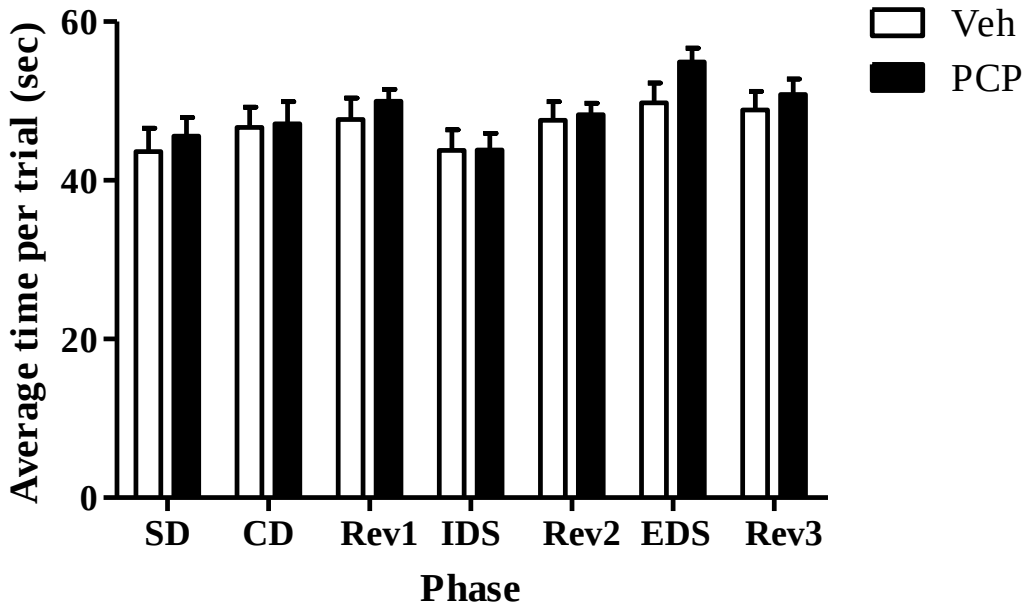


Figure 2.3.4c The average time taken per trial in the attentional set-shifting task. All data are expressed as mean ± SEM (n=8). No significant difference between vehicle and PCP-treated rats at any stage of the task.

Effects of sub-chronic PCP on percentage of errors

A two-way ANOVA using phase as a within-subjects factor and treatment and order of testing (media or odour first) as a between-subjects factors showed a significant interaction ($F_{1,12}$ = 3.12; p<0.05). PCP-treated rats had increased percent errors with respect to trials to criterion in the reversal 1 and reversal 3 stages (p<0.05) and in the EDS phase compared with the vehicle group (p<0.001; Fig. 2.3.4d). There was no effect of order of testing ($F_{1,12}$ = 1.89; p=0.19).

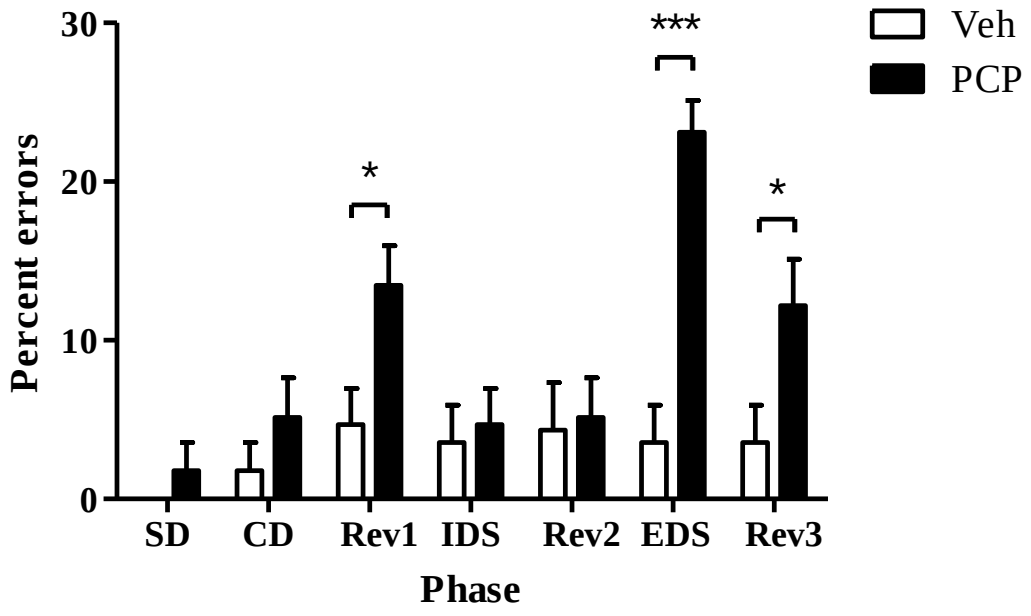


Figure 2.3.4d The percentage of errors made during the attentional set-shifting task. All data are expressed as mean ± SEM (n=8). Percent errors were significantly increased in reversal 1 and 3, *p<0.05; and in the EDS phase, ***p<0.001.

Effects of sub-chronic PCP on exploration of both bowls before digging

A two-way ANOVA using phase as a within-subjects factor and treatment and order of testing (media or odour first) as a between-subjects factors showed an interaction approaching significance ($F_{1,12} = 3.84$; $p=0.07$). Frequency of exploration of both bowls was significantly greater in the PCP-treated rats compared to the vehicle group in the EDS phase ($p<0.05$; Fig. 2.3.4e). There was no effect of order of testing ($F_{1,12} = 1.68$; $p=0.24$).

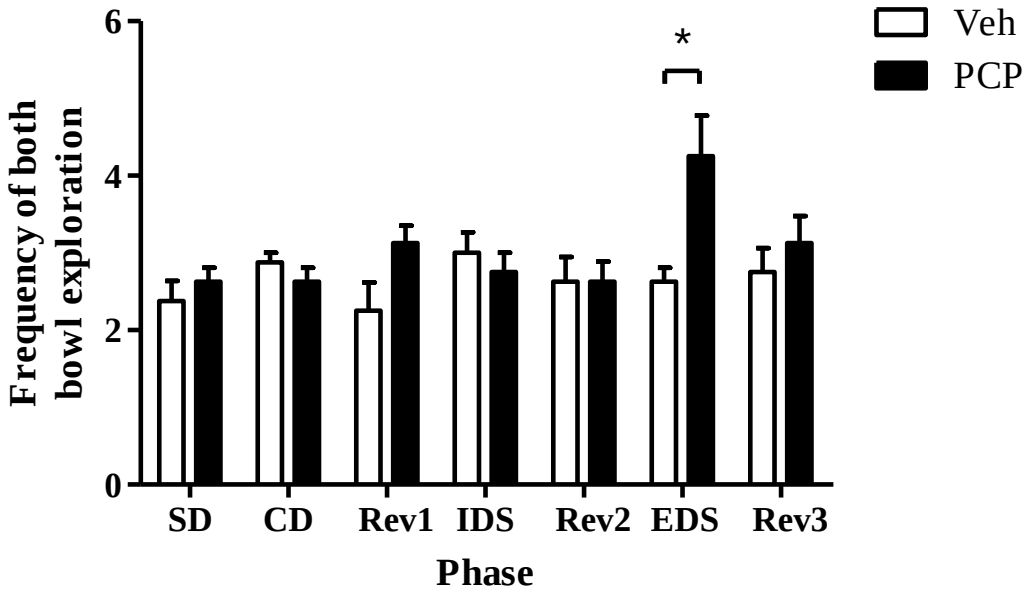


Figure 2.3.4e The total frequency of exploration of both bowls before digging in the attentional set-shifting task. All data are expressed as mean ± SEM (n=8). Frequency of exploration of both bowls was significantly increased in PCP-treated rats compared to the vehicle group; *p<0.05.

2.4 Discussion

The aim of these studies was to examine the effects of isolation rearing and a sub-chronic PCP treatment regimen on attentional set-shifting ability in female rats and to investigate the effects of sub-chronic administration of antipsychotics. An initial study aimed to assess the effect of sub-chronic PCP in male and female rats. The aim was also to investigate the effect of counterbalancing a study and to score the discovery trials to assess whether these could have an impact on the task. The rodent version of this test was adapted by Birrell and Brown (2000) from a previous version used in primates (Roberts *et al.,* 1988) and is a useful analogue of the WCST (Berg, 1948). This task requires rats to initially learn a rule, and then switch their attention to a new stimulus dimension, demonstrating cognitive flexibility.

2.4.1 Experiment 1: Effects of sub-chronic PCP in attentional set-shifting in female and male rats

In female rats there was an increase in trials required to reach criterion in PCP-treated rats for the EDS stage, suggesting sub-chronic PCP causes a deficit in attentional set-shifting ability, although this was not significant. A difference in trials to criterion was not observed in male rats. Female rats also required significantly more time to complete the trials within the EDS phase, suggesting an impairment; again, this effect was not observed in the male rats. The results from experiment 1 suggest that sub-chronic PCP produces more pronounced effects in female rats compared to male rats; this may be due to differing sensitivity to PCP. Therefore, subsequent experiments were only carried out using female rats. A recent study from the laboratory has shown that males are less sensitive to acute PCP treatment compared to females, as measured using the novel object recognition task (Grayson *et al.,* 2005). Several studies have shown female rats

to be more sensitive to the behavioural effects of PCP (Nabeshima *et al.*, 1984a; Nabeshima *et al.*, 1984b; Hönack and Löscher, 1993; Wessinger *et al.*, 1995).

These behavioural differences may be due to differences in pharmacokinetics. Nabeshima *et al.* (1984b) and Gartlon *et al.* (2006) found higher levels of PCP in the plasma and brain tissue of female rats given the same dose as male rats. Shelnutt and colleagues (1999) found significantly lower *in vitro* formation of PCP metabolites in female rats when compared to males. Furthermore pharmacokinetic studies in SD rats showed that, in females, the systemic clearance and non-renal clearance were significantly reduced and terminal elimination half-life was significantly increased when compared to male rats (Shelnutt *et al.*, 1999); this could account for the behavioural differences observed in female rats. Hönack and Löscher (1993) suggested that increased sensitivity to MK-801 in females did not simply reflect differences in drug metabolism but could be due to differences in the number and/or affinity of PCP binding sites, or differences in endogenous modulators of the NMDA receptor. Together, these findings suggest that differential responsiveness to PCP in males and female rodents can be explained by pharmacokinetic and possibly pharmacodynamic differences.

2.4.2 Experiment 2 (and 4): Effects of clozapine, risperidone and haloperidol on sub-chronic phencyclidine-induced deficits in attentional set-shifting

Sub-chronic PCP produced a deficit in the ability to switch from one attentional set to another, indicated by a poor performance in the EDS stage of the task. The PCP-induced deficit was not significant in the first experiment, but there was an increase in trials to reach criterion in the female rats. The lack of a significant deficit in the first experiment may be due to the fact that it was the first time the task had been trialled in

the laboratory. Due to having more experience with the task, in the second experiment the PCP-induced deficit was significant; these deficits were reversed by sub-chronic administration of clozapine and risperidone, but not haloperidol. There was no significant difference in locomotor activity observed between vehicle and sub-chronic PCP-treated groups; therefore it may be assumed that the cognitive deficit produced by sub-chronic PCP was independent of effects on locomotor activity.

In this study rats readily learned to discriminate food bowls based on the digging medium and odour, and also refrained from digging in the un-baited bowls, therefore making few incorrect decisions. In both vehicle and PCP-treated rats the first reversal (R1) required more trials to reach criterion compared to earlier stages of the task. This is supported by data showing a significant increase in trials to criterion in control rats in reversal 1 (Hatcher *et al.,* 2005); however, other studies have shown more modest increases (Birrell and Brown, 2000; Egerton *et al.,* 2005; Fletcher *et al.,* 2005).

Vehicle rats

Vehicle-treated rats required fewer trials to reach criterion in the IDS phase than the EDS phase and completed the IDS trials faster, suggesting that an attentional set was formed. However, lack of a significant increase in trials to criterion from IDS to EDS does not support the idea that an attentional set had been formed in the control rats. One aspect of this study that may have influenced the data set is that, before each new discrimination begins, all rats have 4 discovery trials to learn the new rule; it is therefore possible that vehicle rats learned the EDS rule within these 4 trials thus improving their performance; this may also explain the occurrence of minimum trials to criterion without any apparent errors in certain discriminations.

Therefore, the aim of experiment 4 was to counterbalance a PCP attentional set-shifting study; and to examine the effects of the discovery trials in order to establish if the inclusion of 4 discovery trials at the start of each phase was the reason for the lack of significance in vehicle rats in ID/ED shift. Analysis of the number of correct discovery trials showed that in both vehicle and PCP-treated groups the number of correct discovery trials was reduced when comparing SD to the Rev1 and EDS phases of the task, indicating that these stages are more difficult. Also, in vehicle and PCP-treated rats the number of correct discovery trials was significantly decreased from IDS to EDS. This suggests that if the discovery trials were not included, i.e. scoring started from trial 1, both vehicle and PCP treated rats would find the EDS phase much harder than the IDS phase. This may also suggest that within the discovery trials it is possible for the rats to learn the new correct rule before the scoring starts; therefore the discovery trials could be masking potential errors that would be scored if the discovery trials were omitted.

Effects of PCP

There were no significant differences in time taken to complete the trials at any stage of the test between vehicle and PCP-treated rats. In the EDS phase PCP-treated rats showed an increase in percentage errors and frequency of exploring both bowls before digging, this could be a measure of indecision or could be an indication that they cannot remember which is the correct parameter from the discovery trials. In the literature, set-shifting ability is measured by recording the number of trials to reach criterion, however in the current experiments additional measures (time taken, percentage errors and both bowl exploration) were taken in order to determine whether PCP affected any other behaviours.

There was a significant increase in trials required to reach criterion in PCP-treated rats only in the EDS stage, suggesting that sub-chronic PCP causes a selective deficit in attentional set-shifting ability. These results are supported by a previous study where PCP at 5 mg/kg twice daily for seven days followed by a ten-day drug washout period, in male Long-Evans rats, selectively impaired the EDS without affecting other discrimination tasks or reversals (Rodefer *et al.,* 2005, 2007). Acute PCP (2.58 mg/kg) also produces a deficit in set-shifting ability in rats (Egerton *et al.*, 2005). However, repeated exposure i.e. sub-chronic administration of PCP is believed to mimic schizophrenia more closely than acute administration (Jentsch and Roth, 1999, see chapter 1). The success of the treatment regime used in this investigation is further supported by a comparative study of sub-chronic PCP dosing regimes by Gartlon *et al.* (2006) who reported that a bi-daily (7-day) regime, but not a 26-day intermittent regime, produces cognitive deficits in the NOR task. Furthermore, recent studies have shown that long-term intermittent administration of PCP (3 mg/kg once per day administered Monday, Wednesday and Friday for 5 weeks, and 10 mg/kg daily for 14 days) did not impair set-shifting ability in male rats (Deschênes *et al.,* 2006; Fletcher *et al.,* 2005). These data would suggest that continual dosing with PCP is more effective than an intermittent regime. It is important to note that PCP is not the only pharmacological means of producing schizophrenia-like impairments, for example amphetamine sensitised rats show deficits in sensorimotor gating, in addition to cognitive impairment in tasks such as attentional set-shifting (Fletcher *et al.,* 2005; Tenn *et al.,* 2003).

In experiment 4 PCP-treated rats required more trials to reach criterion in the EDS phase but this effect was also observed in the Rev1 and Rev3 phases. No differences were observed in the time taken per trial. The percent errors were increased in PCP-treated rats in the Rev1, EDS, Rev3 stages when compared to vehicle rats.

PCP-treated rats also explored both bowls before digging more frequently than vehicle-treated rats only in the EDS phase. The results from this experiment closely correspond to those in experiment 2. The only difference is that trials to criterion were increased in two of the reversal phases in the current experiment; this may be due to the orbital PFC being relied upon in reversals as well as the medial PFC in the EDS phase. Sub-chronic PCP does cause reliable deficits in reversal learning in an operant task (Abdul-Monim *et al.*, 2006), so it is not surprising that deficits in reversals are also observed during the attentional set-shifting task. These deficits in operant reversal learning will be explored further in the next chapter.

Effects of antipsychotics

It has been widely reported that atypical antipsychotics have some beneficial effect on cognitive deficits (Buchanan *et al.*, 1994; Hagger *et al.*, 1993; Harvey *et al.*, 2004; Meltzer and McGurk, 1999; Rossi *et al.*, 1997). However, it is important to appreciate the limitations of models such as this one as; although clozapine and risperidone effectively reversed the cognitive deficits observed in this experiment, the CATIE study highlights the fact that these antipsychotics do not provide consistent improvement of cognitive symptoms in patients (Lieberman, 2006). This could indicate a need to develop more robust cognitive deficit models which are only partially improved by currently available antipsychotics, in order to correspond more closely with the clinical situation and allow for assessment of improved therapies. It could also suggest that a full reversal of induced deficits is a limitation of the animal model. Full reversals with current antipsychotics may be gained in animals as compliance is not an issue as it can be in patients, in that it is known exactly how much of the drug is administered and that there are no interactions with other medications as can be the case in patients with

schizophrenia. In addition it is important to note that patients involved in clinical trials can often be on long-term medication which could interfere with the efficacy of potential new therapies.

The use of only one dose of antipsychotics is a limitation of this study, a dose response should ideally have been conducted; however, in order to limit the number of animals used, and length of testing, one active dose of each antipsychotic was carefully selected. The doses of clozapine (2.5 mg/kg) and risperidone (0.2 mg/kg) were chosen on the basis of recent experiments from this laboratory showing efficacy against sub-chronic PCP-induced deficits in reversal learning (Abdul-Monim *et al.,* 2006; Neill *et al.,* 2006) and NOR (Grayson *et al.,* 2007). The dose of haloperidol was chosen on the basis of previous work showing that 0.05 mg/kg haloperidol significantly attenuated a d-amphetamine-induced reversal learning impairment in female hooded-Lister rats (Idris *et al.,* 2005a). It is unlikely that a higher dose of haloperidol would have been any more effective as a dose of 0.075 mg/kg haloperidol was less efficacious to reverse the amphetamine deficit in reversal learning when compared to the dose of 0.05 mg/kg (Idris *et al.,* 2005a) and a dose of 0.1 mg/kg was shown to impair performance (Abdul-Monim *et al.,* 2003). Indeed in this experiment 0.05 mg/kg haloperidol significantly increased the time taken to complete trials in 4 phases of the task. Furthermore, 0.05 mg/kg haloperidol has been shown to occupy 50% of dopamine D_2 receptors (Kapur and Seeman, 2001; Kapur *et al.,* 2003).

The current study shows that the PCP-induced deficit in percentage errors and trials to reach criterion was improved by sub-chronic administration of clozapine and risperidone, but not haloperidol. The increase in frequency of both bowl exploration in PCP-treated rats was also improved by clozapine, although not risperidone. As mentioned above, these results are in agreement with previous studies in this laboratory.

In addition, a recent study has shown that sub-chronic administration of clozapine (5 mg/kg) but not haloperidol (0.05 mg/kg) can prevent the PCP-induced cognitive deficit when administered in conjunction with the daily sub-chronic PCP treatment regimen (Idris *et al.*, 2005b).

Antipsychotics were given sub-chronically as initially a pilot study showed that acute dosing with clozapine and haloperidol prevented rats from being able to complete the task, therefore for rats to complete the task, sub-chronic dosing with clozapine and haloperidol was required. In order to make a direct comparison, risperidone was also given sub-chronically. Didriksen and colleagues used doses of 0.63 and 1.3 mg/kg of clozapine for a reversal learning study as their pilot study showed worsening of performance with higher doses of clozapine in combination with PCP (Didriksen *et al.*, 2007). It has also been shown that tolerance can develop to the sedative effects of clozapine after repeated dosing (Chesler and Salamone, 1996). These data are supported by a study by Hashimoto and colleagues who showed that acute administration of clozapine (5 mg/kg) and haloperidol (0.1 mg/kg) did not improve a sub-chronic PCP-induced cognitive deficit in the NOR task in mice; however, they found that sub-chronic administration of clozapine (5 mg/kg daily for 14 days) significantly improved the deficit, whereas sub-chronic haloperidol (0.1 mg/kg daily for 14 days) did not (Hashimoto *et al.*, 2005).

The results from this experiment show that sub-chronic administration of haloperidol did not significantly improve the deficit in set-shifting performance induced by PCP as indicated by the inability to improve the deficit in trials to reach criterion and the percentage of errors. This is supported by clinical data as patients chronically treated with haloperidol have shown impaired performance in tests of working memory and executive function (Gilbertson and van Kammen, 1997). Haloperidol-treated rats

also required significantly more time to complete their trials in the SD, CD, Rev l and Rev 3 phases of the task, indicating that the rats were impaired behaviourally. The inability of haloperidol to reverse the PCP-induced deficit may be due to its high D_2 receptor affinity and minimal 5-HT_{2A} receptor affinity (Siegfried *et al.,* 2005).

The current study does not provide direct evidence for specific receptor mechanisms involved in mediating the efficacy of the antipsychotics against the PCP effect. Selective agents will be used in chapter 3 to identify which receptors are involved in reversing the PCP-induced deficit in an operant reversal learning task. Data from receptor binding studies in other laboratories allows the suggestion of potential receptor mechanisms which may be involved. Risperidone was highly effective in reversing the PCP-induced deficit and has high 5-HT_{2A} and D_2 receptor affinity (Leysen *et al.,* 1993) as well as high 5-HT_7 affinity (Shahid *et al.,* 2008). 5-HT_7 receptors have been implicated as having a role in learning and memory (Hedlund and Sutcliffe, 2004; Thomas and Hagan, 2004). Risperidone is suggested to be the most effective atypical antipsychotic to improve working memory and executive function in the clinic (Meltzer and McGurk, 1999), although, in this study in rats, clozapine was equally effective. Clozapine has a lower affinity for D_2 receptors than risperidone (Arnt and Skarsfeldt, 1998). It is believed that a 40-80% threshold of D_2 receptor occupancy is required to achieve an antipsychotic response (Kapur *et al.,* 2003). Clozapine is able to reach its antipsychotic effect at 45-65% D_2 receptor occupancy levels (Kapur *et al.,* 2003). Risperidone is only effective at occupancy levels above the threshold of 65%, suggesting that risperidone is more reliant upon its D_2 receptor affinity than clozapine. Although risperidone and clozapine have high affinity for 5-HT_{2A} receptors, it is unlikely that this mechanism alone is responsible for their antipsychotic action, as both have been shown to saturate 5-HT_2 receptors at sub-therapeutic doses (Kapur *et al.,*

1999). Risperidone also has high affinity for D_3, D_4 and α_2-adrenoceptors, which may add to its therapeutic effect (Siegfried *et al.,* 2005). Clozapine has affinity at a wide range of receptors including 5-HT_6 receptors. Activity at 5-HT_6 receptors has been postulated to be involved in cognitive dysfunction (Woolley *et al.,* 2004; Mitchell and Neumaier, 2005). A selective 5-HT_6 receptor antagonist has been shown to improve set-shifting ability (Hatcher *et al.,* 2005), this may be via facilitation of cortical and hippocampal glutamatergic activity (Dawson *et al.,* 2001).

2.4.3 Experiment 3: Effects of isolation rearing and attentional set-shifting

The results demonstrate that isolation reared rats showed hyperactivity when compared to socially housed controls. Since this is in agreement with previous results in this (Smith *et al.,* 1997) and many other laboratories (Bakshi and Geyer, 1999; Lapiz *et al.,* 2003; Elliott and Grunberg, 2005; Bianchi *et al.,* 2006) this validated the isolation rearing paradigm used. It has been widely suggested that increases on LMA in rats reared in isolation may be due to increased dopamine in the mesolimbic pathway (see Fone and Porkess, 2008), with some reported increases in dopamine turnover in the nucleus accumbens (Hall *et al.,* 1998). Although rats reared in isolation demonstrated hyperactivity in the automated photocell cages, there were no significant differences in time taken to complete the trials during attentional set-shifting, except in reversal 3 of the task in which isolation reared rats were quicker to complete the trials. This may be an observed effect as the isolation reared rats appear to be getting progressively quicker over the phases, except in the EDS phase where due to the increased difficulty rats take longer per trial, so that by reversal 3 they are significantly quicker than their social counterparts. The social rats may not complete the trials quicker as they could be less motivated than the isolates as it has been shown that in progressive ratio isolates will

respond much more for food than social animals (Smith *et al.*, 1997). The principal findings show that isolation reared rats exhibit impaired set-shifting ability when compared with socially reared controls. Thus, isolates required significantly more trials to reach criterion selectively at the EDS phase of testing, when compared with socially housed animals indicative of a selective deficit in set-shifting ability. An additional finding in the current study was that isolates explored both bowls more before making a decision to dig. This effect was also observed in PCP-treated rats, demonstrating that it is a consistent finding. This was measured by an increased number of visits to both bowls and could suggest that isolates are more indecisive or could be an indication that they cannot remember which is the correct parameter from the discovery trials. The deficit produced by sub-chronic PCP in experiments 1 and 3 is more robust than the deficit produced by isolation rearing. This may be due to more variation between the isolates as the errors bars are larger in the isolation rearing experiment compared to those in the PCP experiments, and that the isolates are difficult to test due to changes in their behaviour including being more susceptible to distraction produced by housing in isolation.

Patients with schizophrenia also exhibit selective deficits in the ED shift of the ID/ED task and WCST (Haut *et al.*, 1996; Tyson *et al.*, 2004; Jazbec *et al.*, 2007). The data herein show a similar profile in rats reared in isolation. Notably there were no differences between isolates and socials in any other stage of the task including reversal learning, showing that reversal learning is not affected by isolation rearing. This is in agreement with previous data in an operant lever pressing task (Abdul-Monim *et al.*, 2006). This data is also supported by a study using an 8 arm radial maze to assess attentional set-shifting ability showing that isolates performed similarly in the acquisition and reversal phases but were impaired in attentional shifts (Schrijver and

Wurbel, 2001). This may be due to the different regions of the frontal cortex involved in reversal learning and attentional set-shifting. It has been shown that whereas lesions of the orbital prefrontal cortex in rats selectively disrupts reversal learning (McAlonan and Brown, 2003), lesions of the medial prefrontal cortex (mPFC) produce a selective deficit in the EDS phase (Birrell and Brown, 2000). Consistent with a selective deficit in the EDS phase, rats reared in isolation have been shown to exhibit structural abnormalities selectively in the mPFC (Silva-Gomez *et al.,* 2003; Day-Wilson *et al.,* 2006).

It is not surprising that early separation from cage-mates alters brain development as most of the monoamine neurotransmitters and their associated receptor compliment only reach the adult composition between PND 30-50 in the rat (Lapiz *et al.,* 2003). The cognitive deficit shown in this study could be attributed to structural and neurochemical abnormalities previously demonstrated in rats reared in isolation. Isolation rearing decreases dopamine turnover in the medial prefrontal cortex (Heidbreder *et al.,* 2000), which is similar to the hypofrontality in schizophrenia. Schizophrenic patients exhibit decreased dendritic spine density in both the hippocampus and medial prefrontal cortex (Glantz and Lewis, 2000). These structural alterations are also seen in rats reared in isolation (Varty *et al*., 1999; Silva-Gomez *et al.,* 2003; Day-Wilson *et al.,* 2006) and are regions suggested to be involved in the attentional set-shifting task (Birrel and Brown, 2000). Rats reared in isolation also show a decrease in mPFC volume (Day-Wilson *et al.,* 2006).

2.4.4 Conclusions

Sub-chronic PCP administration and isolation rearing impairs attentional set-shifting ability as demonstrated by poor performance in the EDS stage of the task. The findings of this study support NMDA antagonist administration and isolation rearing as useful models for mimicking cognitive dysfunction with relevance to schizophrenia. It was also shown that sub-chronic treatment with the atypical antipsychotics, clozapine and risperidone, is effective in reversing the PCP-induced cognitive deficit in the attentional set-shifting task, and that, the typical antipsychotic, haloperidol, is ineffective. The data from experiment 4 suggests that counterbalancing is not essential but preferred, and analysis of the number of correct discovery trials showed that in both vehicle and PCP-treated groups the number of correct discovery trials was significantly decreased from IDS to EDS. This suggests that, if an attentional shift is to be observed in vehicle rats, the discovery trials should not be disregarded and should be scored and incorporated as normal trials.

This chapter demonstrates that deficits in attentional set-shifting ability can be produced by sub-chronic PCP administration and isolation rearing. However, the deficits produced by PCP in experiments 2 and 4 were more robust than the deficit produced by isolation rearing. Thus, in other tests of cognition it would be more beneficial to use the sub-chronic PCP model to induce cognitive deficits as these appear to be robust and reproducible. It was also shown that clozapine and risperidone improved the PCP-induced deficit; however, this does not give an indication of the specific receptors that are involved in the mediation of the reversal of the PCP-induced deficit. The next chapter will use an operant reversal learning task to elucidate the receptors involved in the PCP-induced deficit and which receptors are important for antipsychotic activity.

Chapter 3

Effects of antipsychotics and receptor selective agents against sub-chronic PCP-induced deficits in reversal learning

3.1 Introduction

The dominant hypothesis of the pathophysiology of schizophrenia until recently was the dopamine hypothesis as dopamine-releasing drugs such as amphetamine were seen to cause psychosis and D_2 antagonists were shown to have some efficacy. However, a purely dopamine hypothesis is limited as negative and cognitive symptoms appear to be untreated by typical antipsychotics. The glutamate hypothesis based on NMDA antagonists, such as phencyclidine (PCP), producing cognitive impairments may be more clinically relevant. The main glutamate hypothesis arises from the NMDA receptor hypofunction hypothesis of schizophrenia of Olney and Farber (1995). They postulated that NMDA hypofunction produces a reduction in inhibitory firing mediated by GABAergic neurotransmission (see chapter 1). In support of this hypothesis it has also been shown that glycine administration to enhance NMDA receptor function can improve schizophrenic symptoms when used in conjunction with other antipsychotic treatment (Rosse *et al.*, 1989).

Sub-chronic phencyclidine (PCP) dosage regimens are believed to induce cognitive impairments to mimic those seen in schizophrenia (Javitt and Zukin, 1991). It was previously shown that PCP selectively impaired performance in the reversal phase of the reversal learning task (Abdul-Monim *et al.*, 2006), and that the atypical antipsychotic ziprasidone, but not the classical antipsychotic haloperidol, was able to prevent the PCP-induced disruption of the task (Abdul-Monim *et al.*, 2006). Sub-chronic PCP has also produced deficits in other cognitive tests such as attentional set-shifting (see chapter 2) and a novel object recognition test (Grayson *et al.*, 2007). In both of these studies the PCP-induced deficit was reversed by atypical antipsychotics, but not classical agents.

In the reversal learning paradigm, animals are required to perform two tasks. The first task requires the animal to inhibit a previously learned rewarded strategy; in the second task, the animal must acquire the new strategy (Mackintosh and Little, 1969). Effective performance requires intact cognitive ability; thus animals are required to demonstrate flexibility, attention, motivation, and ability to suppress a previously learned response and implement a new one (Jones *et al.,* 1991). In rats this reversal learning ability is assessed in operant chambers with two levers, an active lever which when pressed produces food from the magazine, and an inactive lever which has no consequence but to end the trial. The task involves an initial phase for 5 min in which one lever is active; this is followed by a 2 min time-out period. Then in the 5 min reversal phase the opposite lever is now active. The experiments in this chapter utilised an operant reversal learning test and this was combined with our sub-chronic PCP dosage regimen to produce a cognitive deficit similar to those seen in schizophrenia. Similar tests in schizophrenia patients, such as the Wisconsin Card Sorting Test, require intact functioning of the prefrontal cortex (Deicken *et al.,* 1995; Dias *et al.,* 1997). It has been shown more specifically that lesions of the orbital prefrontal cortex impair reversal learning ability (McAlonan and Brown, 2003; Tait and Brown, 2007).

The principal aim of this chapter is to further validate the reversal learning task with sub-chronic PCP and antipsychotics, and to determine which receptor mechanisms may be implicated in cognitive dysfunction associated with schizophrenia by using selective compounds (see chapter 1 section 1.6.3). Receptor affinities of these selective compounds are detailed in table 3.1. The drugs selected were based on the receptor pharmacology of clozapine and risperidone; and on the emerging roles of the dopamine D_1 and α7 nACh receptors in cognitive enhancement in schizophrenia, as well as the conflicting role of 5-HT_{1A} receptors (Gray and Roth, 2007, see chapter 1 table 1.4).

Table 3.1: Receptor affinities for the selective compounds to be used in this chapter.

Drug	Receptor affinity	Dose (mg/kg)	Reference
clozapine	$5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$, $5\text{-}HT_6$, α_1 and α_2-adrenergic, H_1, M_1	5	Siegfreid *et al.*, 2005
risperidone	D_2, D_3, D_4, $5\text{-}HT_{2A}$, $5\text{-}HT_7$, α_1 and α_2-adrenergic, H_1	0.2	Siegfreid *et al.*, 2005
haloperidol	D_2, D_3, D_4, α_1-adrenergic	0.05	Stahl, 2002
SKF-38393	D_1, D_5	0.75, 1.5, 3, 6	Neumeyer *et al.*, 2003, Qandil *et al.*, 2003
SCH-23390	D_1, D_5	0.05	Lawler *et al.*, 1999
buspirone	$5\text{-}HT_{1A}$	0.15625-2.5	Hamik *et al.*, 1990
WAY-100635	$5\text{-}HT_{1A}$	0.3, 1.0	Hamon *et al.*, 1990
77-LH-28-1	M_1	1, 3, 10	Langmead *et al.*, 2008b
SB-243213A	$5\text{-}HT_{2C}$	1, 3, 10	Bromidge *et al.*, 2000
PNU-282987	nicotinic α_7	5, 10, 20	Waring *et al.*, 2008
PheTQS	nicotinic α_7 PAM	3, 10, 30	GSK in house data
pyrilamine (mepyramine)	H_1	10, 20, 40	Soria-Jasso *et al.*, 1997

Table 3.1: See table 3.3 for details of source and administration.

3.2 Materials and Methods

3.2.1 Subjects and housing conditions

Two groups of 50 female hooded-Lister rats (Harlan, UK) housed in groups of four or five were used as subjects. Animals were maintained under standard laboratory conditions at a temperature of 21°C (±2°C) and humidity of 40–50%. They were maintained on a 12 h/12 h light/dark cycle (lights on at 0700 h) and experimental procedures were performed during the light phase. Rats were gradually food deprived to approximately 90% of free-feeding body weight before training; reduced body weight was maintained by restricting the amount of food (standard laboratory chow, Special Diet Services, Essex, UK) given to each rat per day (12 g/day). The availability of water was not restricted. Experiments were conducted in accordance with the Animals Scientific Procedures Act, UK, 1986, and approved by the University of Bradford ethical review process.

3.2.2 Reversal learning training and testing

All rats were tested in one of eight operant chambers (constructed in-house, shown in fig 3.2.2a). Each chamber (29 × 30 × 30 cm) consisted of Plexiglas walls and ceiling, and a metal grid floor over sawdust (see fig 3.2.2b). A hinged Plexiglas panel (6 × 6 cm) provided access to a food hopper containing food pellets (45 mg Noyes pellets, Sandown Scientific, UK). Two retractable levers (4 × 2 cm) were positioned on either side of the food hopper. A light emitting diode (LED) was positioned centrally above each lever and a house light was located in the ceiling of each chamber. The chambers were placed individually within ventilated sound-attenuating hardboard boxes (69 × 38 × 42 cm) containing a Perspex window to allow viewing. A small fan was built into

each chamber to mask external noise. All boxes were controlled by Med-PC software (Version 2.0 for DOS or Med-PC for Windows, Med Associates, Inc. Lafayette, Indiana). Programmes were written using Medstate notation.

Figure 3.2.2a Identical Skinner boxes in test room.

Figure 3.2.2b Individual Skinner box.

Following habituation to the operant chambers, rats were trained on a continuous reinforcement (CR) schedule, whereby a food pellet was delivered every 30 s for a 20 min period. This training period was (for approximately 5 days) followed by the introduction of the two retractable levers using the FR1 (fixed ratio 1) schedule of reinforcement so that one press of either lever resulted in the delivery of a food pellet. This process was conducted for approximately 2 weeks for all rats to acquire the lever pressing response. When responding had stabilised, rats were trained to press either the left or right lever for food delivery according to a visual cue (LED on or off). The experimental session was terminated following a total of 128 lever presses, which took approximately 30 min. The active lever was varied between days using the pseudorandom Gellerman schedule, which randomly assigned either the left or right lever as active, thus avoiding the generation of lever bias. The experimental session began with the illumination of the house-light. After 3 s, the levers were extended to the chamber together with the cue light. Following a lever press the levers were retracted and the house-light was extinguished for a 3 s time-out period. A correct response on the active lever resulted in the delivery of a food pellet, and an incorrect response resulted in no food delivery. The house-light was then illuminated and the cycle was repeated. The experimental session was terminated following 128 lever presses (on either the correct or incorrect levers). Rats were trained once daily and this was repeated until rats had reached criterion, i.e. 90% correct responding for three consecutive days.

The day before each reversal task session, a full 30-min operant training session (as described above) was conducted in order to ensure stable responding, i.e. 90% correct responding. The reversal-learning session involved animals being first exposed to a 5 min period during which the active lever was the same as on the previous training

day. During this period, responses on both correct and incorrect levers were recorded. This part of the session was termed the initial phase. This was followed by a 2 min time-out period, which was signalled by the house light being turned off. In the subsequent 5 min period, the active lever was reversed. Responses made on the correct and incorrect levers were again recorded. This second period was termed the reversal phase. Animals undertook several of these reversal learning sessions before beginning the drug studies in order to ensure that they attained a stable level of performance, i.e. 90% correct responding and at least 25 lever presses in total, in both the initial and reversal phases of the task.

3.2.3 Experimental design

Rats were tested on a cycle of 4 days (see table 3.2.2). On day 1 each animal had a 30-min operant training session. The following day, animals received the appropriate drug(s) and undertook a reversal-learning session. On day 3 and day 4, each animal underwent a further operant training session and reversal task session, respectively, in order to ensure that responding was back to normal after the drug treatment. Following sub-chronic PCP treatment and a 7-day washout period the following drug experiments were carried out. The drug and vehicle treatment given to each PCP-treated rat was pseudo-randomised over the course of these experiments to ensure that the highest dose or vehicle treatment was not given to the same rats in successive experiments. In the experiments in this chapter, normal responding was restored following each drug treatment, if performance was not fully restored, the cycle shown in table 3.2.2 would be repeated with no drug treatments until baseline performance in the reversal test was restored.

Table 3.2.2: The experimental design for each drug study.

Day 1	Day 2	Day 3	Day 4	Day 5 +
Training session	Drug test day, reversal test session. Data shown in results section	Training session	Reversal test session, drug-free	The cycle repeated as needed

3.2.4 Drugs

For each cohort, the study design involved groups of 10 rats initially; rats were given either 2 mg/kg PCP (n = 40) or vehicle (0.9 % saline; n = 10) by the intraperitoneal (i.p.) route twice daily for seven days. Dosing with sub-chronic PCP or vehicle was followed by a washout period of a further seven days. PCP hydrochloride (Sigma, UK) was dissolved in 0.9 % saline. Drugs and respective vehicles used in the experiments are shown in table 3.2.3. The effects of drugs were examined in PCP-treated rats only. The order of experiments is shown is figure 3.2.3.

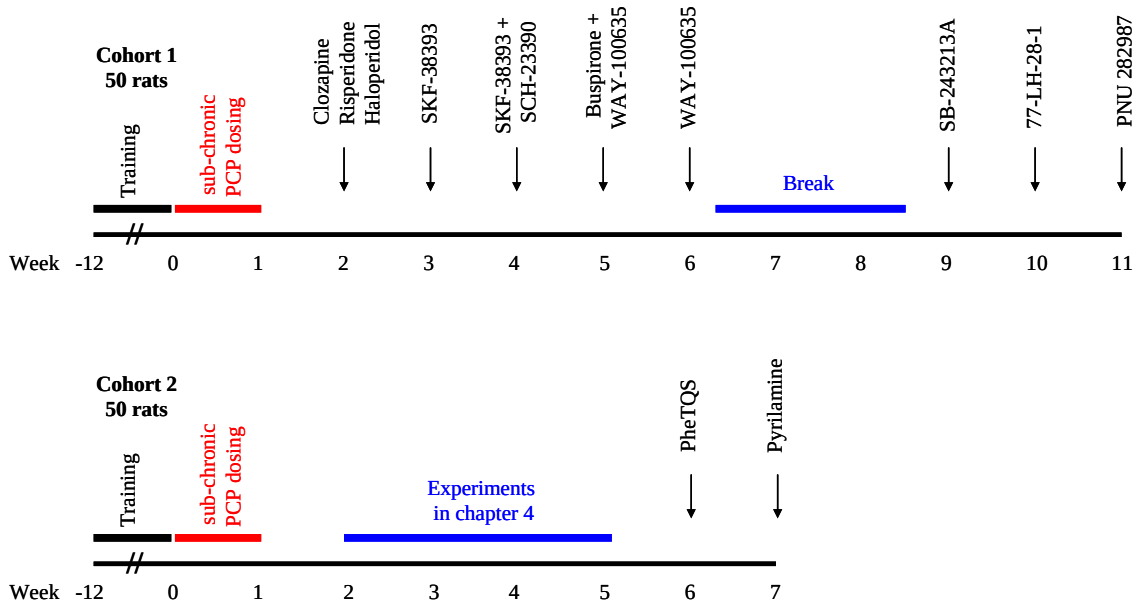


Figure 3.2.3: Timeline of experiments

Experiment 1: Rats were treated with vehicle (0.9% saline), risperidone (0.2 mg/kg), clozapine (5.0 mg/kg), and haloperidol (0.05 mg/kg). Haloperidol (Serenace liquid, 2.0

mg/ml, Baker, UK) was diluted with distilled water. Clozapine (Tocris, UK) and risperidone (provided by GSK, UK) were dissolved in 10µl of acetic acid, made up to volume with distilled water and pH adjusted to 6 with 0.1M NaOH. All drug doses were calculated as base equivalent weight and were administered via the intraperitoneal (i.p.) route in a volume of 1 ml/kg 30 min before testing.

Experiments 2 and 3: A dose-response was carried out to the dopamine D_1 receptor agonist, SKF-38393, and this was followed by a further experiment where the D_1 receptor antagonist, SCH-23390 was also administered in the presence of SKF-38393. The dose-response to SKF-38393 was carried out over 2 experiments, in the first experiment 0.75, 1.5, and 3.0 mg/kg (i.p.) were tested followed by a later experiment with 6.0 mg/kg (i.p.) subsequently the data were combined which meant there were 20 rats in the vehicle and PCP groups. SKF-38393 hydrochloride (Research Biochemicals International, MA, USA) and SCH-23390 hydrochloride (Tocris, UK) were dissolved in saline (0.9 %) and were administered via the i.p. route in a volume of 1 ml/kg. SCH-23390 or vehicle was given 20 min prior to SKF-38393 or vehicle and rats were tested 30 min following this treatment. All drug doses were calculated as base equivalent weight. It was determined that 0.05 mg/kg of SCH-23390 reversed the effect of SKF-38393 in novel object recognition (Idris *et al.*, 2008); therefore this dose was tested in the second reversal learning experiment.

Experiments 4 and 5: The doses of buspirone were selected based on previous dose-response experiments carried out in this reversal learning task. WAY-100635 was shown to have selective 5-HT_{1A} antagonist activity at the doses used in this study (Prinssen *et al.*, 1999). Buspirone hydrochloride (Sigma, UK) and WAY-100635 (Sigma, UK) were dissolved in 0.9% saline and injected in a volume of 1 ml/kg via the i.p. route; buspirone was administered 30 min prior testing and WAY-100635 or vehicle

was administered 30 min prior to buspirone. WAY-100635 alone was administered 60 min prior to testing.

Experiment 6: The doses of SB-243213A (5-methyl-1-[[2-[92-methyl-3-pyridyl0oxy]-5-pyridyl]carbamoyl]-6-trifluoromethylindone hydrochloride) were selected based on effects observed in a study by Wood *et al.,* (2001). SB-243213 was shown to have high affinity for the human 5-HT_{2C} receptor with greater than 100-fold selectivity over 5-HT_{2A} and 5-HT_{2B} receptors (Wood *et al.,* 2001). SB-243213A (generously supplied by GSK, Harlow, UK) was dissolved in vehicle containing cyclodextrin (8% by weight) plus 25mM citric acid, and was administered 30 min prior to testing via the i.p. route in a volume of 2 ml/kg.

Experiment 7: 77-LH-28-1 (1-[3-(4-butyl-1-piperidinyl)propyl]-3,4-dihydro-2(1*H*)-quinolinone) was generously supplied by GSK (Harlow, UK) was prepared in 0.9% saline, given in a volume of 2 ml/kg via the s.c. route, and was administered 30 min before testing. The doses and route of administration of 77-LH-28-1 were selected according to internal GSK DMPK and additional in-house behavioral data.

Experiments 8 and 9: PheTQS ((3aR,4S,9bS)-4-(4-methylphenyl)-3a,4,5,9b-tetrahydro-3H cyclopenta[c]quinoline-8-sulfonamide)) was generously supplied by GSK (Harlow, UK) and was prepared in 1 % methylcellulose, given in a volume of 1 ml/kg via the p.o. route, and was administered 1 hour before testing. The doses and route of administration of PheTQS were selected according to internal GSK DMPK and additional in-house behavioral data. PNU-282987 ([*N*-[(3*R*)-1-Azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide Hydrochloride]) (Tocris, UK) was dissolved in 0.9% saline and given in 2 ml/kg volume via the s.c. route, and was administered 1 hour before testing.

Experiment 10: Pyrilamine maleate salt (Sigma, UK) was prepared in 0.9% saline, given in a volume of 2 ml/kg via the s.c. route, and was administered 45 min before testing.

3.2.5 Data and statistical analysis

Data for percent correct responding in the reversal learning task was calculated using the number of presses on the correct lever divided by the total number of presses multiplied by 100. The percent correct responding data was arcsine transformed and analysed by one-way ANOVA followed by post-hoc Dunnett's t-test. The total number of lever presses was calculated by adding the number of correct and incorrect presses together within the 5 min test session, this was used to assess whether drugs had caused any sedation or behavioural impairment.

Table 3.2.3: Routes of administration and vehicles of the drugs used in this chapter.

Drug	Source	Receptor	Dose (mg/kg)	Volume (ml/kg)	Route	Vehicle	Treatment time (min)
Clozapine	Tocris, UK	See table 3.1	5	1	i.p.	distilled water + 10µl acetic acid and pH balanced with NaOH (1M)	30
Risperidone	GSK, UK	See table 3.1	0.2	1	i.p.	distilled water + 10µl acetic acid and pH balanced with NaOH (1M)	30
Haloperidol	Baker, UK	See table 3.1	0.05	1	i.p.	distilled water	30
SKF-38393	RBI, MA, USA	D_1 agonist	0.75, 1.5, 3, 6	1	i.p.	0.9% saline	30
SCH-23390	Tocris, UK	D_1 antagonist	0.05	1	i.p.	0.9% saline	20 before SKF, 30 before testing
Buspirone	Sigma, UK	5-HT_{1A} partial agonist	0.15625-2.5	1	i.p.	0.9% saline	30
WAY-100635	Sigma, UK	5-HT_{1A} antagonist	0.3, 1.0	1	i.p.	0.9% saline	20 before buspirone, 30 before testing
77-LH-28-1	GSK, UK	M_1 agonist	1, 3, 10	2	s.c.	0.9% saline	30
SB-243213A	GSK, UK	5-HT_{2C} antagonist	1, 3, 10	2	i.p.	0.9% saline containing cyclodextrin (8% by weight) plus 25mM citric acid	30
PNU-282987	Tocris, UK	alpha 7 agonist	5, 10, 20	2	s.c.	0.9% saline	60
PheTQS	GSK, UK	alpha 7 PAM	3, 10, 30	1	p.o.	1% methyl cellulose	60
Pyrilamine	Sigma, UK	H_1 antagonist	10, 20, 40	2	s.c.	0.9% saline	45

Table 3.2.3: Routes of administration: intraperitoneal (i.p.), sub-cutaneous (s.c), per os (p.o).

3.3 Results

3.3.1 Effect of antipsychotics

A one-way ANOVA on the initial phase showed a significant effect of drug ($F_{4,46}$=3.36, P<0.05) and post-hoc Dunnett's t-test showed that haloperidol significantly impaired responding (P<0.01, fig 3.3.1) compared to the vehicle group. A paired t-test showed a significant deficit in the reversal phase in the PCP group compared to the initial phase (P<0.05). A one-way ANOVA on the reversal phase showed a significant effect of drug ($F_{4,46}$=3.77, P<0.01). Post-hoc Dunnett's t-test revealed that risperidone significantly improved performance of PCP-treated rats compared to PCP alone (P<0.05, fig 3.3.1a), while clozapine and haloperidol did not improve performance (P=0.16 and P=1.0 respectively). There was no effect on total lever pressing in the reversal phase; however, in the initial phase clozapine significantly reduced the total lever pressing (P<0.05, table 3.3.1).

Table 3.3.1: The effect of clozapine (5.0 mg/kg), risperidone (0.2 mg/kg), haloperidol (0.05 mg/kg) and sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) on the total number of lever presses in a reversal learning paradigm. Data are expressed as the mean ± SEM total number of lever presses (n=8-10 per group) in the initial and reversal phase of the task. Post-hoc Dunnett's t-test revealed that the total number of lever presses was significantly decreased in the initial phase in clozapine-treated rats when compared to the vehicle-treated group; *P<0.05.

Drug treatment	Initial phase	Reversal phase
vehicle + vehicle	27.1± 0.4	27.6± 0.3
vehicle + sub-chronic PCP 2mg/kg	27.4± 0.5	27.2± 0.4
5.0 mg/kg clozapine + sub-chronic PCP	24.3± 1.4*	26.0± 1.0
0.2 mg/kg risperidone + sub-chronic PCP	26.4± 0.6	26.8± 0.7
0.05 mg/kg haloperidol + sub-chronic PCP	27.5± 0.3	26.9± 0.3

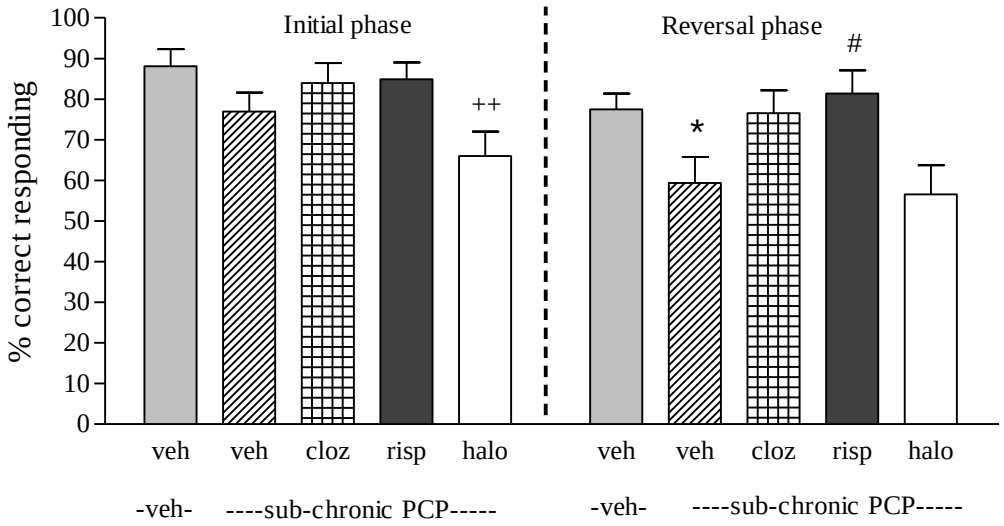


Figure 3.3.1a: The effect of clozapine (5.0 mg/kg), risperidone (0.2 mg/kg) and haloperidol (0.05 mg/kg) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM. percentage correct responding (n=8-10). Post-hoc Dunnett's t-test in the initial phase showed a significant reduction in correct responding in the haloperidol-treated group compared to vehicle; $^{++}P<0.01$. A paired t-test showed a significant deficit in the reversal phase in the PCP-group compared to the initial phase; $^{*}P<0.05$. $^{\#}P<0.05$ Dunnett's t-test showed risperidone significantly improved responding compared to PCP alone.

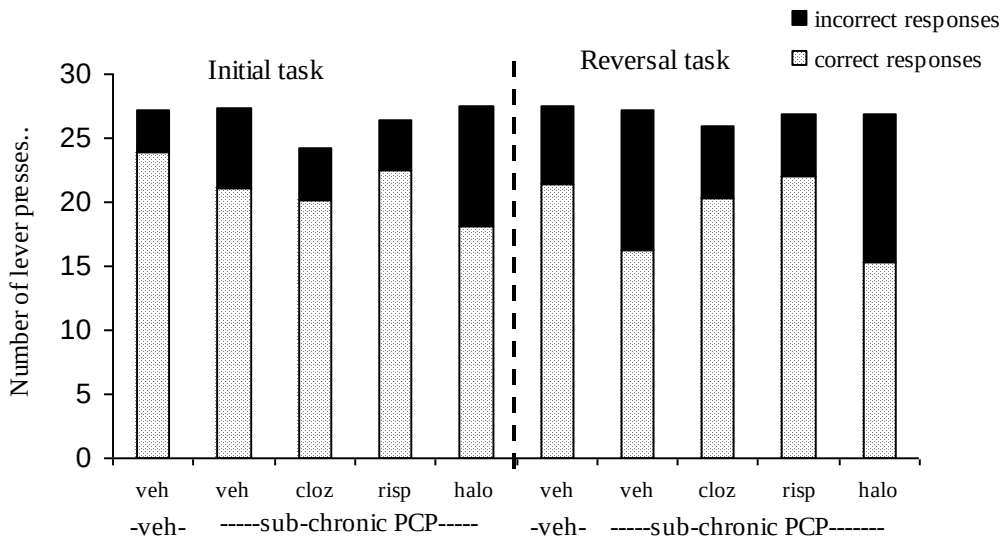


Figure 3.3.1b: The influence of clozapine (5.0 mg/kg), risperidone (0.2 mg/kg) and haloperidol (0.05 mg/kg) on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=8-10).

3.3.2 Effects of the D_1 receptor agonist SKF-38393 alone and in conjunction with the D_1 receptor antagonist SCH-23390

For percent correct responding, a paired t-test showed a significant impairment in responding in the reversal phase compared to the initial phase in the PCP-treated group ($P<0.001$) and following SKF-38393 (0.75, 1.5 mg/kg, i.p.; $P<0.05$). A one-way ANOVA in the reversal phase showed a significant interaction ($F_{5,78} = 7.38$, $P<0.001$). Post-hoc analysis revealed that SKF-38393 at 6.0 mg/kg (i.p.) significantly improved the PCP-induced deficit ($P<0.05$; Fig 3.3.2). There was no significant effect on total lever pressing in the initial or reversal phases, suggesting that neither locomotor capacity nor motivation were affected (table 3.3.2).

In the SKF-38393 and SCH-23390 combination study a paired t-test showed a significant impairment in responding in the reversal phase compared to the initial phase in the PCP-treated group ($P<0.001$; Fig 3.3.3). A one-way ANOVA in the reversal phase showed a significant interaction ($F_{4,47} = 14.63$, $P<0.001$). Post-hoc analysis showed that SKF-38393 (6.0 mg/kg, i.p.) significantly improved the PCP-induced deficit ($P<0.001$), and that SCH-23390 (0.05 mg/kg, i.p.) significantly antagonised this effect ($P<0.001$). A one-way ANOVA on total lever pressing in the reversal phase showed a significant interaction ($F_{4,47} = 6.14$, $P<0.01$) and post-hoc analysis showed a significant ($P<0.001$) reduction in lever pressing following SCH-23390 treatment compared to the vehicle group (table 3.3.3).

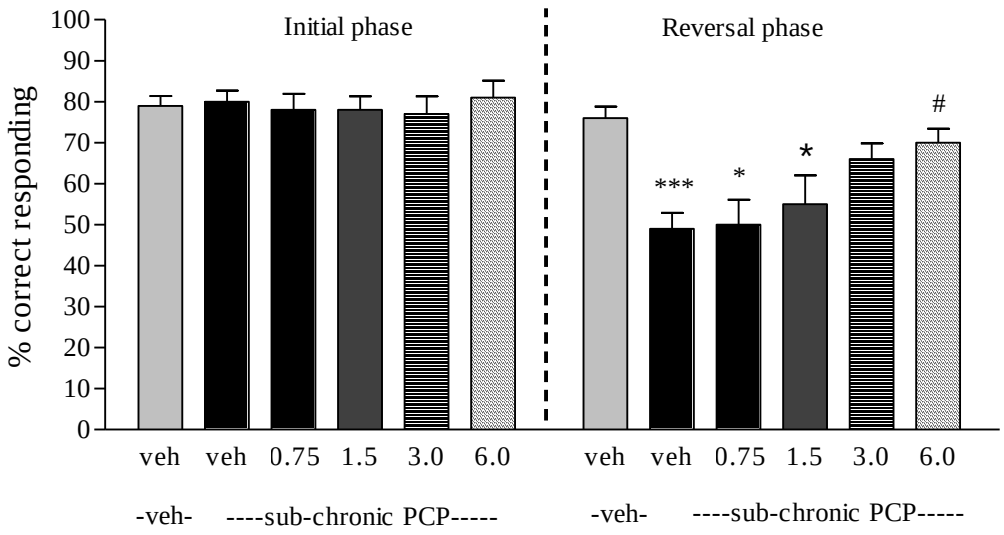


Figure 3.3.2a: The effect of SKF-38393 (0.75, 1.5, 3.0, 6.0 mg/kg) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM. of percent correct responding (n=20 for vehicle and PCP+vehicle, n=9 for all doses of SKF-38393). A paired t-test showed a significant reduction in performance of the reversal phase compared with the initial phase; ***P<0.001, *P<0.05. Dunnett's t-test showed significant improvement in responding compared to PCP alone in the reversal phase at 6.0 mg/kg of SKF-38393; $^{\#}$P<0.05.

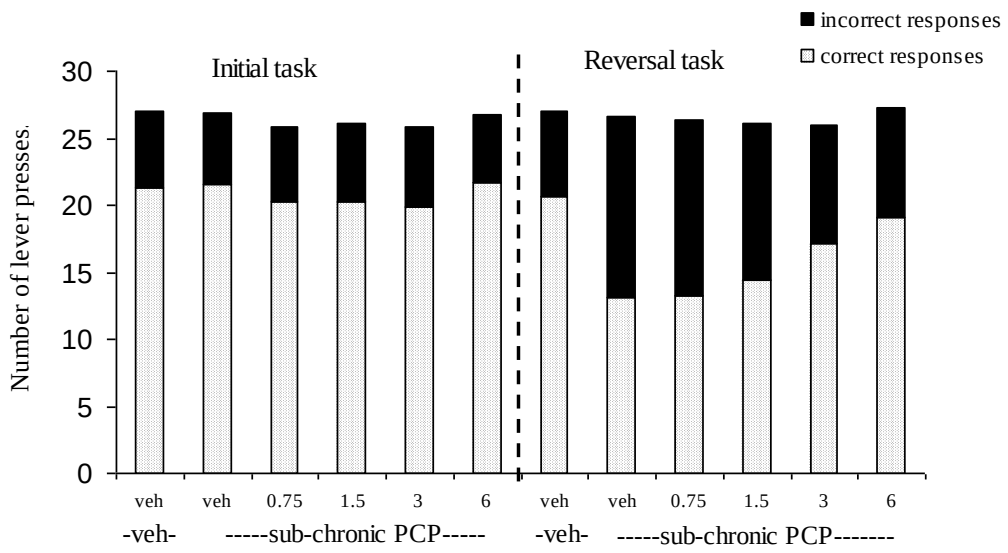


Figure 3.3.2b: The influence of SKF-38393 (0.75, 1.5, 3.0, 6.0 mg/kg) on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=20 for vehicle and PCP+vehicle, n=9 for all doses of SKF-38393).

Table 3.3.2: The effect of sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) and SKF-38393 (0.75, 1.5, 3.0, 6.0 mg/kg) and on total lever pressing. Data are expressed as the mean ± SEM total number of lever presses (n=20 for vehicle and PCP+vehicle, n=9 for all doses of SKF-38393) in the initial and reversal phase of the task.

Drug treatment	Initial phase	Reversal phase
vehicle + vehicle	27.0± 0.3	27.0± 0.2
vehicle + sub-chronic PCP	26.9± 0.2	26.6± 0.2
0.75 mg/kg + sub-chronic PCP	25.9± 0.3	26.3± 0.2
1.5 mg/kg + sub-chronic PCP	26.1± 0.2	26.1± 0.2
3.0 mg/kg + sub-chronic PCP	25.8± 0.3	26.0± 0.1
6.0 mg/kg + sub-chronic PCP	26.8± 0.5	27.3± 0.4

Table 3.3.3: The effect of sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.), SKF-38393 (6.0 mg/kg) and SCH-23390 (0.05 mg/kg) on total lever pressing. Data are expressed as the mean ± SEM total number of lever presses (n=9-10 per group) in the initial and reversal phase of the task. Dunnett's t-test showed a significant reduction in lever pressing compared to vehicle (*P<0.05, ***P=0.001).

Drug treatment	Initial phase	Reversal phase
vehicle + vehicle	27.9± 0.5	26.9± 0.3
vehicle + sub-chronic PCP	27.0± 0.4	26.9± 0.4
6.0 mg/kg SKF-38393 + sub-chronic PCP	26.1± 0.6	27.8± 0.4
0.05 mg/kg SCH23390 + sub-chronic PCP	24.2± 1.5*	20.2± 2.1***
0.05 mg/kg SCH23390 + 6.0 mg/kg SKF-38393 + sub-chronic PCP	25.0± 0.9	24.6± 1.6

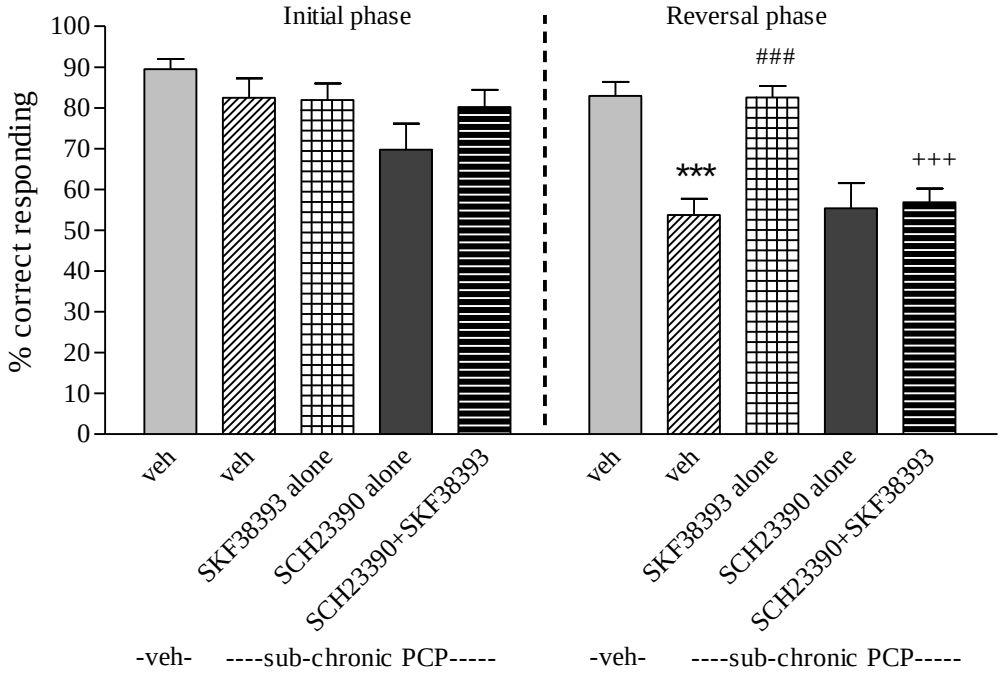


Figure 3.3.3a: The effect of SKF-38393 (6.0 mg/kg) and SCH-23390 (0.05 mg/kg) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM of percent correct responding (n=9-10). Significant reduction in performance of the reversal phase compared with the initial phase (***P<0.001). Post-hoc Bonferroni's multiple comparison test showed that SKF-38393 (6.0 mg/kg) significantly improved the PCP-induced deficit ($^{###}$P<0.001), and that this deficit was significantly antagonised by SCH-23390 (0.05 mg/kg); $^{+++}$P<0.001.

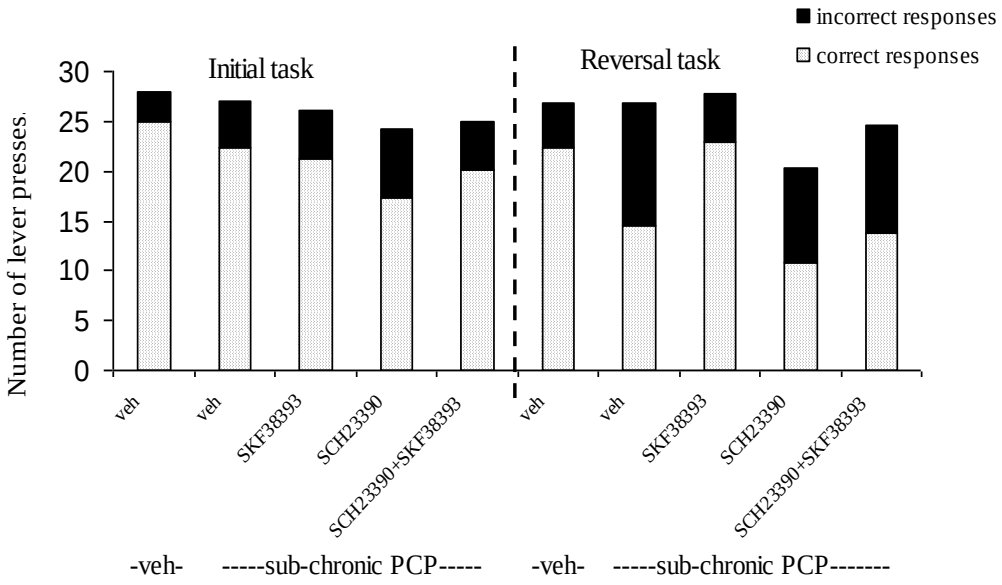


Figure 3.3.3b: The influence of SKF-38393 (6.0 mg/kg) and SCH-23390 (0.05 mg/kg) on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=9-10).

3.3.3 Effects of the 5-HT$_{1A}$ receptor partial agonist, buspirone, alone and in conjunction with the antagonist, WAY-100635

For percent correct responding, a paired t-test showed a significant impairment in responding in the reversal phase compared to the initial phase in the PCP-treated group ($P<0.001$), and following treatment with buspirone at 0.15625 mg/kg ($P<0.05$). A one-way ANOVA in the reversal phase showed a significant interaction ($F_{6,75} = 5.70$, $P<0.001$). Post-hoc analysis revealed that buspirone at 0.3125 and 0.625 mg/kg significantly improved reversal learning compared to the PCP alone group ($P<0.01$; fig 3.3.4). Pre-treatment with WAY-100635 (0.3 and 1.0 mg/kg) partially antagonised the effect of buspirone (0.3125 mg/kg), but this effect was not significant as buspirone in the presence of WAY-100635 did not significantly improve the PCP-induced deficit ($P=0.208$ and $P=0.082$ respectively). There was no significant effect on total lever pressing in the initial or reversal phases (table 3.3.4).

Table 3.3.4: The effect of sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.), buspirone (0.15625, 0.3125, 0.625 mg/kg), and WAY-100635 (0.3, 1.0 mg/kg) against buspirone (0.3125 mg/kg) on total lever pressing in the reversal learning paradigm. Data are expressed as the mean ± SEM (n=20 for vehicle, PCP + vehicle and PCP + buspirone at 0.3125 mg/kg, n=8-10 for other doses of buspirone and WAY-100635 groups) in the initial and reversal phase of the task.

Drug treatment	Initial phase	Reversal phase
vehicle + vehicle	27.4± 0.2	27.8± 0.1
vehicle + sub-chronic PCP	26.9± 0.4	26.8± 0.2
0.15625 mg/kg + sub-chronic PCP	27.6± 0.3	27.0± 0.2
0.3125 mg/kg + sub-chronic PCP	27.4± 0.4	27.4± 0.2
0.625 mg/kg + sub-chronic PCP	26.6± 0.7	27.1± 0.5
0.3125 mg/kg + WAY-100635 (0.3 mg/kg) + sub-chronic PCP	28.8± 0.3	27.6± 0.3
0.3125 mg/kg + WAY-100635 (1.0 mg/kg) + sub-chronic PCP	27.6± 0.3	27.0± 0.3

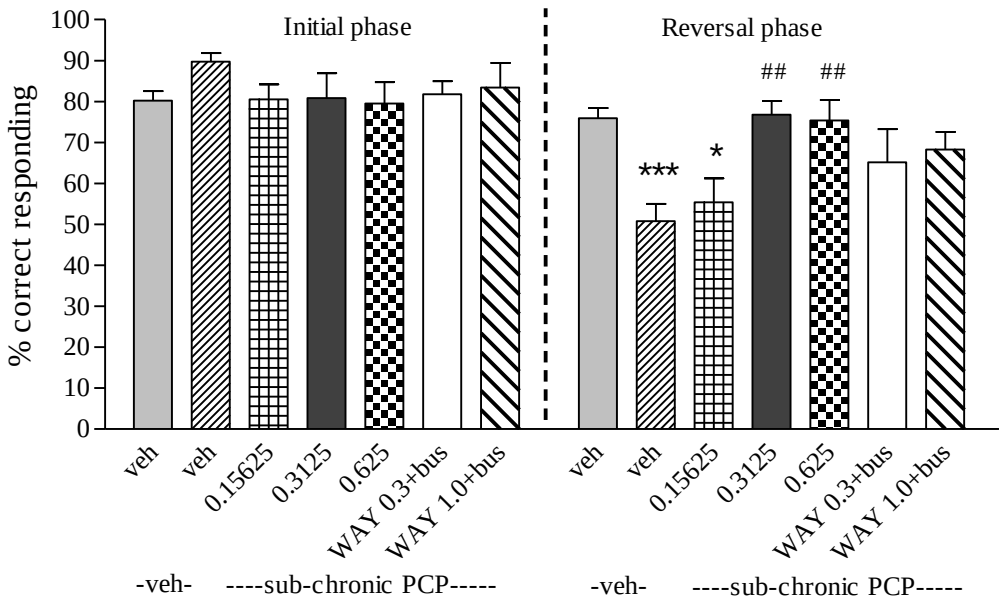


Figure 3.3.4a: The effect of buspirone (0.15625, 0.3125, 0.625 mg/kg), and WAY-100635 (0.3, 1.0 mg/kg) in combination with buspirone (0.3125 mg/kg) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM percentage correct responding (n=20 for vehicle, PCP + vehicle and PCP + buspirone at 0.3125 mg/kg, n=8-10 for other doses of buspirone and WAY-100635 groups). A paired t-test showed a significant reduction in performance of the reversal phase compared with the initial phase; ***P<0.01, *P<0.05. Dunnett's t-test showed significant improvement in responding compared to PCP alone in the reversal phase at 0.3125 mg/kg and 0.625 mg/kg; $^{\#\#}$P<0.01. Pretreatment with WAY-100635 at 0.3 and 1.0 mg/kg produced a partial reversal of buspirone but did not significantly alter the effect of 0.3125 mg/kg buspirone (P=0.208 and P=0.082 respectively).

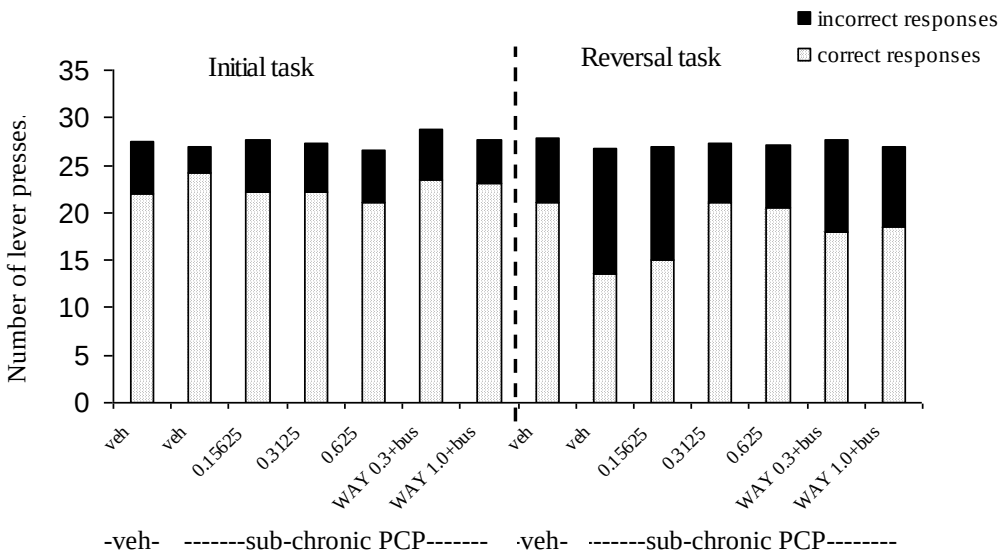


Figure 3.3.4b: The influence of buspirone (0.15625, 0.3125, 0.625 mg/kg), and WAY-100635 (0.3, 1.0 mg/kg) against buspirone (0.3125 mg/kg) on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=20 for vehicle, PCP + vehicle and PCP + buspirone at 0.3125 mg/kg, n=8-10 for other doses of buspirone and WAY-100635 groups).

3.3.4 Effect of WAY-100635 alone

For percent correct responding, a paired t-test showed a significant reduction in performance of the reversal phase compared with the initial phase in the PCP-group ($P<0.001$), and in both WAY-100635 groups ($P<0.05$). A one-way ANOVA in the reversal phase showed a significant interaction ($F_{3,33} = 4.58$, $P<0.01$). Post-hoc analysis revealed that WAY-100635 at 0.3 and 1.0 mg/kg did not significantly improve performance compared to PCP alone in the reversal phase ($P=0.541$ and $P=0.422$ respectively, fig 3.3.5). There was no significant effect on total lever pressing in the initial phase of the task, however, WAY-100635 at 1.0 mg/kg did significantly reduce lever pressing in the reversal phase of the task ($P<0.01$, table 3.3.5).

Table 3.3.5: The effect of WAY-100635 (0.3, 1.0 mg/kg), and sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) on total lever pressing in the reversal learning paradigm. Data are expressed as the mean ± SEM (n=8-10) in the initial and reversal phase of the task.

Drug treatment	Initial phase	Reversal phase
vehicle + vehicle	27.5± 0.2	27.9± 0.2
vehicle + sub-chronic PCP 2mg/kg	27.4± 0.3	26.7± 0.3
0.3 mg/kg + sub-chronic PCP	28.3± 0.4	27.2± 0.3
1.0 mg/kg + sub-chronic PCP	27.8± 0.2	26.0± 0.8**

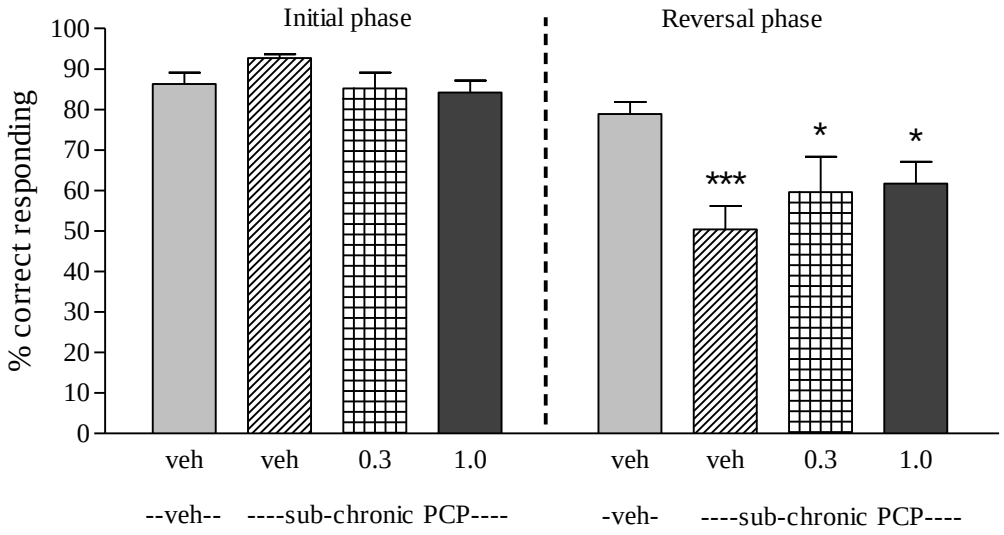


Figure 3.3.5a: The effect of WAY-100635 (0.3, 1.0 mg/kg) alone on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM percentage correct responding (n=8-10). A paired t-test showed a significant reduction in performance of the reversal phase compared with the initial phase; ***P<0.001, *P<0.05. Dunnett's t-test showed no significant improvement in responding compared to PCP alone in the reversal phase at 0.3 mg/kg and 1.0 mg/kg WAY-100635 (P=0.541 and P=0.422 respectively).

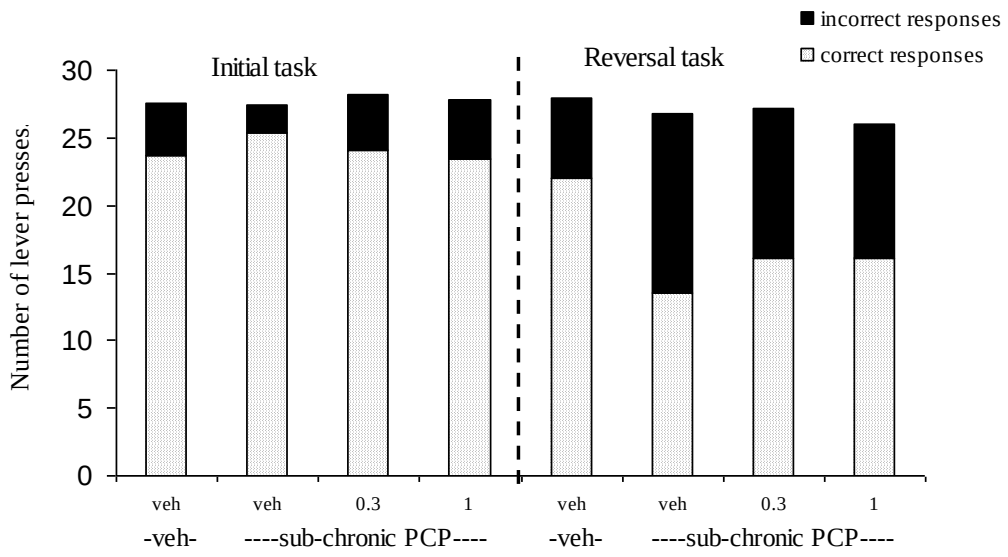


Figure 3.3.5b: The influence of WAY-100635 (0.3, 1.0 mg/kg) alone on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=8-10).

3.3.5 Effects of the 5-HT_{2C} receptor antagonist, SB-243213A

For percentage correct responding, a paired t-test showed a significant impairment in responding in the reversal phase compared to the initial phase in the PCP-treated group ($P<0.001$), 1.0 mg/kg SB-243213A treated group ($P<0.01$) and 3.0 mg/kg SB-243213A treated group ($P<0.001$). A one-way ANOVA in the reversal phase revealed a significant interaction ($F_{4,49} = 7.27$, $P<0.001$). Post-hoc analysis revealed that SB-243213A at 10 mg/kg significantly ($P<0.05$) improved the PCP-induced deficit (fig 3.3.6). There was no significant effect on total lever pressing in the initial or reversal phases, suggesting that neither locomotor capacity nor motivation were affected (table 3.3.6).

Table 3.3.6: The effect of SB-243213A (1.0, 3.0, 10.0 mg/kg) and sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) on the total number of lever presses in a reversal learning paradigm. Data are expressed as the mean ± SEM total number of lever presses (n=10) in the initial and reversal phase of the task.

Drug treatment	Initial phase	Reversal phase
vehicle + vehicle	28.2± 0.4	27.6± 0.3
vehicle + sub-chronic PCP 2mg/kg	27.2± 0.3	27.5± 0.3
1.0 mg/kg + sub-chronic PCP	28.8± 0.3	26.9± 0.9
3.0 mg/kg + sub-chronic PCP	28.2± 0.4	27.4± 0.4
10.0 mg/kg + sub-chronic PCP	27.8± 0.4	27.4± 0.3

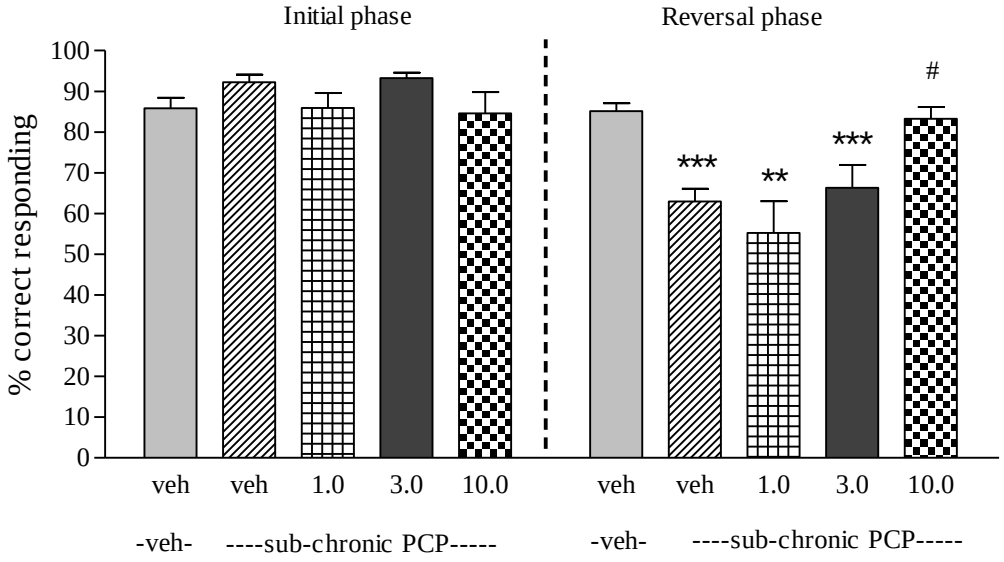


Figure 3.3.6a: The effect of SB-243213A (1.0, 3.0, 10.0 mg/kg) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM percentage correct responding (n=10). Paired t-tests showed a significant deficit in the reversal phase compared to the initial phase; **P<0.01, ***P<0.001. Post-hoc Dunnett's t-test showed that SB-243213A at 10.0 mg/kg significantly improved responding compared to PCP alone in the reversal phase; $^{\#}$P<0.05.

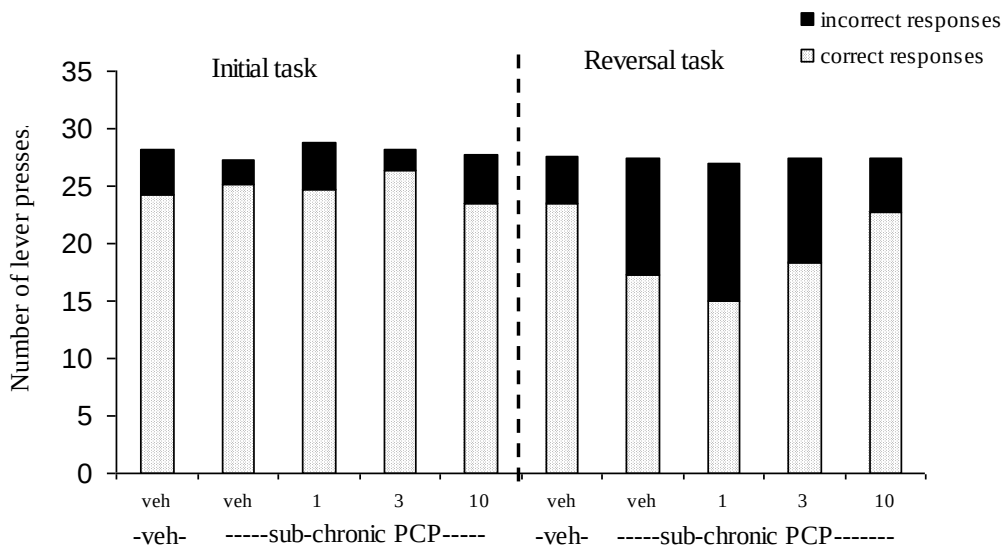


Figure 3.3.6b: The influence of SB-243213A (1.0, 3.0, 10.0 mg/kg) on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=10).

3.3.6 Effect of the M_1 receptor agonist, 77-LH-28-1

For percent correct responding, a paired t-test showed a significant impairment in responding in the reversal phase compared to the initial phase in the PCP-treated group ($P<0.05$), 1.0 mg/kg treated group ($P<0.05$), 3.0 mg/kg treated group ($P<0.05$) and 10.0 mg/kg treated group ($P<0.01$). A one-way ANOVA in the reversal phase showed a significant interaction ($F_{4,41} = 4.21$, $P<0.01$); however, post-hoc analysis did not reveal any significant improvements with 77-LH-28-1 (fig 3.3.7). There was no significant effect on total lever pressing in the initial or reversal phases, suggesting that neither locomotor capacity nor motivation were affected (table 3.3.7).

Table 3.3.7: The effect of 77-LH-28-1 (1.0, 3.0, 10.0 mg/kg) and sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) on total lever pressing. Data are expressed as the mean ± SEM total number of lever presses (n=7-10) per group) in the initial and reversal phase of the task.

Drug treatment	Initial phase	Reversal phase
Vehicle + vehicle	27.0± 0.5	27.7± 0.2
Vehicle + sub-chronic PCP 2mg/kg	27.1± 0.4	28.8± 0.3
1.0 mg/kg + sub-chronic PCP	27.6± 0.4	28.0± 0.6
3.0 mg/kg + sub-chronic PCP	26.4± 0.6	26.0± 1.6
10.0 mg/kg + sub-chronic PCP	24.9± 2.2	26.0± 1.0

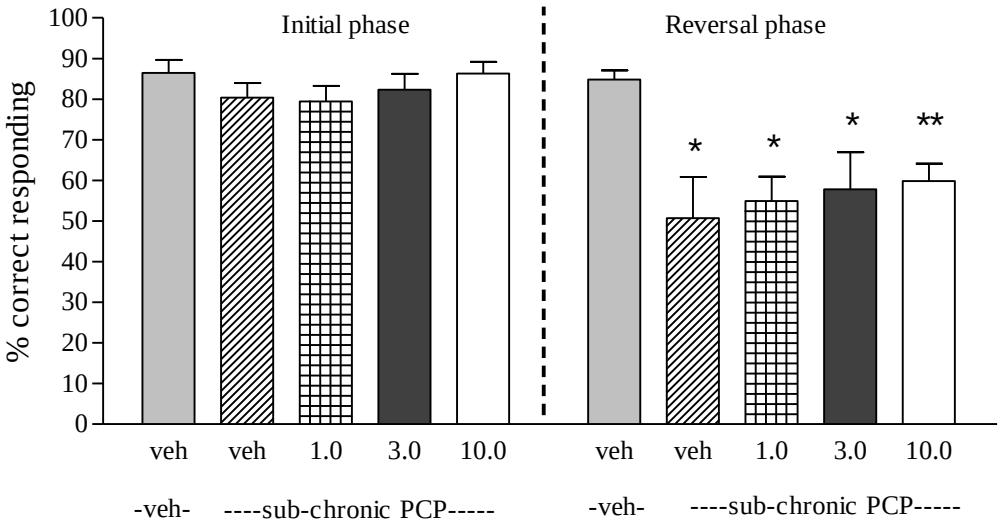


Figure 3.3.7a: The effect of 77-LH-28-1 (1.0, 3.0, 10.0 mg/kg) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM percentage correct responding (n=7-10). Significant reduction in performance of the reversal phase compared with the initial phase; *P<0.05, **P<0.01.

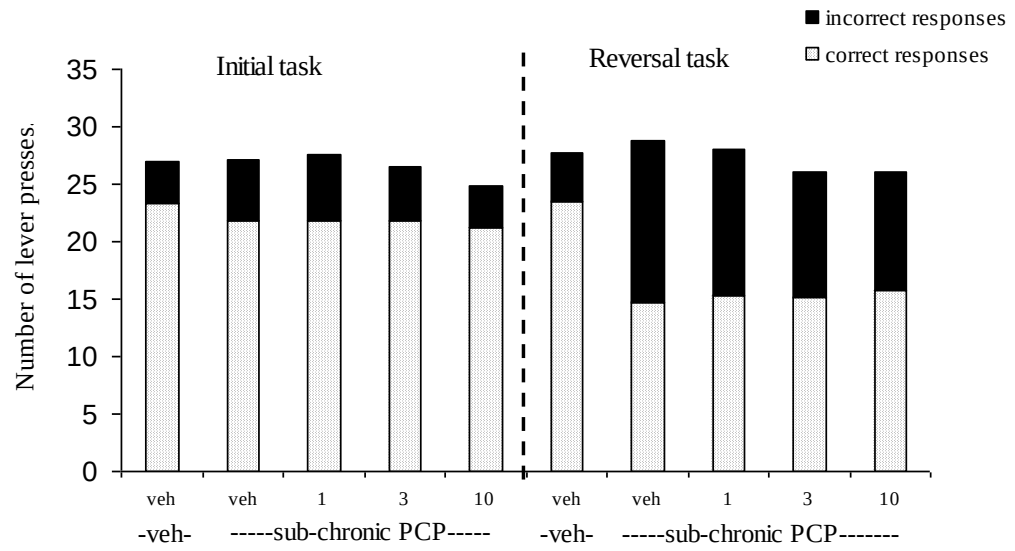


Figure 3.3.7b: The influence of 77-LH-28-1 (1.0, 3.0, 10.0 mg/kg) on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=7-10).

3.3.7 Effects of activating α7 nAChR with PheTQS and PNU-282987

In the PheTQS experiment, a paired t-test showed a significant impairment in responding in the reversal phase compared to the initial phase in the PCP-treated group ($P<0.05$) and 10 mg/kg treated group ($P<0.05$). A one-way ANOVA in the reversal phase showed a significant interaction ($F_{4,46} = 5.82$, $P<0.01$). Post-hoc analysis revealed that PheTQS at 30 mg/kg significantly ($P<0.01$) improved the PCP-induced deficit (fig 3.3.8a). There was no significant effect on total lever pressing in the initial or reversal phases, suggesting that neither locomotor capacity nor motivation were affected (table 3.3.8). In the PNU-282987 experiment, a paired t-test showed a significant impairment in responding in the reversal phase compared to the initial phase in the PCP-treated group ($P<0.001$). A one-way ANOVA in the reversal phase showed a significant interaction ($F_{4,47} = 10.69$, $P<0.001$). Post-hoc analysis revealed that PNU-282987 significantly improved the PCP-induced deficit at 10 mg/kg ($P<0.01$) and 20 mg/kg ($P<0.001$, fig 3.3.9a). There was no significant effect on total lever pressing in the initial or reversal phases, suggesting that neither locomotor capacity nor motivation were affected (table 3.3.9).

Table 3.3.8: The effect of PheTQS (3.0, 10.0, 30.0 mg/kg; p.o.) and sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) on total lever pressing. Data are expressed as the mean ± SEM total number of lever presses (n=9-10) in the initial and reversal phase of the task.

Drug treatment	Initial phase	Reversal phase
Vehicle + vehicle	27.6± 0.2	27.3± 0.3
Vehicle + sub-chronic PCP	27.2± 0.4	27.6± 0.5
3.0 mg/kg + sub-chronic PCP	27.9± 0.3	27.0± 0.8
10.0 mg/kg + sub-chronic PCP	28.3± 0.5	28.1± 0.3
30.0 mg/kg + sub-chronic PCP	28.3± 0.4	28.1± 0.2

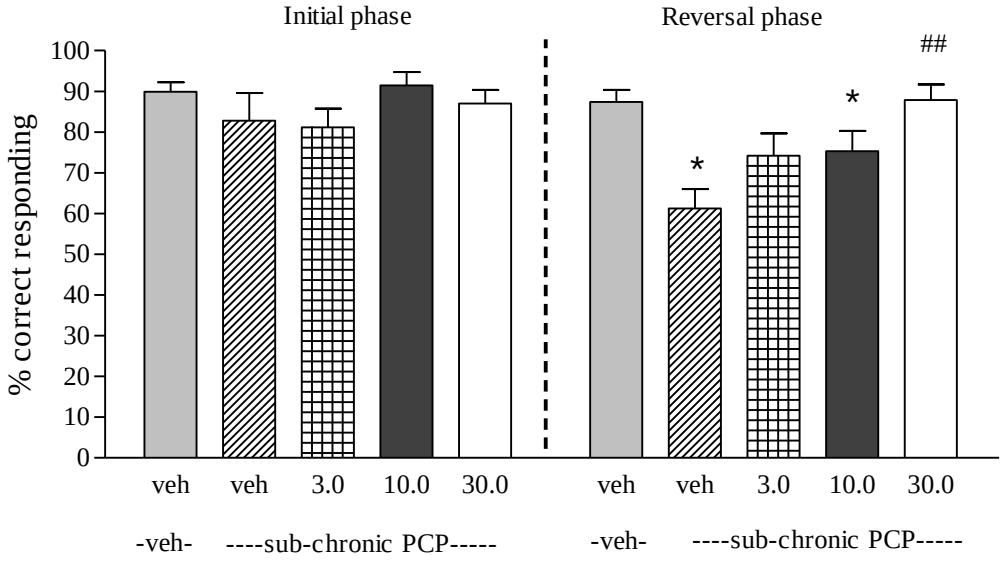


Figure 3.3.8a: The effect of PheTQS (3.0, 10.0, 30.0 mg/kg; p.o.) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM percent correct responding (n=9-10). Paired t-test showed a significant deficit in the reversal phase compared to the initial phase; *P<0.05. Post-hoc Dunnett's t-test showed a significant improvement in responding compared to PCP alone in the reversal phase at 30.0 mg/kg ($^{\#\#}$P<0.01).

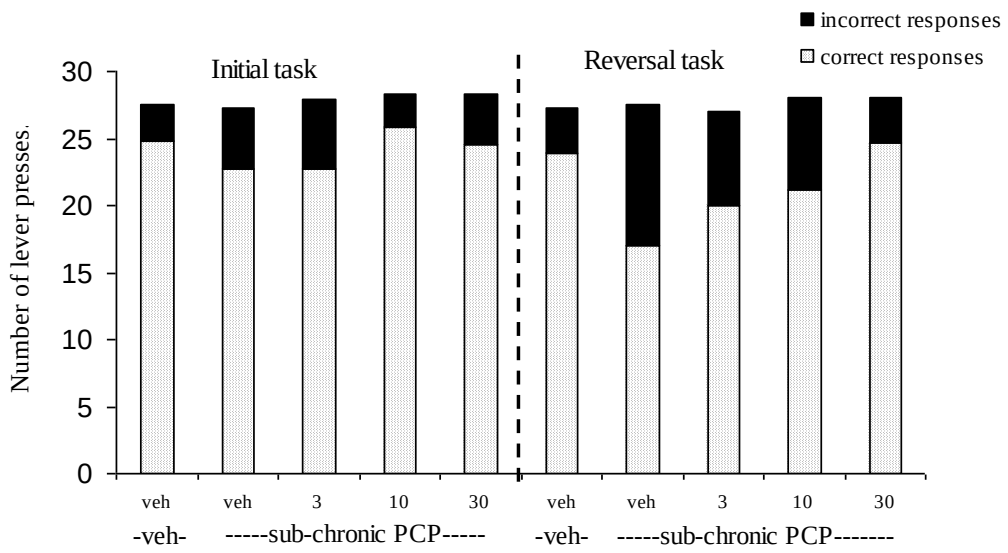


Figure 3.3.8b: The influence of PheTQS (3.0, 10.0, 30.0 mg/kg; p.o.) on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=9-10).

Table 3.3.9: The effect of PNU-282987 (5.0, 10.0, 20.0 mg/kg; s.c.) and sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) on total lever pressing. Data are expressed as the mean ± SEM total number of lever presses (n=9-10) in the initial and reversal phase of the task.

Drug treatment	Initial phase	Reversal phase
Vehicle + vehicle	27.6± 0.4	27.5± 0.2
Vehicle + sub-chronic PCP	27.8± 0.1	28.0± 0.3
5.0 mg/kg + sub-chronic PCP	27.1± 0.3	27.6± 0.4
10.0 mg/kg + sub-chronic PCP	27.1± 0.3	27.4± 0.3
20.0 mg/kg + sub-chronic PCP	27.3± 0.3	27.4± 0.4

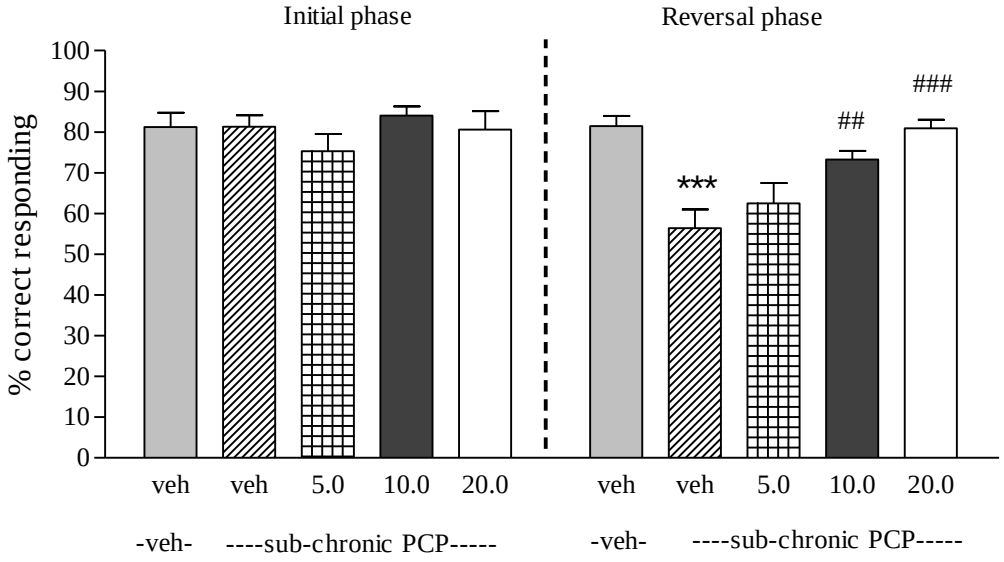


Figure 3.3.9a: The effect of PNU-282987 (5.0, 10.0, 20.0 mg/kg; s.c.) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM percentage correct responding (n=9-10). Paired t-test showed a significant deficit in the reversal phase compared to the initial phase; ***P<0.001. Post-hoc Dunnett's t-test showed PNU-282987 significantly improved responding compared to PCP alone in the reversal phase at 10.0 mg/kg ($^{\#\#}$P<0.01) and 20.0 mg/kg ($^{\#\#\#}$P<0.001).

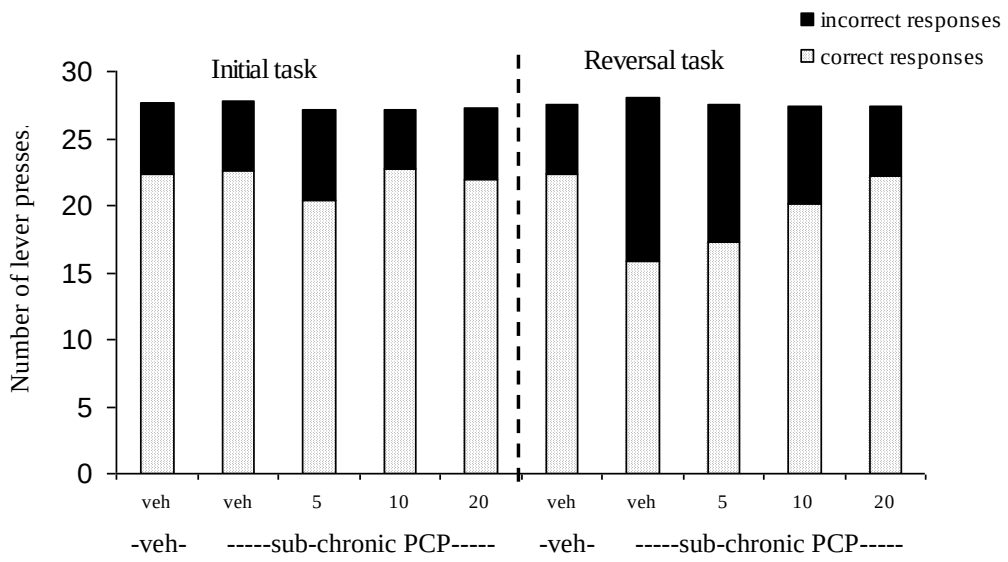


Figure 3.3.9b: The influence of PNU-282987 (5.0, 10.0, 20.0 mg/kg; s.c.) on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=9-10).

3.3.8 Effect of the H_1 receptor antagonist, pyrilamine

For percent correct responding, a paired t-test showed a significant impairment in responding in the reversal phase compared to the initial phase in the PCP-treated group ($P<0.01$). A one-way ANOVA in the reversal phase showed a significant interaction ($F_{4,45} = 3.50$, $P<0.05$); however, post-hoc analysis did not reveal any significant improvements with pyrilamine (fig 3.3.10). A one-way ANOVA revealed a significant effect of pyrilamine on total lever pressing in the initial and reversal phases ($F_{(4,45)}=10.35$, $P<0.001$) and ($F_{(4,45)}=5.55$, $P<0.01$) respectively. Post-hoc Dunnett's test showed that pyrilamine at 40.0 mg/kg significantly reduced lever pressing compared to vehicle-treated rats in the initial and reversal phases ($p<0.001$; table 3.3.10).

Table 3.3.10: The effect of pyrilamine (10.0, 20.0, 40.0 mg/kg; s.c.) and sub-chronic PCP (2.0 mg/kg, twice a day for 7 days, i.p.) on total lever pressing. Data are expressed as the mean ± SEM total number of lever presses (n=8-10) in the initial and reversal phase of the task. Post-hoc Dunnett's t-test revealed that the total number of lever presses was significantly decreased in rats treated with 40 mg/kg when compared to the vehicle-treated group; ***P<0.001.

Drug treatment	Initial phase	Reversal phase
vehicle + vehicle	27.2± 0.2	28.1± 0.2
vehicle + sub-chronic PCP 2mg/kg	26.7± 0.3	27.3± 0.3
10.0 mg/kg + sub-chronic PCP	27.4± 0.3	27.2± 1.0
20.0 mg/kg + sub-chronic PCP	25.3± 0.9	26.4± 0.8
40.0 mg/kg + sub-chronic PCP	16.4± 3.4***	22.3± 1.9***

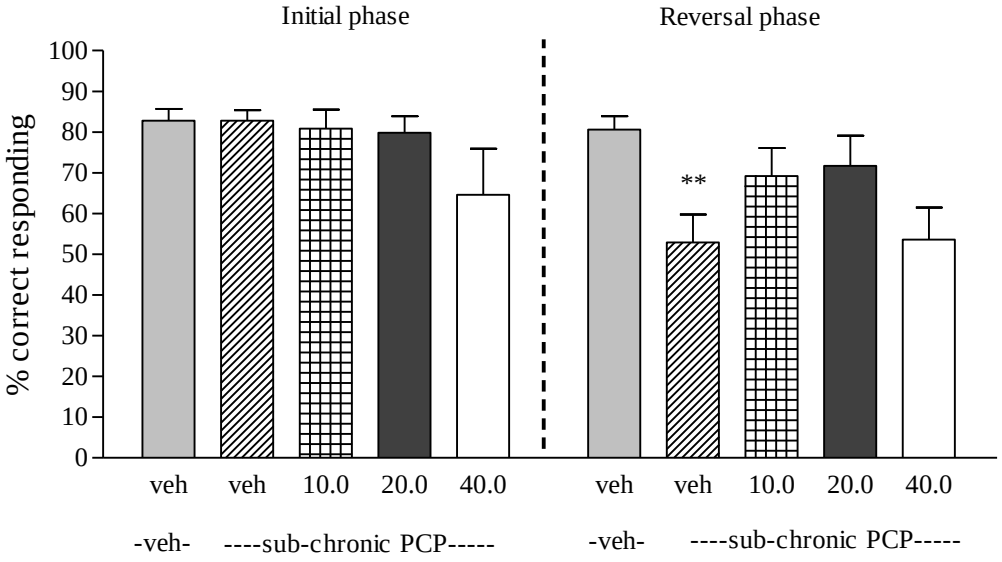


Figure 3.3.10a: The effect of pyrilamine (10.0, 20.0, 40.0 mg/kg; s.c.) on the deficit produced by sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM percentage correct responding (n=8-10). Paired t-test showed a significant deficit in the reversal phase compared to the initial phase; **P<0.01. Post-hoc Dunnett's t-test showed no significant improvement following drug treatment.

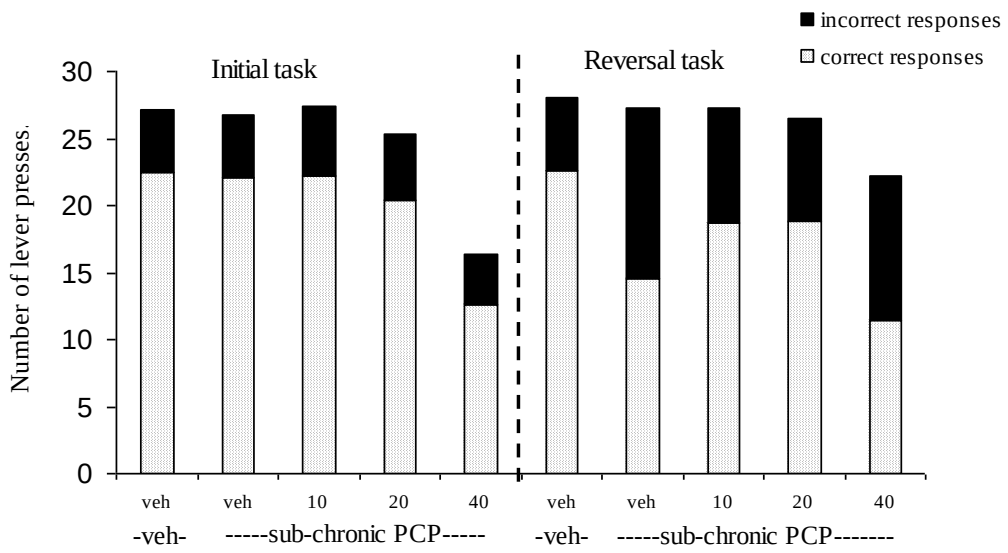


Figure 3.3.10b: The influence of pyrilamine (10.0, 20.0, 40.0 mg/kg; s.c.) on the effect of sub-chronic PCP (2.0 mg/kg, twice daily for 7 days, i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=8-10).

3.4 Discussion

In the reversal learning task, the PCP-treated rats were unaffected in the initial phase, but demonstrated reduced correct responding in the reversal phase, suggesting that when the rule changes PCP-treated rats do not switch to respond on the now correct lever. This is supported by previous results using this sub-chronic PCP model (Abdul-Monim *et al.*, 2006; Abdul-Monim *et al.*, 2007). The advantages of using this sub-chronic model and the pathological effects have previously been discussed in chapters 1 and 2.

3.4.1 PCP-induced disruption and antipsychotic reversal

The present results show that vehicle-treated rats can be trained to an equivalent level of accuracy in both the initial and reversal phases of the task. Following treatment with sub-chronic PCP rats continually demonstrated reduced performance which was selective for the reversal phase. This is in agreement with other results from this laboratory (Abdul-Monim *et al.*, 2006) and also this effect is observed following acute treatment with PCP (Abdul-Monim *et al.*, 2003). Sub-chronic PCP treatment did not affect total lever pressing, suggesting locomotor activity nor motivation were affected, thus the apparent cognitive deficits in the reversal phase were not due to any other non-specific effects of PCP. Results from other laboratories have also shown deficits in reversal learning following sub-chronic PCP administration (Jentsch and Taylor, 2001). The use of sub-chronic PCP treatment is believed to more closely mimic cognitive deficits associated with schizophrenia compared to acute treatment (Jentsch and Roth, 1999).

The single doses of clozapine and risperidone were chosen on the basis of previous work showing efficacy against sub-chronic PCP-induced deficits in reversal learning (Abdul-Monim *et al.*, 2006; Neill *et al.*, 2006) and NOR (Grayson *et al.*, 2007).

The effect of clozapine to improve the PCP-induced deficit in this study failed to reach statistical significance; however, risperidone was effective in reversing the PCP-induced deficit at 0.2 mg/kg. Clozapine did in fact improve performance in sub-chronic PCP-treated rats to an equivalent level as the vehicle rats, with vehicle-treated rats performing at 77% and clozapine rats performing at 76% correct responding. However, the performance of clozapine was not sufficient to reach statistical significance compared to the PCP-treated rats, which responded at a level of 59%. Whereas the level of correct responding following risperidone treatment improved the performance of PCP-treated rats to a higher level than the vehicle group. Risperidone is suggested to be the most effective atypical antipsychotic to improve working memory in the clinic (Meltzer and McGurk, 1999) and unlike in the attentional set-shifting study (chapter 2) where clozapine was equally effective, these results from reversal learning would appear to be consistent with the clinic. As in chapter 2 the inability of haloperidol to reverse the PCP-induced deficit may be due to its high D_2 receptor affinity and minimal 5-HT_{2A} receptor affinity (Siegfried *et al.*, 2005). A selective 5-HT_{2A} receptor antagonist was shown to be efficacious in this model (Neill *et al.*, 2008).

3.4.2 Dopamine D_1 receptor compounds

Both ligands shall be referred to as D_1-like throughout as SKF-38393 and SCH-23390 have been reported to have similar K_i values at D_1 and D_5 receptors; SKF-38393 having reported K_i values of 26nM and 80nM at D_1 and D_5 receptors respectively (Neumeyer *et al.*, 2003; Qandil *et al.*, 2003), whilst SCH-23390 has reported K_i values of 0.37nM and 0.47nM for D_1 and D_5 receptors respectively (Lawler *et al.*, 1999). The data show that SKF-38393 significantly improved a sub-chronic PCP-induced deficit in the reversal learning test, an improvement which was subsequently antagonised by the D_1-like

receptor antagonist, SCH-23390. Hersi and colleagues (1995) showed that a dose of 3.0 mg/kg (i.p.) of SKF-38393 in male Long-Evans rats increased acetylcholine release by two-fold over baseline, thus dose response was carried out with 3.0 mg/kg as the middle dose (0.75, 1.5, 3.0, 6.0 mg/kg) in the reversal learning task. Experiment 2 showed that 6.0 mg/kg was the most efficacious dose, and was also shown to improve a scopolamine-induced impairment in a T-maze working memory test (Amico *et al.*, 2007). 0.05 mg/kg SCH-23390 has also been shown to inhibit acetylcholine release in the hippocampus in male Long-Evans rats (Hersi *et al.*, 1995).

This data is in agreement with a study by Granon and colleagues (2000), whereby infusions into the PFC of SKF-38393 (0.06 μg/side) and SCH-23390 (0.3 μg/side) improved and then respectively attenuated accuracy in the five-choice attentional task in male Lister-hooded rats. Hersi and co-workers (1995) found that SKF-38393 improved cognitive performance in the Morris water maze in rats with age-related impairments. In the present experiments we have used a sub-chronic PCP regimen to induce schizophrenia-like deficits in cognition, however, amphetamine can also induce cognitive impairments in tests of working memory (Castner *et al*., 2005), attentional set-shifting (Fletcher *et al.*, 2005), and reversal learning (Idris *et al.*, 2005a). A study by Fletcher and colleagues found that amphetamine-induced impairments were reversed by infusing SKF-38393 into the medial PFC (Fletcher *et al.*, 2007). This effect of SKF-38393 to improve cognitive function in amphetamine-treated rats is in agreement with the effect observed in our sub-chronic PCP-treated rats.

It is important to note that the effect of SCH-23390 alone in PCP-treated rats was investigated in order to eliminate the possibility that SCH-23390 could have affected cognitive performance alone. It appears that SCH-23390 (0.05 mg/kg) impaired performance in the initial phase, an effect not observed following sub-chronic

PCP treatment alone, although this effect was not statistically significant. This trend was supported by a study showing that intracortical infusions of SCH-23390 impaired performance accuracy in the five-choice attentional task (Granon *et al.,* 2000). Therefore, blockade of D_1-like receptors with SCH-23390 alone may impair cognition, but this was not observed in the current experiments.

SKF-38393 also has an affinity for 5-HT_{2C} receptors (then classified as 5-HT_{1C}; Briggs *et al.,* 1991), albeit reduced affinity compared with D_1-like receptors. It is unlikely that 5-HT_{2C} receptors play a critical role in the effects of SKF-38393 as its effect was fully reversed by SCH-23390 which has been shown to be selective for D_1-like receptors, however further experiments would be required to investigate this possibility since 5-HT_{2C} receptor agonists do show efficacy in pre-clinical tasks of cognition (Siuciak *et al.,* 2007) and in the current studies the 5-HT_{2C} antagonist, SB-243213A significantly improved the PCP-induced deficit. It was previously shown that 3.0 mg/kg of SKF-38393 increased acetylcholine (ACh) release by two-fold over baseline (Hersi *et al.,* 1995). However, the most efficacious dose in the reversal learning experiment was 6.0 mg/kg; this dose was also shown to improve a scopolamine-induced impairment in a T-maze working memory test (Amico *et al.,* 2007). The dose of SCH-23390 used here (0.05 mg/kg) has been shown to transiently inhibit acetylcholine release (Hersi *et al.,* 1995).

Cognitive impairments observed in the Wisconsin Card Sorting Task (Kashima, 1991) have been positively correlated with a down-regulation of D_1 receptor binding in the PFC of treatment-free/-naïve schizophrenic patients (Okubo *et al.,* 1997); thus identifying the D_1 receptor as a particularly relevant target for improving cognition in schizophrenia (Gray and Roth, 2007). As previously mentioned in chapter 1, the prefrontal cortex is critically involved in reversal learning (McAlonan and Brown, 2003;

Tait and Brown, 2007). Damage to the PFC has also been shown to impair performance in recognition memory tasks (Kolb *et al.,* 1994; Meunier *et al.,* 1997). The hippocampus is also a critical brain region for learning and memory. The density of D_1 receptors is considerably higher than D_2 receptors throughout the cortex with differential distribution in the rat mPFC (Vincent *et al.,* 1995), and low levels of D_2 receptors have been detected in the hippocampus (Meador-Woodruff *et al.,* 1989; Levey *et al.,* 1993). The majority of dopamine receptors in the hippocampus are from the D_1-like subfamily of receptors (Dawson *et al.,* 1986), with a high density of the D_5 receptor (Ciliax *et al.,* 2000). Furthermore, it has been reported that administration of SKF-38393 increased hippocampal acetylcholine (ACh) release in young and aged rats (Hersi *et al.,* 1995), which improved performance in the Morris Water Maze, thus suggesting a mechanism for the cognitive enhancing effects of SKF-38393. Dihydrexidine, a full D_1-like receptor agonist has been shown to increase extracellular concentrations of cortical ACh to around 300% of baseline values; an effect which was completely blocked by SCH-23390, and coincided with an improvement in cognitive performance assessed using a passive-avoidance paradigm (Steele *et al.,* 1997). Indeed, it has also been demonstrated that atypical antipsychotics such as clozapine, olanzapine, risperidone and ziprasidone can increase levels of ACh in the mPFC (Ichikawa *et al.,* 2002). This suggests a beneficial mechanism of action in that D_1-like agonists could improve cognition in a similar manner to some atypical antipsychotics.

Positive symptoms are induced by elevation of dopamine in the limbic system, whereas negative and cognitive symptoms are due, at least in part, to decreased prefrontal dopamine function (Davis *et al.,* 1991). As agents which increase prefrontal dopamine turnover may have beneficial effects on cognition it would seem profitable to explore direct stimulation of the D_1 receptor in the PFC. Indeed, D_1 agonists have been

highlighted as a molecular target for cognitive enhancement in schizophrenia (see Gray and Roth, 2007). Subsequently, a single administration of the full D_1-like receptor agonist, dihydrexidine, produced increased perfusion in D_1 rich PFC regions in patients with schizophrenia (Mu *et al.,* 2007); suggesting that agents increasing transmission at dopamine D_1 receptors could have potential in the pharmacotherapy of schizophrenia through a variety of mechanisms. However, the use of D_1 receptor agonists as therapeutic agents pose difficulties for pharmacologists as dopamine function in the PFC seems to follow an "inverted-U" dose-response relationship whereby increases or decreases from an optimal level result in cognitive impairment (Goldman-Rakic *et al.,* 2000). In addition, chronic treatment with D_1 agonists may lead to the down-regulation of D_1 receptors which could in turn worsen cognition.

3.4.3 5-HT_{1A} receptor agents

In the current experiments, the efficacy of buspirone to improve the PCP-induced reversal learning deficit was partially reversed by the 5-HT_{1A} antagonist, WAY-100635. There is evidence to show that 5-HT_{1A} receptor density is high in the frontal cortices of patients with schizophrenia (Burnet *et al.,* 1997) and a subsequent PET study has shown an increase in cortical 5-HT_{1A} receptor binding in schizophrenia (Tauscher *et al.,* 2002). Post-mortem studies have also indicated abnormalities in 5-HT_{1A} receptor-mediated transmission in the frontal cortex of patients with schizophrenia (Sumiyoshi *et al.,* 1996).

Buspirone is a 5-HT_{1A} receptor partial agonist with weak D_2 receptor antagonist properties compared to typical antipsychotic drugs, with K_i values of 20nM and 240nM respectively (Hamik *et al.,* 1990). WAY-100635 may not have fully antagonised the effects of buspirone as buspirone is rapidly converted to its metabolite 1-(2-pyrimidinyl)-piperazine (1-PP; Caccia *et al.,* 1986), a potent α_2-adrenoceptor antagonist,

which is able to stimulate cortical dopamine release (Gobert *et al.*, 1999). The effect of buspirone is unlikely to be due to D_2 receptor blockade as haloperidol is ineffective in reversing the PCP-induced deficit (Abdul-Monim *et al.*, 2006). It is important to highlight that WAY-100635 administered alone at both 0.3 and 1.0 mg/kg did not improve the PCP-induced deficit. This is in agreement with a study in mice using a novel object recognition task whereby WAY-100635 at 1.0 mg/kg did not produce any improvement in the PCP-induced cognitive deficit (Hagiwara *et al.*, 2008). However, there are conflicting studies surrounding the effects of WAY-100635 and other 5-HT_{1A} receptor antagonists; Wedzony *et al.* (2000) reported that WAY-100135 attenuated the deficit induced by MK-801 in tests of working memory and selective attention in rats. In addition, WAY-100635 has been shown to prevent learning deficits induced by blockade of AMPA receptors (Schiapparelli *et al.*, 2006). An explanation for the conflicting effects of WAY-100635 and WAY-100135 could be due to the acute administration of MK-801 (an NMDA receptor antagonist) and NBQX (an AMPA receptor antagonist) in comparison to the sub-chronic PCP administration in this thesis, and also both of these studies were performed in male Wistar rats compared to the female hooded-Lister rats used throughout this thesis.

The antipsychotics clozapine, quetiapine, and ziprasidone have partial agonist properties at 5-HT_{1A} receptors, like buspirone (Newman-Tancredi *et al.*, 1998; Ichikawa *et al.*, 2001). There are conflicting pre-clinical results with 5-HT_{1A} compounds with selective agonists, partial agonists, and antagonists being highlighted as potential molecular targets for cognitive enhancement in schizophrenia (see Gray and Roth, 2007). As previously mentioned there is a high concentration of 5-HT_{1A} receptors on cortical and hippocampal pyramidal neurons (Azmitia *et al.*, 1996). There is also a population of 5-HT_{1A} receptors on raphe neurons, where 5-HT_{1A} receptors function as

somatodendritic autoreceptors providing inhibitory feedback control of 5-HT release (Barnes and Sharp, 1999). It is thought that this is the mechanism by which 5-HT_{1A} antagonists improve cognition, while the beneficial effects on cognition in animal studies of 5-HT_{1A} partial agonists are thought to be mediated via an action at pyramidal neurons (Roth *et al.*, 2003; Schechter *et al.*, 2002). In schizophrenic patients, the 5-HT_{1A} agonist, tandospirone, has been shown to improve executive function and verbal memory when added to ongoing typical antipsychotic treatment (Sumiyoshi *et al.*, 2001).

The results from the present experiments are supported by another investigation in a social interaction test (Snigdha and Neill, 2008b). It was demonstrated that co-administration of WAY-100635 with aripiprazole, another antipsychotic with partial 5-HT_{1A} agonist properties (Shapiro *et al.*, 2003), successfully prevented the beneficial effects of aripiprazole in PCP-treated rats following the same regime in a social interaction model of negative symptoms. It was suggested that 5-HT_{1A} receptor activation selectively augments cortical dopamine levels which results in improvement in the social behaviour deficits. This suggestion is in agreement with a previous report by Ichikawa and colleagues (2001) suggesting that 5-HT_{1A} induced dopamine release in the mPFC may be the potential basis for the mechanism of action of at least some of the atypical antipsychotics.

3.4.4 5-HT_{2C} receptor antagonist

SB-243213A was effective in reversing the sub-chronic PCP-induced deficit at 10 mg/kg, without having any adverse effects on behaviour. SB-243213A is a selective 5-HT_{2C} receptor antagonist (Bromidge *et al.*, 2000). 5-HT_{2C} receptors are located in a variety of brain regions including the neocortex, amygdala, hippocampus, nucleus

accumbens, substantia nigra and the ventral tegmental area (Pompeiano *et al.,* 1994; Abramowski *et al.,* 1995; Eberle-Wang *et al.,* 1997). 5-HT_{2C} receptor activation has been suggested to reduce dopamine release in the nucleus accumbens and prefrontal cortex (Di Matteo *et al.,* 2001). The 5-HT_{2C} antagonist, SB242084, enhances dopamine release in the nucleus accumbens (Di Matteo *et al.,* 1999) and cortex (Gobert *et al.,* 2000). Antagonism at 5-HT_{2C} receptors by various atypical antipsychotics (e.g. clozapine, risperidone, sertindole and ziprasidone; Roth *et al.,* 2003) may play a role in their beneficial effects and their diminished likelihood to produce to neurological adverse effects (Meltzer, 1995; 1999). However, antagonism at the $5HT_{2C}$ receptor can increase food intake and body weight in rats (Bonhaus *et al.,* 1997), thus this may not be an advantageous strategy for treatment in patients as weight gain is a major adverse effect and could affect compliance (see chapter 1 section 1.6.3).

SB-243213 is active in preclinical models of anxiety and has an improved anxiolytic profile compared with benzodiazepines (Wood *et al.,* 2001; Blackburn *et al.,* 2002). SB-243213A may also have antidepressant-like activity as it increased deep slow wave sleep quantity and reduced paradoxical sleep quantity with similar efficacy to paroxetine (Smith *et al.,* 2002). There is limited data concerning the efficacy of SB-243213A in tests of cognition. The current data show that SB-243213A at 10.0 mg/kg selectively and significantly improved the PCP-induced deficit in the reversal phase of the task. This is supported by recent data showing improvements in spatial learning by the 5-HT_{2C} antagonist, SB242084, suggesting that the facilitatory effects of 5-HT_{2C} antagonists are mediated by the OFC (Boulougouris *et al.,* 2008).

3.4.5 Activation/ positive modulation of α7 nACh receptors

The most prevalent nicotinic acetylcholine receptors nAChRs are the α4β2 and α7 subtypes, both of which have been shown to have reduced numbers in schizophrenia (Freedman *et al.,* 1995; Breese *et al.,* 2000). It has been suggested that these receptor subtypes are involved in cognition (Schreiber *et al.,* 2002; Chan *et al.*, 2007; Gray and Roth, 2007).

Much of the α7 nAChR research *in vivo* has focused on sensory gating with antagonists at the α7 nAChR inducing deficits (Luntz-Leybam *et al.,* 1992), while agonists at this receptor have been shown to normalise sensory gating in rodents (Simosky *et al.,* 2001). Both PheTQS and PNU-282987 attenuated the sub-chronic PCP-induced deficit in the reversal phase of the task. PheTQS significantly reversed the PCP-induced deficit at the highest dose of 30 mg/kg, and did not have any effect on lever pressing, indicating that PheTQS did not cause sedation. PNU-282987 dose-dependently attenuated the impairment produced by PCP with significant improvements in performance at 10 and 20 mg/kg without causing any impairment in lever pressing.

The role of α7 nACh receptors in cognition is supported by evidence showing that α7 knock-out mice were impaired in 5-choice serial reaction time task (Hoyle *et al.,* 2006). The α7 nAChR agonist AR-R 17779 improved scopolamine-induced social recognition deficits in rats; in addition, this reversal was blocked by the α7 nAChR antagonist methyllycaconitine (van Kampen *et al.,* 2004). SSR180711 is a selective α7 nAChR partial agonist which was shown to enhance object recognition memory following a 1 hour inter-trial interval (at 0.3 mg/kg) and at 1-3 mg/kg SSR180711 was shown to reverse deficits in the Morris water maze induced by MK-801 and PCP (Pichat *et al.,* 2007). Furthermore, SSRI80711 (1 mg/kg) was found to increase extracellular dopamine in the PFC (Pichat *et al.,* 2007).

PNU-282987 has been shown to enhance amphetamine-induced theta and gamma oscillations in the CA3 region of the hippocampus and enthorinal cortex (Hajós *et al.,* 2005; Hoffmann *et al.,* 2005). PNU-282987 has also been shown to increase c-Fos dose-dependently in the PFC and the NAc shell, while the NAc core and the dorsolateral striatum were unaffected (Hansen *et al.,* 2007).

3.4.6 Activation of muscarinic M_1 receptors

The M_1 agonist, 77-LH-28-1, was ineffective in improving the PCP-induced deficit at 1.0, 3.0 and 10.0 mg/kg. There was also no significant effect on lever pressing behaviour, although in the initial phase there does appear to be a difference at 10.0 mg/kg compared to vehicle, this effect was not statistically significant. The M_1 receptor is located primarily in postsynaptic terminals in the cortex, hippocampus and striatum. Furthermore, the M_1 receptor subtype is the most abundant muscarinic receptor in the forebrain and hippocampus (Levey *et al.,* 1991; Wei *et al.,* 1994).

There is an emerging role for M_1 receptors in cognition. It has been shown that M_1 deficient mice demonstrate deficits in working and reference memory (Miyakawa *et al.,* 2001; Anagnostaras *et al.,* 2003). In addition, a major metabolite of clozapine, N-desmethylclozapine, is a muscarinic M_1 agonist (Davies *et al.,* 2005). N-desmethylclozapine has been found to increase acetylcholine and dopamine release in the mPFC and hippocampus, an effect which is believed to contribute to the ability of N-desmethylclozapine and clozapine to improve cognitive dysfunction in patients with schizophrenia (Weiner *et al.,* 2004; Li *et al.,* 2005).

Xanomeline, a potent $M_{1/4}$ receptor agonist (Shannon *et al.,* 1994), has been reported to improve cognition in Alzheimer's disease, and to improve cognition and decrease psychotic symptoms in a small placebo-controlled trial in patients with

schizophrenia (Bodick *et al.*, 1997; Bymaster *et al.*, 2002). AC260584 is a potent muscarinic M_1 receptor agonist and a newly developed atypical antipsychotic (Spalding *et al.*, 2006). AC260584 significantly increased acetylcholine and dopamine in the mPFC and hippocampus, these increases were significantly blocked by telenzepine, a M_1 antagonist (Li *et al.*, 2007).

Although the M_1 receptor is a target for the treatment of cognitive dysfunction, many of the developed M_1 receptor agonists, including sabcomeline and xanomeline, actually demonstrate little selectivity for the M_1 receptor subtype amongst the other mACh receptors (see Langmead *et al.*, 2008a). Therefore, the identification of a selective M_1 receptor agonist would be an advantage for mACh receptor pharmacology. Spalding *et al.* (2002) described a novel compound, AC-42, which activated human recombinant M_1 receptors without activating hM_2-hM_5 receptors. Subsequently it was found that the regions of the M_1 receptor required for the activation by AC-42 were regions distinct from the orthosteric site of the M_1 receptor. This newly described binding site was termed the allosteric site (Langmead *et al.*, 2006).

77-LH-28-1 is a structural analogue of the allosteric M_1 receptor agonist AC-42, and has selective agonist activity for the M_1 receptor subtype in both human recombinant and naïve rat tissue (Langmead *et al.*, 2008b). Furthermore, 77-LH-28-1 at 3.0 mg/kg (s.c.) was shown to increase network oscillations relevant to cognitive processing (Langmead *et al.*, 2008b). However, in contrast to other M_1 receptors agonists, in the current experiment, 77-LH-28-1 (1.0, 3.0, 10.0 mg/kg s.c.) did not reverse the sub-chronic PCP-induced deficit in reversal learning. The pharmacokinetic profile of 77-LH-28-1 suggested that the s.c. route was preferable to the i.p. route, and that brain concentrations of the drug were highest at 15 min after administration. Therefore, it may have been necessary to test the rats 15 min after dosing rather than 30

min. Furthermore, the pharmacokinetic profile of 77-LH-28-1 was carried out in male Sprague-Dawley rats, whereas the reversal learning experiments was conducted using female hooded-Lister rats, therefore the lack of behavioural effect may be due to differences in metabolism or pharmacokinetic profile of the drug.

3.4.7 Activation of histamine H_1 receptors

Pyrilamine was selected for use in these experiments due to many antipsychotic having affinity for the histamine H_1 receptor, including clozapine, risperidone, olanzapine and quetiapine (Siegfreid *et al.,* 2005). Pyrilamine at 40 mg/kg caused a significant reduction in lever pressing in both the initial and reversal phases of the task. In support of this finding, it has been suggested that sedation induced by clozapine and risperidone at higher doses is attributed to their affinity for the H_1 receptor (Richelson and Souder, 2000). At 10 and 20 mg/kg pyrilamine does appears to produce a slight improvement of the PCP-induced deficit in the reversal phase, however this does not reach statistical significance.

On the basis of their pharmacology and signal transduction mechanisms histamine receptors have been subdivided into H_1, H_2 and H_3 subtypes (Hill *et al.,* 1990). Activation of histamine H_1 receptors leads to the breakdown of phosphatidylinositol 4,5-biphosphate (PIP_2) into inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (Hill *et al.,* 1990). Histaminergic neurons are exclusively located in the tuberomamillary nucleus (TMN) of the posterior hypothalamus (Watanabe *et al.,* 1984). A high density of H_1 receptors are found in the frontal and temporal cortex, hippocampus, thalamus and limbic system (Kanba and Richelson, 1984; Yanai *et al.,* 1992).

In the present study, 10 and 20 mg/kg pyrilamine does appear to produce a slight improvement of the PCP-induced deficit in the reversal phase; however this did not

reach statistical significance. Pyrilamine at 40 mg/kg did not affect the PCP-induced deficit. Pyrilamine alone at 10, 20 and 40 mg/kg did not impair PPI; in addition it was shown that 10 and 20 mg/kg reversed a dizocilpine-induced deficit in PPI (Roegge *et al.*, 2007). The same study found that 20 and 40 mg/kg impaired reference memory in the radial arm maze and 40 mg/kg impaired working memory (Roegge *et al.*, 2007). In support of this, chronic treatment with pyrilamine (20 mg/kg) impaired reference and working memory in the radial arm maze (Chen *et al.*, 2001). These data suggest that pyrilamine alone may have an impairing effect on cognition, but may improve NMDA-induced deficits, although the reversal in the current experiment was not significant.

3.4.8 Conclusions

From the experiments in this chapter it is evident that D_1 receptor agonists, 5-HT_{1A} receptor partial agonists, 5-HT_{2C} receptor antagonists and positive modulators of the alpha 7 nACh receptor are able to reverse the sub-chronic PCP-induced deficit in reversal learning. Although many antipsychotics have affinity for muscarinic M_1 and histamine H_1 receptors, selective agents at these receptors were not able to improve the PCP-induced deficit. From previous experiments in the literature it is possible that these agents may improve cognition via increasing dopamine or acetylcholine levels in the prefrontal cortex (see fig 3.4), the region thought to be involved in reversal learning ability.

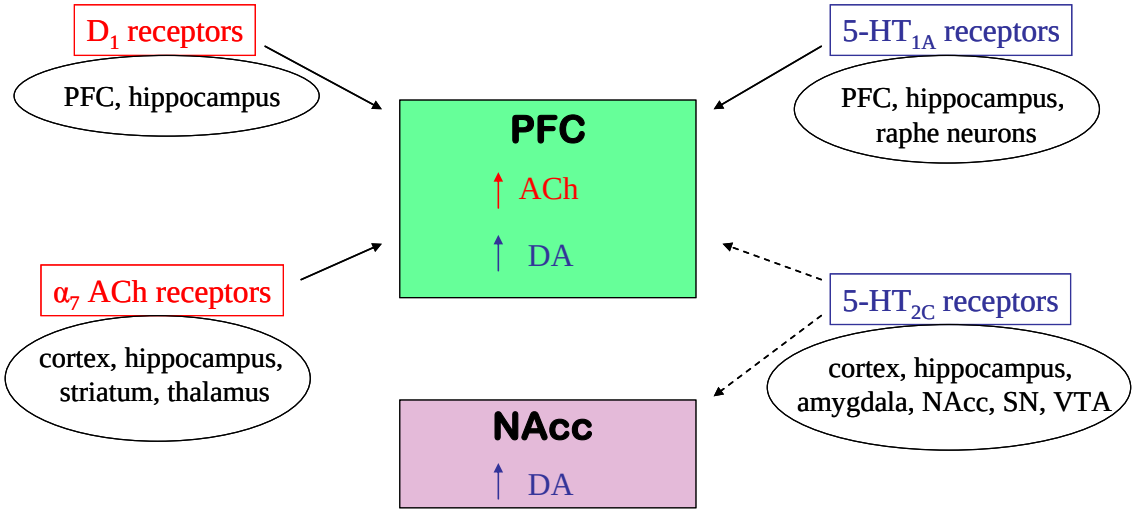


Figure 3.4: Summary diagram of the possible mechanisms involved in the attenuation of the sub-chronic PCP-induced deficit in reversal learning. Abbreviations: acetylcholine (ACh), dopamine (DA), prefrontal cortex (PFC); nucleus accumbens (NAcc), substantia nigra (SN), ventral tegmental area (VTA). Solid lines indicate agonism or activation, dotted lines indicate antagonism. Red highlights increases in ACh and blue highlights increases in DA.

This chapter has demonstrated that deficits in reversal learning are robust and very reproducible using sub-chronic PCP administration; the results with antipsychotics support the findings of the previous attentional set-shifting chapter. This chapter has indicated possible receptors involved in the PCP-induced deficit. The next chapter will aim to validate the operant reversal learning task to investigate whether the task can be developed to increase the difficulty of the task, therefore making the deficit harder to reverse by antipsychotics. The aim is also to investigate the temporal effects of compounds to potentially distinguish antipsychotics by efficacy.

Chapter 4

Validation of the reversal learning task

4.1 Introduction

The previous chapter established that sub-chronic PCP produces robust deficit in reversal learning. Selective compounds suggested that D_1, 5-HT_{1A}, 5-HT_{2C} and nicotinic α_7 acetylcholine receptors are involved in the attenuation of this PCP-induced deficit. Following the demonstration of these deficits in reversal learning in the previous chapter, the aim of this chapter was to validate the reversal learning task and to improve our understanding of how sub-chronic PCP produces the cognitive deficits observed in chapter 3. The atypical antipsychotics risperidone and clozapine were shown to fully reverse and partially reverse (respectively) the sub-chronic PCP induced deficit. However, the UK Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS; Jones *et al.,* 2006; Lewis *et al.,* 2006) and the CATIE study (Keefe *et al.,* 2007) highlight the fact that atypical antipsychotics do not provide consistent improvement of cognitive symptoms in patients. Furthermore, clinical studies have in fact noted that classical antipsychotics such as haloperidol can impair working memory performance (Gilbertson and van Kammen, 1997). However, like many pre-clinical studies, the effect of the antipsychotics in drug naïve rats on baseline levels of performance has not been previously investigated. Therefore, the first aim was to investigate the effect of clozapine, risperidone and haloperidol on reversal learning ability in naïve rats.

The second aim was to explore the nature of the sub-chronic PCP-induced deficit. In the reversal learning task used in chapter 3, there is a 2 min time-out period prior to the reversal phase, which is believed to act as a cue. Orbitofrontal cortex (OFC) damage in varying species causes reversal learning deficits while preserving the normal ability to learn initial contingencies (McAlonan and Brown, 2003; Chudasama and Robbins, 2003; Dias *et al.,* 1996; Izquierdo *et al.,* 2004). There is a hypothesis that the

OFC drives behaviour according to associative information representing the significance of a particular cue (Rolls *et al.*, 1996; Thorpe *et al.*, 1983). An aim of this chapter is to assess performance of vehicle and sub-chronic PCP-treated rats in the absence of the 2 min cue normally employed between the initial and reversal phases of the reversal learning task.

The temporal profile of the sub-chronic PCP-induced deficit in this model will also be determined by extending the reversal phase of the experiment and recording the results at each minute, this would allow determination of how long it takes the rats to adapt to the new rule. Following this, a double reversal experiment will also be carried out in an attempt to make the task more difficult, or rather more difficult to reverse by antipsychotics. Olanzapine will also be tested in this double reversal as it has also been shown to improve a sub-chronic PCP-induced deficit (Abdul-Monim *et al.*, 2006). Therefore, the aim of this chapter is two-fold; A) to test antipsychotics in naïve rats and B) to explore the nature of the sub-chronic PCP-induced deficit.

4.2 Material and Methods

4.2.1 Subjects and housing conditions

One cohort of thirty and a second cohort of fifty female hooded-Lister rats (Harlan, UK) housed in groups of four or five were used as subjects. Animals were housed and food restricted as in chapter 3.

4.2.2 Reversal learning

Rats were trained as detailed in chapter 3.

4.2.3 Antipsychotic validation experiments

Cohort 1 was tested once weekly with a vehicle control group and dose responses to the antipsychotics risperidone (0.1, 0.2, 0.4 mg/kg), clozapine (1.25, 2.5, 5.0 mg/kg) and haloperidol (0.025, 0.05, 0.1 mg/kg) were carried out. The drug treatment and vehicle treatment given to each rat (and within each home cage) over the course of these experiments was pseudo-randomised to ensure the highest dose of drug and vehicle were not given to the same rats in successive experiments. The order of experiments is shown in figure 4.2.1.

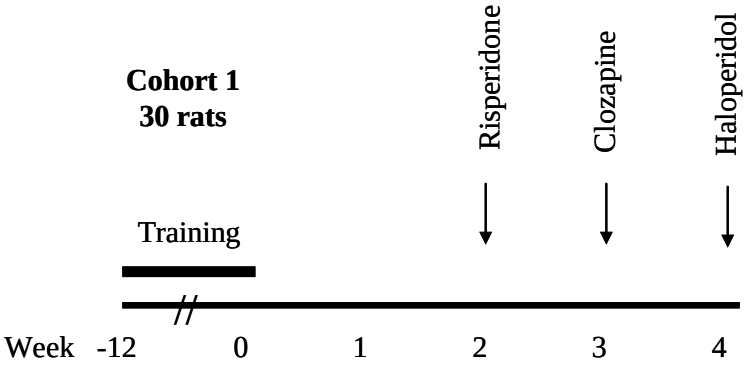


Figure 4.2.1: Timeline of experiments in cohort 1.

4.2.4 Experiments with sub-chronic PCP

Cohort 2 was trained to perform the reversal learning task and then sub-chronically dosed with PCP (see chapter 2). Training was not continued throughout the PCP dosing or the 7 day washout period. Following the washout period rats underwent the time-out experiment, this involved a 5 min initial phase followed by a 2 min time-out period (or not in the time-out experiment) and then a 5 min reversal phase. The order of experiments is shown in figure 4.2.2. Cohort 2 were tested using a cross-over study as there were only 10 vehicle-treated rats, i.e. in the first week 5 vehicle rats and 5 PCP-treated rats were given the 2 min time-out period and the other 5 of each group went straight into the reversal phase without the time-out (shown in table 4.2.1). In the second week the same rats were used but whether the rats received the time-out or not was reversed from the previous week, this gave a total of 10 per group.

Table 4.2.1: Cross-over study for the time-out experiment. Rats in red were tested with a time-out in week 1 and without the time-out in week 2. Rats in blue were tested vice versa.

Experiment	Time-out	No time-out
Week 1	5 Vehicle + 5 PCP	5 Vehicle + 5 PCP
Week 2	5 Vehicle + 5 PCP	5 Vehicle + 5 PCP

The extended reversal experiment involved a 5 min initial phase followed by a 2 min timeout period, and then the reversal phase was extended for 15 min and recordings of correct and incorrect lever presses were carried out at every minute of this reversal phase. Minute by minute recordings were only carried out in the reversal phase and not in the initial phase, as results from the previous chapter showed that the deficit is only present in the reversal phase.

The double reversal experiment involved a 5 min initial phase followed by a 2 min time-out period and a 5 min reversal phase followed by a 4 min time-out period and a second 5 min reversal. A 4 min time-out was selected in an attempt to make the second reversal more difficult. Rats were treated with vehicle (0.9% saline), risperidone (0.2 mg/kg), clozapine (5.0 mg/kg), olanzapine (1.5 mg/kg) and haloperidol (0.05 mg/kg). The drug treatment given to each rat (and within each home cage) over the course of these experiments was randomised. The doses of antipsychotics were selected based on previous studies in our laboratory (Abdul-Monim *et al.*, 2006; Garyson *et al.*, 2007; Idris *et al.*, 2005a).

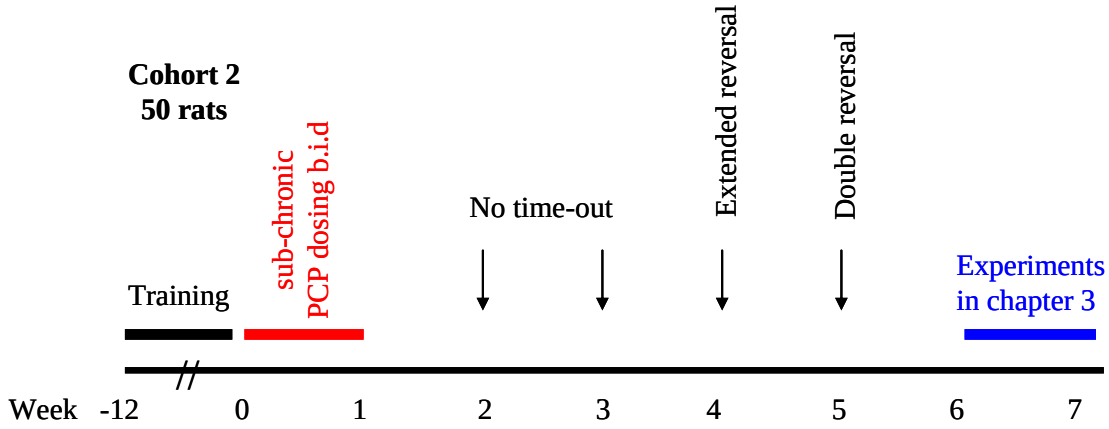


Figure 4.2.2: Timeline of experiments in cohort 2.

4.2.5 Drugs

Cohort 1 were injected with increasing doses of clozapine (Tocris, UK), risperidone (provided by GSK, UK) and haloperidol (Serenace liquid, 2.0 mg/ml, Baker, UK) based on doses used in previous experiments (Idris *et al.*, 2005a; Abdul-Monim *et al.*, 2006; Neill *et al.*, 2006; Grayson *et al.*, 2007; chapter 2). These doses have also been shown to correspond to clinical doses (Kapur *et al.*, 2003). Clozapine and risperidone were

dissolved in 10µl of acetic acid, made up to volume with distilled water and pH adjusted to 6 with 0.1M NaOH. Haloperidol was diluted with distilled water.

From cohort 2 40 rats were dosed 2 mg/kg PCP and 10 with vehicle by the intraperitoneal (i.p.) route twice daily for seven days. Dosing with sub-chronic PCP or vehicle was followed by a washout period of a further seven days. PCP hydrochloride (Sigma, UK) was dissolved in 0.9 % saline. For the double reversal experiment doses of antipsychotics were selected based on previous experiments and from the data from cohort 1. Olanzapine (provided by GSK, UK) was made as described for clozapine and risperidone. All drug doses were calculated as base equivalent weight and were administered via the i.p. route in a volume of 1 ml/kg 30 min before testing.

4.2.6 Data and statistical analysis

Data for percent correct responding was calculated using the number of presses on the correct lever divided by the total number of presses multiplied by 100. The percent correct responding data was arcsine transformed and analysed by one-way ANOVA followed by post-hoc Dunnett's t-test. The total number of lever presses was calculated by adding the correct and incorrect presses together within the 5 min test session, this was used to assess whether drugs had caused any sedation or behavioural impairment.

Paired t-tests were used to compare performance in the reversal phase to the initial phase. For the extended reversal experiments data for percent correct responding at each time point in the reversal learning task was calculated. Area under the curve was calculated for the extended reversal phase and was analysed by a one-way ANOVA followed by post-hoc Dunnett's t-test.

4.3 Results

4.3.1 Antipsychotics in vehicle rats

Effect of risperidone alone

There were no significant effects of risperidone on reversal learning performance in the initial or reversal phases of the task (fig 4.3.1a). However, a one-way ANOVA revealed a significant effect of risperidone on total lever pressing in the initial and reversal phases ($F_{(3,28)}$=6.36, P<0.01) and ($F_{(3,28)}$=13.61, P<0.001) respectively. Post-hoc Dunnett's test showed that risperidone at 0.4 mg/kg significantly reduced lever pressing compared to vehicle-treated rats in the initial (P<0.01; table 4.1) and reversal phases (P<0.001; table 4.3.1).

Table 4.3.1: The effect of risperidone (0.1, 0.2, 0.4 mg/kg; i.p.) on the total number of lever presses in a reversal learning paradigm. Data are expressed as the mean ± SEM total number of lever presses (n=7-8) per group) in the initial and reversal phase of the task. Significant reduction in lever pressing compared with vehicle group; **P<0.01, ***P<0.001.

Drug treatment	Initial phase	Reversal phase
Vehicle	27.7± 0.2	28.4± 0.2
0.1 mg/kg	26.3± 0.4	27.8± 0.4
0.2 mg/kg	27.1± 0.3	27.6± 0.2
0.4 mg/kg	21.0± 2.4**	20.9± 1.9***

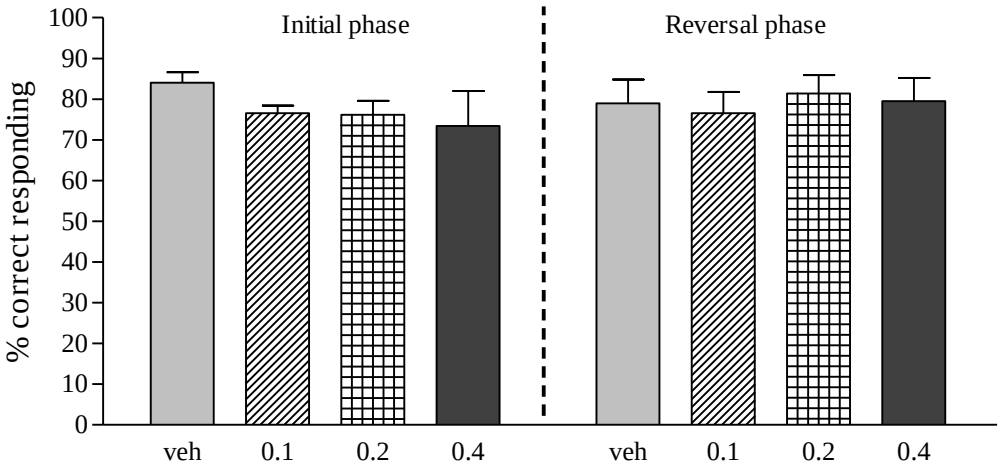


Figure 4.3.1a: The effect of risperidone (0.1, 0.2, 0.4 mg/kg; i.p.) on performance of the initial and reversal phases of the reversal learning task. Data are shown as mean ± SEM percent correct responding (n=7-8).

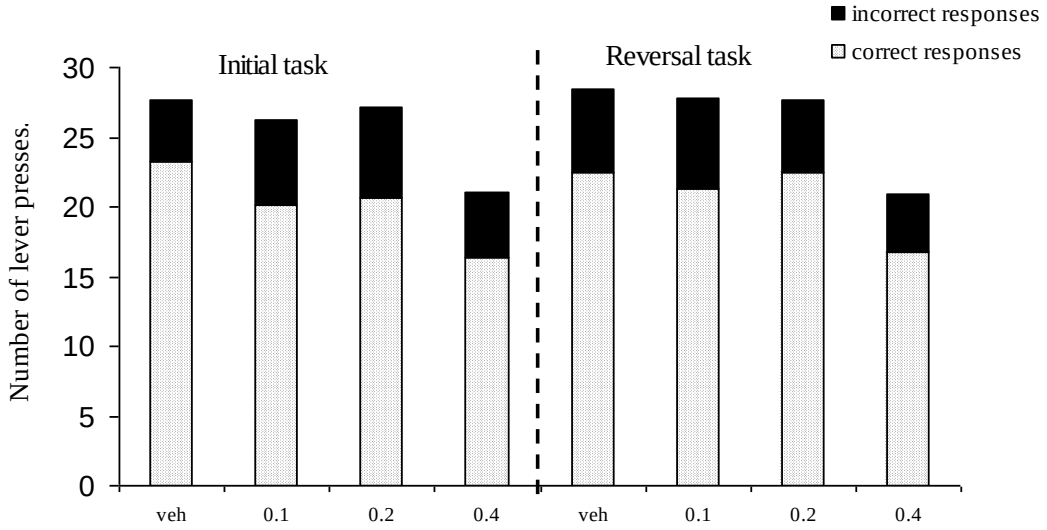


Figure 4.3.1b: The influence of risperidone (0.1, 0.2, 0.4 mg/kg; i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=7-8).

Effect of clozapine alone

There were no significant effects of clozapine on reversal learning performance in the initial or reversal phases of the task (fig 4.3.2a). However, a one-way ANOVA revealed a significant effect of clozapine on total lever pressing in the initial and reversal phases ($F_{(3,28)}$=6.67, P<0.01) and ($F_{(3,28)}$=4.60, P<0.05) respectively. Post-hoc Dunnett's t-test showed that clozapine at 5.0 mg/kg significantly reduced lever pressing compared to vehicle-treated rats in the initial (P<0.01; table 4.3.2) and reversal phases (P<0.05; table 4.3.2).

Table 4.3.2: The effect of clozapine (1.25, 2.5, 5.0 mg/kg; i.p.) on the total number of lever presses in a reversal learning paradigm. Data are expressed as the mean ± SEM total number of lever presses (n=7-8 per group) in the initial and reversal phase of the task. Significant reduction in lever pressing compared with vehicle group; *P<0.05, **P<0.01.

Drug treatment	Initial phase	Reversal phase
Vehicle	28.0± 0.2	28.0± 0.4
1.25 mg/kg	27.4± 0.4	27.5± 0.3
2.5 mg/kg	24.9± 1.2	27.3± 0.6
5.0 mg/kg	23.0± 1.3**	20.6± 3.3*

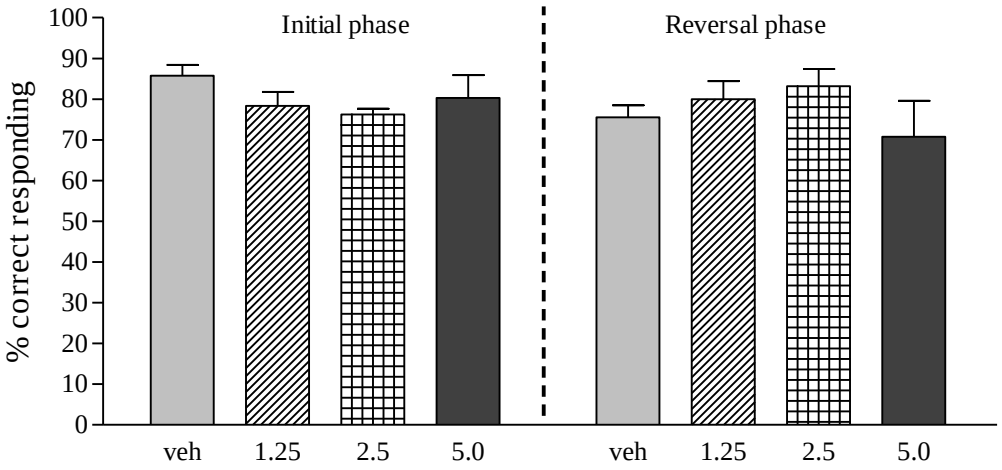


Figure 4.3.2a: The effect of clozapine (1.25, 2.5, 5.0 mg/kg; i.p.) on performance of the initial and reversal phases of the reversal learning task. Data are shown as mean ± SEM percent correct responding (n=7-8).

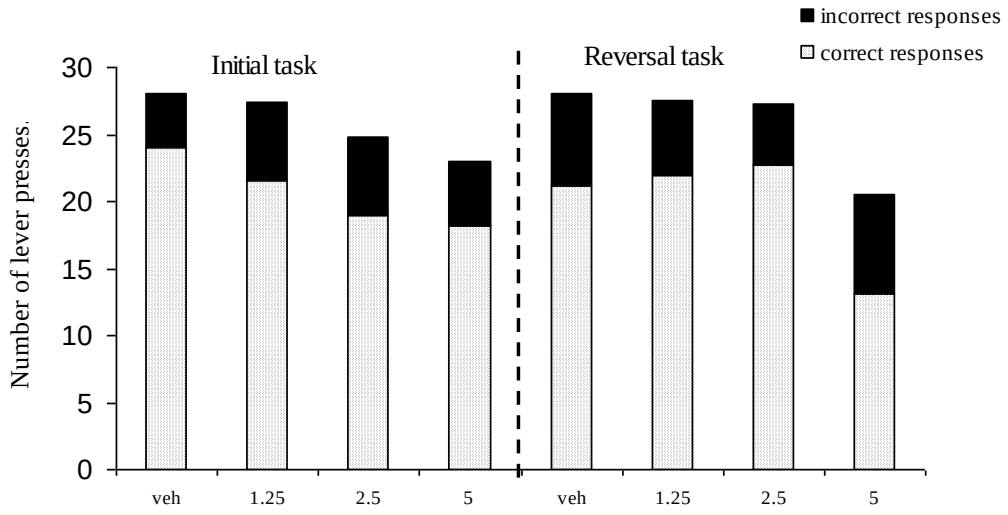


Figure 4.3.2b: The influence of clozapine (1.25, 2.5, 5.0 mg/kg; i.p.) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=7-8).

Effect of haloperidol alone

There were no significant effects of haloperidol in the initial phase ($F_{(3,28)}$=2.64, P = 0.071). However, a one-way ANOVA in the reversal phase showed a significant effect ($F_{(3,28)}$=3.54, P<0.05;). Post-hoc Dunnett's t-test revealed that haloperidol at 0.1 mg/kg significantly impaired reversal learning performance compared to vehicle rats (P<0.05; fig 4.3.3a). A one-way ANOVA on total lever pressing in the initial phase showed a significant interaction ($F_{(3,28)}$=27.76, P<0.001) and Dunnett's post-hoc t-test revealed that haloperidol at 0.1 mg/kg significantly reduced the total lever pressing compared to vehicle-treated rats (P<0.001; table 4.3.3). A one-way ANOVA also showed that haloperidol had a significant effect on total lever pressing in the reversal phase ($F_{(3,28)}$=31.39), P<0.001) and Dunnett's t-test again revealed that the significant reduction in lever pressing occurred a dose of 0.1 mg/kg of haloperidol (P<0.001; table 4.3).

Table 4.3.3: The effect of haloperidol (0.025, 0.05, 0.1 mg/kg; i.p.) on the total number of lever presses in a reversal learning paradigm. Data are expressed as the mean ± SEM total number of lever presses (n=6-8) per group) in the initial and reversal phase of the task. Significant reduction in lever pressing compared with vehicle group; ***P<0.001.

Drug treatment	Initial phase	Reversal phase
Vehicle	27.6± 0.4	27.6± 0.3
0.025 mg/kg	27.3± 0.5	28.1± 0.3
0.05 mg/kg	27.1± 0.3	26.7± 0.4
0.1 mg/kg	17.3± 2.0***	16.5± 2.1***

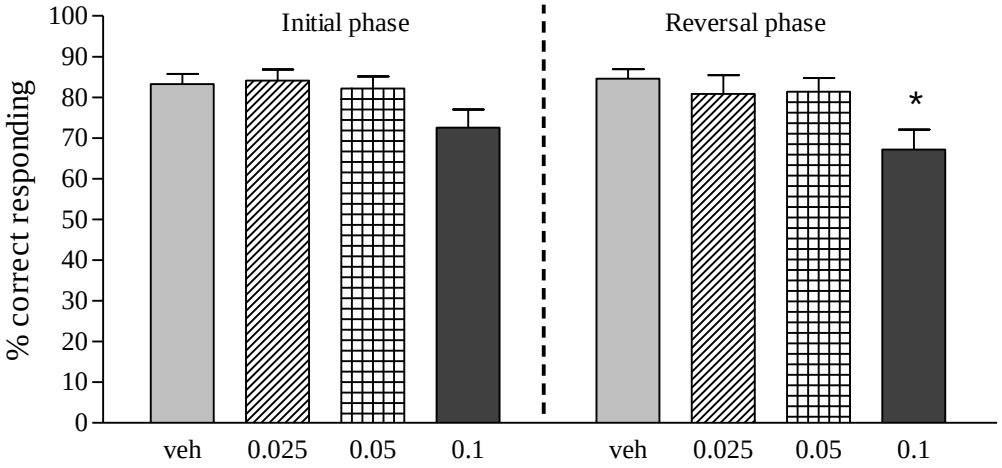


Figure 4.3.3a: The effect of haloperidol (0.025, 0.05, 0.1 mg/kg; i.p) on performance of the initial and reversal phases of the reversal learning task. Data are shown as mean ± SEM percent correct responding (n=6-8). Significant impairment in responding compared to vehicle group; *P<0.05.

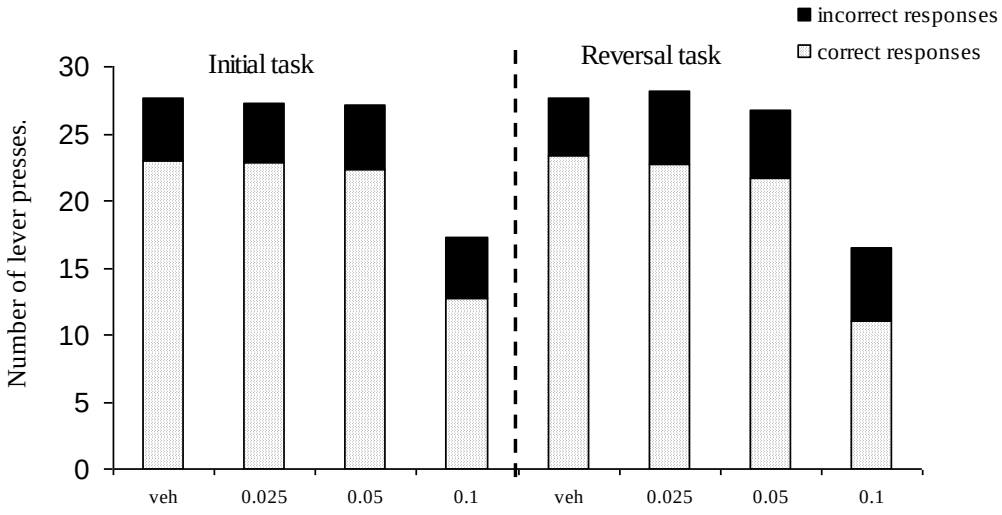


Figure 4.3.3b: The influence of haloperidol (0.025, 0.05, 0.1 mg/kg; i.p) on performance of the initial and reversal phase of the reversal learning task. Data are shown as the mean total number of lever presses, correct responses are shown as clear bars and incorrect responses are shown as shaded bars (n=6-8).

4.3.2 Experiments with sub-chronic PCP

Time-out experiment

Paired t-tests showed a significant reduction in performance of the reversal phase in PCP-treated rats compared with the initial phase following a 2 min time-out ($P<0.01$; fig 4.3.4) and without the time-out period ($P<0.001$; fig 4.3.4). Interestingly, a paired t-test showed a reduction in performance of the reversal phase in vehicle-treated rats compared with the initial phase which approached statistical significance following the absence of the time-out period ($P=0.06$). However, within the reversal phase, the performance of both sets of rats appears unaffected by the absence of the time-out cue. Paired t-tests comparing with and without the 2 min time-out in the reversal phase showed no significant difference in vehicle rats ($P=0.13$) or PCP rats ($P=0.08$). Total lever pressing was unaffected (table 4.3.4).

Table 4.3.4: The effect of sub-chronic PCP on total lever pressing with and without a 2 min time-out period (n=8 per group).

Drug treatment	Initial phase	Reversal phase
Vehicle + time out	27.5± 0.2	28.9± 0.4
PCP + time out	27.5± 0.3	28.6± 0.3
Vehicle + without time out	27.4± 0.4	27.4± 0.2
PCP + without time out	27.4± 0.2	27.9± 0.3

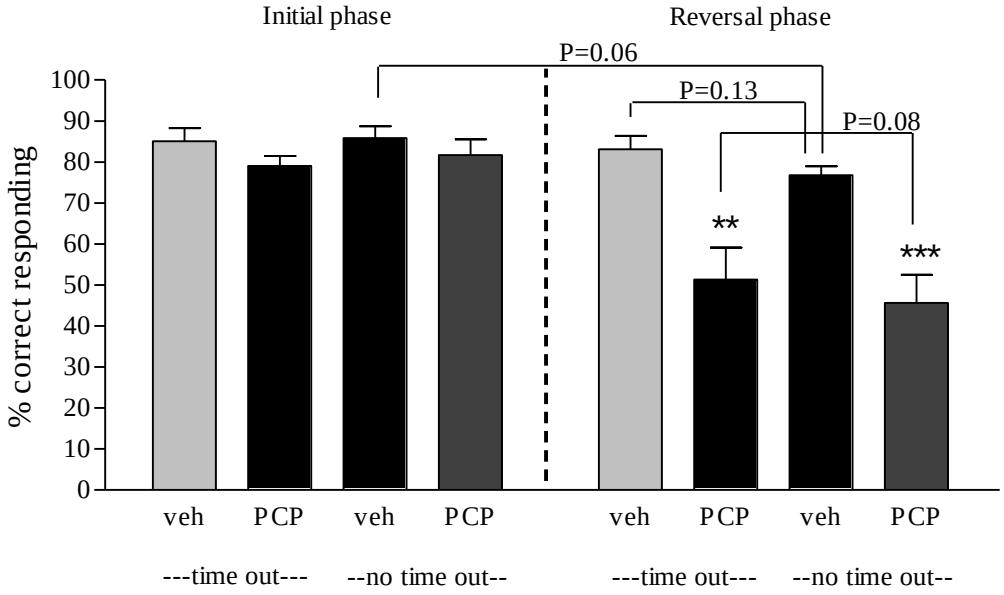


Figure 4.3.4: The effect of sub-chronic PCP on performance of the reversal phase of the reversal learning task with and without a 2 min time-out period. Data are shown as mean ± SEM percent correct responding (n=8). Paired t-test showed a significant reduction in performance of the reversal phase compared with the initial phase (**P<0.01, ***P<0.001).

Extended reversal experiment

Figure 4.3.5a shows the percent correct responding after 5 and 15 min of the reversal phase. In the first 5 min of the reversal phase PCP produced a selective deficit in the reversal phase of the task, a paired t-test showed that percent correct responding was reduced in the reversal phase compared to the initial phase ($P<0.01$; fig 4.3.5a). However, after 15 min of the reversal phase this deficit in PCP-treated rats was no longer present ($P=0.36$). Within the reversal phase of the task, performance of vehicle and PCP-treated rats improved with time. Paired t-tests showed significant improvements after 15 min compared to 5 min in vehicle rats ($P<0.01$) and sub-chronic PCP-treated rats ($P<0.001$). Vehicle rats also showed improved performance after 15 min of the reversal phase compared to their initial phase performance ($P<0.05$). Following 15 min of the reversal phase although the PCP-treated rats had improved their level of responding up to that in the initial phase, vehicle-treated rats performed at a significantly higher level ($P<0.01$).

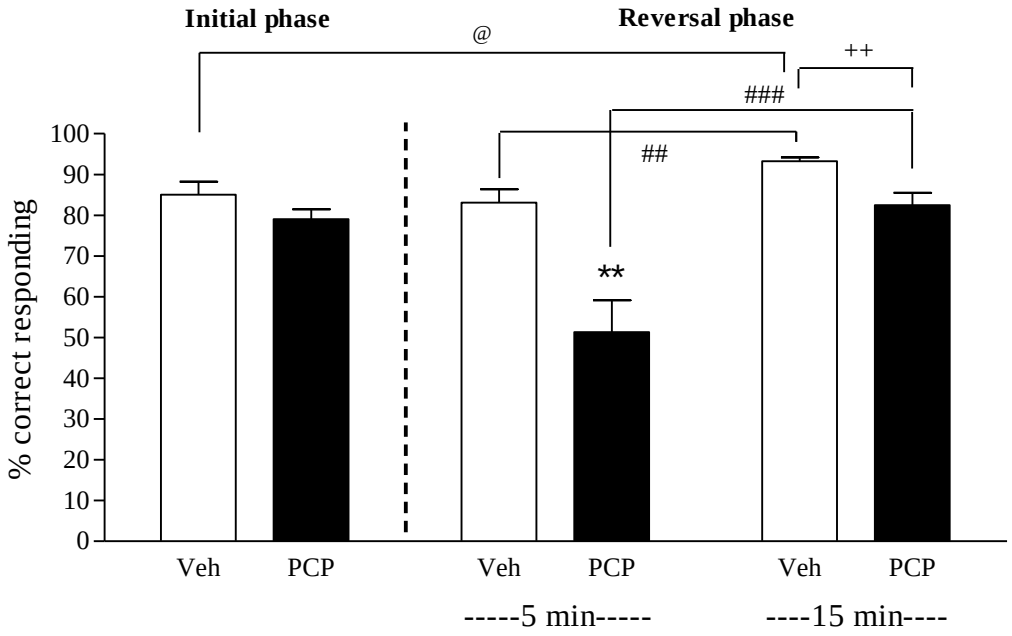


Figure 4.3.5a: The effect of sub-chronic PCP on performance of the reversal phase of the reversal learning task at 5 and 15 min. Data are shown as mean ± SEM percent correct responding (n=8). Significant reduction in performance of the reversal phase compared with the initial phase; **P<0.01 Paired t-test showed a significant deficit in the reversal phase after 5 min in the PCP-group compared to the initial phase. $^{@}$P<0.05 Paired t-test showed a significant improvement in the reversal phase after 15 min in the veh-group compared to the initial phase. Paired t-tests also showed significant improvements after 15 min compared to 5 min of the reversal phase $^{\#\#}$P<0.01; $^{\#\#\#}$P<0.001. An independent t-test showed that PCP-treated rats were impaired compared to the vehicle group following 15 min of the reversal phase $^{++}$P<0.01.

The temporal analysis of the percent correct responding data over every min of the 15 min reversal phase show that PCP and vehicle rats both improve over time with vehicle and sub-chronic PCP-treated rats reaching 93% and 82% correct responding after 15 min respectively (fig 4.3.5b). The area under the curves (AUC) show reduced percent correct responding in PCP-treated rats over the 5 min extended reversal (P<0.05; fig 4.3.5c) with an AUC of 193 compared to 290 in vehicle rats. Following 15 min of the

extended reversal the AUC also showed a reduction in correct responding in PCP-treated rats (P<0.01; fig 4.3.5c) with an AUC of 915 compared to 1189 in vehicle rats.

The correct and incorrect lever presses shows at which point the rats begin to make more correct than incorrect responses. As a result, in vehicle rats this cross-over point is reached before 1 min, while in sub-chronic PCP-treated rats this point is not reached until 4-5 min has elapsed (fig 4.3.5d).

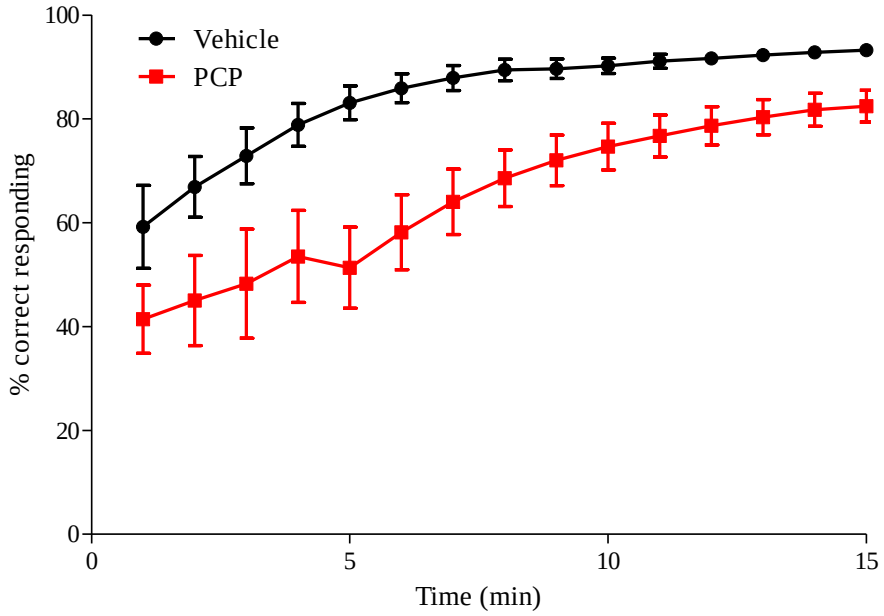


Figure 4.3.5b: The effect of sub-chronic PCP on performance of the reversal phase of the reversal learning task at 1 min intervals for 15 min. Data are shown as mean ± SEM percent correct responding (n=8).

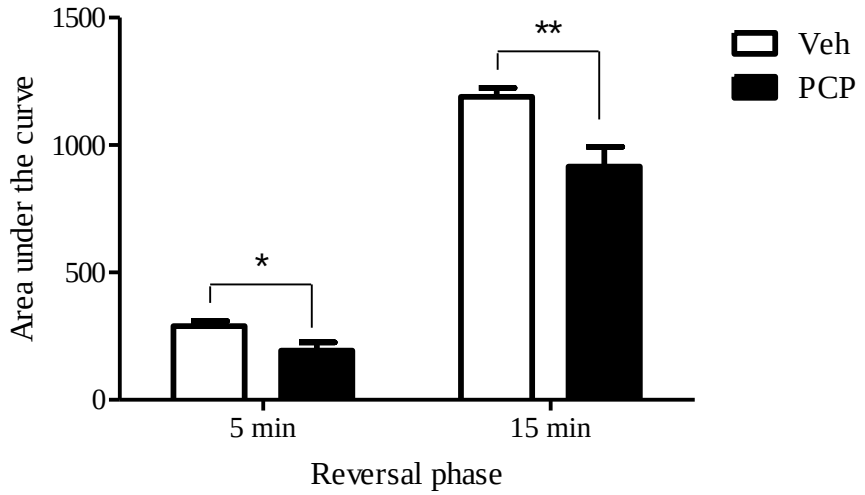


Figure 4.3.5c: The effect of sub-chronic PCP on area under the curve of percent correct responding over a 15 min period. Data are shown as mean ± SEM percent correct responding (n=8). Significant reduction in PCP-treated rats; *P<0.05; **P<0.01 independent t-test.

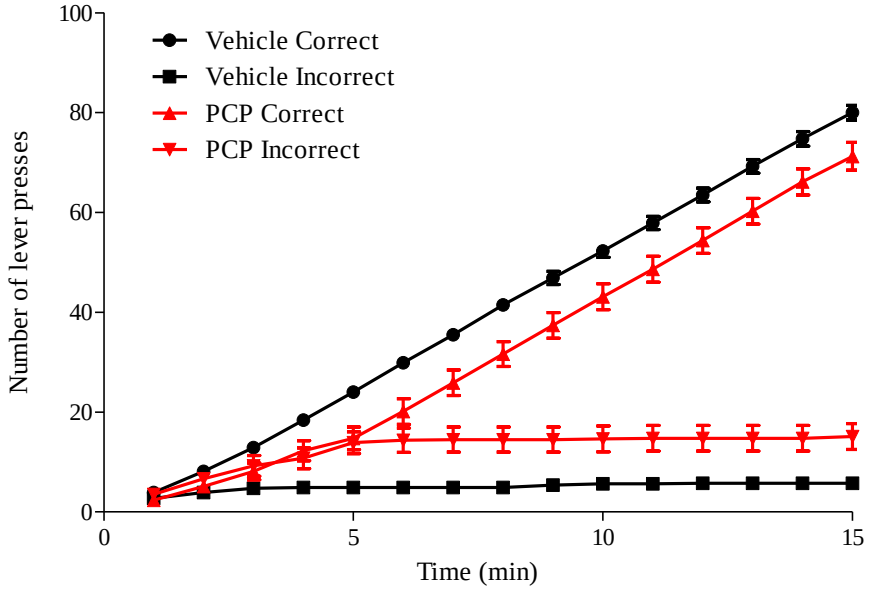


Figure 4.3.5d: The effect of sub-chronic PCP on performance of the reversal phase of the reversal learning task at 1 min intervals for 15 min. Data are shown as mean ± SEM for correct and incorrect responses (n=8).

Effect of double reversal

There were no differences in performance of the initial phase (fig 4.3.6). Paired t-tests showed that the PCP-treated group and the PCP-haloperidol rats were significantly impaired in reversal 1 compared to their initial phase performance ($P<0.05$ and $P<0.01$ respectively; fig 4.3.6). A one-way ANOVA in reversal 1 revealed a significant effect of drug treatment ($F_{(5,49)}=5.49$, $P<0.01$). Post-hoc Dunnett's test showed that clozapine (5.0 mg/kg), risperidone (0.2 mg/kg) and olanzapine (1.5 mg/kg) significantly reversed the sub-chronic PCP-induced deficit ($P<0.05$-0.01; fig 4.3.6). Paired t-tests showed that the PCP-treated group, olanzapine-treated and haloperidol-treated rats were significantly impaired in reversal 2 compared to their initial phase performance ($P<0.05$; fig 4.3.6). A one-way ANOVA in reversal 2 revealed a significant effect of drug treatment ($F_{(5,49)}=5.47$, $P<0.01$). Post-hoc Dunnett's test showed that clozapine (5.0 mg/kg) and risperidone (0.2 mg/kg) significantly reversed the sub-chronic PCP-induced deficit ($P<0.05$ and $P<0.01$ respectively; fig 4.3.6). However, olanzapine (1.5 mg/kg) no longer reversed the effect of PCP ($P=0.86$). Furthermore, a paired t-test revealed that in reversal 2, performance was significantly impaired compared to reversal 1 in olanzapine-treated rats ($P<0.05$). There were no significant effects on total lever pressing (table 4.3.6).

Table 4.3.6: The effect of clozapine (5.0 mg/kg; i.p.), risperidone (0.2 mg/kg; i.p.), olanzapine (1.5 mg/kg) and haloperidol (0.05 mg/kg; i.p.) and sub-chronic PCP on the total number of lever presses in a reversal learning paradigm. Data are expressed as the mean ± SEM total number of lever presses (n=8-10) in the initial and reversal phase of the task.

Drug treatment	Initial phase	Reversal phase 1	Reversal phase 2
vehicle + vehicle	26.3± 0.2	26.3± 0.2	26.3± 0.2
vehicle + sub-chronic PCP	26.3± 0.3	26.1± 0.2	26.1± 0.2
Clozapine + sub-chronic PCP	26.4± 0.2	26.0± 0.3	26.3± 0.3
Risperidone + sub-chronic PCP	25.8± 0.2	26.1± 0.4	26.3± 0.2
Olanzapine + sub-chronic PCP	25.9± 0.3	25.3± 0.8	25.5± 0.4
Haloperidol + sub-chronic PCP	26.1± 0.1	25.9± 0.3	25.9± 0.4

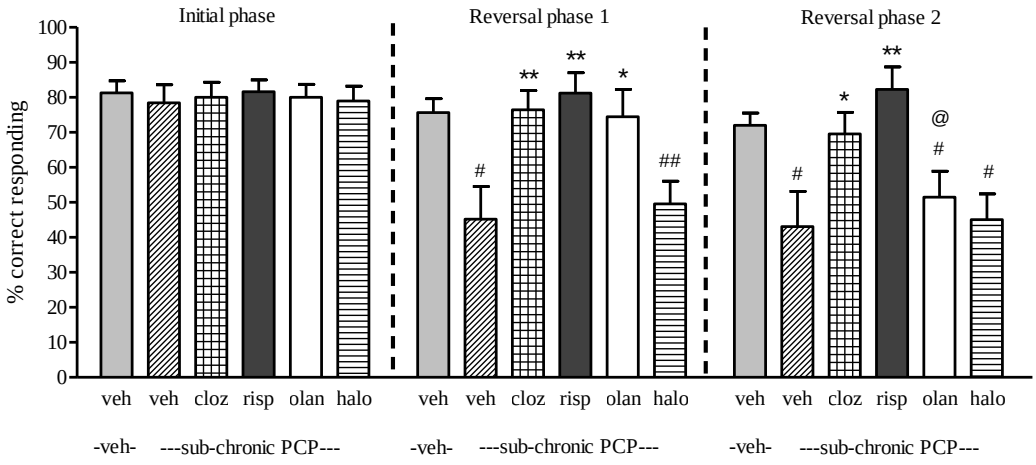


Figure 4.3.6: The effect of clozapine (5.0 mg/kg; i.p.), risperidone (0.2 mg/kg; i.p.), olanzapine (1.5 mg/kg) and haloperidol (0.05 mg/kg; i.p.) on the deficit produced by sub-chronic PCP on performance of the reversal phase of the reversal learning task. Data are shown as mean ± SEM percent correct responding (n=8-10). Paired t-tests showed a significant deficit in the reversal phases compared to the initial phase; $^{\#\#}P<0.01$, $^{\#}P<0.05$. Post-hoc Dunnett's t-test on each reversal phase showed a significant improvement following drug treatment compare to the PCP-veh group (**P<0.01, *P<0.05). A paired t-test showed that olanzapine-treated rats performed significantly worse in reversal 2 compared to reversal 1 ($^{@}P<0.05$).

4.4 Discussion

4.4.1 Antipsychotics alone

The present results demonstrate that vehicle-treated rats can be trained to an equivalent level of accuracy in both the initial and reversal phases of the reversal learning task. In vehicle-treated rats clozapine and risperidone did not produce any impairment in percent correct responding. The highest dose of each drug did, however, produce slight sedation measured by significantly reduced total lever pressing. This was also observed at the highest dose of haloperidol. Sedation induced by clozapine and risperidone could be due to affinity for histamine H_1 receptors with K_d values of 3.1nM and 5.2nM respectively (see Richelson and Souder, 2000). However, haloperidol does not have high affinity for the H_1 receptor with a K_d value of 260nM (Richelson and Souder, 2000), as a result sedation with haloperidol is more likely to be due to high D_2 receptor affinity. Haloperidol at 0.1 mg/kg reduced percent correct responding in the reversal phase indicative of cognitive impairment; this is supported by clinical data as patients treated with haloperidol have shown impaired performance in tests of working memory and executive function (Gilbertson and van Kammen, 1997).

In contrast to these results with clozapine and risperidone, impairments have been shown in other studies; Didriksen and colleagues used low doses (0.63 and 1.3 mg/kg) of clozapine for a reversal learning study, as a pilot study showed worsening of performance with higher doses of clozapine in combination with PCP (Didriksen *et al.,* 2007). A recent study also showed that clozapine (2.5 mg/kg) but not haloperidol (0.025 mg/kg), disrupted performance in an attentional task in drug naïve rats (Martinez and Sarter, 2008). Amitai and colleagues (2007) found that clozapine (3.0 mg/kg) and

risperidone (0.3 mg/kg) impaired performance, but haloperidol (0.05 mg/kg) did not, in the 5-choice serial reaction time task as measured by a decrease in the percent of correct responses. These doses of clozapine and risperidone were shown to induce some sedation in the rats as measured by an increase in correct latency (Amitai *et al.,* 2007). Although the doses of clozapine and risperidone used by Martinez and Sarter (2008) and Amitai and co-workers (2007) are comparable to present doses used, the dose at which an impairment was observed in haloperidol-treated rats was considerably higher (0.1 mg/kg) in the current experiment. In summary, clozapine and risperidone in drug naïve rats did not impair reversal learning, in marked contrast, haloperidol at 0.1 mg/kg impaired performance in this reversal learning task.

4.4.2 Sub-chronic PCP experiments

Following treatment with sub-chronic PCP, rats continually demonstrated a reduction in responding which was selective for the reversal phase (chapter 3). This is in agreement with other results from this laboratory (Abdul-Monim *et al.,* 2006) and also this effect is observed following acute treatment with PCP (Abdul-Monim *et al.*, 2003; Idris *et al.*, 2005a). Sub-chronic PCP treatment did not affect total lever pressing, suggesting locomotor activity nor motivation were affected. Results from other laboratories have also shown deficits in reversal learning following sub-chronic PCP administration (Jentsch and Taylor, 2001). The use of sub-chronic PCP treatment is believed to more closely mimic cognitive deficits associated with schizophrenia compared to acute treatment (Jentsch and Roth, 1999).

In the time-out experiment sub-chronic PCP-treated rats were impaired following the time-out but were also equally impaired in the absence of the time-out. Vehicle-treated rats performed slightly worse without the time-out compared to with the

time-out, although this effect was not statistically significant (P=0.13). This finding was also observed in the PCP-treated rats, but again was not statistically significant (P=0.08). In vehicle rats in the experiment without the time-out, performance was impaired compared to the initial phase performance, and this affect was close to reaching significance (P=0.06). Although the aforementioned differences were not statistically significant they do suggest that the 2 min time-out is needed as a cue. The orbitofrontal cortex (OFC) is believed to control reversal learning ability (McAlonan and Brown, 2003). There are several hypotheses regarding the role of the OFC in cognitive flexibility. One explanation is that the OFC normally acts to inhibit prepotent responding that has become inappropriate after reversal (Jones and Mishkin, 1972). Animals lacking OFC function would therefore perseverate after reversal because they cannot inhibit the previous response. A second account of the role of the OFC in reversal learning is that it drives behaviour according to associative information representing the significance of a particular cue (Rolls *et al.*, 1996; Thorpe *et al.*, 1983). An alternative explanation for the importance of OFC to reversal learning and to cognitive flexibility more generally, lies in its ability to signal the value of an expected outcome, rather than simply to drive behaviour based on the associative history of a particular cue. Neuronal recordings and functional imaging in the OFC, have demonstrated that activity there tracks with and anticipates the value of rewarding or punishing outcomes (Blair *et al.*, 2006; Feierstein *et al.*, 2006; Furuyashiki *et al.*, 2008; Roberts, 2006). Under this hypothesis, OFC-lesioned animals would perseverate because they continue to encode the old contingencies, or fail to encode the new ones, in other associative learning areas. The results in this chapter show that PCP affects reversal learning which is believed to be controlled by the OFC (McAlonan and Brown, 2003), in future studies it would be beneficial to investigate the neurobioloical

differences in PCP-treated rats. It would be advantageous to carry out lesions of the OFC in our reversal learning task to determine the brain region involved.

The differences in the vehicle-treated and sub-chronic PCP-treated rats with or without the time-out were not significant but suggest that the 2 min time-out could be important as a cue. It was hypothesised that the lack of a time-out period would make the task much more difficult. Though there was not an intentional time-out given in the no time-out experiment, to record the number of lever presses in the initial phase and to switch the active lever took approximately 15 sec. Between food pellets the house-light is out for only 3 sec, in the no time-out experiment the house-light was out for approximately 15 sec, which may still have acted as a cue. The rats are also very highly trained to perform reversals to achieve 90% correct, therefore this may be difficult to disrupt. It may be advantageous to test the rats before they are so highly trained to observe the affect of removing the cue in the training phase.

In the double reversal experiment, clozapine and risperidone were efficacious in reversing the sub-chronic PCP-induced deficit. Clozapine was effective in attenuating the reversal learning deficit produced by sub-chronic PCP in both reversal 1 and reversal 2. However, in chapter 3 clozapine did not significantly improve the performance of PCP rats despite restoring performance to the level of the vehicle group. The difference between the effects of clozapine in these two chapters may be that the PCP-induced deficit in this experiment impaired the rats' performance to 45% compared to 59% in chapter 3; therefore, there was more capacity for improvement as the PCP rats were performing to a lower level. Olanzapine was also effective in reversing the deficit in reversal 1. This is also supported by a recent study showing that the same dose (1.5 mg/kg) of olanzapine was effective in reversing the PCP-induced deficit (Abdul-Monim *et al.,* 2006). Olanzapine has been shown to improve cognition

in the clinic in schizophrenic patients (Stratta *et al.,* 2005), and the dose used in this experiment (1.5 mg/kg) is clinically comparable in terms of D_2 receptor occupancy (Kapur *et al.,* 2003). As in chapter 2 the inability of haloperidol to reverse the PCP-induced deficit may be due to its high D_2 receptor affinity and minimal 5-HT_{2A} receptor affinity (Siegfried *et al.,* 2005).

Following the second reversal phase the clozapine-treated rats performed slightly less well than in the first reversal, although this effect was not significantly different. However, the olanzapine-treated rats performed significantly less well in the second reversal compared to the first reversal, suggesting that olanzapine is not as efficacious as clozapine or risperidone. Olanzapine was also shown to impair responding at 2.0 mg/kg but this effect was only observed in the reversal phase, suggesting that rats were no longer attending to the task (Abdul-Monim *et al.,* 2006). This could be similar to the effect observed in the second reversal in the current experiment. A recent study showed that olanzapine was less efficacious than clozapine and risperidone in attenuating PPI deficits (Cilia *et al.,* 2009). It has been suggested in the clinic that risperidone is more effective in improving cognition than clozapine (Keefe *et al.,* 2007). Olanzapine has higher affinity for D_1 receptors than clozapine and risperidone, and has comparable affinity for D_2, 5-HT_{2A} and 5-HT_{2C} receptors (Siegfried *et al.,* 2005; Hertel *et al.,* 2007). The main difference in receptor affinities between olanzapine and clozapine and risperidone appears to be olanzapine's lack of affinity for 5-HT_{1A} receptors (Siegfried *et al.,* 2005; Hertel *et al.,* 2007). The 5-HT_{1A} receptor may be important in reversal learning as buspirone, a partial 5-HT_{1A} agonist, was shown to reverse the sub-chronic PCP-induced deficit in chapter 3. Olanzapine may have been less efficacious than clozapine and risperidone in the second reversal due to its higher affinity for D_1 receptors. The results from the previous chapter showed that activation

of D_1 receptors is effective in improving reversal learning performance, however, it has been suggested that over activation of D_1 receptors can have a detrimental effect on cognition (Goldman-Rakic *et al.,* 2000). The second reversal phase may also incorporate an attention aspect of cognition as rats have to maintain attention on the task for a period that is longer than normal and following a longer (4 min) time-out. This would therefore make the reversal learning task more difficult, and could provide a method of distinguishing between antipsychotics.

From the temporal data in the extended reversal phase experiment, both the vehicle and the sub-chronic PCP-treated rats showed improved performance over time. PCP produces a selective deficit within the reversal phase over the first 5 min test period, however, after 15 min this deficit is no longer observed. Previously in reversal learning experiments (chapter 3) data would only be obtained on the performance of the rats in the first 5 min of the reversal phase, and it would not be determined at which point the PCP loses its detrimental affect, or at which point antipsychotic compounds reverse the PCP-induced deficit. The extended reversal phase used in the current experiment would also allow the investigation of the temporal effects of antipsychotics or selective compounds.

During the first 5 min of the reversal phase it is evident that, in vehicle-treated rats, the curve of percent correct responding is steeper than that of the sub-chronic PCP-treated rats, suggesting that vehicle-treated rats learn the new rule faster. Pre-clinical time course data for antipsychotic drug effects is often shown as the effect on each day, or across training sessions (Li *et al.,* 2007), and in reversal learning tasks is usually shown as trials to criterion or correct responding for a total reversal learning session (Boulougouris *et al.,* 2008). Analysis of the number of correct and incorrect responses reveals a "cross-over" point at which the rats begin to make more correct than incorrect

responses. In vehicle-treated rats this cross-over point is reached before 1 min of the reversal phase, therefore the vehicle-treated rats have the ability to adapt to the new rule immediately. In PCP-treated rats this point is not reached until 4-5 min has elapsed, which, when the reversal phase would normally be stopped after 5 min, would give a percent correct responding value of approximately 50%. This is a novel measure that could potentially differentiate between the mechanism of action and efficacies of different antipsychotics.

4.4.3 Conclusions

In conclusion, the experiments in naïve rats suggest that the atypical antipsychotics clozapine and risperidone when given alone have no effect on reversal learning. Haloperidol when given to naïve rats impaired performance at the highest dose.

Sub-chronic PCP administration impairs reversal learning ability as demonstrated by reduced percent correct responding in the reversal phase of the reversal learning task. The 2 min time-out could be important as a cue for the reversal phase. Following a double reversal, olanzapine-treated rats lose the ability to switch between the rules, indicating it is not as efficacious as clozapine or risperidone in reversing the PCP-induced deficit. Using double or even multiple reversals could provide an approach of distinguishing efficacy between antipsychotics. The extended reversal phase showed that PCP caused a deficit after 5 min but this effect was lost after 15 min. Using an extended reversal would also allow the investigation of the temporal effects of antipsychotics or selective compounds.

This chapter reiterates that this sub-chronic PCP dosage regime reliably produces cognitive deficits in this reversal learning task, and offers insight into the temporal profile of the PCP-induced deficit. Having aimed to explore the mechanism of the behavioural deficit induced by sub-chronic PCP it is now necessary to investigate the nature of the PCP-induced neurobiological deficits that accompany the behaviour. These deficits will be explored using electrophysiology and immunohistochemistry in the PFC and hippocampus. The PFC will encompass both the medial and orbital regions on which the behavioural tasks in chapters 2 and 3 (respectively) are based. The hippocampus will be investigated as a control region for electrophysiology and it is a region implicated in learning and memory.

Chapter 5

The effect of sub-chronic PCP on gamma oscillations and parvalbumin expression

5.1 Introduction

Post mortem studies in patients with schizophrenia have revealed robust pathology involving GABAergic signalling (see chapter 1; Perry *et al.,* 1979; Bird, 1985; Benes and Berretta, 2001; Reynolds *et al.,* 2002; Zhang and Reynolds, 2002; Lewis *et al.,* 2005). These GABAergic deficits are largely restricted to GABAergic interneurons containing the Ca^{2+} binding protein parvalbumin (PV; Lewis *et al.,* 2005). Importantly these interneurons have been shown to synapse onto pyramidal cells (Hendry *et al.,* 1989), and so are positioned to regulate pyramidal cell output (see figure 5.1). This decrease in PV interneuron functionality would not only produce a decrease in inhibitory control over pyramidal cell activity (Olney and Farber, 1995), but would also reduce co-ordinated activity of brain networks. Pyramidal cells drive oscillations among inhibitory neurons, which in turn modulate the firing rates of pyramidal cells, leading to synchronised activity. Oscillations reflect this synchronised activity of neuronal populations and within the cortex and hippocampus there is emerging data suggesting the importance of fast-spiking interneurons in the generation of these oscillatory potentials (Whittington *et al.,* 1995; Bartos *et al.,* 2002; Freund and Katona, 2007).

Gamma-frequency oscillations (20-80 Hz) are prevalent in active cortical networks, and are important for cognition, learning and memory (Engel and Singer, 2001). Gamma oscillations arise from networks of PV interneurons in the middle cortical layers, whereas GABA neurons containing PV and calbindin give rise to theta frequency (4-7 Hz) oscillations (Blatow *et al.,* 2003). Theta and gamma oscillations induced in the prefrontal cortex during cognitive tasks, are reduced in patients with schizophrenia, who also perform poorly in these tasks (González-Hernández *et al.,* 2003; Cho *et al.,* 2006). Patients with schizophrenia have shown reductions in gamma power,

which may reflect cognitive and negative symptoms, whereas positive symptoms may be related with increases in gamma power (Light *et al.*, 2006).

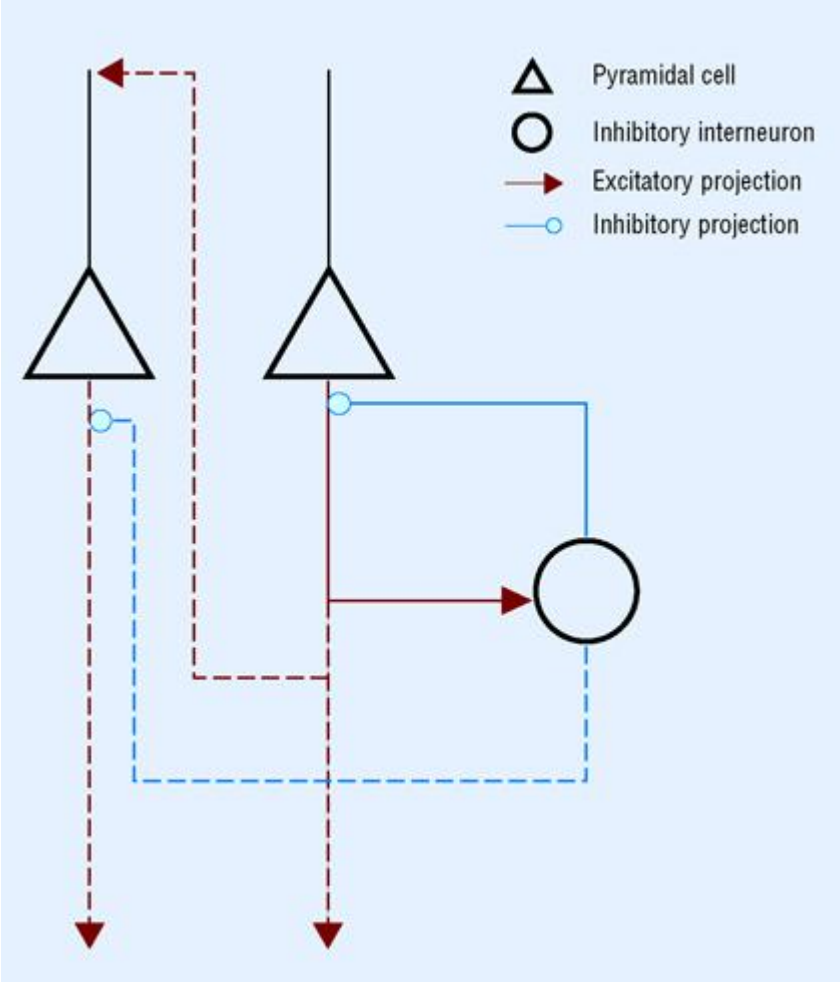


Figure 5.1: Simplified diagram of a neuronal circuit. Pyramidal cells send excitatory (glutamatergic) projections to neighbouring pyramidal cells, other brain regions and local inhibitory interneurons. These interneurons in turn send inhibitory (GABAergic) projections back to the pyramidal cells, enabling the generation of synchronous oscillations. The basic pyramidal-interneuron- pyramidal circuit is shown in solid lines, other connections are shown by dashed lines. Diagram taken from Spencer and McCarley, 2006.

Following the discovery of suitable induction protocols it is now possible to induce gamma oscillation in *in vitro* slice preparations. Gamma oscillations are mainly induced by 2 methods: (1) induction by electrical stimulation (Traub *et al.,* 1996), which generates transient gamma oscillations, and (2) induction by pharmacologically activating muscarinic receptors (Fisahn *et al.,* 1998) or kainate receptors (KARs) (Hormuzdi *et al.,* 2001), which results in sustained gamma oscillations. Genetic deletion of a receptor subtype contributing to the excitation of pyramidal neurons and/or interneurons prevents the induction of gamma oscillations by the respective agonists (Fisahn *et al.*, 2004). Kainate receptors are widely expressed in the hippocampus, with five subunits (GluR5-7 and KA1-2; Bureau *et al.,* 1999). While NMDA and AMPA receptors are predominantly post-synaptic, kainate receptors are also located pre-synaptically at many synapses where they can modulate transmitter release (Lerma, 2003). Muscarinic receptors are widely expressed in the CNS; however, it is the M_1 and M_4 subtypes that are predominant in the frontal cortex and hippocampus (Levey *et al.*, 1991; Wei *et al.,* 1994). Gamma oscillations are generated in the CA3 region but not CA1 as the network architecture between pyramidal cells and interneurons is highly recurrent, thus generating strong rhythmic activity (Amaral *et al.,* 1990).

The aim of this study was to investigate whether the cognitive deficits induced by the sub-chronic PCP dosage regimen in chapters 2, 3 and 4 are underpinned by differences in gamma oscillations in the prefrontal cortex (PFC) and CA3 region of the hippocampus. Expression of parvalbumin-containing interneurons will also be investigated to establish the role of GABAergic transmission in gamma oscillations and sub-chronic PCP-induced deficits in cognition. A PCP sensitisation experiment will be carried out in order to establish that the sub-chronic PCP dosing regimen was effective.

5.2 Materials and Methods

5.2.1 Subjects and sub-chronic PCP treatment

Fifty-two adult female hooded-Lister rats (Charles River, UK) were housed in groups of four and weighed 190-240 g at the start of the experiment. Animals were maintained under standard laboratory conditions at a temperature of 21°C (±1°C) and humidity of 55±5%. They were maintained on a 12 h/12 h light/dark cycle (lights on at 0700 h) and experimental procedures were performed during the light phase. Rats had free access to food and water. Experiments were conducted in accordance with the Home Office under the Animals (Scientific Procedures) Act, UK, 1986, and approved by the GlaxoSmithKline Procedure Review Panel.

Rats were treated with 2 mg/kg PCP (n = 26) or vehicle (0.9 % saline; n = 26) by the intraperitoneal (i.p.) route in a volume of 1 ml/kg twice daily for seven days. Dosing with sub-chronic PCP or vehicle was followed by a washout period of a further seven days.

5.2.2 Order of experiments (see figure 5.2.1)

Following seven-day sub-chronic PCP treatment, 20 rats from the cohort (10 vehicle and 10 sub-chronic PCP-treated) were used for electrophysiology experiments, these were carried out following the seven day washout period. Electrophysiology was carried out for approximately 6 weeks, using 1 rat on 3 days per week.

Thirty-two rats (16 vehicle and 16 sub-chronic PCP-treated) were used to assess PCP sensitisation to ensure the sub-chronic PCP regimen as effective. These experiments were carried out on days 17 and 18 (10 days after the final sub-chronic PCP dose).

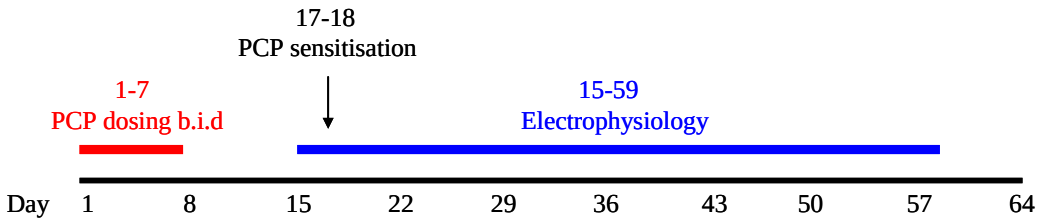


Figure 5.2.1: Timeline of experiments carried out in one cohort of rats

5.2.3 PCP sensitisation

Thirty-two rats (16 vehicle and 16 sub-chronic PCP-treated) were used to assess PCP sensitisation to ensure the sub-chronic PCP regimen as effective. Locomotor activity (LMA) response to a novel environment was monitored using automated photocell cages. For details of LMA apparatus and software see chapter 2 (2.2.2). Rats were placed in the LMA boxes for a period of 3 hours. After 1 hour rats were either given an acute dose of PCP (2 mg/kg, i.p.) or vehicle (0.9% saline, i.p.) and were placed back in the LMA boxes immediately after dosing. The acute dose of PCP was chosen based on a previous study showing that 2 mg/kg can produce cognitive deficits without affecting locomotor activity (Grayson, unpublished findings). Therefore, there were 4 test groups (all n=8) sub-chronic vehicle-vehicle, sub-chronic vehicle-PCP, sub-chronic PCP-vehicle, sub-chronic PCP-PCP. Activity was monitored every 10 min over a 180-min period.

5.2.4 Electrophysiology

The remaining 20 rats from the cohort (10 vehicle and 10 sub-chronic PCP-treated) were used for electrophysiology experiments. Rats were anesthetised with inhaled isoflurane (Abbott, UK), immediately followed by an intramuscular injection of ≥ 100 mg/kg ketamine hydrochloride (Vetalar™, Pfizer, UK) and ≥ 10 mg/kg xylazine

hydrochloride (Rompun™, Bayer, UK). Animals were perfused intracardially with ~50 ml of modified sucrose-containing artificial CSF (aCSF), which was composed of the following (in mM): 189 sucrose, 25 KCl, 1.2 NaH_2PO_4, 26 $NaHCO_3$, 5 $MgCl_2$, 0.1 $CaCl_2$, and 10 glucose. All salts were obtained from BDH Chemicals (Poole, UK). Although this was a terminal procedure through exsanguination, death was further confirmed by decapitation. The brain was removed and submerged in cold (4–5°C) aCSF during dissection. The dorsal surface of the brain was fixed to a cutting block with Vetbond (3M). The cutting block was then lowered into the cutting chamber and submerged in ice cold sucrose-containing aCSF; the temperature was maintained at ~3°C by a cooling unit (CU65, Microm). Horizontal slices (400 μm) containing hippocampus and prefrontal cortex (PFC) were prepared using a Vibraslice (HM650 V, Microm) from vehicle and sub-chronic PCP-treated rats.

Slices were cut and transferred to an interface recording chamber (Harvard apparatus, MA, USA; see figure 5.2.2). Slices were placed on a layer of lens tissue in a Perspex trough within the chamber (see figure 5.2.3). Oxygenated (95% O_2/5% CO_2) aCSF [containing the following (in mM): 124 NaCl, 3 KCl, 1 NaH_2PO_4, 26 $NaHCO_3$, 2 $MgCl_2$ (for CA3, 1mM for PFC), 2 $CaCl_2$, and 10 glucose] was continuously pumped into the chamber which was maintained at 30±1°C. Slices were permitted to equilibrate for 1 hour before any recordings commenced. CA3 slices were taken from bregma -6.8 to -5.0 mm and PFC cortex slices were taken from bregma -4.0 to -2.5 mm. Remaining slices were transferred to a holding chamber containing cold oxygenated (95% O_2/5% CO_2) aCSF. If required, a slice from the holding chamber was transferred to the interface chamber and incubated as above before recording.

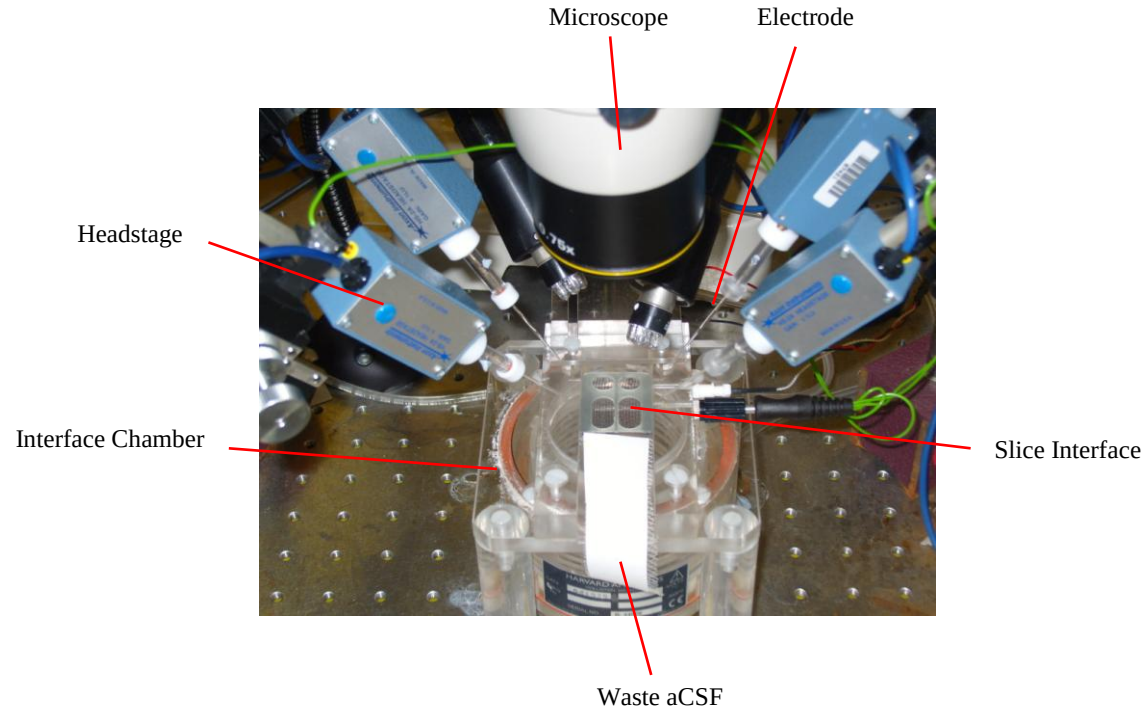


Figure 5.2.2: Photograph of the electrophysiology rig showing the interface chamber and headstages.

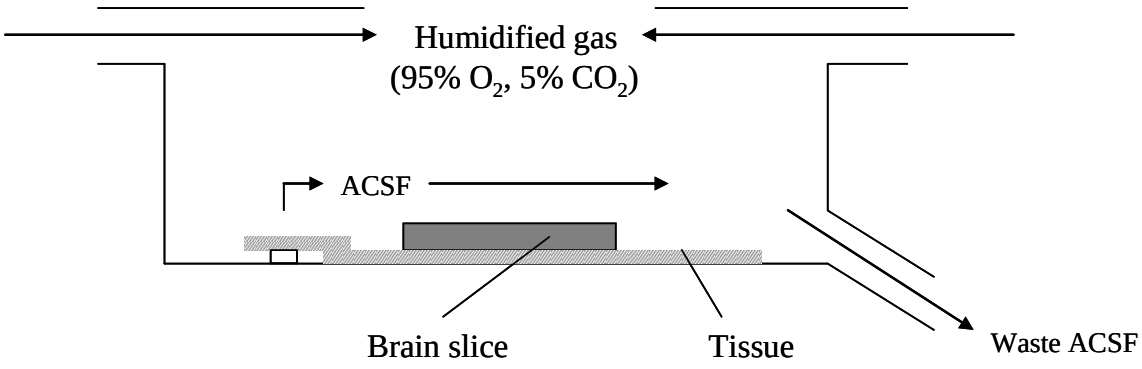


Figure 5.2.3: Schematic diagram of the slice interface.

Recording electrodes were pulled from borosilicate glass (Harvard Apparatus, Kent, UK) using a horizontal micropipette puller (DMZ-Universal Puller, Zeitz-Instrumente, Munich, Germany), filled with ACSF, and had resistances in the range of ~2MΩ. The recording electrodes were fixed into the headstages (HS-2A headstage, Axon

Instruments, USA) and were connected to an Axoclamp 2B Amplifier (Axon Instruments, UK) by a silver chloride wire. Electrodes were manually positioned using coarse control micro-manipulators (Narishige, Japan) on the surface of the CA3 and PFC in the position shown in red in figures 5.2.4 and 5.5.5. A schematic diagram of the rig and recording equipment is shown in figure 5.2.6.

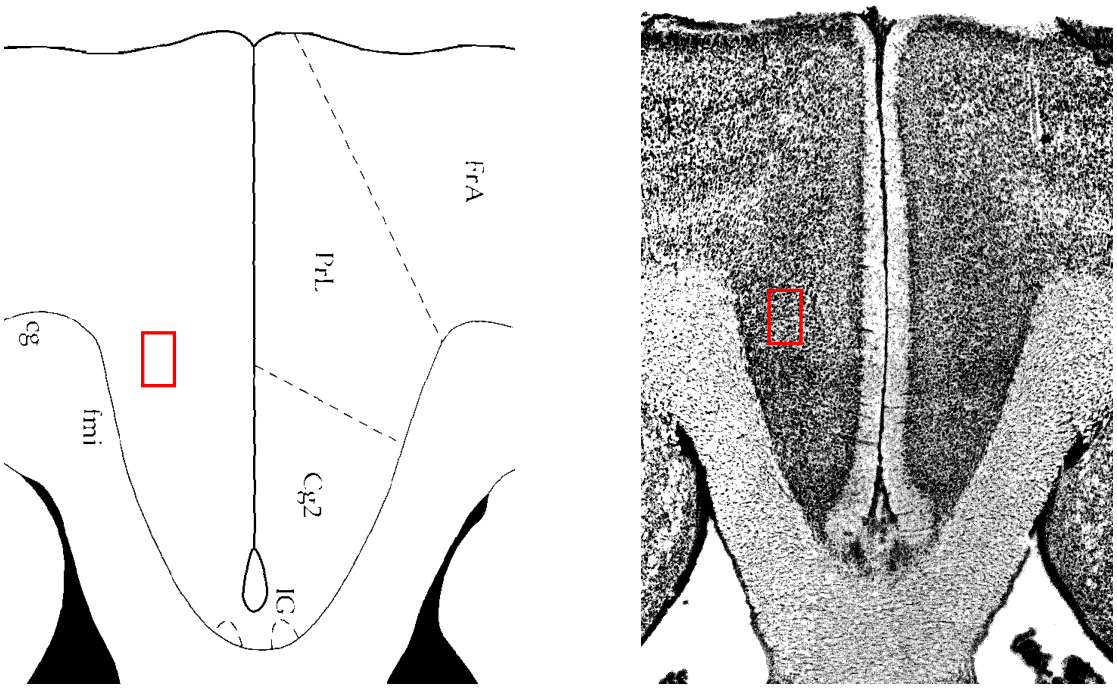


Figure 5.2.4: Example of a section of horizontal prefrontal cortex used as a slice. This example shows the PFC at Bregma -3.38mm (taken from Watson and Paxinos, 1998). Electrodes were positioned in the region highlighted in red. FrA frontal association cortex, PrL prelimbic cortex, Cg2 cingulate cortex area 2, IG indusium griseum, fmi forceps minor corpus callosum, cg cingulum.

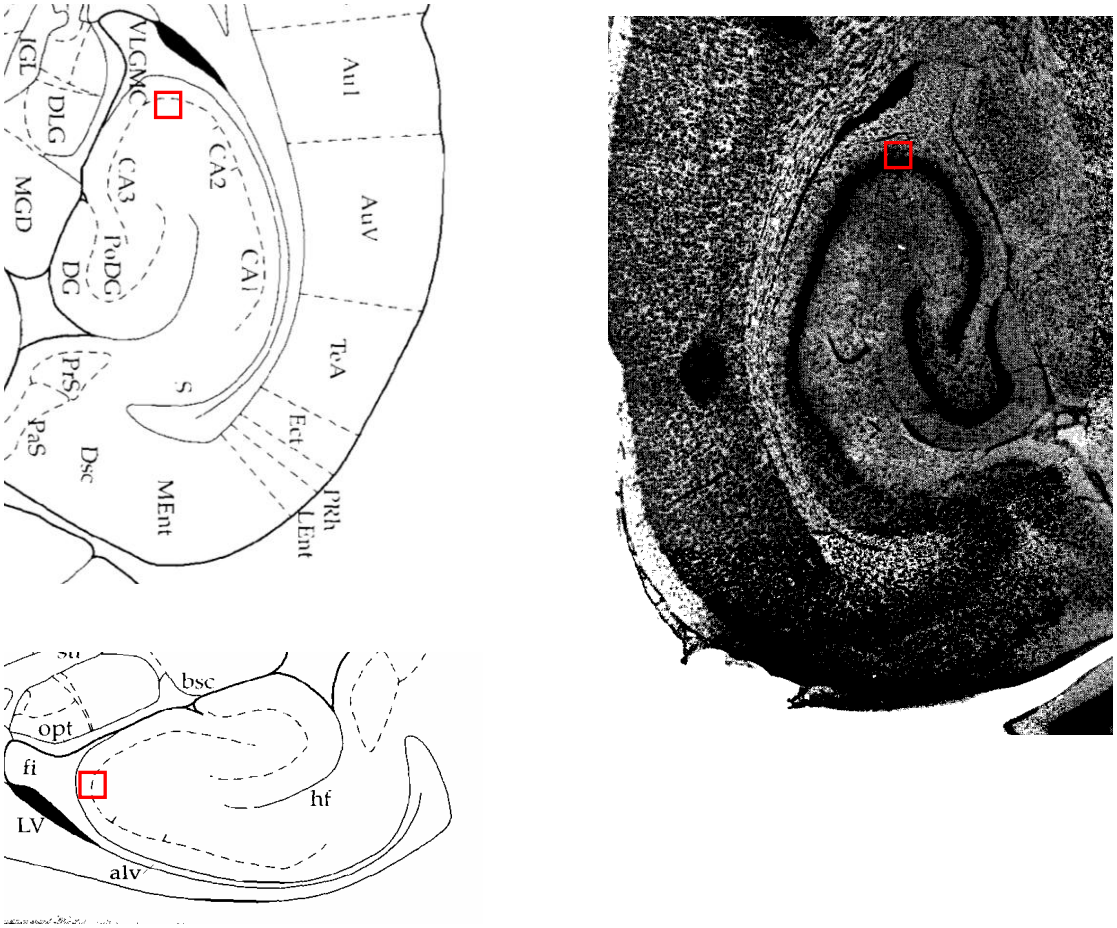


Figure 5.2.5: Example of a section of horizontal hippocampus used as a slice. This example shows the hippocampus at Bregma -5.32mm (taken from Watson and Paxinos, 1998). Electrodes were positioned in the CA3 region, highlighted in red. Lower left panel shown for clarity. CA1, 2, and 3 fields of hippocampus, PoDG polymorph layer dentate gyrus, DG dentate gyrus, S subiculum, hf hippocampal fissure.

The following drugs were added to the perfusion medium for various experimental conditions: kainic acid (KA, 100-400 nM), carbachol (10 µM). Pilot experiments were carried out to determine the concentrations of the agonists required to induce gamma oscillations. For CA3 slices gamma oscillations were induced with 100 nM KA and for PFC oscillations were induced by 400 nM KA and 10 µM carbachol. In both cases

oscillations were permitted to stabilise (for approximately 3 hours) before measurements were taken.

Peak frequency and peak power values were obtained from power spectra generated by Fast Fourier Transform (FFT) analysis in Spike2 version 6 (Cambridge Electronic Design). The FFT generated a power spectrum at 0.512 sec which gave a resolution of 1.9 Hz. Power was determined as the area under the peak in the power spectra between 20 and 80 Hz for gamma frequency oscillations. All values are given as the mean ± S.E.M. Power spectra were constructed offline from digitised data, using a 60 s epoch of recorded activity, the mean was taken over 5-10 min of recordings.

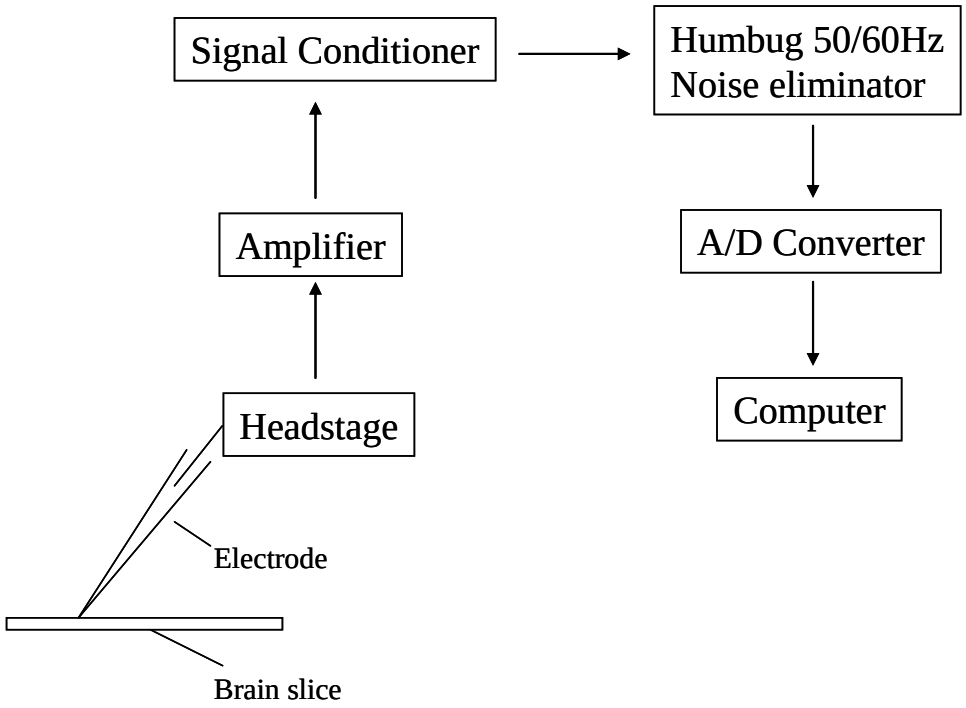


Figure 5.2.6: Schematic diagram of rig. The recording electrodes were fixed into the headstages (HS-2A headstage, Axon Instruments) and were connected to an Axoclamp 2B Amplifier (Axon Instruments) by a silver chloride wire. The signal was then further amplified or conditioned (Brownlee Precision model 440). 50Hz noise was eliminated using a Humbug (Quest Scientific), and the analogue signal was then converted to digital (Micro 1401, Cambridge Electronic Design). This is signal was then fed into the computer for analysis.

5.2.5 Drugs

PCP hydrochloride (Sigma, UK) was dissolved in 0.9% saline and the dose was calculated at base equivalent. Kainic acid (100-400 nM) and carbachol (10 µM), from Sigma (Poole, UK), were dissolved in distilled water.

5.2.6 Immunohistochemistry

In a separate cohort, 32 female hooded-Lister rats (Charles River, UK) were dosed with either PCP (n=16) or vehicle (n=16) for 7 days (see section 5.2.1).

5.2.6.1 Brain preparation and fixation

Two and eight weeks post sub-chronic PCP treatment, vehicle-treated (n=8) and sub-chronic PCP-treated animals (n=8) were deeply anesthetised with sodium pentobarbitone (Euthatal, Merial Animal Health Ltd, Harlow, UK). See figure 5.2.7 for the timeline of dosing and brain preparation. Animals were then perfused intracardially with ~200 ml of phosphate buffered saline (PBS, Sigma, UK) followed by ~200 ml of 4% paraformaldehyde (PFA, Sigma, UK), the brains were removed and cut into 2 large coronal sections and placed in 4% PFA prior to immunohistochemical analyses. This was to prevent post-mortem brain changes in the tissue and to protect against shrinkage and distortion during dehydration, embedding and sectioning.

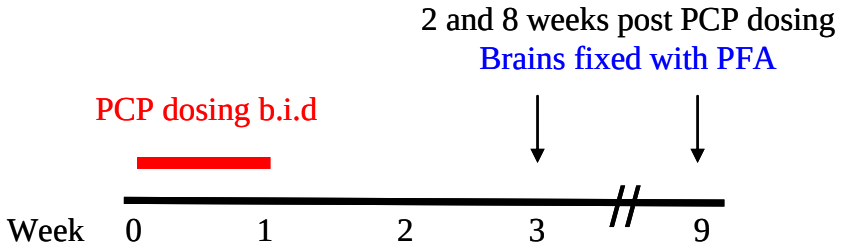


Figure 5.2.7: Timeline of PCP dosing and tissue collection for immunohistochemistry.

5.2.6.2 Wax embedding

The brain samples were rinsed in distilled water four times. The tissue was then dehydrated using increasing concentrations of ethanol: 50% (2 x 1 h), 70% (2 x 1 h), 90% (2 x 1 h) and 100% (2 x 1 h). Brains were then immersed in Histoclear clearing agent (Fisher Scientific, UK) overnight. Subsequently the brain samples were placed in 3 changes 60ºC heated paraffin wax (TissuePrep®2, Fisher Scientific, UK; MP=55-57ºC; 1 x 2 h without pressure and 2 x 2 h under vacuum). Samples were blocked individually in fresh wax. Each brain section was placed face-down onto a plastic tray (3 x 2.5 x 0.5 cm deep) which was then partially filled with molten wax (shown in figure 5.2.8). A perforated plastic holder was then positioned over the tray and molten wax added to attach the holder to the wax. The trays were allowed to solidify overnight. The holder plus wax containing the brain section was then removed from the tray (shown in figure 5.2.8, bottom).

Figure 5.2.8: Photograph of the plastic tray used to wax-embed brain sections (top). The removed tissue holder plus wax containing the brain section is shown in the bottom of the picture.

5.2.6.4 Sectioning

The excess wax was cleared from the outside of the plastic holder in which the tissue was embedded. The sample was clamped into a microtome (Reichert-Jung, model 2030, MI, USA) and serial slices were cut coronally at 10 µm of the dorsal hippocampus (around Bregma -3.3 mm). Samples were placed into a beaker of distilled water within a waterbath, and were then mounted onto slides coated with Poly-L-Lysine. Samples were then left to dry overnight.

5.2.6.5 Parvalbumin staining

Sections were de-waxed in Histoclear for 5 min. The sections were then re-hydrated for 5 min in each of the following solutions: 100%, 90%, 70% ethanol and distilled water.

- *Antigen retrieval*: Slides were placed in a 0.1 M sodium citrate solution (Sigma, UK) and were placed into a microwave for 15 min; at 5 min intervals the sodium citrate solution was topped up. The solution was left to cool for 20 min. Antigen retrieval is needed with previously paraffin embedded tissue sections. Protein cross-links mask the antigenic sites in the tissue, thereby giving weak or false negative staining for detection of proteins. Antigen retrieval breaks protein cross links, therefore unmasking the antigens and epitopes in paraffin embedded tissue sections, thus enhancing the staining intensity of the antibodies.
- *Peroxidase blocking*: Sections were placed into a solution containing: 0.6% H_2O_2 (Sigma, UK), 10% methanol, 0.1% Triton X-100 (Fluka Biochemika, Switzerland), 8.8% phosphate buffer and 80.5% water. The sections were incubated with this peroxidise block for 30 min and were then placed into a 0.01 M phosphate buffer wash for 5 min. Perioxidase blocking denatures

endogenous peroxidase enzymes which would otherwise lead to non-specific staining of the tissue.

- *Protein blocking*: Sections were then placed into a protein block containing: 5% horse serum (Vector Laboratories, USA), 0.4% Triton X-100, 9.5% phosphate buffer and 85.1% water for 60 min. Sections were then washed with 0.01 M phosphate buffer for 5 min. This process blocks non-specific binding of immunoglobulin.
- *Primary antibody*: Sections were then incubated with monoclonal anti-parvalbumin (Swant, Switzerland) at a dilution of 1:5000 in 5% horse serum, 0.4% Triton X-100, 9.5% phosphate buffer and 85.1% water for 36 hours at 4ºC. Sections were washed in 0.01 M phosphate buffer wash for 5 min twice. See step 1 in figure 5.2.9.
- *Secondary antibody*: The bound primary antibody is detected with a secondary antibody labelled with biotin. Sections were incubated for 2 hours with secondary antibody, biotinylated anti-mouse IgG (Vector Laboratories, USA) at 1:200 dilution in 2% horse serum, 0.1% Triton X-100, 9.7% phosphate buffer and 88.2% water. Sections were washed in a 0.01 M phosphate buffer for 5 min. See step 2 in figure 5.2.9.
- *ABC Kit*: This is an immunoperoxidase procedure which increases the sensitivity of the staining. The ABC Kit (Vector Laboratories, USA) contains avidin DH and a biotinylated enzyme complex. Avidin has four binding sites for biotin. The biotinylated enzyme forms a complex with the avidin before being added to the tissue. Once added to the tissue the remaining biotin binding sites on the avidin molecule bind to the biotinylated secondary antibody that is already bound to the tissue. This results in an amplification of the concentration

of enzyme at the antigenic site and therefore increases the staining intensity. The sections were processed by the ABC Kit for 2 hours at room temperature before being washed in 0.01 M phosphate buffer for 5 min. See step 3 in figure 5.2.9.

- *DAB Kit*: The peroxidase was then visualised by incubation with a substrate for the enzyme. In this case the chromagen diaminobenzadine (DAB) kit (Vector Laboratories, USA) was used. Tissue sections were incubated for 15 min at room temperature and were then washed with distilled water before mounting. See step 4 in figure 5.2.9.

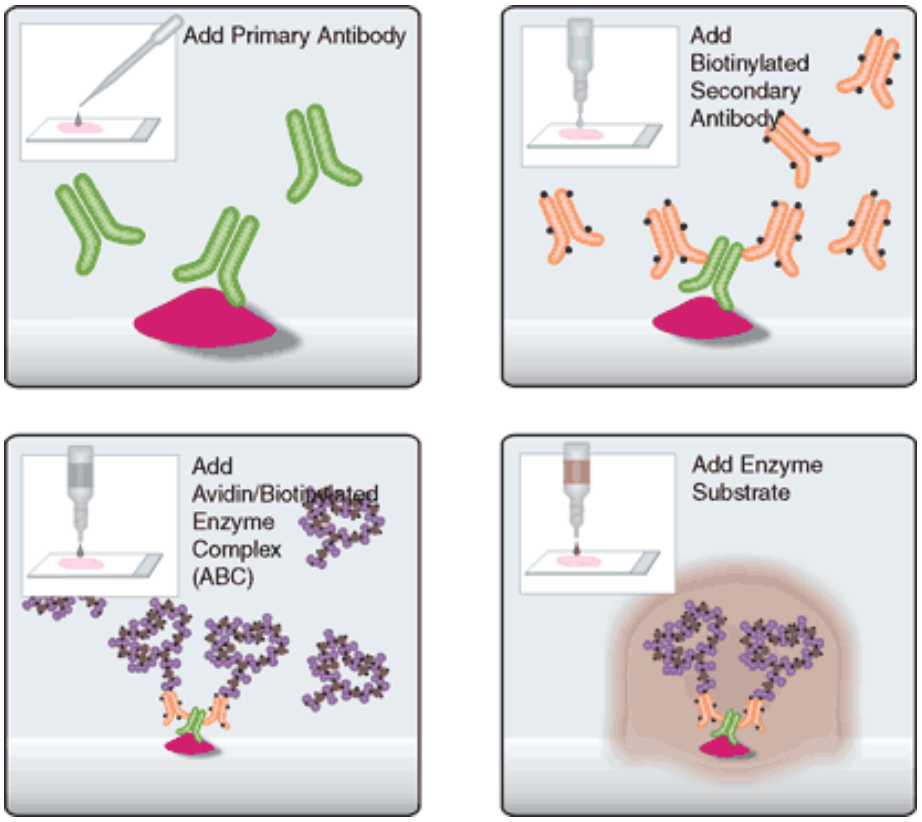


Figure 5.2.9: Schematic diagram of parvalbumin staining detailed in section 5.2.6.5 (diagram from vectorlabs.com).

5.2.6.7 Counting and analysis

Stained sections were scanned at 4x magnification using an Olympus BX51 microscope interfaced to an Image ProPlus (version 6.3) analysis system (Media Cybernetics, USA) via a JVC 3-CCD video camera. Estimations of neuronal density (cells/mm^2) were carried out in 6 sections per animal (~60 µm apart), using both images of left and right hippocampus. Immunostained neurons were counted at a higher magnification in defined complete sub-fields of each region according to the Atlas of Paxinos and Watson (1998). Within the hippocampal formation, the CA2 plus CA3 region was examined. Images and cells were scanned and counted blind to the experimenter to avoid bias.

5.2.7 Data and statistical analysis

Area under the curve was calculated for the LMA data (for the 2 hour period following the acute challenge) using the trapezoid rule, and analysed using a one-way ANOVA followed by post-hoc Bonferroni's multiple comparison test. For electrophysiology the area under the curve, peak power and peak frequency of the generated power spectra were analysed by Student's Independent t-tests. Parvalbumin-containing cell density data is expressed as mean ± SEM. Differences between sub-chronic vehicle and PCP treated animals were statistically analysed by Student's Independent t-test.

5.3 Results

5.3.1 PCP sensitisation

Treatment had no effect on the habituation stage (first hour) of the sensitisation experiment. A one-way ANOVA on the area under the curves and total LMA count over the 2 hour period following the acute challenge revealed a significant effect of treatment ($F_{3,31}$=21.96, P<0.001) and ($F_{3,31}$=20.46, P<0.001) respectively. Bonferroni's multiple comparison test on the area under the curves (figure 5.3.1a) and the total LMA counts (figure 5.3.1b) showed that following an acute challenge with PCP (2 mg/kg) the PCP-PCP group was significantly more active compared to veh-veh, veh-PCP and PCP-veh (P<0.001) groups. There were no significant differences between any other groups.

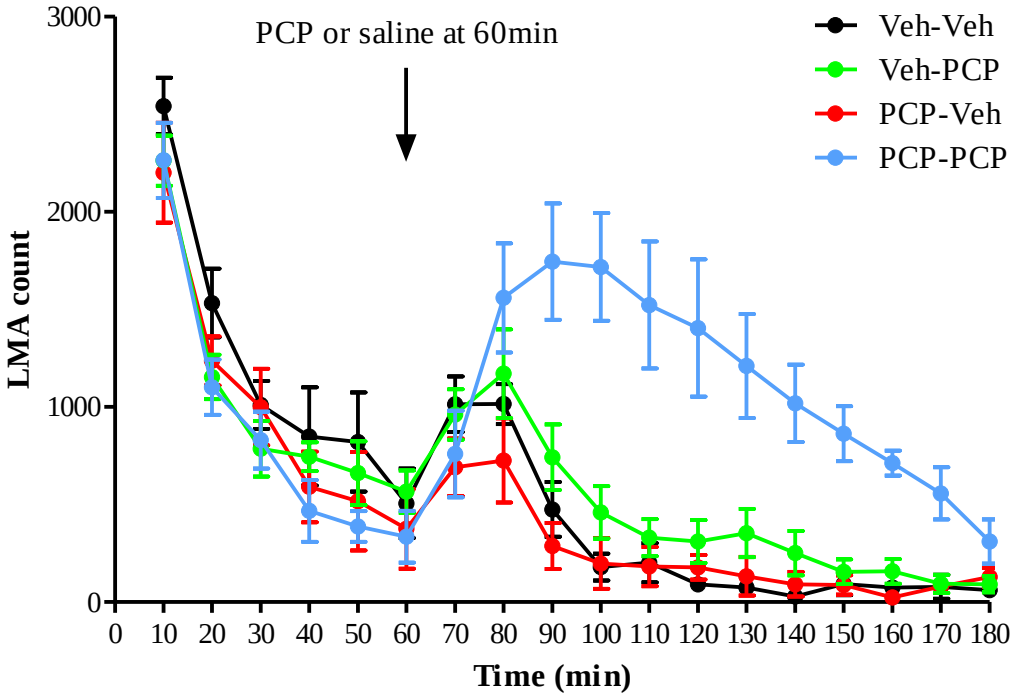


Figure 5.3.1a Locomotor activity in vehicle or sub-chronic PCP-treated rats following acute challenge with PCP (2 mg/kg) measured over a 3 hour period at 10-min intervals.

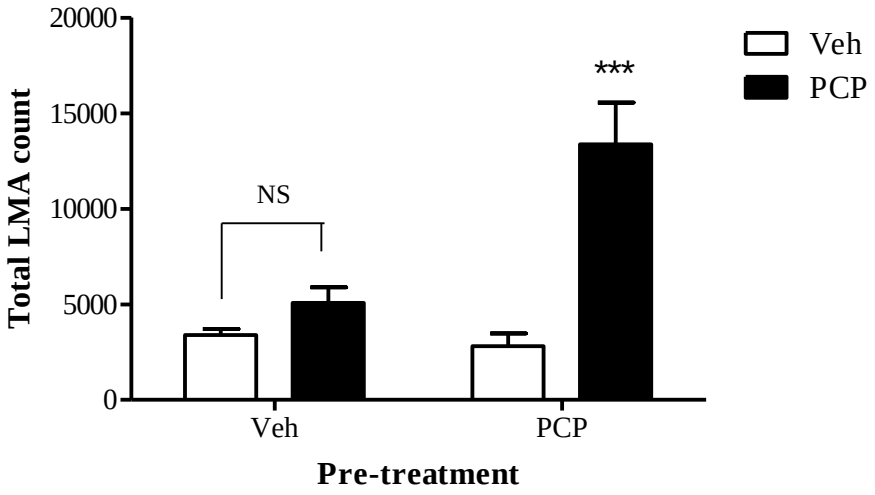


Figure 5.3.1b: Locomotor activity in vehicle or sub-chronic PCP-treated rats following acute challenge with PCP (2 mg/kg) for the 2 hour period following the acute challenge (n=8). Post-hoc Bonferroni's multiple comparison test showed that the PCP-PCP group was significantly more active compared to the veh-veh, veh-PCP and PCP-veh (***P<0.001) groups. Acute administration of PCP in vehicle rats did not induce significant hyperactivity.

5.3.2 Electrophysiology pilot studies

Pilot studies were carried out in 6 rats dosed with saline (1 ml/kg, i.p.) for 7 days followed by a 7-day washout period. Slices were discarded and were not included in the analysis if there was no apparent peak on the power spectrum. Initially it was necessary to determine which concentration of the agonist would be required to evoke stable oscillations in the two regions of interest i.e. CA3 and PFC. Increasing concentrations of kainate were bath applied to the slice until a maximum power was reached. An example trace showing increasing power with increasing concentrations of kainate in the CA3 is shown in figure 5.3.2a and the power spectrum is shown in figure 5.3.2b.

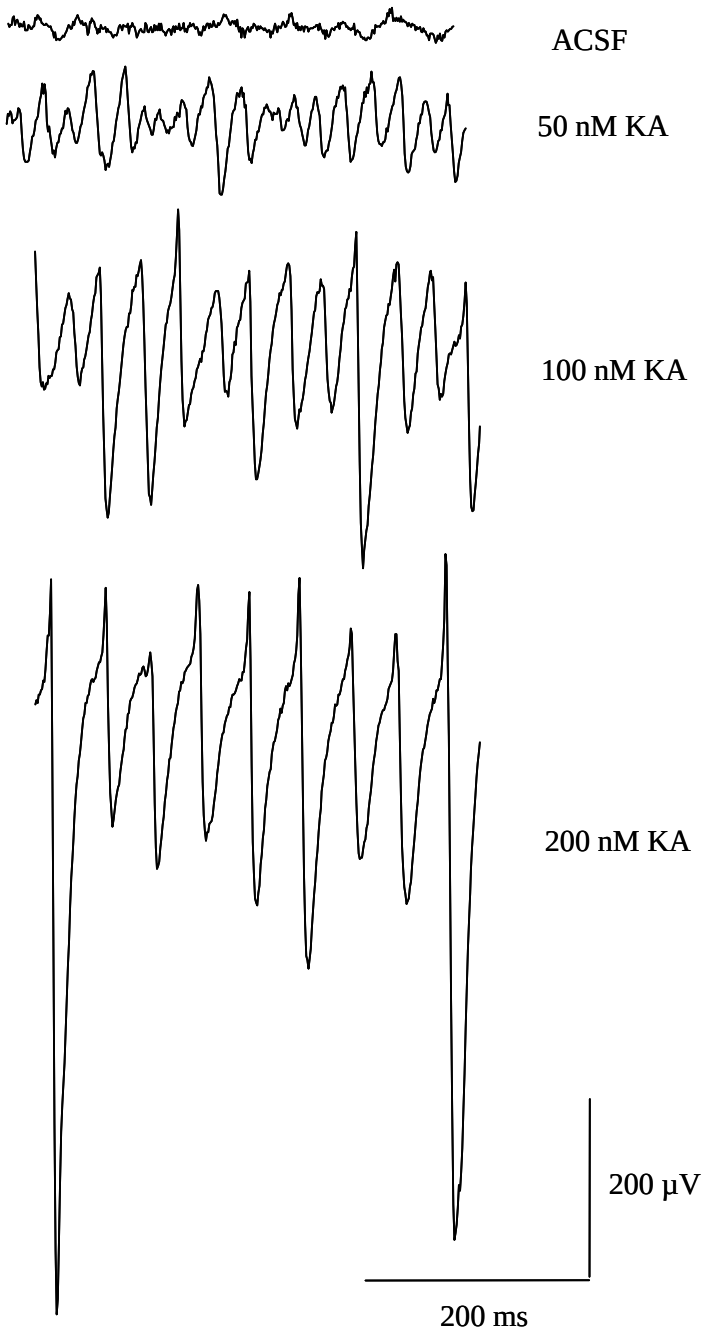


Figure 5.3.2a: Gamma oscillations driven by kainite recorded in the CA3. The traces show recordings from 1 slice in response to increasing concentration of kainate.

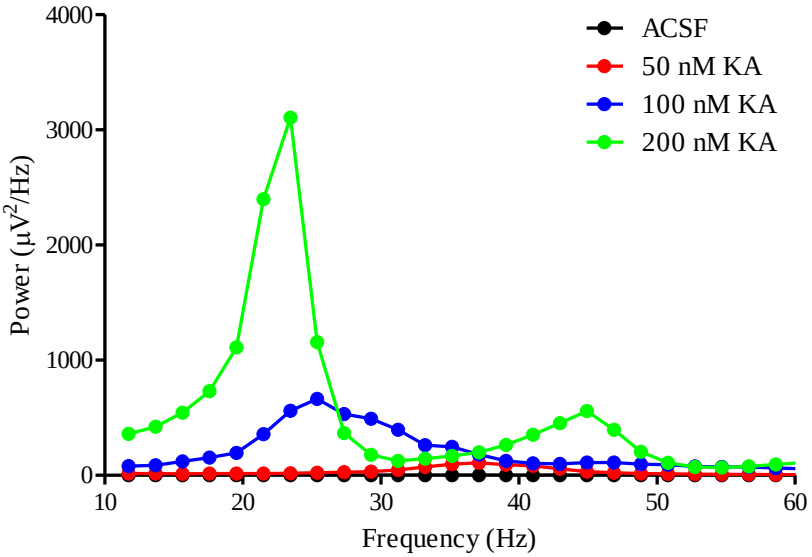


Figure 5.3.2b: Power spectral analysis of gamma oscillations from 1 slice in the CA3 induced by increasing concentrations of kainate.

The traces show that the power was increased at 200 nM kainite, however, these oscillations did not remain stable over a 3 hour period therefore a lower concentration of 100 nM was used routinely in this region.

The same procedure was repeated in PFC slices and it was determined that 400 nM kainate and 10 μM carbachol was required to induce stable oscillations, these experiments were conducted in different slices. Increasing concentrations of kainate were bath applied to the slice until a maximum power was reached. Following this carbachol was also bath applied in increasing concentrations. An example trace showing increasing power with increasing concentrations of in kainate in the PFC is shown in figure 5.3.3a and the power spectrum is shown in figure 5.3.3b. Once it was determined that 400 nM kainate was needed, increasing concentrations of carbachol were added, this example trace is shown in 5.3.4a and the power spectrum is shown in figure 5.3.4b.

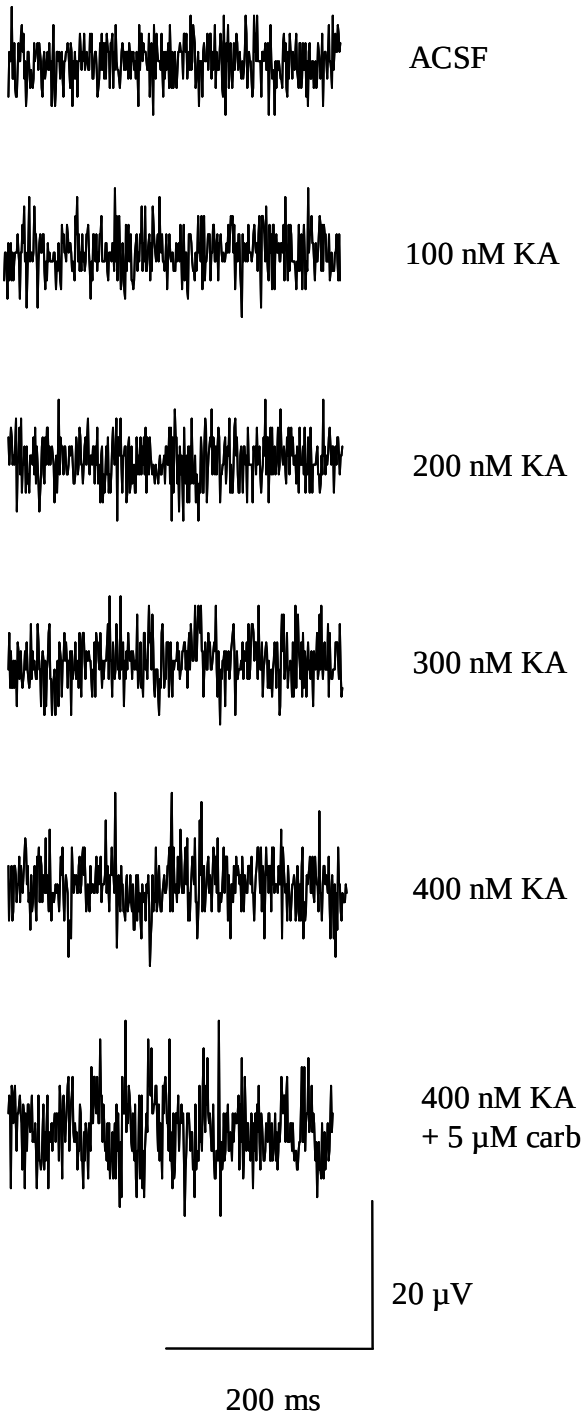


Figure 5.3.3a: Gamma oscillations driven by kainate recorded in the PFC. The traces show recordings from 1 slice in response to increasing concentrations of kainate and then 5 µM carbachol.

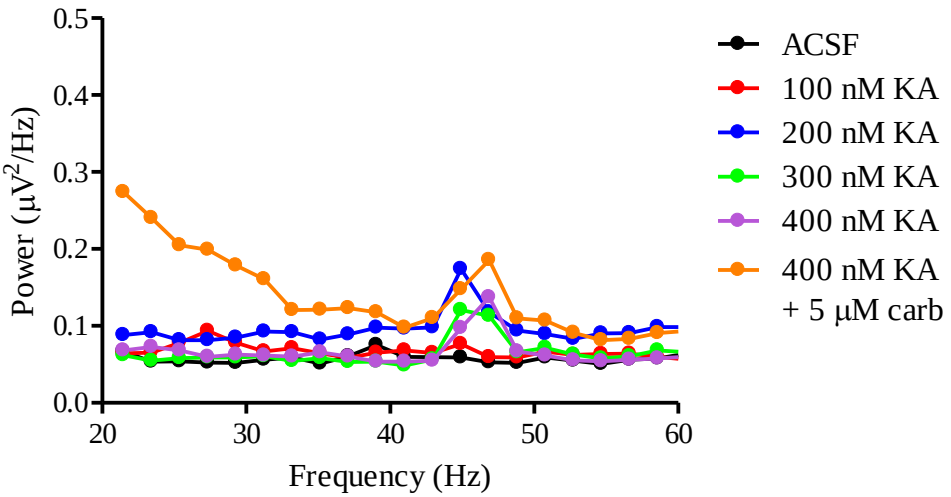


Figure 5.3.3b: Power spectral analysis of gamma oscillations from 1 slice in the PFC induced by increasing concentrations of kainate and then 5 µM carbachol.

The traces show that the power was increased at 200 nM kainate, compared to 400 nM kainate alone, however, these oscillations did not remain stable over a 3 hour period. Whereas oscillations induced with 400 nM kainate did remain stable, therefore this concentration of 400 nM was used routinely in this region. It also appeared that carbachol (5 µM) in combination with 400 nM kainate produced an enhancement of these oscillations; subsequently increasing concentrations of carbachol were tested.

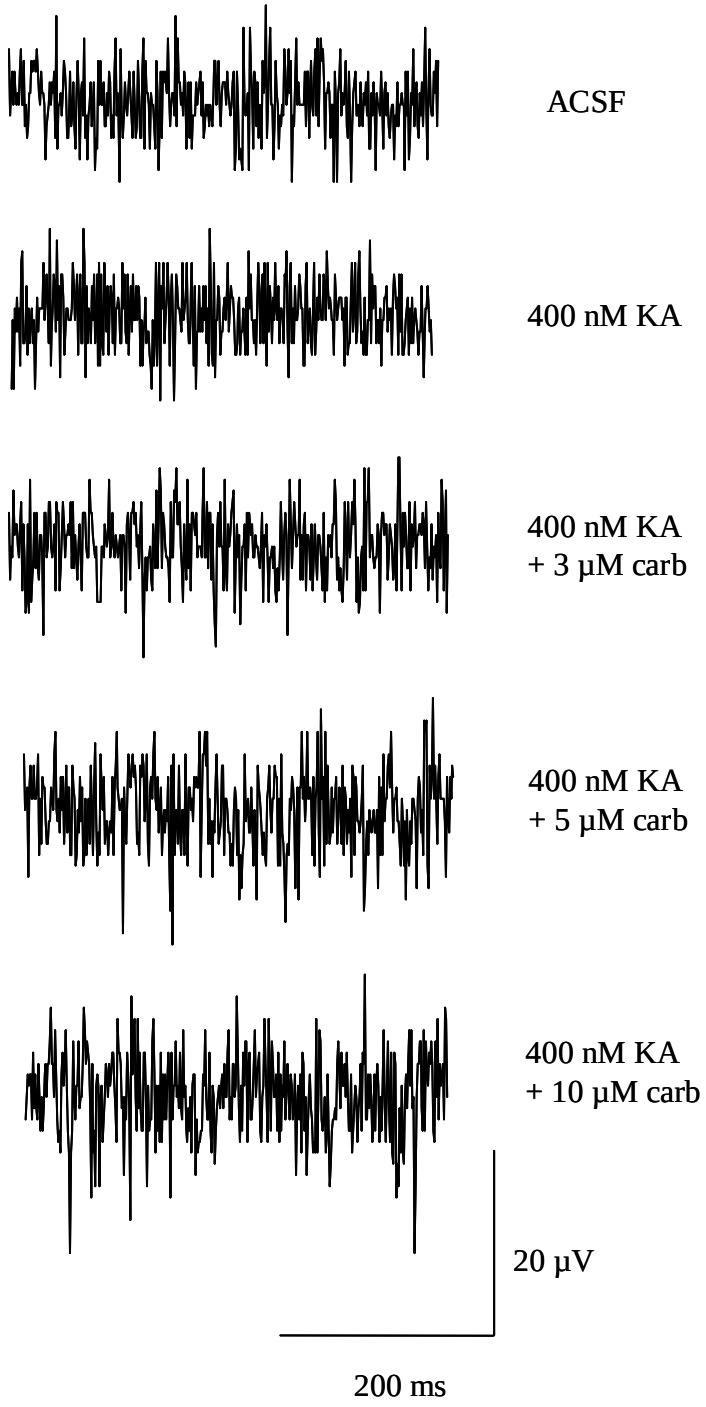


Figure 5.3.4a: Gamma oscillations driven by 400 nM kainate and carbachol recorded in the PFC. The traces show recordings from 1 slice in response to increasing concentrations of carbachol.

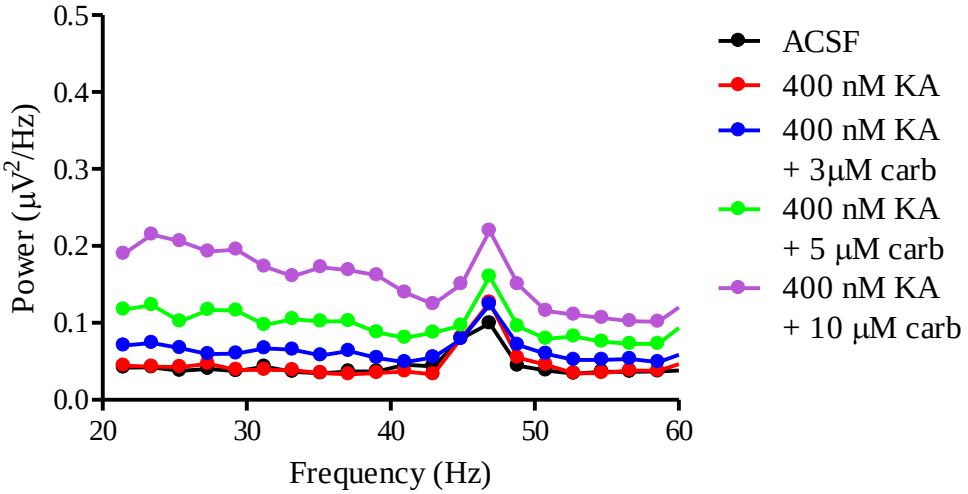


Figure 5.3.4b: Power spectral analysis of gamma oscillations from 1 slice in the PFC induced by 400 nM kainate and increasing concentrations of carbachol.

In all cases the experiments were carried out blind to the treatment group to avoid bias. The treatment group remained blinded until the end of each day.

Careful observation throughout the duration of the 6-week electrophysiology experiments revealed a differential effect of PCP over time. Therefore, the data was analysed in 2 groups, with data from 2-5 weeks after PCP treatment, and then 6-8 weeks grouped together for CA3 and weeks 3-5 and 6-8 were grouped together for PFC. Experiments in week 2 (following PCP treatment) were not carried out in the PFC as the second rig was in the process of being developed.

5.3.3. Gamma Oscillations in CA3

Gamma oscillations were obtained from 39/39 slices from the vehicle-treated group, and 40/44 slices from the PCP-treated group. Power spectra were generated between 20 and 80 Hz. The power spectra for weeks 2-5 are shown in figure 5.3.5a and weeks 6-8 are shown in weeks 5.3.5.b. The difference between the time bins in vehicle-treated rats is shown in figure 5.3.6a and figure 5.3.6b shows the difference in PCP-treated rats.

Representative traces of the oscillations are shown in figure 5.3.7. The traces selected were form the slice with largest oscillations in all cases.

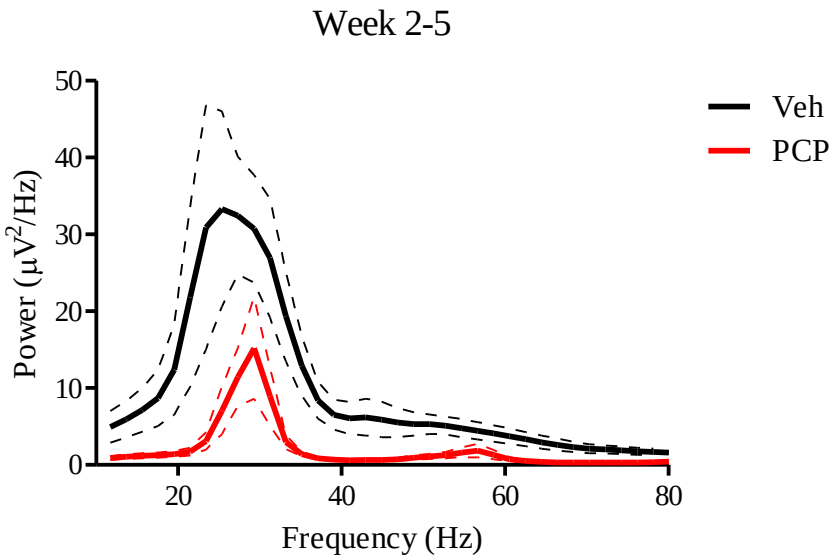


Figure 5.3.5a: Power spectra for vehicle and PCP-treated rats between 20 and 80 Hz for weeks 2-5 in the CA3. Solid lines show the mean and dotted lines show the SEM.

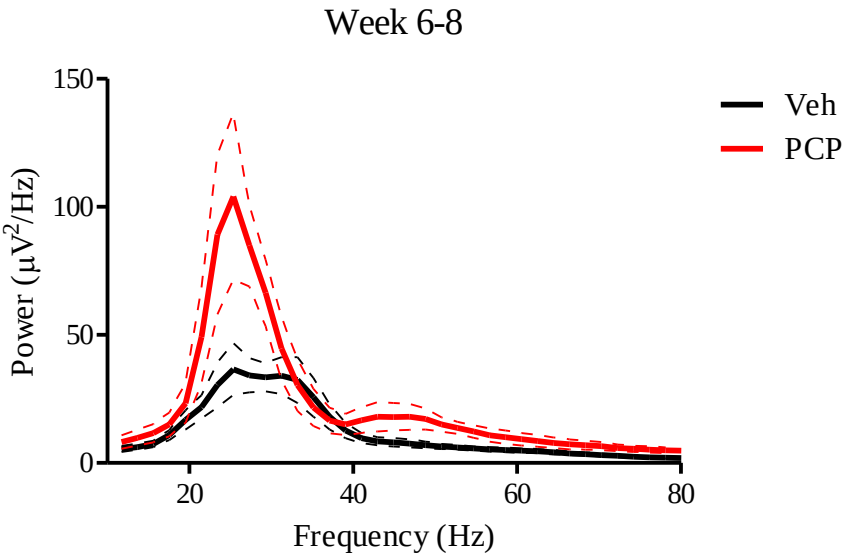


Figure 5.3.5b: Power spectra for vehicle and PCP-treated rats between 20 and 80 Hz for weeks 6-8 in the CA3. Solid lines show the mean and dotted lines show the SEM.

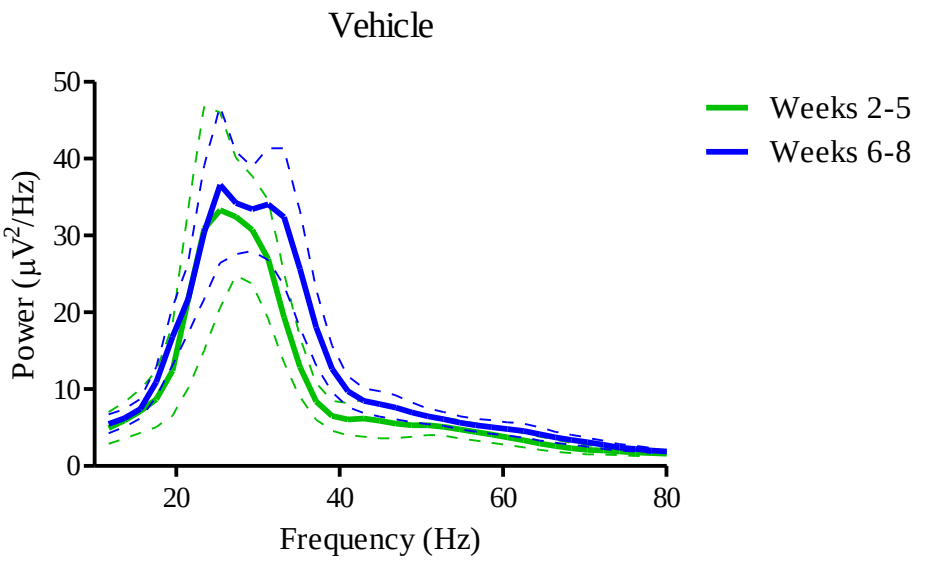


Figure 5.3.6a: Power spectra between 20 and 80 Hz for vehicle-treated rats in the CA3, comparing weeks 2-5 with weeks 6-8. Solid lines show the mean and dotted lines show the SEM.

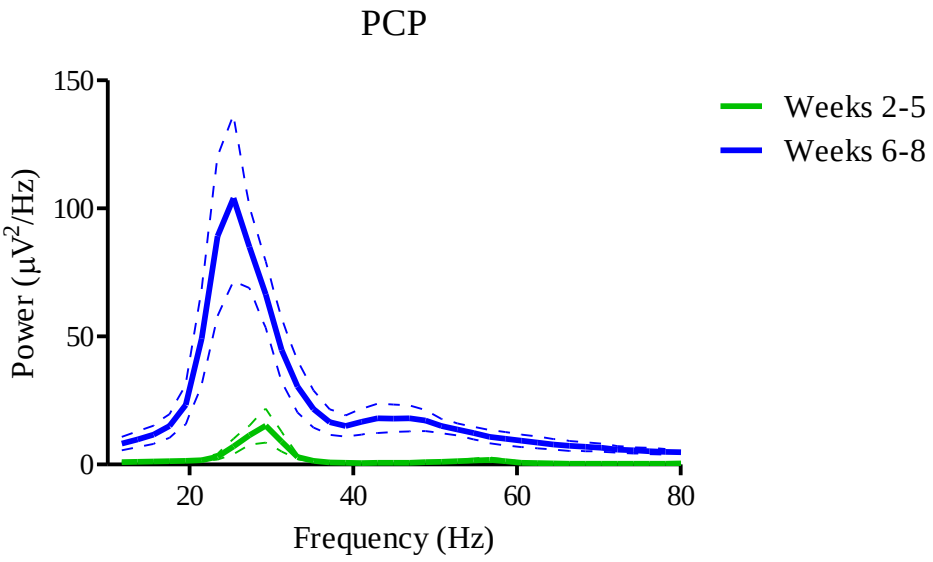


Figure 5.3.6b: Power spectra between 20 and 80 Hz for PCP-treated rats in the CA3, comparing weeks 2-5 with weeks 6-8. Solid lines show the mean and dotted lines show the SEM.

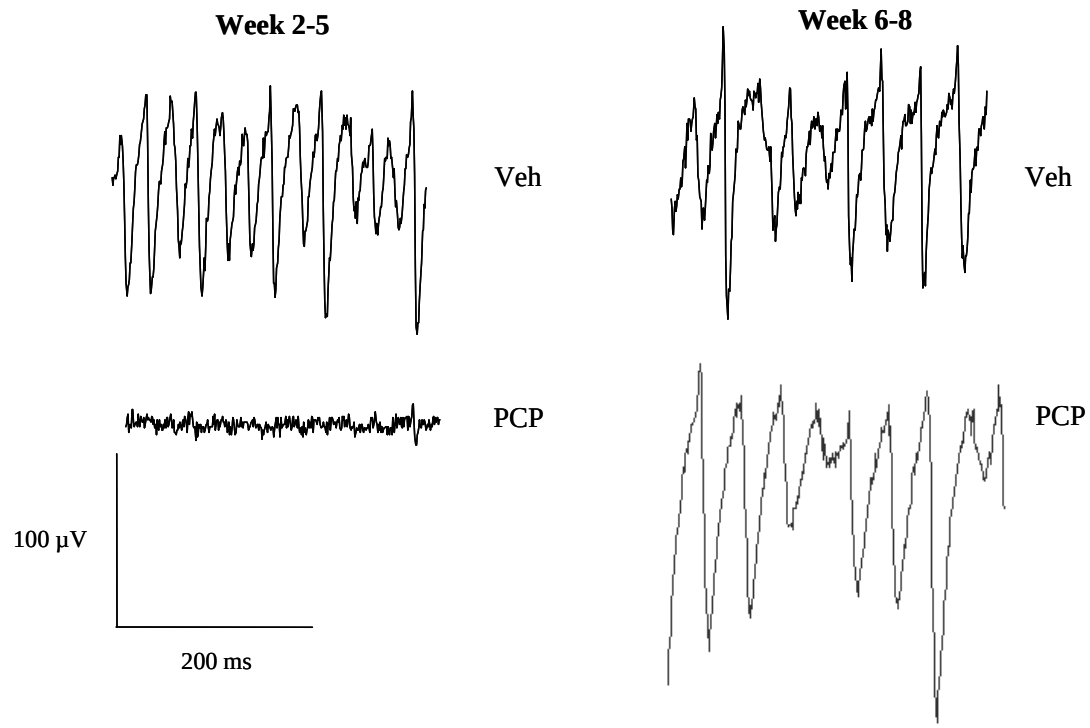


Figure 5.3.7: Representative traces showing oscillations in the CA3 in vehicle and PCP-treated rats in weeks 2-5 and 6-8.

The area under the curve of the power spectrum is shown in figure 5.3.8a. Independent t-tests on the area under the curve of the power spectra in weeks 2-5 showed that PCP-treated rats were significantly lower than vehicle-treated rats ($P<0.05$), and in weeks 6-8 PCP-treated rats were significantly higher compared to the vehicle group ($P<0.05$). This effect is also shown by the power spectra in figures 5.3.5a and 5.3.5b. There was no significant difference in area under the curve between vehicle-treated rats in weeks 2-5 compared to weeks 6-8 ($P=0.54$), however, in PCP-treated rats there was a significant increase in the area under the curve of the power spectra in weeks 6-8 compared to weeks 2-5 (*$P<0.001$; figure 5.3.8a). This effect is also clear from the power spectrum in figure 5.3.6b.

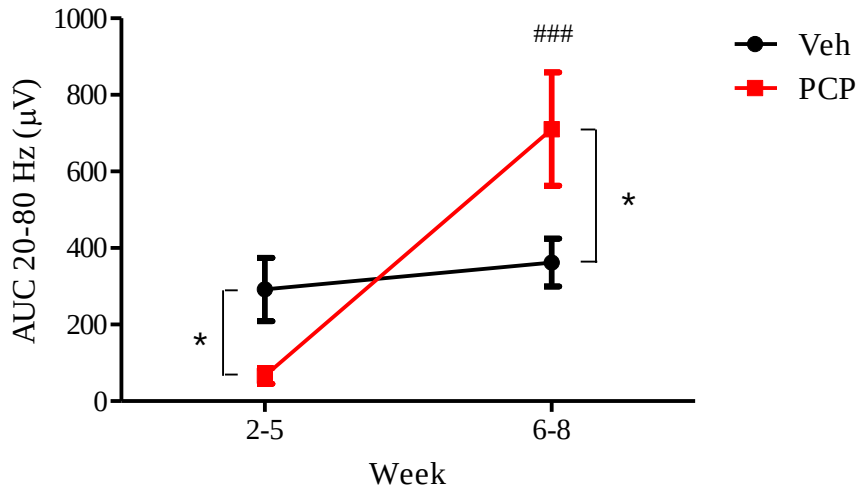


Figure 5.3.8a: Area under the curve of the power spectra for vehicle and PCP-treated rats. Recordings taken from the CA3. Data is shown as mean ± SEM. Independent t-tests showed that PCP-treated rats were significantly different to the vehicle group (*$P<0.05$). $^{###}P<0.001$ indicates a significant difference in PCP-treated rats between 2-5 weeks and 6-8 weeks.

There were no significant differences in peak frequency of the power spectra between vehicle and PCP-treated rats at weeks 2-5 or weeks 6-8, P=0.89 and P=0.49 respectively (figure 5.3.8b). There were also no differences between the time points in vehicle or PCP-treated rats, P=0.70 and P=0.10 respectively.

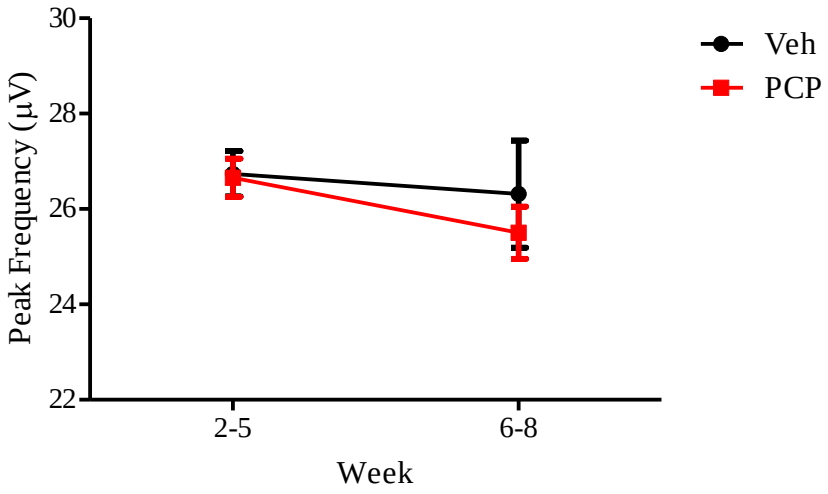


Figure 5.3.8b: Peak frequency of the power spectra for vehicle and PCP-treated rats. Recordings taken from the CA3. Data is shown as mean ± SEM. Independent t-tests revealed no significant differences.

The peak power of the power spectra is shown in figure 5.3.8c. Independent t-tests on the peak power in weeks 2-5 showed no significant difference between vehicle and PCP-treated rats (P=0.07), however in weeks 6-8 PCP-treated rats had significantly higher power compared to the vehicle group (P<0.05). There was no significant difference in peak power between vehicle-treated rats in weeks 2-5 compared to weeks 6-8 (P=0.92), however, in PCP-treated rats there was a significant increase in the peak power of the power spectra in weeks 6-8 compared to weeks 2-5 (P<0.001; figure 5.3.8c).

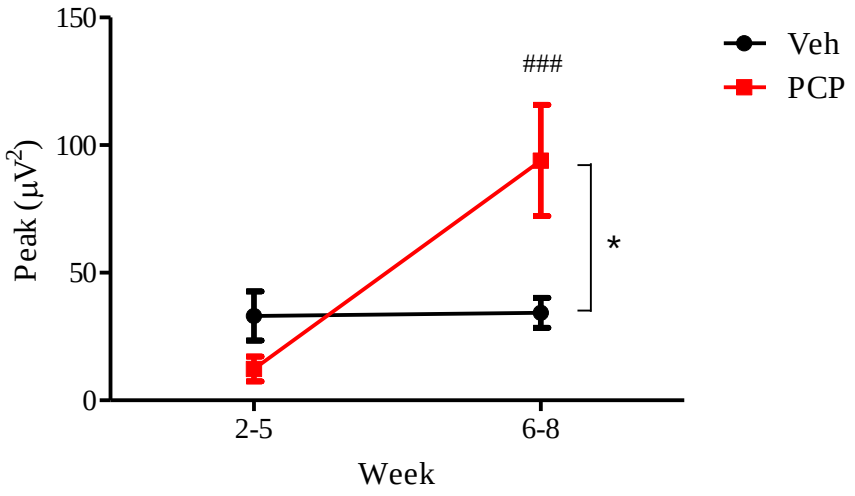


Figure 5.3.8c: Peak power of the power spectra for vehicle and PCP-treated rats. Recordings taken from the CA3. Data is shown as mean ± SEM. Independent t-tests showed that PCP-treated rats were significantly different to the vehicle group at weeks 6-8 (*P<0.05), and that there was a significant difference in PCP-treated rats between 2-5 weeks and 6-8 weeks ($^{###}$P<0.001).

5.3.4. Gamma Oscillations in PFC

Gamma oscillations were obtained from 21/22 slices from the vehicle-treated group, and 34/34 slices from the PCP-treated group. Power spectra were generated between 20 and 80 Hz. The power spectra for weeks 3-5 are shown in figure 5.3.9a and weeks 6-8 are shown in weeks 5.3.9b. The difference between the time bins in vehicle-treated rats is shown in figure 5.3.10a and figure 5.3.10b shows the difference in PCP-treated rats. In all cases there were no significant differences between vehicle and PCP-treated rats, and no differences between the time points. Figure 5.3.11 show representative traces of the oscillations. The traces selected were form the slice with largest oscillations in all cases.

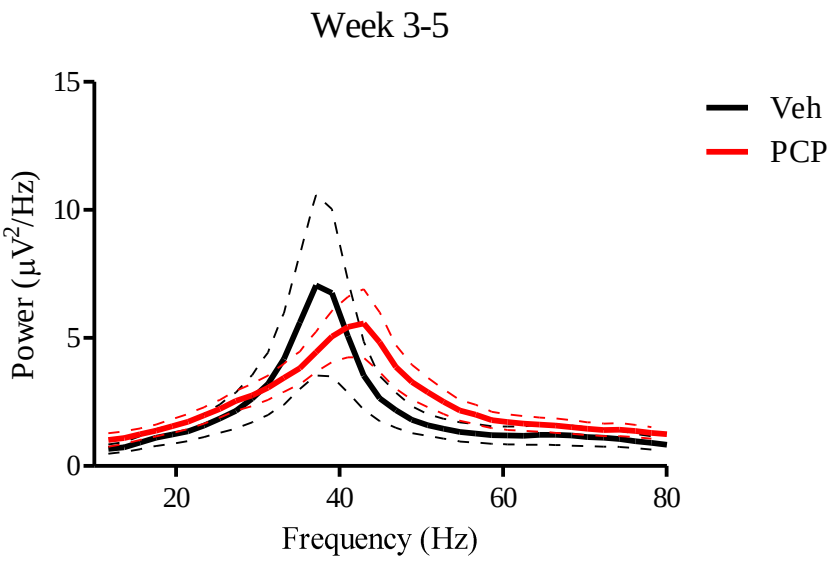


Figure 5.3.9a: Power spectra for vehicle and PCP-treated rats between 20 and 80 Hz for weeks 3-5 in the PFC. Solid lines show the mean and dotted lines show the SEM.

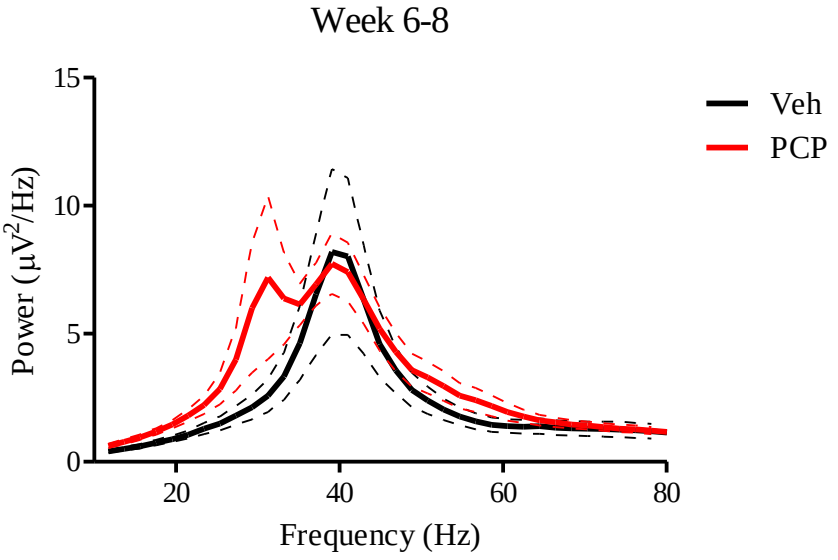


Figure 5.3.9b: Power spectra for vehicle and PCP-treated rats between 20 and 80 Hz for weeks 6-8 in the PFC. Solid lines show the mean and dotted lines show the SEM.

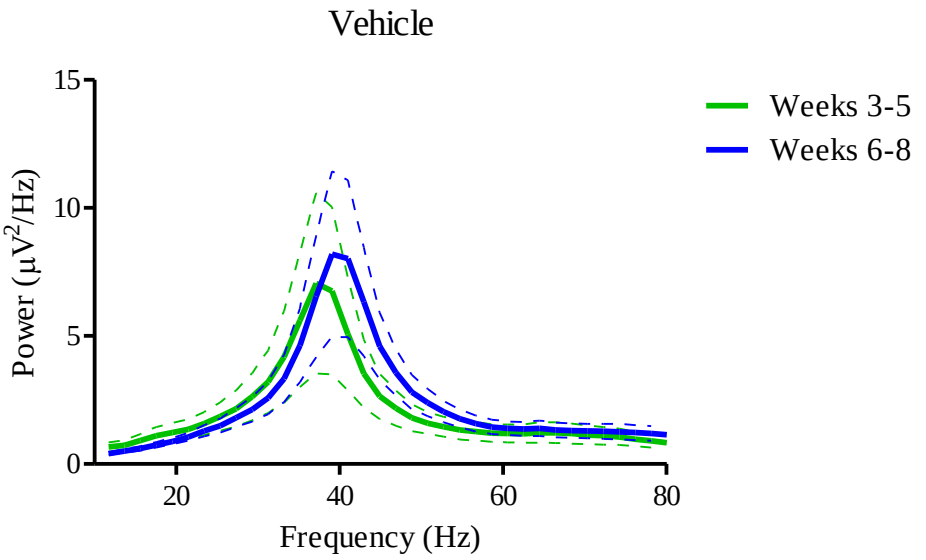


Figure 5.3.10a: Power spectra between 20 and 80 Hz for vehicle-treated rats in the PFC, comparing weeks 3-5 with weeks 6-8. Solid lines show the mean and dotted lines show the SEM.

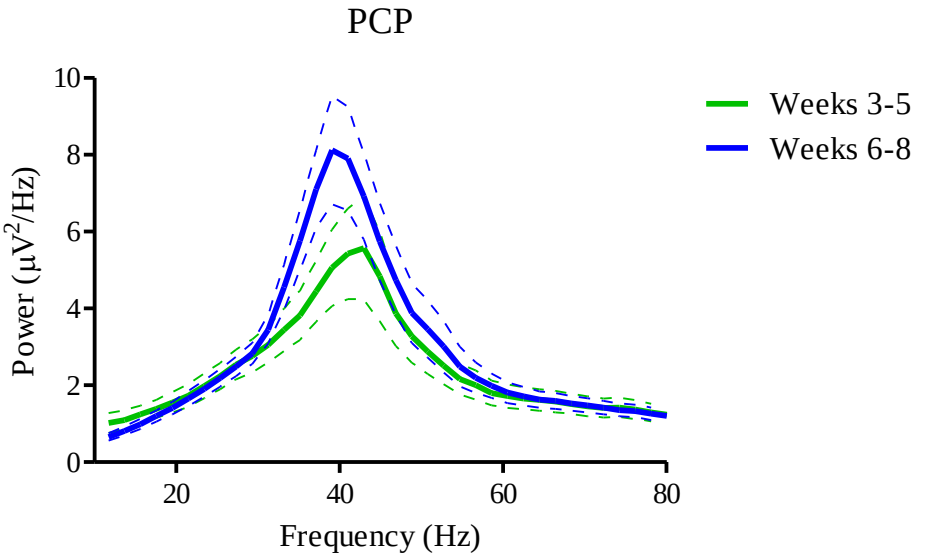


Figure 5.3.10b: Power spectra between 20 and 80 Hz for PCP-treated rats in the PFC, comparing weeks 3-5 with weeks 6-8. Solid lines show the mean and dotted lines show the SEM.

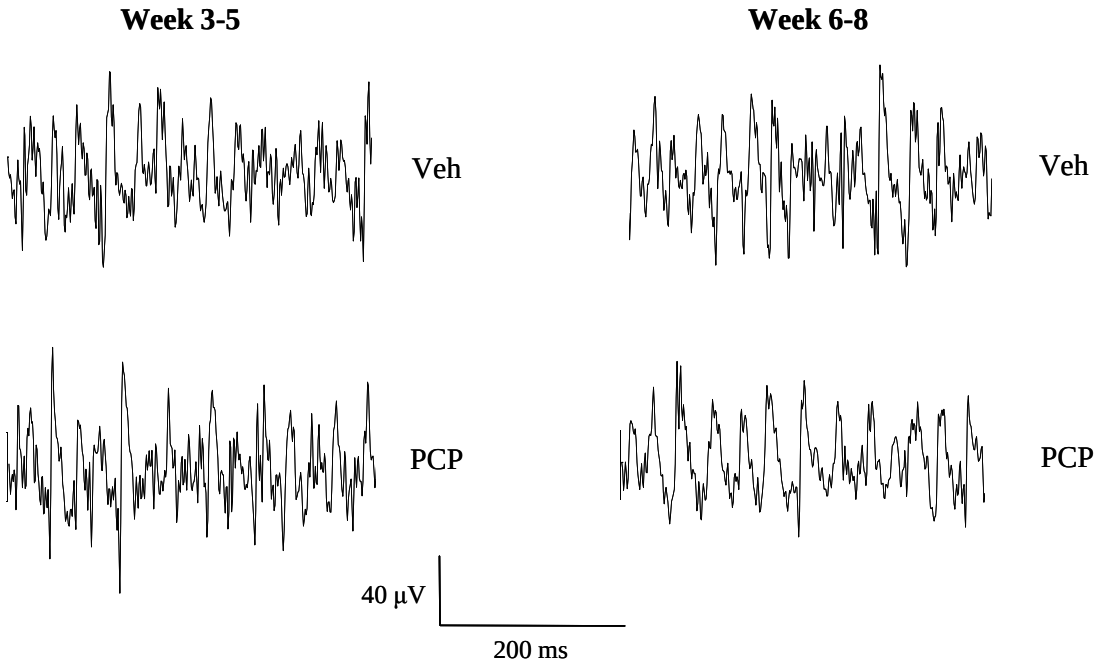


Figure 5.3.11: Representative traces showing oscillations in the PFC in vehicle and PCP-treated rats in weeks 2-5 and 6-8.

The area under the curve of the power spectrum is shown in figure 5.3.12a. The peak frequency is shown in figure 5.3.12b and the peak power is shown in figure 5.3.12c. Independent t-tests revealed no significant difference between treatment groups or time points in vehicle or PCP-treated rats.

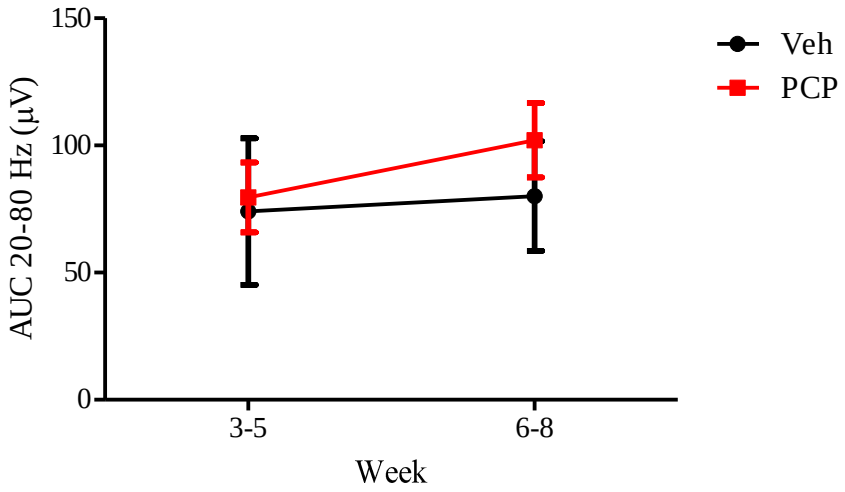


Figure 5.3.12a: Area under the curve of the power spectra for vehicle and PCP-treated rats. Recordings taken from the PFC. Data is shown as mean ± SEM.

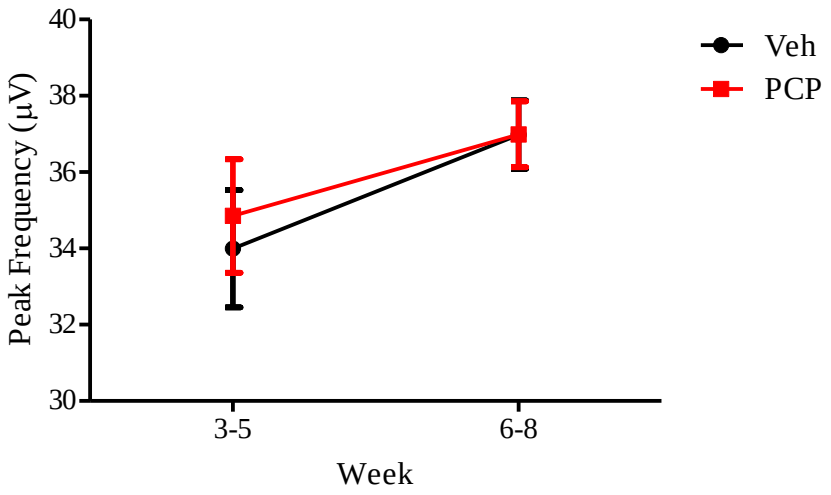


Figure 5.3.12b: Peak frequency of the power spectra for vehicle and PCP-treated rats. Recordings taken from the PFC. Data is shown as mean ± SEM.

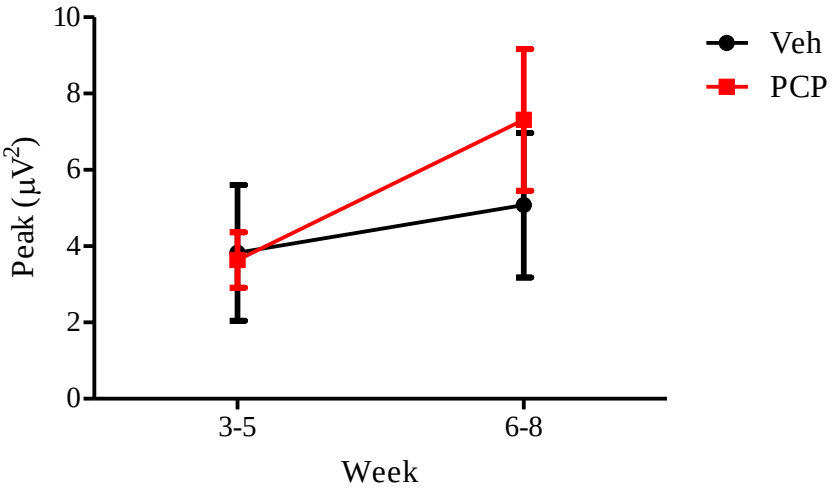


Figure 5.3.12c: Peak power of the power spectra for vehicle and PCP-treated rats. Recordings taken from the PFC. Data is shown as mean ± SEM.

5.3.5 Immunohistochemistry for parvalbumin

Following the *in vitro* electrophysiology, it was decided to examine parvalbumin expression in the CA2/3 region of the hippocampus. Figure 5.3.13 shows the distribution of parvalbumin immunoreactivity in a coronal section of the region at 4× magnification. *In vitro* analysis of rat brains 2 weeks post PCP treatment showed a reduction in parvalbumin immunoreactive cell density in the CA2/3 region of the hippocampus of PCP-treated rats (P=0.058; figure 5.3.14). This reduction was not observed 8 weeks following sub-chronic PCP treatment (P=0.981). There were no differences between the vehicle groups at 2 and 8 weeks (P=0.777) nor in the PCP-treated groups (P=0.141).

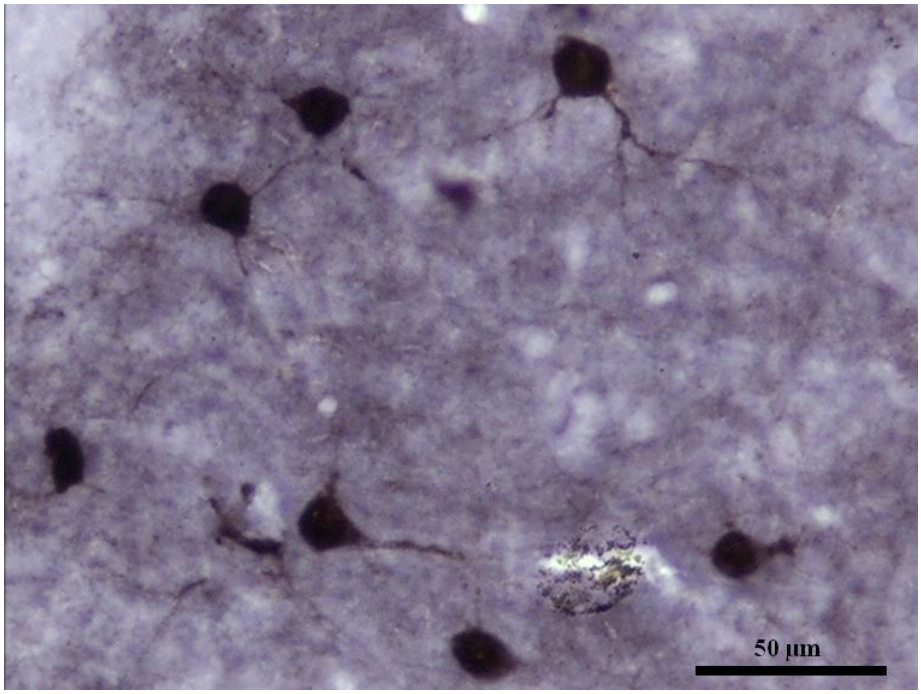


Figure 5.3.13: Parvalbumin immunoreactivity in the CA2/3 region of the hippocampus. Brightfield photomicrograph of a coronal section showing the distribution of parvalbumin immunoreactivity throughout the region at 4× magnification.

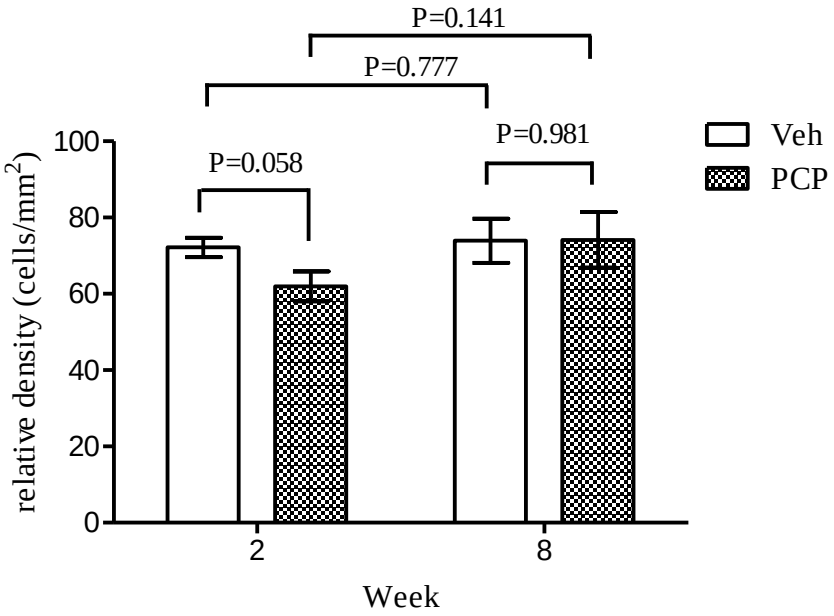


Figure 5.3.14: Relative density (cells/mm^2) of parvalbumin immunoreactive neurons in the CA2/3 region of the hippocampus of vehicle and sub-chronic PCP-treated rats at 2 and 8 weeks post treatment. Data are expressed as mean density ± SEM. Week 2: vehicle *n* = 6, PCP-treated *n* = 7, week 8: vehicle *n* = 5, PCP-treated *n* = 6. *N* indicates the number of rats (means were calculated from 6 sections per animal). P values indicate the results of independent t-tests.

5.4 Discussion

The principal findings from this chapter are that sub-chronic PCP treatment has no effect on gamma oscillations in the PFC. In the CA3 region of the hippocampus, oscillations were initially significantly reduced in the sub-chronic PCP group compared to the vehicle-treated group; however, in the second half of the experiment there was a significant increase in gamma oscillations compared to the vehicle group. Following the *in vitro* electrophysiology results, it was decided to investigate parvalbumin immunoreactive (IR) cell density in the CA2/3 region of the hippocampus. It was found that sub-chronic PCP-treated rats showed reduced parvalbumin IR cell density at 2 weeks following PCP treatment, however, cell density was unchanged 8 weeks following PCP treatment.

5.4.1 PCP sensitisation

Following an acute challenge with PCP, an increase in locomotor activity in the sub-chronic PCP treated rats was observed, with no effect in vehicle treated animals. Previous studies in male rats, using similar treatment regimes at comparable doses have reported similar findings (Kalinichev *et al.*, 2008). This sensitisation in the sub-chronic PCP treated animals may be related to the disruption of GABAergic interneurons in the medial prefrontal cortex (Abekawa *et al.*, 2007) leading to dysregulation of the striatal dopaminergic system (see Jentsch and Roth, 1999). Taken together these results indicate that the sub-chronic PCP regimen was successful in producing behavioural changes in these animals thus validating the treatment prior to electrophysiology experiments.

5.4.2 PCP effects on gamma oscillations in the CA3 region of the hippocampus

Gamma oscillations are generated in the CA3 region but not the CA1 region of the hippocampus, which lacks recurrent connectivity and the high level of kainate receptors (KAR) expression in area CA3 (Bureau *et al.*, 1999; Fisahn, 1999). It has been widely reported that gamma oscillations are decreased in patients with schizophrenia, and stimulation or working memory load-dependent increases in gamma oscillations in healthy controls are absent in patients during cognitive tasks (see Başar and Günterkin, 2008). However, it has been suggested that gamma band activity in schizophrenia patients is complex, region-dependent and symptom specific. It has further been suggested that negative symptoms are associated with decreases in left hemisphere synchrony, while positive symptoms were found to be associated with increased gamma band power in right hemisphere regions (Lee *et al.*, 2003a, b).

The results presented in this chapter demonstrate that in the CA3 region of the hippocampus oscillations were initially reduced in the sub-chronic PCP group, however, in the second half of the experiment there was a significant increase in gamma oscillations. The rodent data investigating PCP and gamma oscillation is minimal. However, studies have been performed using other NMDA antagonists, with varying results. To our knowledge this is the first study to examine the effects of sub-chronic PCP on gamma oscillations and this is also the first slice preparation experiment in animals to carry out a time-course experiment following PCP treatment.

It was shown *in vivo* using EEG recordings that rats which were pre-treated with acute PCP or methamphetamine exhibited increased gamma band power in the hippocampus 2 hours following drug administration (Ma and Leung, 2000). It has been postulated that acute block of NMDA receptors will dampen the activity of interneurons (Homayoun and Moghaddam, 2007) and thereby disinhibit pyramidal neurons (Grunze

et al., 1996). Consistent with this theory firing rates of pyramidal neurones were potentiated in the PFC in freely moving rats following acute treatment with MK-801 (Jackson *et al.,* 2004). Chronic ketamine was shown to reduce power and amplitude of gamma oscillations in the PFC; slices were prepared following the final dose of ketamine (Zhang *et al.,* 2008). These authors attribute this effect to a different mechanism that reduced GAD67 (Zhang *et al.,* 2008; Lisman *et al.,* 2008). The downregulation of GAD67 produced by ketamine is associated with accumulation of superoxide; inhibition of the superoxide-synthesising enzyme prevents the loss of GAD67 immunoreactivity in parvalbumin interneurons (Behrens *et al.,* 2007). Taken together the results from rodent studies with NMDA antagonists are inconsistent, but the alteration in gamma oscillations could depend on whether the treatment with NMDA antagonists is acute or sub-chronic. All of the aforementioned studies have used different species of rats and have been males; therefore, differences in the present data may be observed due to the use of female hooded-Lister rats, and also due to the seven day sub-chronic PCP dosage regimen.

Studies involving transgenic mouse models of psychiatric disorders have also been carried out. Alterations in gamma oscillations have been reported in Alzheimer's disease (Koenig *et al.,* 2005; Uhlhaas and Singer, 2006). Mice over-expressing human amyloid precursor protein (APP) exhibited a reduction in hippocampal gamma activity at 8 months of age compared to age-matched wild-type mice (Driver *et al.,* 2007). Sensorimotor-gating deficits and neurochemical changes resembling those in schizophrenia are observed in lysophosphatidic acid 1 receptor (LPA-1) knock-out mice (Harrison *et al.,* 2003). Lysophosphatidic acid 1 receptor (LPA-1) knock-out mice were shown to have reduced gamma oscillations and a decrease in parvalbumin neurons in the medial entorhinal cortex (Cunningham *et al.,* 2006).

5.4.3 PCP effects on gamma oscillations in the PFC

Sub-chronic PCP treatment had no apparent effect on gamma oscillations in the PFC, and there were no significant differences over time in either the vehicle or sub-chronic PCP-treated rats. In another study, chronic ketamine was shown to reduce power and amplitude of gamma oscillations in the PFC (Zhang *et al.*, 2008). Gamma oscillations in the present study in the PFC were approximately 5 times smaller than in the CA3 region of the hippocampus. Due to this very small signal, it may not have been possible to observe a difference between the treatment groups. The majority of the literature concerning gamma oscillations is focused on the hippocampus; this is likely due to the network architecture between pyramidal cells and interneurons being highly recurrent, thus generating strong rhythmic activity (Amaral *et al.*, 1990). As the architecture is less recurrent in the PFC it may be more beneficial to investigate gamma oscillation *in vivo* rather than in a slice preparation. This was also the first study to be carried out using prefrontal slices in the laboratory; therefore, it may be that the method of inducing the oscillations needs improvement. For example, the method of inducing the oscillations may need refinement, and/or the location of the electrode placement may need altering.

5.4.4 PCP effects on parvalbumin IR cell density in the CA2/3 region of the hippocampus

Following the *in vitro* electrophysiology results, it was decided to investigate parvalbumin immunoreactive (IR) cell density in the CA2/3 region of the hippocampus. Post mortem studies in patients with schizophrenia have revealed robust pathology involving GABAergic signalling (see section 5.1). Benes *et al.* (1998) reported a

reduced density of GABA interneurons in the CA2/3 region of the hippocampus, the same region in which a reduction in parvalbumin IR cell density was observed in sub-chronic PCP treated rats. Calcium binding proteins (CBP), namely parvalbumin (PV), calbindin (CB) and calretinin (CR) have been used as markers of specific subpopulations of non-overlapping GABAergic interneurons in the brain. The GABAergic deficits found in schizophrenia are largely restricted to the PV-containing neurons (Lewis *et al.,* 2005). Deficits in PV IR cells have been reported in both the frontal cortex (Beasley and Reynolds, 1997; Beasley *et al.,* 2002) and hippocampus (Zhang and Reynolds, 2002) in schizophrenia. Whether these studies reflect deficits in the density of PV interneurons or that the interneurons are present but PV is not expressed was unanswered. In a more recent study, it was reported that, at the cellular level, a decrease in signal intensity for PV mRNA was attributable principally to a reduction in PV mRNA expression per neuron rather than by a decreased density of PV mRNA-positive neurons (Hashimoto *et al.,* 2003). Furthermore, it was reported that treatment of PV interneurons in culture with ketamine resulted in a concentration dependent decrease in PV, and at the highest concentration of ketamine cell death was not observed (Kinney *et al.,* 2006).

The results presented here show a reduction in parvalbumin IR cell density in sub-chronic PCP-treated rats at 2 weeks following PCP treatment. These results are supported by other studies using a sub-chronic PCP treatment regime that have reported deficits in PV-immunoreactive neurons in the hippocampus; these reductions have occurred alongside cognitive and behavioural alterations (Abdul-Monim *et al.,* 2006; Jenkins *et al.,* 2008). Deficits in the attentional set-shifting task following acute PCP treatment were also accompanied by a reduction in PV mRNA in the reticular thalamus with no change in the prefrontal cortex (Egerton *et al.,* 2005). However, using a regime

of chronic intermittent exposure to PCP, Cochran and co-workers (2003) reported decreases in PV mRNA expression in both the rat prefrontal cortex and reticular nucleus of the thalamus. A recent study found that repeated PCP administration (10 mg/kg for 10 days) impaired performance in a working memory test and reduced PV mRNA expression in the PFC (Thomsen *et al.,* 2009). Furthermore, it was shown that co-administration of SSR180711, an α7 nAChR agonist, prevented the PV and behavioural deficits (Thomsen *et al.,* 2009). Other studies using the NMDA antagonists MK-801 and ketamine have also showed deficits in PV interneurons in the hippocampus (Keilhoff *et al.,* 2001; Braun *et al.,* 2007; Rujesca *et al.,* 2006). It is also important to note that deficits in PV-immunoreactive neurons are also observed in neurodevelopmental models of schizophrenia such as post-natal administration of PCP (Wang *et al.,* 2008), the MAM model (Penschuck *et al.,* 2006) and isolation rearing (Harte *et al.,* 2007).

5.4.5 Time-dependent changes in the PCP-induced effects on gamma oscillation and parvalbumin IR cell density in the CA2/3 region of the hippocampus

The literature is lacking data studying the time-course effects of PV and gamma oscillations, therefore it is difficult to postulate how these effects could be changed over time. Parvalbumin IR cell density was unchanged at 8 weeks post PCP treatment, suggesting a compensatory mechanism could be increasing PV. A previous study in our laboratory found reductions in PV IR cell density at 6 weeks post PCP treatment in the CA2/3 region (Abdul-Monim *et al.,* 2007). It is not clear from immunohistochemistry whether these studies initially reflected a decreased number of interneurons or that the interneurons were present but PV was not detectable. It was reported that treatment of PV interneurons in culture with ketamine resulted in a concentration-dependent

decrease in PV, and at the highest concentration of ketamine cell death was not observed (Kinney *et al.,* 2006). Therefore, it is suggested that blocking NMDA receptors on interneurons changes the phenotype of the neurons. If the PV interneurons are still present but are not expressing parvalbumin, this may allow PV expression to be increased with time due to compensatory mechanisms, such a mechanism may involve the increase in NMDA receptors or NMDA receptor subunits. PV expression is thought to be activity dependent (Philpot *et al.,* 1997); therefore, an increase in functional NMDA receptors could result in an increase in PV expression and so increase release of GABA and glutamate within the network. This could also account for the increase in gamma oscillations, as the system would be more synchronous. Although the gamma oscillations began to increase over time, the fact that oscillations were increased compared to vehicle-treated rats still represents an abnormal system of firing. Gamma oscillations may be further increased to above a normal level because NMDA receptors could also be increased on pyramidal cells which could also disrupt the synchronicity of the system. It is also unclear whether the levels of parvalbumin return to normal (as in vehicle-treated rats) or whether these levels are further increased, as immunohistochemistry only allows us to count whether the cells express PV or not, it does not quantify the levels of PV within each neuron.

Although the effects in this study appear to be time-dependent, the evidence here and in the literature does suggest a role of GABAergic neurotransmission in schizophrenia. It should be an aim of antipsychotic treatment to reverse the deficits in PV expression. In a rodent model of schizophrenia using chronic PCP treatment it was shown that clozapine reversed the PCP-induced deficits in PV expression (Cochran *et al.,* 2003). Furthermore, it was shown that co-administration of SSR180711, an α7 nAChR agonist, prevented the PV and behavioural deficits (Thomsen *et al.,* 2009).

Indeed, the $GABA_A$ receptor could be a novel target for cognitive dysfunction associated with schizophrenia. For example the positive allosteric modulator of $GABA_A$ receptors, MK-0777, was shown to have high selectivity for the α2/α3 subunits and was found to improve working memory in schizophrenia patients; furthermore it was also shown to increase gamma power during the Preparing to Overcome Prepotency task (Lewis *et al.,* 2008). This cognitive task is a cued stimulus-response reversal paradigm that requires increases in cognitive control through the maintenance and use of context information to overcome prepotent response tendencies (Cho *et al.,* 2006).

5.4.6 Conclusions

There was no difference in gamma oscillations at either time point in the PFC. The results from this chapter demonstrate a reduction in gamma oscillations in the CA3 region of the hippocampus following PCP treatment that was paralleled by a deficit in parvalbumin IR cell density, at a similar time point (2-5 weeks post PCP-treatment). In contrast, a time-dependent increase in gamma oscillations was observed (6-8 weeks post PCP-treatment), at which point parvalbumin IR cell density was unchanged. To our knowledge this is the first study to investigate the effects of sub-chronic PCP on gamma oscillations *in vitro*. These experiments demonstrate a link between altered gamma-frequency oscillations and abnormalities in parvalbumin interneurons, which may underlie some of the cognitive deficits previously reported in this animal model of schizophrenia.

This chapter demonstrates differences in gamma oscillations in the CA3 region of the hippocampus; however, the behavioural tasks employed in this thesis are believed to be

primarily based on the PFC. Due to the small signal obtained from the PFC, it could be concluded that this protocol is not the ideal method for exploring changes in the PFC. Therefore, the next chapter will aim to investigate differences in the PFC using *in vivo* microdialysis.

Chapter 6

Effect of sub-chronic PCP on dopamine release in the prefrontal cortex during a novel object recognition test

6.1 Introduction

The previous chapters have principally explored two behavioural tests of cognition, i.e. the attentional set-shifting task and the reversal learning test. The current chapter aims to utilise a quicker, more ethologically relevant task, namely the novel object recognition task.

6.1.1 Novel object recognition (NOR) task

As early as 1950, Berlyne found that rats spent significantly more time exploring a novel object than two familiar objects (Berlyne, 1950). Subsequently the novel object recognition (NOR) task was developed, based on the natural propensity of rats to explore novel objects (Ennaceur and Delacour, 1988). It is a non-rewarded, ethologically relevant, relatively simple test (Puma *et al.,* 1998). Rats would be expected typically to respond to changes in their environment, in this case this would be shown by preferential exploration of a novel object compared to a familiar object. Such tests are increasingly being used to study and screen potential novel antipsychotic drugs. Indeed, NOR has been listed by the TURNS initiative as relevant for studying visual learning and memory deficits in schizophrenia (TURNS.ucla.edu). This model is relevant to the disease since visual recognition memory is impaired in schizophrenic patients (Calkins *et al.,* 2005).

It has been shown that female rats can perform better in NOR compare to males, and that there is no effect of stage of oestrus cycle on ability to perform in the NOR task (Sutcliffe *et al.,* 2007). Sub-chronic PCP has previously been reported to induce a long lasting robust deficit in novel object recognition, an effect which can be reversed by the atypical antipsychotics clozapine and risperidone (Grayson *et al.,* 2007). The NOR task

may therefore provide a relatively quick and simple means of evaluating novel therapies for aspects of cognition in schizophrenia. Furthermore, the efficacy of the atypical antipsychotics clozapine, risperidone, sertindole, and the ampakine, farampator, was demonstrated in this model against PCP (Grayson *et al.*, 2007; Neill *et al.*, 2007; Idris *et al.*, 2009).

6.1.2 Brain regions involved in NOR

Studies of primates and rodents have shown the importance of the parahippocampal regions of the temporal lobe (namely perirhinial, entorhinal, and inferior temporal cortices) in visual object recognition memory (Gilbert and Kesner, 2003; Murray *et al.*, 2000). Excitotoxic lesions of the perirhinal cortex in rats have been shown to disrupt object recognition memory (Aggleton *et al.*, 1997; Liu and Bilkey, 2001). Furthermore, additional studies in rats and primates have suggested that it is cortical rather than hippocampal neurons that are involved in object recognition tasks (Brown and Aggleton, 2001; Wan *et al.*, 1999; Xiang and Brown, 1999). However, some human and primate studies have shown that hippocampal lesions result in impaired object recognition memory (Beason-Held *et al.*, 1999; Cave and Squire, 1991; Reed and Squire, 1997; Zola *et al.*, 2000). It is thought that the brain regions involved in object recognition memory depend upon the length of the inter-trial interval. Rats with hippocampal lesions exhibited impairments in object recognition following long inter-trial intervals (>15 min), but not short intervals of <15 min (Clark *et al.*, 2000). In support of this, intra-hippocampal administration of the NMDA antagonist, APV, was reported to impair object recognition memory with a long (3 hour), but not short (5 min) inter-trial interval (Baker and Kim, 2002).

Though much of the evidence indicates a critical role of the perirhinal cortex in object recognition memory (Gaffan and Murray, 1992; Meunier *et al.*, 1993; Ennaceur *et al.*, 1996; Brown and Aggleton, 2001; Hannesson *et al.*, 2004), evidence also suggests that the medial PFC may also contribute to recognition memory. PFC neurons have been shown to carry information concerning the relative familiarity of individual stimuli (Miller *et al.*, 1996; Xiang and Brown, 2004), and damage to this area has been shown to impair recognition memory tasks (Bachevalier and Mishkin, 1986; Kolb *et al.*, 1994). Therefore, recognition memory following a short inter-trial interval may involve a large network of cortical connections that include the perirhinal cortex and the prefrontal cortex.

6.1.3 NMDA receptor antagonists and the role of dopamine

It is suggested that interaction with novelty may activate rewarding mechanisms in rats, and that novelty seeking behaviour is controlled by the dopaminergic system (Besheer *et al.*, 1996; Peters *et al.*, 2007; Rebec *et al.*, 1997). As yet, the role of dopamine in object recognition remains less well established. Thus, it would be particularly useful to gain an insight into dopaminergic changes *in vivo* during the novel object recognition task.

Several lines of evidence suggest that current antipsychotics have one common factor regarding their mechanism of action, in that they all interfere with the dopaminergic system in the brain (see Arnt and Skarsfeldt, 1998). Dopamine hypofunction in the PFC is thought to have a major role in the aetiology of negative symptoms and cognitive dysfunction of schizophrenia (Abi-Dargham and Moore, 2003; Stone *et al.*, 2007). It has been shown that the atypical antipsychotics, sertindole and

risperidone, increase extracellular dopamine in the rat mPFC and nucleus accumbens (Mork *et al.,* 2009).

There are several studies which demonstrate that NMDA antagonists produce psychotic and neurocognitive disturbances similar to those observed in schizophrenia (Enomoto *et al.,* 2007; Javitt, 2007; Krivoy *et al.,* 2008; Stone *et al.,* 2007; Tan *et al.,* 2007). Moreover, PCP has been shown to disturb dopamine levels in the PFC and nucleus accumbens (Jentsch *et al.,* 1997). The reduction of dopamine levels in the PFC is associated with negative and cognitive symptoms of schizophrenia (Jentsch *et al.,* 1999), while an increase in dopamine in the nucleus accumbens is associated with hyperlocomotion often seen in PCP-treated animals (Jentsch *et al.,* 1998). Acute dosing with PCP has limitations in modelling the chronic psychotic illness or the persistent cognitive deficits of schizophrenic patients. Alternatively, as previously discussed in prior chapters, repeated sub-chronic exposure to PCP is believed to mimic schizophrenia more accurately than acute dosing (Jentsch and Roth, 1999). Examples of these changes include reduced frontal blood flow and glucose utilisation (Hertzman *et al.,* 1990; Wu *et al.,* 1991) and reduced dopamine utilisation in the PFC (Jentsch *et al.,* 1997). These data indicate that dopamine function is significantly altered in the PFC by sub-chronic treatment with PCP.

It has been suggested that the brain areas involved in recognition memory include the PFC and perirhinal cortex (Miller *et al.,* 1996; Xiang and Brown, 2004; Winters and Bussey, 2005). In addition, there is also evidence that the effects of NMDA receptor antagonists are more robust in the PFC compared with other regions having major dopaminergic innervation (Verma and Moghaddam, 1996). Although a single injection of MK-801 has been reported to increase extracellular levels of glutamate in the mPFC (Lopez-Gil *et al.,* 2007; Zuo *et al.,* 2006), repeated

administration of MK-801 has been shown to decrease glutamate levels in the mPFC (Zho *et al.,* 2006). Furthermore, basal dopamine levels seem to be reduced in the rat PFC following sub-chronic PCP administration (Jentsch and Roth, 1999).

6.1.4 Control anxiety testing

As the NOR task involves rats being placed in an open arena it would be valuable to assess whether there were any differences in anxiety between vehicle and sub-chronic PCP treated rats, which could then confound results in the NOR task. Therefore, rats were tested in two anxiety-like behaviour tests, namely the elevated plus maze (Pellow *et al.,* 1985) and open field (Walsh and Cummins, 1976) tests. The elevated plus-maze (Pellow *et al.,* 1985) involves placing a naive rat in the centre of an elevated plus-maze with two open and two enclosed arms and allowing it to freely explore (Rodgers and Cole, 1993). It has been suggested that the reluctance of rats to explore the open arms of the maze is caused by fear of open spaces rather than the novelty of the maze or its height (Pellow *et al.,* 1985). The open-field test is also a frequently used test of anxiety (Gray, 1979). Both tests have been pharmacologically validated with anxiolytic compounds increasing and conversely anxiogenic compounds decreasing the percentage of time spent in the open areas (Pellow *et al.,* 1985; Gentsch *et al.,* 1987; Cole *et al.,* 1995).

6.1.5 Aims

The aim of this chapter was to investigate the role of prefrontal dopamine in specific phases of the novel object recognition (NOR) task using *in vivo* microdialysis during the behavioural task. Predominantly, in our laboratory the NOR task is carried out using a 1 min inter-trial interval; however, due to the methodological constraints of

microdialysis these experiments were carried out using a 10 min inter-trial interval. In addition, as a control experiment, anxiety tests were carried out to ensure that any differences in novel object recognition in PCP-treated rats were not due to anxiety or neophobia.

6.2 Materials and Methods

The main technique used in this chapter was *in vivo* microdialysis. The development of brain sampling techniques was limited by the large sample previously required for the identification and quantification of neurotransmitters. The development of highly sensitive analytical techniques, such as HPLC, has made it possible for the development of micro-collection techniques such as *in vivo* microdialysis (Benveniste and Huttemeier, 1990; Ungerstedt, 1984). An important advantage of high-liquid performance chromatography (HPLC) is rapid separation, which takes minutes rather than hours for each sample unlike previous methods of detection.

Microdialysis is a technique that allows both administration and collection of substances from remote brain regions with a high level of accuracy. Sampling can be done on a continuous basis in freely moving animals, limited only by the amount of time needed to fill the sample loop of the HPLC injector. Microdialysis is based on the principal of dialysis, in which a semi-permeable membrane separating two solutions allows some diffusion to occur. The movement of fluid through the probe carries the substance of interest to the sampling site for analysis.

6.2.1 Experiment 1: PCP and anxiety

6.2.1.1 Subjects and drug treatment

Forty adult female hooded-Lister rats weighing 200-250 g were housed in groups of five in standard laboratory conditions with free access to food and water. Light intensity in the holding and behavioural testing rooms was 400-500 lx. All experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act, UK (1986)

and were approved by the University of Bradford ethical review process. At adulthood 20 rats received sub-chronic PCP (2 mg/kg) and 20 rats received vehicle (0.9% saline, 1 ml/kg) as previously detailed in chapter 2.

6.2.1.2 Locomotor activity (LMA)

The LMA response to a novel environment was monitored using automated photocell cages. The movement of each animal was monitored in a Plexiglas chamber (16 x 26 x 19 cm) covered with a compatible Plexiglas lid using AM1052 Activity Monitor (Linton Instrumentation). Rats were habituated to the cages for 2 hours on the day prior to testing. On the test day, rats were either given an acute dose of PCP (2 mg/kg, i.p.) or vehicle (0.9% saline, i.p.) and were placed in the LMA boxes immediately after dosing. The acute dose of PCP was chosen based on a previous study showing that 2 mg/kg can produce cognitive deficits without affecting locomotor activity (Grayson, unpublished findings). Therefore, there were 4 test groups (all n=10) sub-chronic vehicle-vehicle, sub-chronic vehicle-PCP, sub-chronic PCP-vehicle, sub-chronic PCP-PCP. Counts were recorded by AmLogger software (supplied by GSK, Harlow, UK) by means of photo beam interruptions within the chamber. Activity was monitored every 5min over a 120 min period. Rats were challenged to ensure that the sub-chronic PCP regimen was effective. The rats that did not receive the PCP challenge (veh-veh (n=10) and PCP-veh (n=10)) then went on to be tested in the elevated plus maze and open field paradigms.

6.2.1.3 Elevated plus maze

The elevated plus maze (constructed in house) consisted of four arms elevated 50 cm above the floor and is shown in figure 6.2.1.3. Each arm (12 cm wide, 46 cm long) was joined to the others by a central square (12 cm ×12 cm) in a cross-like disposition. A wall (12 cm in height) enclosed two opposite arms, while the other two arms were open. Placing the rat on the platform facing an open arm started the test. The arms of the maze were cleaned between the tests. The experimenter monitored the movements of the rats via a video camera (JVC, TK/C1480E) mounted above the maze, and recordings were scored using Hindsight version 1. During the 10 min test session, the number of entries into the open or closed-arms and the time spent in the open or closed-arms were recorded. The rats were considered to have entered an arm when all four limbs were located in an arm of the maze. The latency to enter the open arm was also scored.

Figure 6.2.1.3: The elevated plus maze

6.2.1.4 Open field

The open field arena (constructed in house) consisted of an open box (52 cm wide by 40 cm high by 52 cm long) with black Perspex sides, a white Perspex base and black grid lines dividing the arena into 9 equally sized squares (see figure 6.2.1.4). No additional illumination was placed on the box and the box was placed in the experimental room in such a position where no shadows fell. Placing the rat in the centre square started the test. The arena was cleaned in between tests. The experiment was recorded and scored as described previously for the elevated plus maze. During the 10 min test session, the number of entries into the centre square and the time spent in the centre square were recorded. The rats were considered to have entered a square when all four limbs were located in that square of the arena.

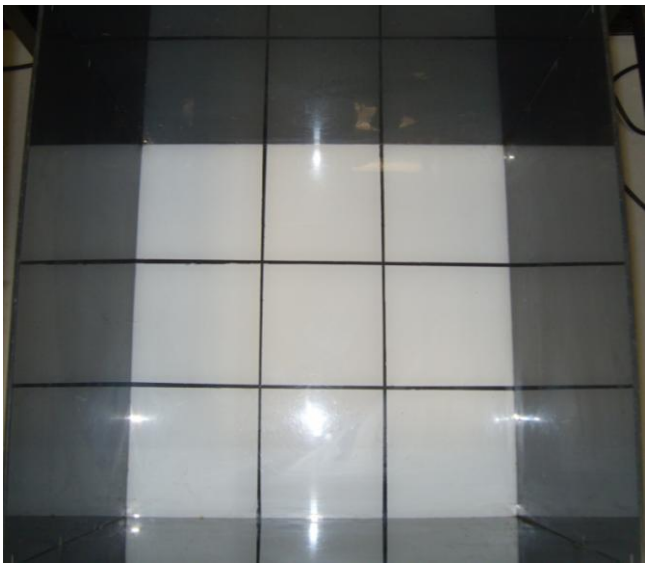


Figure 6.2.1.4: The open field arena

6.2.1.5 Data and statistical analysis

Area under the curve was calculated for the LMA data using the trapezoid rule, and analysed using post-hoc Bonferroni's multiple comparison test. Independent Student's t-tests were carried out on the elevated plus maze and open field data between the vehicle and PCP treated groups.

6.2.2 Experiment 2: novel object recognition and microdialysis

6.2.2.1 Subjects and drug treatment

Twelve adult female hooded-Lister rats (Charles River, UK) were housed in groups of two to three and weighed 200-220 g at the start of the experiment. Animals were maintained under standard laboratory conditions at a temperature of 20°C (±1°C) and humidity of 50±5%. They were maintained on a 12-h/12-h light/dark cycle (lights on at 0700 hours) and experimental procedures were performed during the light phase. Rats had free access to food and water. Rats were treated with 2 mg/kg PCP (n = 5) or vehicle (0.9 % saline; n = 7), twice daily for 7 days. All experiments were performed with appropriate project and personal license authority under the Animals (Scientific Procedures) Act, 1986, and with approval of the University of Leicester Animal Ethics Committee.

6.2.2.2 Surgery

Prior to surgery rats were administered buprenorphine (0.05 mg/kg, s.c; Vetergesic®, Reckitt Benckiser, UK). Rats were anaesthetised using isoflurane (3% isoflurane in O_2: 1 litre/min) in an induction chamber. Once fully anaesthetised, the head was shaved and swabbed with an iodine solution and the animal was mounted in a Kopf stereotaxic

frame, incorporating a gaseous anaesthetic delivery mask (Stoelting, USA), and was maintained at 1-2% isoflurane in 1 litre/min oxygen. Exhaust gas was filtered through a scavenger unit (Fluovac Aldasorber, Aldred and Co, UK). The head was positioned such that the incisor bar was 3.3 mm below the intra-aural line (Paxinos and Watson, 1998). Blunt ear bars were used to minimise trauma to the ear canal, and a topical local anaesthetic (EMLA cream containing 25 mg lidocaine and 25 mg prilocaine, AstraZeneca, UK) was applied to the ear bars before insertion into the ear canal; an ocular lubricant (Lacri-lube, Allergan, UK) was placed onto the eyes.

The skin overlying the surface of the skull was removed to reveal the area to be covered by the cap. A midline incision was made in the pericranium overlying the skull, which was scraped away laterally and removed to fully expose the bone. The horizontal and transverse stereotaxic coordinates of bregma were recorded. The coordinates of the implants were then calculated and the position marked on the skull surface (PFC: H, +3.2; Tr, -0.5 and NAc: H, +1.6; Tr, +1.4, relative to bregma; (Paxinos and Watson, 1998)). Holes were drilled in the skull using a hand-held drill (bit size 1.18 mm diameter) at the locations of the implants and two additional holes in the left and right parietal bones. Anchor screws (3.2 mm shaft length, 1.57 mm shaft diameter, 2.5 mm head diameter Plastics One, USA) were inserted into the holes in the parietal bones. The stainless steel guide cannulae (o.d., 890 μm; i.d., 685 μm; length 10 mm: Coopers Needle Works, Birmingham, UK) were mounted onto an implanter device and were then lowered into their respective holes until the surface of the brain was reached. At this point the vertical coordinate was recorded and the vertical position of the implanted cannula was calculated (PFC: V, -1.4; NAc: V, -6.0, relative to dura). The guide cannula was raised and the dura was pierced with a needle before the cannula could be lowered into position. The guide cannula was then secured in place using dental acrylic

(DuraLay, BAS, UK). Once dry, the implanter was raised leaving the guide in position. This was repeated for the other region. Stainless steel stylettes (10 mm length, o.d., 0.64 μm, top 2 mm bent over) were then inserted into the guides and secured in place with a small amount of silicon rubber compound (Hombase, UK). Rats received 2-3 sutures below the cap using FS-3 coated vicryl polyglactin (Ethicon®, Johnson and Johnson, Belgium). Finally, a topical antibiotic (cloxacilin benzathine, Orbenin dry cow®, Pfizer, UK) was applied around the wound to aid healing. Following surgery animals were singly housed in order not to cause damage to each others implants and were fed a mixture of standard chow and chow softened by pre-soaking for 2 hours. Animals were closely monitored until behavioural testing, they were weighed daily, and the amount of food and water intake was recorded.

After recovery from surgery (≥7 days), animals were again anaesthetised with isoflurane and the stylettes mounted in the silicon rubber were removed. The dialysis probes (see below) were inserted into the guide cannulae to lie in the PFC (tip position H, +3.2; Tr, -0.5; V, -5.4) and NAC (tip position H, +1.6; Tr, +1.4; V, -8.5). The probes were then fixed in place using dental acrylic. Following the insertion of the probes the wire rack lid of the home-cage was replaced with a clear Perspex lid with holes for ventilation, to ensure that the probes were not damaged.

6.2.2.3 Microdialysis probes

Microdialysis probes were constructed in house (by Dr Andrew Young) as previously described (Young *et al.*, 1998). The dialysis membrane (o.d. 350 μm; Cuprophan, COBE laboratories, Gloucester, UK), sealed at one end with epoxy resin and with two fused silica capillaries (o.d. 170 μm, SGE, Milton Keynes, UK) inserted, was mounted into a stainless steel tube (o.d. 640 μm, Coopers Needle Works, Birmingham, UK) with

a 2.5 or 4.0 mm length of membrane exposed. The open ends of the capillaries were each inserted into a 6 mm length of stainless steel tube (o.d. 640 μm) with a 5 mm length of silicon rubber tube on the other end to act as a connector. The joint was secured with epoxy resin. A schematic diagram of the probe is shown in figure 6.2.2.3. All probes used in the experiments were checked for flow rate integrity and leaks before implantation. The recovery of the probes *in vitro* was 10-15%.

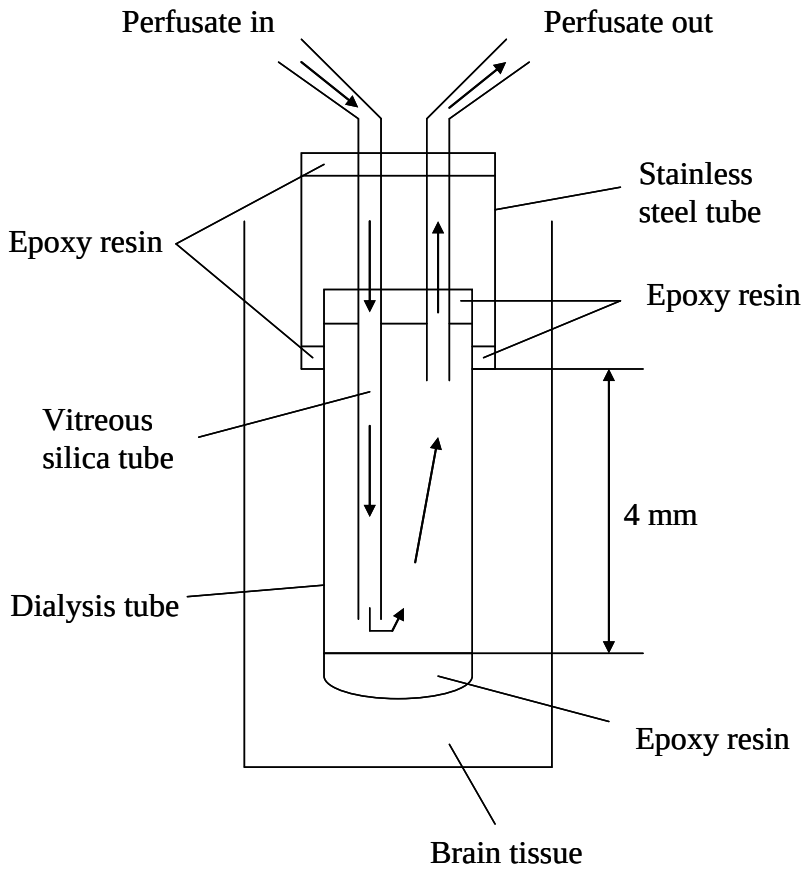


Figure 6.2.2.3: A schematic diagram of the microdialysis probes used for the PFC.

6.2.2.4 Delivery system

Delivery tubes comprised four 20 cm lengths of 'Portex' polythene tubing (o.d. 0.61 mm, Anachem, Luton, UK), mounted in a 17 cm length of stainless steel spring (Plastics One, USA). Two tubes (inlet) were connected via a two channel liquid swivel (Eicom, Japan), to a two channel syringe pump (CMA, Sweden). The other two tubes (output) went from the top end of the spring into the collector tubes mounted on the spring, such that they rotated with the animal. The length of the output line was adjusted to give a 30 min dead time from probe tip to collection at a flow rate of 2 µl/min. Custom built gantry was curved to allow movement (20 gauge steel wire). The delivery system set-up is shown with NOR apparatus in figures 6.2.2.4a and b.

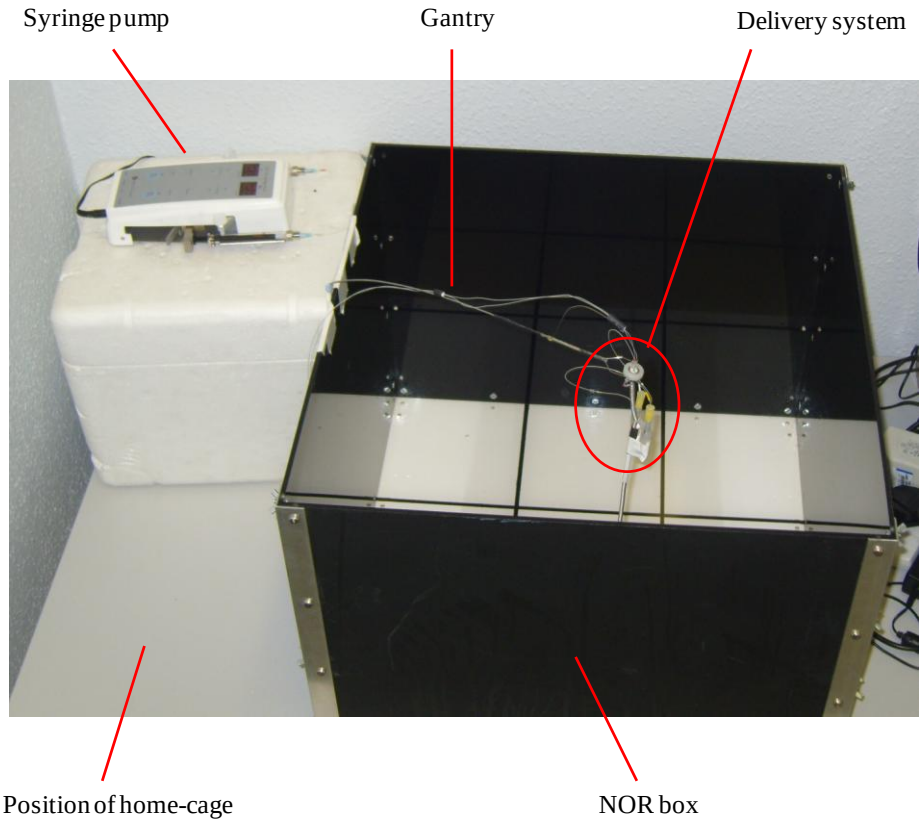


Figure 6.2.2.4a: Labelled photograph of the delivery system set-up shown attached to the NOR box

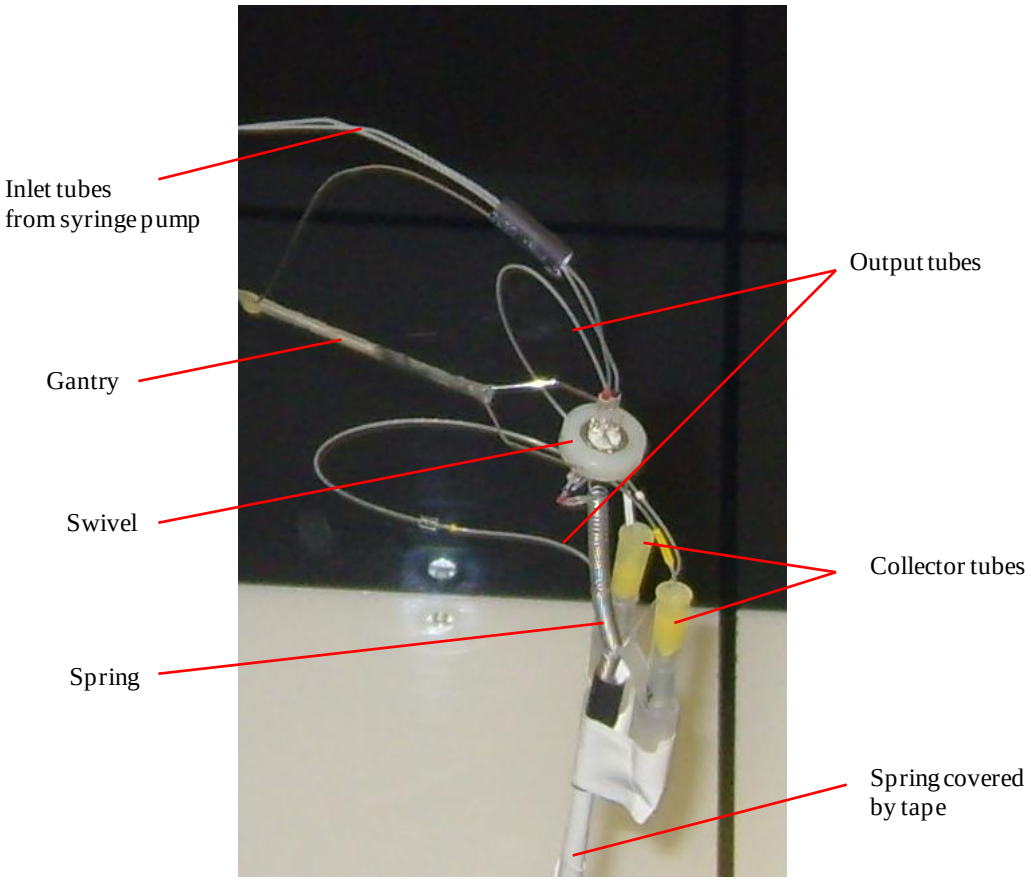


Figure 6.2.2.4b: Labelled photograph of the delivery system, the gantry was attached to the NOR box and the spring leads to the implanted probes

6.2.2.5 Microdialysis procedure during NOR

At least one hour after implantation of the probe, animals were connected up for microdialysis, and perfusion with artificial cerebrospinal fluid (mM: NaCl, 145; KCl, 3.3; $MgSO_4$, 2.4; KH_2PO_4, 1.25; $CaCl_2$, 1.85: 2 µl/min) commenced immediately. Following equilibration for 1 hour, dialysate samples were collected consecutively for 10 min into 2 µl of 1.0 M H_3PO_4 (to minimise oxidation). The details of sample collection throughout the experiment are shown in table 6.2.2.5. The first four samples (40 min) were used to determine basal dopamine levels in the dialysates.

Table 6.2.2.5: Shows the collection time points of the samples. Collection was offset by 30 min to allow for 30 min dead time within the tubes.

Time (min)	Behaviour	Sample
0	Home cage	Clearing dead volume
10	Home cage	
20	Home cage	
30	Home cage	Dialysis Equilibration
40	Home cage	
50	Home cage	
60	Baseline in home cage	
70	Baseline in home cage	
80	Baseline in home cage	
90	Baseline in home cage	S1 - Baseline
100	**Habituation in NOR box**	S2 - Baseline
110	**Acquisition in NOR box**	S3 - Baseline
120	**ITI**	S4 - Baseline
130	**Retention test**	S5 - Habituation
140	Re-baseline in home cage	S6 - Acquisition
150	Re-baseline in home cage	S7 - ITI
160	Re-baseline in home cage	S8 - Retention
170	Remain in home cage and continue perfusion	S9 - Re-baseline
180		S10 - Re-baseline
190		S11 - Re-baseline

Dopamine content in every sample (S1-11) was assessed by high performance liquid chromatography (HPLC) with electrochemical detection. On completion of the dialysis collection, animals were disconnected from the delivery tubes and returned to their home cage. At the end of the collection period, animals were killed by anaesthetic overdose (sodium pentobarbitone 400 mg, JML, Southampton, UK) and cervical dislocation, and the brains were removed and stored in 4% formalin for subsequent histological verification of cannulae placement.

6.2.2.6 Microdialysis probe location - Cresyl Violet staining

Brain samples were embedded in wax (see chapter 5 section 5.2.6.2). Samples were sectioned (see chapter 5 section 5.2.6.4) as the probe location was observed, and were then mounted onto slides coated with Poly-L-Lysine (see chapter 5 section 5.2.6.3). Samples were then left to dry overnight. Sections were de-waxed in Histoclear clearing agent (Fisher Scientific, UK) for 10 min. The sections were then re-hydrated for 5 min in each of the following solutions: 100%, 90%, 70% ethanol and distilled water. The tissue was then stained with cresyl violet acetate (Fluka Biochemika, Switzerland) for 10 min. Slides were washed with distilled water until the water ran clean. Sections were then subjected to the same process as re-hydration in reverse, i.e. 5 min in each of the following: distilled water, 70%, 90%, 100% ethanol. Sections were then placed into Histoclear for 5 min. Slides were then mounted in DPX (BDH, UK) and covered with a coverslip.

6.2.2.7 NOR apparatus

The apparatus consisted of an open box made of Plexiglas (52 cm L; 52 cm W; 31 cm H) and was positioned 60 cm above the floor on a table. The tube delivery system was mounted on one side of the box. The NOR box is shown in figure 6.2.2.7a. The walls of the box were black and the objects to be discriminated (in triplicate) were cola cans or Frijj® bottles. The size of the objects was similar (8-10 cm) and they were heavy enough not to be displaced by the animals, to achieve this, the bottles were filled with NaCl. The objects used in these experiments are shown in figure 6.2.2.7b. Objects were positioned away from the walls of the box, in opposite corners. After each trial, 10% ethanol was used to clean the objects in an attempt to remove any lingering

olfactory cues on the objects and in the box. The familiar and novel objects were counterbalanced to the left and right position to prevent bias for a particular location.

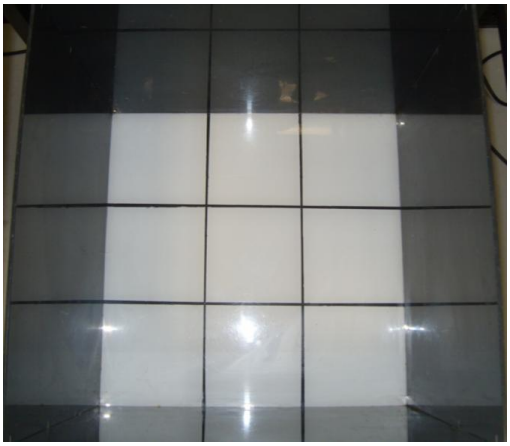


Figure 6.2.2.7a: Photograph of the NOR box

Figure 6.2.2.7b: Objects used in the NOR task

6.2.2.8 Habituation

During the week prior to the first behavioural testing procedure, all rats were handled daily. For three days prior to behavioural testing, rats were given daily 30 min exploration periods in the NOR box to ensure habituation to the empty apparatus and test room environment.

6.2.2.9 Behavioural testing

The order of testing is shown in appendix 2. The test was divided into 3 phases, the acquisition trial, an inter-trial interval and a retention trial proceeded by a 10 min habituation.

- Acquisition trial: each rat was placed into the NOR box and exposed to two identical objects (A1 and A2) for a period of 10 min.
- Inter-trial interval: the rats were then returned to their home cage for a 10 min inter-trial interval. Both objects were removed and the entire NOR box was then cleaned.
- Rats were returned to the NOR box to explore a familiar (a triplicate of those used in the acquisition phase to minimise olfactory cues) and a novel object (B) for a 10 min retention trial. The location of the novel object in the retention trial was randomly assigned for each rat.

All experiments were video recorded (JVC, TK/C1480E) for subsequent blind behavioural analysis. Object exploration was defined as the rats sniffing, licking or touching the objects with forepaws whilst sniffing but not by leaning against, turning around, standing or sitting on the objects. The exploration time (s) of each object in each trial was recorded manually using two stopwatches and the following factors were calculated.

- Discrimination index (DI) = (time exploring the novel object (s) – time exploring the familiar object) / total time exploring both novel and familiar objects. A value above zero indicates that the rats have explored the novel object more than the familiar object. A value below zero indicates that the rats have explored the familiar object more than the novel object.
- The total exploration time of objects in both the acquisition and retention trials.

Locomotor activity (LMA) was also recorded; this was evaluated by scoring the total number of sectors or line crossings by the animal in both acquisition and retention trials. The rat was only considered to have crossed the line once all four paws had crossed the line. A schematic diagram of the task is shown in figure 6.2.2.9.

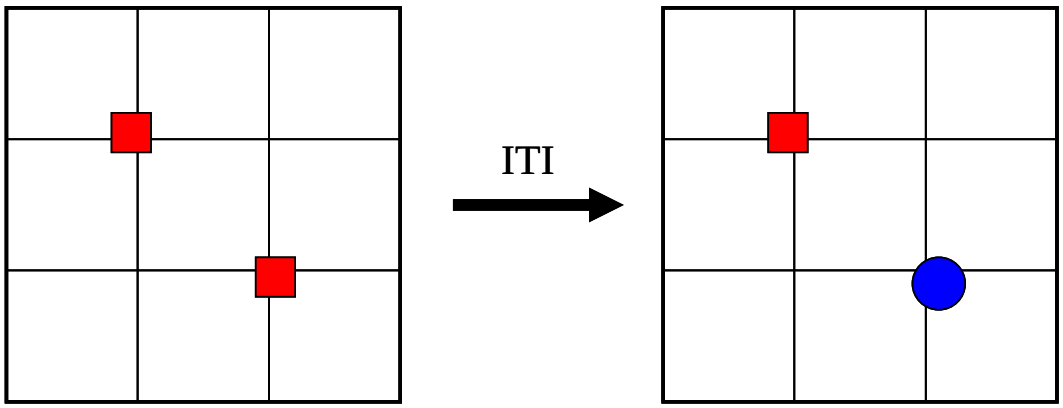


Figure 6.2.2.9: Schematic diagram of the novel object recognition task. The left panel shows the position of the objects in the acquisition trial followed by the inter-trial internal, and then the position of the familiar and novel objects in the retention trial.

6.2.2.10 HPLC detection of dopamine

After collection, dialysate samples were analysed to determine the dialysate concentration of dopamine in each sample by HPLC with electrochemical detection. Samples (15 µl) were injected onto the column using a Spark Triathlon refrigerated autosampler (Presearch, UK). The mobile phase (75 mM NaH_2PO_4, 1.1 mM octanesulfonic acid, 1 mM EDTA, 10% methanol, pH 3.7), was pumped at 110 µl/min using a Rheos 4000A pump (Presearch, UK), and separation was achieved with a 150 mm × 1.0 mm LUNA C18(2) 5 µm column (Phenomenex, UK). The separation was previously optimised for dopamine (see appendix 3). Dopamine, with a retention time of approximately 12.1 min was clearly separated from the other main constituents of the samples which had the following retention times (min): DOPAC, 5.9; 5-hydroxyindole

acetic acid, 10.2; HVA, 14.4. The concentrations were measured using an ANTEC Intro electrochemical detector incorporating a VT-03 low volume flow cell (Presearch, UK) with the working electrode set at 700 mV relative to a silver/silver chloride reference electrode, and data were collected and analysed using Chrom Perfect Analysis v5.5.4 (Justice laboratories, NJ, USA) PC-based integrator. The detection limit for dopamine under these conditions was 4.5 fmol per injection (equivalent to 15 µl injection of 0.3 nM). All chemicals were supplied by Sigma Chemicals (Poole, UK) and were HPLC grade.

6.2.2.11 Data and statistical analysis

The NOR data are expressed as mean ± SEM. Student's paired t-test was performed to compare the effect of treatment on the time spent exploring the familiar versus the novel object. LMA data are expressed as mean ± SEM of the total number of lines crossed during the acquisition and retention trials. Analysis of the total exploration values, LMA and DI data were performed using unpaired t-tests.

Dopamine concentrations were calculated with reference to standards at 1, 10, and 100 nM. Data are expressed as concentrations (nM) in the dialysates and percent of basal. The basal value was calculated from the four samples taken prior to behavioural testing. Microdialysis data were analysed using repeated measures two-way ANOVA with stage of task as a within subjects factor (basal, habituation, acquisition, ITI, retention, 3 post-test) and treatment (vehicle or PCP) as a between subjects factor. This was followed by post-hoc Bonferroni pair-wise comparisons. All statistical analyses were performed in SPSS (version 15). The DI from the NOR data and the percent of basal dopamine in the retention stage were assessed for correlation using Pearson's correlation (Graph Pad, Prism version 5).

6.3 Results

6.3.1 Experiment 1: Verification of PCP dosing regimen and anxiety tests

Bonferroni's multiple comparison test on the area under the curves (figure 6.3.1a) and the total LMA counts (figure 6.3.1b) showed that the PCP-PCP group was significantly more active compared to veh-veh ($P<0.05$), veh-PCP ($P<0.01$) and PCP-veh ($P<0.01$) groups. There were no significant differences between the other groups.

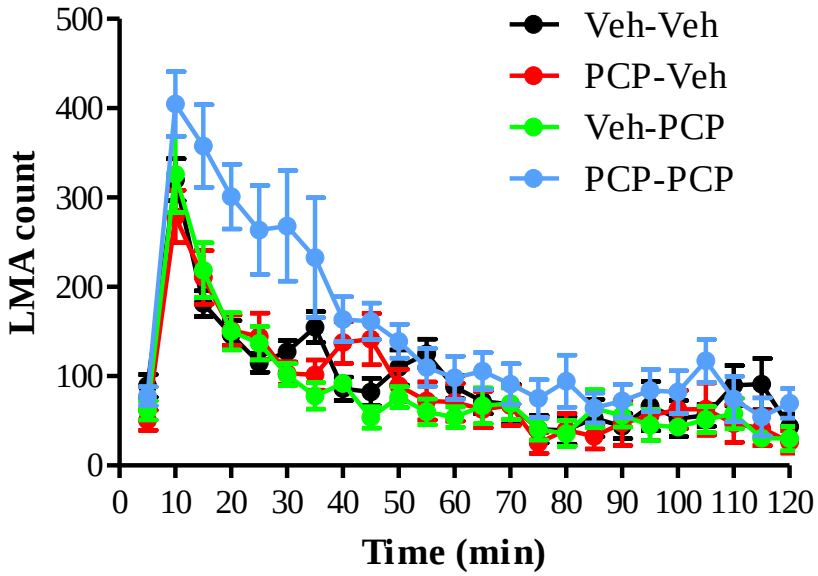


Figure 6.3.1a: Locomotor activity in vehicle or sub-chronic PCP-treated rats (2 mg/kg twice daily for seven days) following acute challenge with PCP (2 mg/kg) measured over a 2-hour period at 5-min intervals (n=10). Post-hoc Bonferroni multiple comparison test on the area under the curves showed that the PCP-PCP group was significantly more active compared to veh-veh ($P<0.05$), and veh-PCP and PCP-veh ($P<0.01$) groups.

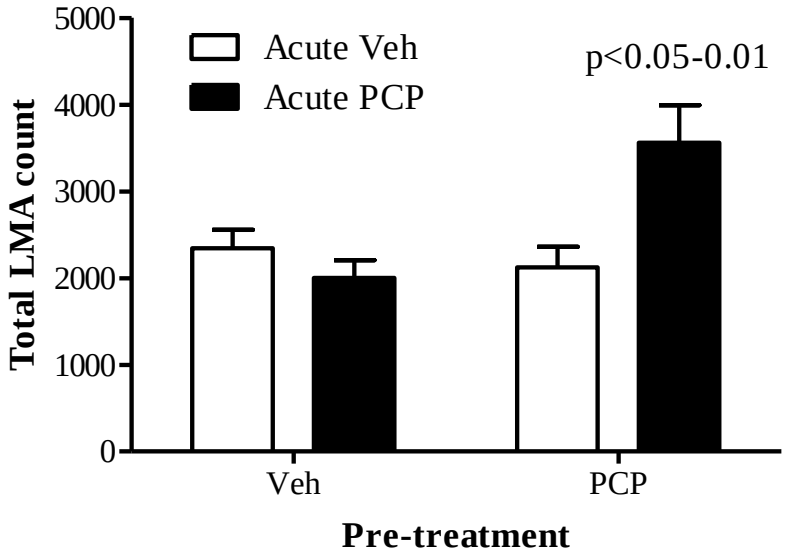


Figure 6.3.1b: Locomotor activity in vehicle or sub-chronic PCP-treated rats (2 mg/kg twice daily for seven days) following acute challenge with PCP (2 mg/kg) measured over a 2-hour period (n=10). Post-hoc Bonferroni multiple comparison test showed that the PCP-PCP group was significantly more active compared to veh-veh ($P<0.05$), and veh-PCP and PCP-veh ($P<0.01$) groups.

Rats which had received an acute dose of vehicle were then tested in the behavioural paradigms (vehicle and PCP n=10). Rats were excluded from the elevated plus maze study if they did not remain on the maze for the full test session, therefore groups sizes for the elevated plus maze experiment were n=8. Behaviour in the open field and on the elevated plus maze was unaffected by sub-chronic PCP with no significant differences observed between groups in time spent in the open/closed arms and number of entries into the arms, or time spent and entries into the centre square of the open field (table 6.3.1a). The ratio of open/closed arm duration and entries were calculated and are shown in table 6.3.1b; these also showed no significant effect of PCP treatment.

Table 6.3.1a: Behaviour of vehicle and PCP-treated rats on the elevated plus maze and open field tests.

Behaviour	Vehicle	PCP	P value
Elevated plus maze			
Open arm duration (s)	109.8 ± 11.3	94.2 ± 10.0	0.31
Closed arm duration (s)	377.5 ± 18.0	368.9 ± 14.3	0.71
Centre duration (s)	112.7 ± 22.2	131.0 ± 7.9	0.45
Open arm entries	6.8 ± 0.7	6.6 ± 0.5	0.89
Closed arm entries	12.9 ± 0.7	15.5 ± 1.1	0.07
Centre entries	19.8 ± 1.3	22.3 ± 1.3	0.20
Open arm latency (s)	28.8 ± 6.8	17.4 ± 7.0	0.26
Open field test			
Centre square duration (s)	25.2 ± 5.9	27.1 ± 4.9	0.80
Centre square entries	9.1 ± 1.5	11.1 ± 1.8	0.32
Line crossings	111.4 ± 5.9	111.1 ± 8.1	0.98

Table 6.3.1a: Behaviour of vehicle and sub-chronic PCP-treated rats on the elevated plus maze (n=8) and open field tests (n=10). Data are means ± SEM. P values were obtained from Independent Student's t-test comparing PCP to vehicle-treated group.

Table 6.3.1b: Ratios of open/closed arm duration and number of entries on the elevated plus maze in vehicle and PCP-treated rats.

	Open/closed ratio		
Behaviour	Vehicle	PCP	P value
Duration (s)	0.29 ± 0.03	0.27 ± 0.04	0.59
Entries	0.53 ± 0.05	0.44 ± 0.03	0.14

Table 6.3.1b: Ratios of open/closed arm duration and number of entries on the elevated plus maze in vehicle and PCP-treated rats (n=8). Data are means ± SEM. P values were obtained from Independent Student's t-test comparing PCP to vehicle-treated group.

6.3.2 Experiment 2: Microdialysis and NOR

Initially the groups consisted of 5 vehicle-treated and 5 PCP-treated rats (2 vehicle rats were excluded during behavioural testing). Bodyweights were monitored for a week following surgery. This data is shown in figure 6.3.2, day 0 represents the day of surgery. A two-way repeated measures ANOVA with day as the within factor and treatment as the between factor revealed no significant interaction ($F_{(1,8)}$=0.27; P=0.62).

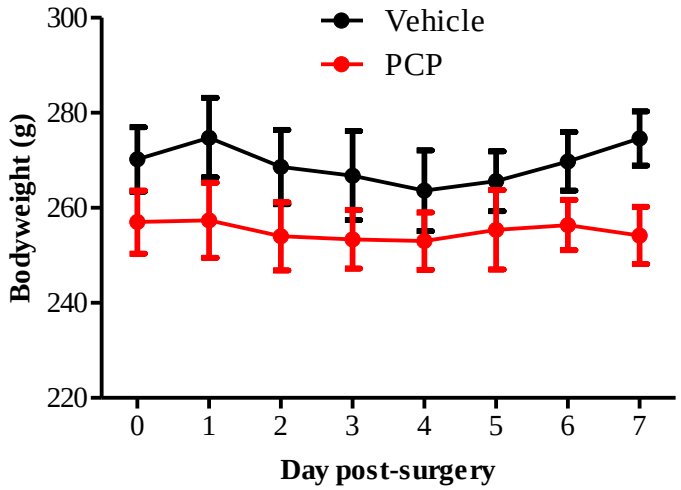


Figure 6.3.2: The bodyweights of sub-chronic vehicle and PCP-treated rats following surgery to insert the guide cannulae (n=5). Day 0 represents the day of surgery. There was no significant difference between the vehicle and PCP-treated groups.

6.3.2.1 NOR results

Animals were excluded if there was a lack of exploration in the behavioural task. Therefore, the groups for behaviour were vehicle (n=5) and PCP (n=5). The total time spent exploring both objects in the acquisition and retentions trials is shown in table 6.3.2.1. Student's independent t-test showed that there was no significant difference between vehicle and PCP-treated rats on total object exploration in the acquisition

(P=0.56) and retention trials (P=0.44). Paired t-tests revealed that there was no significant difference in the time spent exploring the two identical objects during the acquisition trial in either vehicle (P=0.83) or PCP-treated rats (P=0.73; figure 6.3.2.1a). In the retention trial (figure 6.3.2.1b), vehicle-treated rats explored the novel object more than the familiar object, although this effect was not significant (P=0.11; there was no difference in exploration of the novel and familiar objects in sub-chronic PCP-treated rats (P=0.09). An independent t-test revealed a significant difference in the discrimination index (DI, P<0.05). The DI for the PCP-treated group was significantly reduced to 0.25 from 0.65 in the vehicle treated group (Fig 6.3.2.1c). There was no effect on locomotor activity assessed by the number of line crossings in the acquisition and retention trials (P=0.45; Fig 6.3.2.1d).

Table 6.3.2.1: Total exploration time (sec) in the acquisition and retention trials for vehicle (n=5) and PCP-treated (n=5) rats.

	Total exploration time (sec)	
	Acquisition Trial	**Retention Trial**
Vehicle	40.5 ± 11.2	30.7 ± 13.3
PCP	49.0 ± 7.0	44.2 ± 9.4

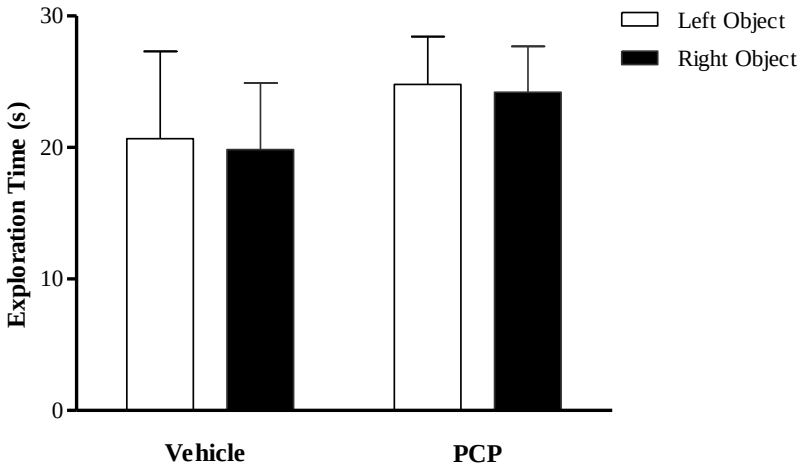


Figure 6.3.2.1a: The effect of treatment with vehicle or sub-chronic PCP (2 mg/kg, twice daily for 7 days, i.p.) in the acquisition phase of the novel object recognition task. Data are shown as mean ± s.e.m of time spent exploring the objects (n=5 per group).

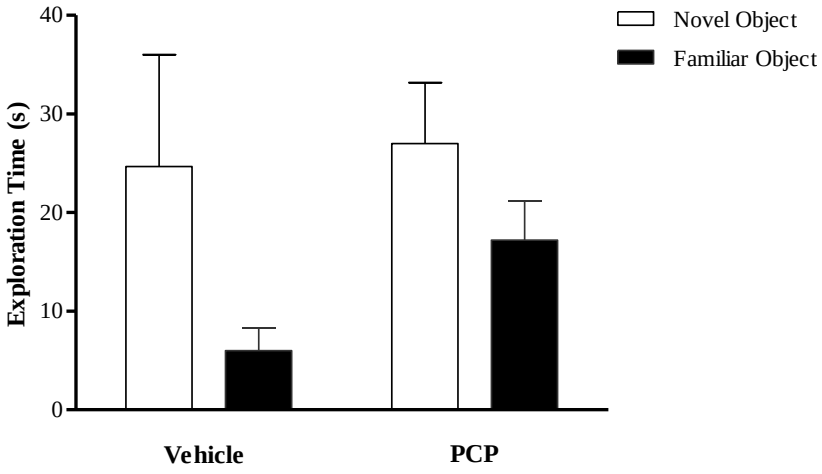


Figure 6.3.2.1b: The effect of treatment with vehicle or sub-chronic PCP (2 mg/kg, twice daily for 7 days, i.p.) in the retention phase of the novel object recognition task. Data are shown as mean ± s.e.m of time spent exploring the objects (n=5 per group).

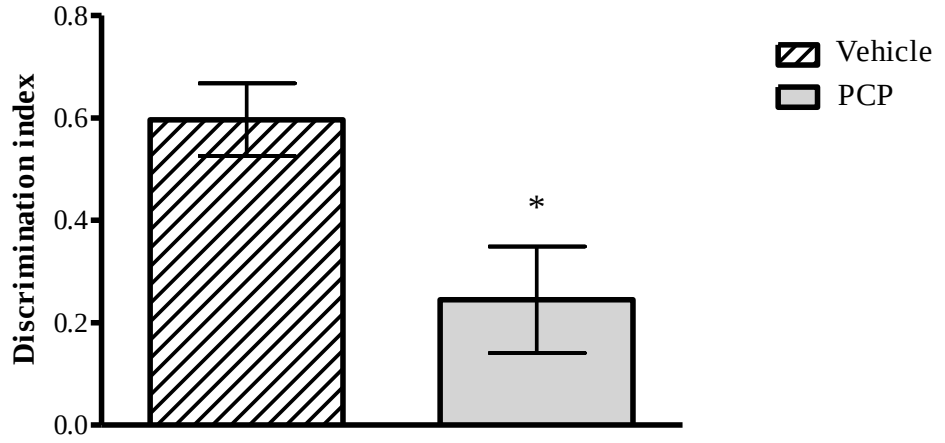


Figure 6.3.2.1c: The effect of treatment with vehicle or sub-chronic PCP (2 mg/kg, twice daily for 7 days, i.p.) in the novel object recognition task. Data are shown as mean DI ± s.e.m (n=5 per group). The DI for the PCP-treated group was significantly impaired compared to the vehicle group (*P<0.05).

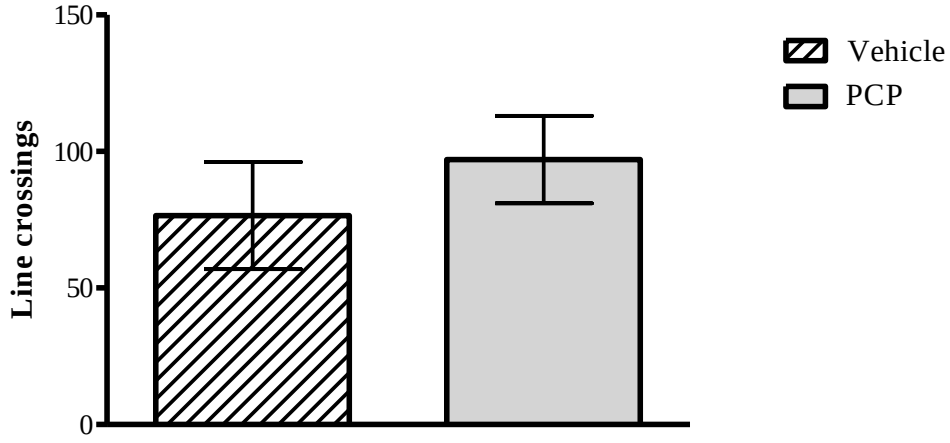


Figure 6.3.2.1d: The effect of treatment with vehicle or sub-chronic PCP (2 mg/kg, twice a day for 7 days, i.p.) on locomotor activity (line crossings) in the novel object recognition task. Data are shown as mean line crossings ± s.e.m (n=5 per group).

6.3.2.2 Dopamine results

Following the behavioural experiments, HPLC was carried out, therefore the groups for dopamine analysis were vehicle (n=5) and PCP (n=5). The raw values obtained for each rat over the baseline measurements and in the retention trial of the NOR task are shown in table 6.3.2.2a, the data expressed as percent of basal are shown in table 6.3.2.2b. An example chromatogram for the baseline values of a vehicle and PCP rats is shown in appendix 4.

Table 6.3.2.2a: Raw values of relative dopamine concentrations (nM) in the PFC for vehicle and sub-chronic PCP-treated rats. The data shows the mean basal concentrations and retention trial concentrations.

	Dopamine concentration (nM)			
	Vehicle		**PCP**	
Rat	**Mean basal**	**Retention**	**Mean basal**	**Retention**
1	0.260	0.198	3.229	3.437
2	10.391	6.109	4.223	4.587
3	4.897	38.173	3.844	4.148
4	0.354	0.741	2.290	4.162
5	0.456	1.509	1.020	0.617
Mean	*3.272*	*9.346*	*2.921*	*3.390*
SEM	*1.812*	*6.658*	*0.577*	*0.717*

Table 6.3.2.2b: Retention trial PFC dopamine data expressed as percent of basal for vehicle and sub-chronic PCP-treated rats. The data shows the mean basal concentrations and retention trial concentrations.

Rat	Vehicle	PCP
1	76.1	106.5
2	58.8	108.6
3	779.6	107.9
4	209.2	181.7
5	330.8	60.5

The mean of the 4 baseline concentrations prior to behavioural testing was calculated and data in figure 6.3.2.2a were expressed as percent of basal. A two-way repeated measures ANOVA with stage of test as the within subjects factor and treatment as the between subjects factor revealed no significant interaction ($F_{(7,56)}$=1.26; NS). However, planned pair-wise Bonferroni comparisons revealed a selective and significant increase in dopamine levels in vehicle treated rats in the retention phase (P<0.01), however, this effect was not found in PCP-treated rats.

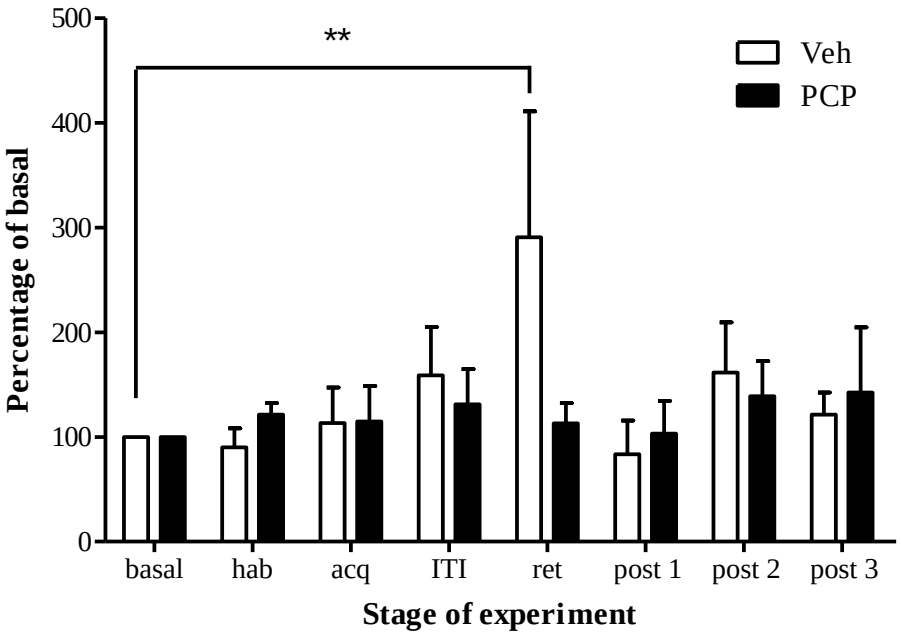


Figure 6.3.2.2a: Relative concentrations of dopamine in the PFC expressed as percent basal in vehicle and sub-chronic PCP-treated rats in each stage of the behavioural task. Data are shown as mean concentration ± S.E.M (n=5). **P<0.01 significant increase in dopamine in the retention phase compared to baseline levels.

The line of best fit was plotted for the DI and percent of basal dopamine in the retention stage (figure 6.3.2.2b), and a correlation analysis revealed that the line was not significant from zero (P=0.18).

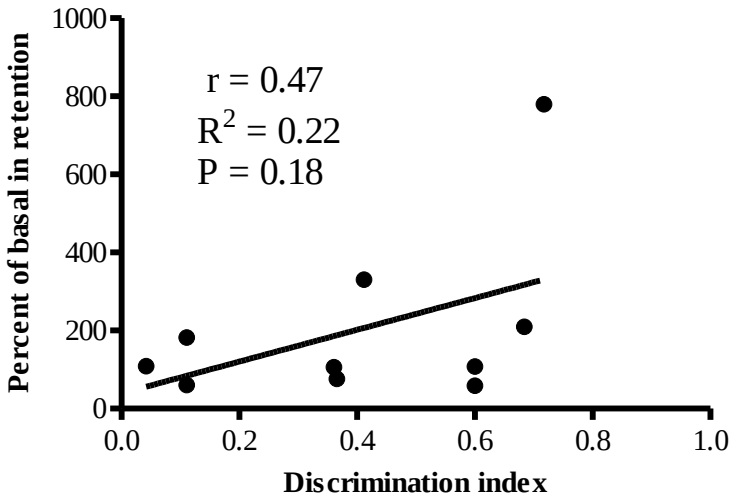


Figure 6.3.2.2b: Plot of DI and percent basal dopamine in the retention trial in NOR with the line of best fit plotted. Correlation analysis revealed a correlation coefficient of 0.47 and a coefficient of determination of 0.22; this was not significant from zero.

6.3.2.3 Microdialysis probe placement

Following Cresyl violet staining, the locations of the probes were verified. All probes were located within the PFC at Bregma 2.7 to 3.2 mm. An example image of probe location is shown in figure 6.3.2.3.

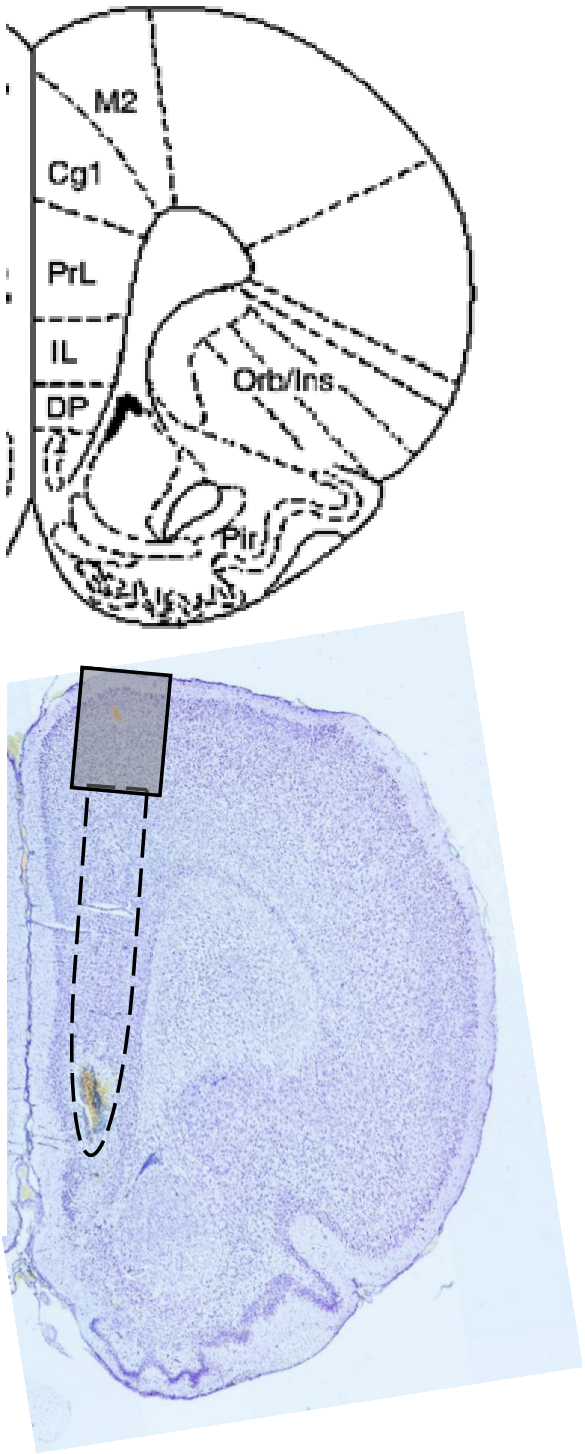


Figure 6.3.2.3: Microdialysis probe placement. *Top*: coronal image from Watson and Paxinos (1998), Bregma 2.7 mm showing PFC regions. *Bottom*: Photograph taken at x4 magnification following Cresyl violet staining. The solid shaded rectangle represents the position of the cannula; the dotted line represents the tip position.

6.4 Discussion

6.4.1 PCP sensitisation and anxiety

A component of this study examined whether sub-chronic PCP would induce anxiety-like behaviour in the adult rat. Sub-chronic PCP had no effect on behaviour in the open field or elevated plus maze. Following an acute challenge with PCP, there was an observed increase in locomotor activity in the sub-chronic PCP treated rats, with no effect in vehicle treated animals. This effect was also seen in chapter 5. Previous studies in male rats, using similar treatment regimes at comparable doses have reported similar findings (Kalinichev *et al.,* 2008). This sensitisation in the sub-chronic PCP treated animals may be related to the disruption of GABAergic interneurons in the medial prefrontal cortex (Abekawa *et al.,* 2007). Taken together these results indicate that the sub-chronic PCP regimen was successful in producing behavioural changes in these animals thus validating the treatment prior to testing in the anxiety paradigms.

In the present study there were no significant differences between vehicle and PCP-treated rats in time spent in each arm or number of entries on the elevated plus maze. There were also no differences in open field behaviour with rats spending a similar duration in the centre square and making a similar number of entries into the centre. These data suggest that sub-chronic PCP does not have an anxiogenic effect as assessed by the elevated plus maze or open field test. These findings are supported by studies using a perinatal (du Bois *et al.,* 2008) and sub-chronic pubertal (Schwabe *et al.,* 2006) regimen of PCP dosing which showed no difference in elevated plus maze behaviour in adult female and male rats respectively, suggesting that these PCP regimes do not produce anxiety-like behaviour. However, these data are in contrast with a recent study by Audet and co-workers. They found that sub-chronic PCP followed by a week washout period resulted in male rats spending less time in the lit area of a brightly

lit/dark arena (Audet *et al.,* 2007). However, it should be noted that they used a different strain and sex of rat and a higher dosing regimen of 5 mg/kg twice daily for 7 days, compared to our regimen of 2 mg/kg twice daily for the same duration in female rats. It is important to note that results concerning anxiety and neophobia vary between laboratories and differences are observed between sexes (see Toufexis *et al.,* 2006) and strains (Hall *et al.,* 2000; Rex *et al.,* 2004).

6.4.2 Novel object recognition

In the NOR task, the time spent exploring both objects in the acquisition trial was not significantly different between vehicle and PCP-treated rats. There was also no significant difference in total exploration between the groups in the retention trial; this suggests that neither group of rats were experiencing neophobia when confronted with a novel object in the retention trial or that PCP rats found the open field anxious. This result is in agreement with previous results from our laboratory (Grayson *et al.,* 2007 Grayson *et al.,* 2008; Idris *et al.,* 2009).

In the acquisition trial of the NOR task, there was no difference in the exploration time of the two identical objects in either vehicle or PCP-treated rats. In the retention trial, the discrimination index revealed that vehicle-treated rats could discriminate the novel from familiar object in the retention trial, suggesting they recognised the familiar object. In the PCP-treated rats, the discrimination index revealed that rats could not distinguish between the novel and familiar objects, suggesting that PCP-treated rats did not remember the familiar object. Although the time exploring the novel and familiar objects in the retention trial was not significant, this is likely to be due to the low numbers in the group (vehicle n=5 and PCP n=5). Previous studies in this laboratory have shown a significant deficit with sub-chronic

PCP in NOR when using 8-10 rats (Grayson *et al.,* 2007; Grayson *et al.,* 2008; Idris *et al.,* 2009). These results have also been supported by Hashimoto *et al.,* (2005) who showed that a sub-chronic PCP dosing regimen of 10 mg/kg, s.c. once daily for 10 days, administered on days 1-5 and 8-12 produced a deficit in NOR, and that clozapine (5 mg/kg) but not haloperidol (0.1 mg/kg) when administered once daily for 2 weeks, attenuated the deficit in object recognition in mice. Additionally, in mice, it has been shown that PCP (10 mg/kg) administration for 14 days produced a deficit in NOR, an effect which was ameliorated by single (1 mg/kg) and repeated (0.3 and 1 mg/kg, for 7 days) treatment with the antipsychotic aripiprazole (Nagai *et al.,* 2009). Furthermore, the attenuating effect of aripiprazole to reverse the PCP-induced deficit in object recognition memory was blocked by co-treatment with dopamine D_1 receptor antagonist, SCH23390, and the serotonin 5-HT_{1A} receptor antagonist, WAY-100635 (Nagai *et al.,* 2009). Results from our laboratory have also suggested a role of D_1 receptors, in that the D_1 receptor agonist, SKF-38393, was shown to improve a PCP-induced deficit in the NOR task (Idris *et al.,* 2009b). The mechanism for this effect of PCP is not established yet, however, recent work in our laboratory showed that the PCP-induced deficit is maintained following a 1 hour inter-trial interval and may be due to increased susceptibility to distraction during the inter-trial interval (Grayson *et al.,* 2008).

6.4.3 Dopamine levels during NOR

The concentration of dopamine in the PFC revealed that the basal levels in vehicle and PCP-treated rats were similar. The concentrations were also comparable in all stages of the NOR task with the exception of the retention trial, in which a large increase in dopamine was observed in the vehicle treated group. This increase was absent in the PCP-treated group. This finding is comparable with the percent of basal data, where in

the retention trial; dopamine in vehicle rats was increased to 225% of baseline, an increase which was statistically significant from the basal level. In comparison, the PCP-treated rats remained at 113% of baseline. Correlation analysis, however, did not reveal a significant correlation between the discrimination index and percent of basal dopamine in the retention phase.

These results demonstrate a significant increase in dopamine observed during the retention trial of the NOR task in vehicle-treated rats. This result is supported by similar data showing a significant increase in dopamine during the retention trial of the task (Snigdha *et al.,* sfn abstract). The increase in dopamine in the current study was selective for the retention trial. It must be noted that within the vehicle treated group there is one very high value that may be skewing the data; more animals would be needed to confirm this finding. This may suggest that the act of recalling the familiar object and therefore recognising the novel object as new is accompanied by an increase in dopamine in the PFC. Thus, PCP-treated rats do not recruit prefrontal dopamine in the retention trial of the task and therefore perform poorly. This study implies that a lack of prefrontal dopamine when required may underlie the observed cognitive deficit in novel object recognition. It has been suggested that the negative and cognitive symptoms of schizophrenia may arise from a dopaminergic deficit in the prefrontal cortex (hypofrontality), whereas the positive symptoms may be related to hyperdopaminergic activity in mesolimbic dopaminergic neurones (Davis *et al.,* 1991; Laruelle *et al.,* 2003; this will be further discussed in chapter 7).

This requirement for prefrontal dopamine is supported by a previous study from our laboratory showing that the sub-chronic PCP-induced deficit in NOR was fully attenuated by the dopamine D_1 receptor agonist SKF-38393 (Idris *et al.,* 2009). Additionally, the dopamine D_1 receptor agonist, SKF-81297, has been shown to

improve object recognition following a 4 hour delay (Hotte *et al.,* 2005). Furthermore, chronic PCP treatment (5 mg/kg for 14 days) was shown to dramatically decrease the D_1 dopamine receptor agonist-mediated activation of G-proteins in the PFC, indicating a dysfunction of D_1 receptors in the PFC in chronic PCP treated rats (Guo *et al.,* 2009). As previously described in chapter 3, D_1 receptors are coupled to adenylyl cyclase, which results in an increase in cAMP and activation of protein kinase A (PKA). Hotte and coworkers (2006) attribute the improvement in object recognition memory to an increase in the level of phosphorylation of both cAMP response element binding protein (CREB) and DARPP-32 in the prefrontal cortex. CREB is a transcription factor believed to be important in the cascade produced by activation of D_1 receptors (Lamprecht, 1999; Liu and Graybiel, 1996). DARPP-32 is a major downstream target for dopamine signaling and PKA activation, and has been established as a crucial mediator of the biochemical, electrophysiological, and behavioural effects of dopamine (Svenningsson *et al.,* 2004).

6.4.4 Limitations

It was originally planned to also measure dopamine from the nucleus accumbens (NAc) during the NOR task. However, there were several methodological issues with the NAc data. Firstly, in two of the rats the probes were not in the correct region and in other rats there were problems with the flow through the probe on the day of the experiment. Problems with the HPLC were also encountered with the NAc samples. Subsequently, there was insufficient data to report the NAc results.

Two vehicle-treated rats were excluded for behavioural reasons i.e. one rat jumped out of the NOR box and one rat did not explore the objects, these rats were subsequently also excluded from the bodyweight and HPLC analysis as the data was to

be analysed for a correlation between the DI and dopamine levels in the retention trial. The final number of rats (5 vehicle and 5 PCP) used in the microdialysis experiment is also a limitation of the study. It would have been more useful to include more rats; however, due to time constraints this was not possible.

The NOR task was performed using 10 min trials and a 10 min inter-trial interval. This was to ensure that an adequate sample volume (20 µL) could be collected for detection by HPLC (15 µL injected onto the column). In our laboratory this task is usually carried out using 3 min trials with a 1 min inter-trial interval, as generally most object exploration occurs within the first 2 min. In the microdialysis experiment, potential increases in dopamine caused by novel object exploration may have been undetected within the sample as it was likely that this exploration occurred within the first 2-3 min; therefore there was potentially another 7-8 min of collection time in which dopamine levels may have returned to normal, thus diluting any increase in dopamine in the sample. Sampling has been carried out over 1 min periods previously (Young *et al.*, 2004); however, this method was considered too complex to carry out within the behavioural experiments detailed in this current study.

Probe *in vitro* recovery was found to be 10-15%, which merely provides an indication of how the probe was functioning *in vitro*. It is not appropriate to apply this *in vitro* recovery as a conversion factor to calculate the concentration of dopamine *in vivo* as the probe is possibly acting differently in an *in vivo* situation. Therefore, concentrations can only be compared to that animals' baseline thus these concentrations remain relative, and do not give an accurate measure of the actual brain concentrations *in vivo*.

6.4.5 Conclusions

In summary, the novel object recognition task is a test of short term episodic memory, a cognitive function which was impaired (as measured by the DI) following sub-chronic PCP treatment. These data demonstrate an increase in dopamine during the retention trial in vehicle rats which was not observed in PCP-treated rats, suggesting recruitment of prefrontal dopamine may be necessary for object recognition memory; however, more animals would be required to confirm this proposal. This is impaired in PCP-treated rats providing good validation of the sub-chronic PCP model for cognitive dysfunction in schizophrenia.

Chapter 7

General Discussion

7.1 General Discussion

The work presented in this thesis forms part of a larger effort by researchers working on schizophrenia to identify an animal model that mimics the behavioural aspects of the disorder and also aims to investigate the underlying neurobiology. One of these models is the sub-chronic PCP model in rodents which induces disruption in behavioural tasks of relevance to schizophrenia. The data presented here describes robust and reproducible sub-chronic PCP-induced deficits in rodent cognitive tasks. Furthermore, studies were then undertaken to investigate the neurobiological changes produced by sub-chronic PCP in an attempt to develop an understanding of the circuit dysfunction underpinning the disorder.

A summary of the behavioural results with sub-chronic PCP in this thesis are shown in table 7.1.1. The effect of isolation rearing in attentional set-shifting was also examined. The principal findings show that isolation reared rats exhibit impaired set-shifting ability when compared with socially reared controls (see chapter 2). Thus isolates required significantly more trials to reach criterion selectively at the EDS phase of testing, when compared with socially housed animals indicative of a selective deficit in set-shifting ability. However, the deficits produced by sub-chronic PCP were more robust than the deficit produced by isolation rearing; therefore this model was further investigated. Following on from the behavioural tasks, the results shown from *in vitro* electrophysiology, *ex vivo* immunohistochemistry and *in vivo* microdialysis are shown in table 7.1.2.

Table 7.1.1: Effects of sub-chronic PCP on behaviour.

Paradigm	Chapter	Brain Region	Result
Attentional set-shifting	2	mPFC	Deficits in EDS with PCP
Reversal learning	3, 4	PFC, orbital	Deficit in reversal phase with PCP
Novel object recognition	6	PFC, perirhinal cortex, entorhinal cortex	Deficit in novel object recognition during the retention trial in PCP-treated rats.
Elevated plus maze	6	Limbic structures e.g. amygdala	No effect of PCP
Open field	6	Limbic structures e.g. amygdala	No effect of PCP

Table 7.1.2: Effects of sub-chronic PCP in non-behavioural techniques.

Technique	Chapter	Brain Region	Result
Electrophysiology (*in vitro*)	5	CA3, PFC	Reduction in gamma oscillations 2 weeks following PCP treatment in the CA3. Increase in gamma oscillations 8 weeks following PCP treatment. No change in PFC at either time point.
Immunohistochemistry (*ex vivo*)	5	CA2/3	Reduction in parvalbumin IR cell density in the CA2/3 2 weeks post PCP treatment. No difference 8 weeks following PCP treatment.
Microdialysis (*in vivo*)	6	PFC	Vehicle rats show an increase in dopamine during the retention trial of the NOR task. This increase in dopamine was absent in PCP-treated rats.

The chief findings are summarised below:-

(1) Sub-chronic PCP produces more pronounced effects in female rats compared to male rats; this may be due to differing sensitivity to PCP. Sub-chronic PCP

administration was shown to impair set-shifting performance selectively in the EDS phase, a deficit which was significantly attenuated by sub-chronic administration of clozapine (2.5 mg/kg) and risperidone (0.2 mg/kg), but not haloperidol (0.05 mg/kg). A deficit in set-shifting ability was also observed in isolation reared rats. This chapter demonstrated that deficits in attentional set-shifting ability can be produced by sub-chronic PCP administration and isolation rearing. However, the deficits produced by PCP were more robust than the deficit produced by isolation rearing. Therefore, the sub-chronic PCP model was pursued in other cognitive tests.

(2) Sub-chronic PCP administration impairs reversal learning ability as demonstrated by reduced percent correct responding in the reversal phase of the reversal learning task. It was found that D_1 agonists, 5-HT_{1A} partial agonists, 5-HT_{2C} antagonists and positive modulators of the α7 nACh receptor are able to reverse the sub-chronic PCP-induced deficit in reversal learning. Although many antipsychotics have affinity for muscarinic M_1 and histamine H_1 receptors, selective agents at these receptors were not able to improve the PCP-induced deficit. From previous experiments in the literature it is possible that these agents may improve cognition via increasing dopamine or acetylcholine levels in the prefrontal cortex (see fig 7.2), the region thought to be involved in reversal learning ability. For detailed discussion of the receptor pharmacology refer to chapter 3.

(3) The results from chapter 4 show that the atypical antipsychotics, clozapine and risperidone, when given alone to naïve rats have no effect on reversal learning. Haloperidol when given to naïve rats impaired performance at the highest dose (0.1 mg/kg). Sub-chronic PCP was again found to impair reversal learning performance. The differences in the vehicle-treated and sub-chronic PCP-treated rats with or without the 2 min time-out were not significant but suggested that the 2 min time-out could be

important as a cue. Following a double reversal, olanzapine-treated rats lose the ability to switch between the rules, possibly indicating it is not as efficacious as clozapine or risperidone in reversing the PCP-induced deficit. The extended (15 min) reversal phase showed that PCP caused a deficit after 5 min but this effect was lost after 15 min. Using an extended reversal would also allow the investigation of the time-course effects of antipsychotics or selective compounds.

(4) The studies presented in chapter 5 found a reduction in gamma oscillations following sub-chronic PCP treatment (2-5 weeks post treatment) that was paralleled by a deficit in parvalbumin immunoreactive cell density, at a similar time point (2 weeks post treatment). In contrast, a time-dependent increase in gamma oscillations was observed (6-8 weeks post treatment), at which point parvalbumin IR cell density was unchanged (8 weeks post treatment). These studies demonstrate a link between altered gamma-frequency oscillations and abnormalities in parvalbumin interneurons, which may underlie some of the cognitive deficits previously reported in this animal model of schizophrenia.

(5) Sub-chronic PCP treatment impaired rats to distinguish between a novel and a familiar object following a 10 min inter-trial interval (chapter 6). In vehicle-treated rats an increase in dopamine was observed in the PFC during the retention trial of the behavioural task. In PCP-treated rats the levels of dopamine were unchanged in the retention trial. This study may suggest that the recruitment of prefrontal dopamine is needed to distinguish between the novel and familiar objects.

7.2 The validity of the sub-chronic PCP model to mimic cognitive dysfunction observed in patients with schizophrenia and the relevance of the cognitive behavioural tasks

Animal models are critical in understanding the mechanisms underlying many human diseases, and in particular psychiatric disorders. However, an animal model of schizophrenia has proved to be very difficult to develop as it is mainly a thought disorder involving complex cortical processes which may not be expected to be found in lower mammals. It is therefore unrealistic to expect a single animal model to mimic all aspects of schizophrenia; however, it is possible to mimic certain symptom clusters. Table 7.1.3 summarises the behavioural changes induced by different PCP dosage regimens and their relation to the clinical symptoms of schizophrenia.

Table 7.1.3: Comparison of clinical symptoms of schizophrenia with the schizophrenia-like behaviour induced by PCP (table adapted from Lipska and Weinberger, 2000).

Clinical symptom	Behavioural change	Reference
Psychotic symptoms	An increase in locomotor activity	See chapters 5 and 6
Stereotyped behaviours	Repetitive sniffing, face washing etc	Sams-Dodd, 1998; Linn *et al.*, 2007
Vulnerability to stress	Changes in locomotor activity or behaviour induced by stress	Jentsch *et al.*, 1998
Information processing deficits	Deficits in PPI	Egerton *et al.*, 2008
Attentional deficits	Deficits in 5-choice serial reaction time task	Amitai *et al.*, 2007; Amitai and Markou, 2009
Cognitive deficits	Deficits in attentional set-shifting, reversal learning and novel object recognition memory	See chapters 2,3 and 6 of this thesis
Social withdrawal	Deficits in social interaction	Sams-Dodd, 1998; Snigdha and Neill, 2008a,b

Whilst impairments have been reported across all of the cognitive domains identified by the MATRICS initiative (see chapter 1), impairments in executive function have long been considered to be a core feature of schizophrenia (Elvevag and Goldberg, 2000; Nuechterlein and Dawson, 1984; Sullivan *et al.*, 1994). One aspect of executive function is the ability to modify behaviour in response to the changing relevance of a stimulus; this is commonly assessed in patients using the Wisconsin Card Sorting Test (WCST) (Berg, 1948). The attentional set-shifting task represents a rat analogue of the human Wisconsin Card Sorting Task (WCST, Berg, 1948) and CANTAB ID/ED task (Downes *et al.,* 1989) in which patients with schizophrenia exhibit impaired set-shifting (Kolb and Wishaw, 1983; Haut *et al.*, 1996; Pantelis *et al.,* 1999; Tyson *et al.,* 2004). It was shown that clozapine and risperidone, but not haloperidol reversed the sub-chronic PCP-induced deficit in the EDS phase of the attentional set-shifting task (chapter 2). It was also shown that clozapine, olanzapine (chapter 4) and risperidone (chapters 3 and 4) reversed the PCP-induced deficit in the reversal learning task. Effective performance in the reversal learning task requires intact cognitive ability; thus animals are required to demonstrate flexibility, attention, motivation, and ability to suppress a previously learned response and implement a new one (Jones *et al.*, 1991). Both the reversal learning and attentional set-shifting tasks have been identified by the TURNS initiative as tests that can be used to determine the reasoning and problem solving deficits described in the MATRICS cognitive battery (www.turns.ucla.edu).

Deficits in the novel object recognition task (Ennaceur and Delacour, 1988) were also observed following sub-chronic PCP treatment. The MATRICS initiative categorised the novel object recognition task as relevant to visual learning and memory. It has been shown that visual recognition memory is impaired in patients with

schizophrenia (Calkins *et al.,* 2005). Data in this thesis may suggest a role for dopamine in object recognition memory as an increase in prefrontal dopamine was found in the retention trial of the task in vehicle-treated rats (chapter 6); however, more animals would be required to confirm this proposal. The effect of antipsychotics was not investigated in this study, but other results from our laboratory have found clozapine and risperidone to improve the PCP-induced deficit (Grayson *et al.,* 2007). Furthermore, it was shown that clozapine and risperidone increased dopamine acetylcholine in the mPFC, but only sertindole increased glutamate levels (see Mork *et al.,* 2009).

The results from this thesis suggest that the sub-chronic PCP model is able to induce cognitive deficits similar to those seen in patients with schizophrenia. Sub-chronic PCP has also altered gamma oscillations and parvalbumin IR cell density, suggesting GABAergic deficits, as well as dopamine hypofunction in the PFC during the novel object recognition task. It was also shown that atypical but not typical antipsychotics were able to reverse the PCP-induced deficits in attentional set-shifting and reversal learning, therefore providing predictive validity. However, the Clinical Antipsychotic Trials of Invention Effectiveness (CATIE) study revealed that atypical antipsychotics, such as clozapine, show little improvement compared to typical antipsychotics, against cognitive symptoms (Lieberman, 2006; Keefe *et al.,* 2007). Although this is a valid concern, it is important to note that this study did not incorporate young patients or patients with first episode psychosis. Therefore, it may be possible that atypical antipsychotics could show improvement if given early. It is also important to remember that we are using animals to mimic aspects of a uniquely human condition; humans who may have a genetic and neurodevelopmental predisposition for the disorder. Patients are also prescribed a considerable amount of medication and are abusing numerous psychoactive agents, including being avid smokers and have varied

and often traumatic life experiences. In marked contrast, our animals have no genetic predisposition (unless transgenic mice are used), no previous drug history and no current drug abuse. Instead they are genetically identical (if using an inbred strain) and live in optimum conditions (lighting, noise level, humidity etc) for that species with minimum stress. Subsequently this makes them ideal for our research but also makes it even harder to fully mimic a human disorder (particularly a psychiatric disorder) and this caveat is important to recognise.

7.3 The possible mechanism of action of PCP

The behavioural deficits in this thesis are possibly due to decreased dopamine in the PFC. In particular, this was demonstrated in the novel object recognition study. Treatment with NMDA antagonists induces disruption of the glutamatergic system. However, unlike acute treatment which increases glutamate release in the brain, sub-chronic treatment with NMDA antagonists induces a decrease in glutamate release in the frontal cortex (Zuo *et al.,* 2006). In addition, sub-chronic PCP treatment produces hypofunction of mesocortical dopamine neurons and hyperactivity of mesolimbic dopamine neurons (Jentsch *et al.,* 1998). Similar changes in the dopaminergic and glutamatergic systems have been found in schizophrenia patients (Kim *et al.,* 1980; Tsai *et al.,* 1995; Breier *et al.,* 1997). The mechanism by which prolonged or sub-chronic PCP treatment leads to dopaminergic dysfunction is currently unknown, but it is suggested that subcortical efflux of dopamine along with cortical dopamine hypofunction is responsible for the cognitive deficits induced by NMDA receptor antagonists such as PCP and ketamine (Jentsch *et al.,* 1997). It has been suggested that acute PCP increases dopamine via the GABAergic disinhibition of dopaminergic and glutamatergic neurons (see figure 7.1 bottom panel). Sub-chronic PCP is then thought

to show reduced levels of dopamine and glutamate due to compensatory changes, such as alteration in postsynaptic receptor density (see Jentsch and Roth, 1999). In addition to the dopaminergic and glutamatergic systems, PCP has also been shown to affect the serotonergic system. Systemic administration of acute PCP was shown to increase 5-HT release in the nucleus accumbens (Millan *et al.,* 1999), suggesting that PCP (acutely) can enhance the serotonergic function, which can subsequently be blocked by 5-HT_{2A} receptor antagonist (Idris *et al.,* 2008); an important pharmacological aspect of antipsychotics (Schmidt *et al.,* 1995; Meltzer, 1989). It has also been shown that repeated PCP administration results in an increase in 5-HT utilisation in the PFC under stress (Noda *et al.,* 2000). Results from this thesis (chapter 3) have shown that serotonergic compounds improved the sub-chronic PCP-induced deficit in reversal learning. The discussion of chapter 3 suggests that these compounds improve cognition by increasing dopamine in the PFC and nucleus accumbens (Ichikawa and Meltzer, 1990; Di Matteo *et al.,* 2001), which would presumably reverse the changes induced by sub-chronic PCP in figure 7.1 (bottom panel).

Results from chapter 5 showed significant decreases in both gamma frequency oscillations and parvalbumin IR cell density in the CA2/3 region of the hippocampus at 2-5 weeks and 2 weeks following PCP treatment respectively. In a normal brain these interactions between interneurons and pyramidal cells are perfectly balanced and synchronised. Reductions in gamma oscillations and parvalbumin IR density are also observed in patients with schizophrenia (Benes and Berretta, 2001; Reynolds *et al.,* 2002; Zhang and Reynolds, 2002; Lewis *et al.,* 2005; González-Hernández *et al.,* 2003; Cho *et al.,* 2006). Therefore, it could be suggested that these neurobiological changes induced by sub-chronic PCP may underly the cognitive deficits also observed in schizophrenia and the sub-chronic PCP animal model. The cognitive deficits observed

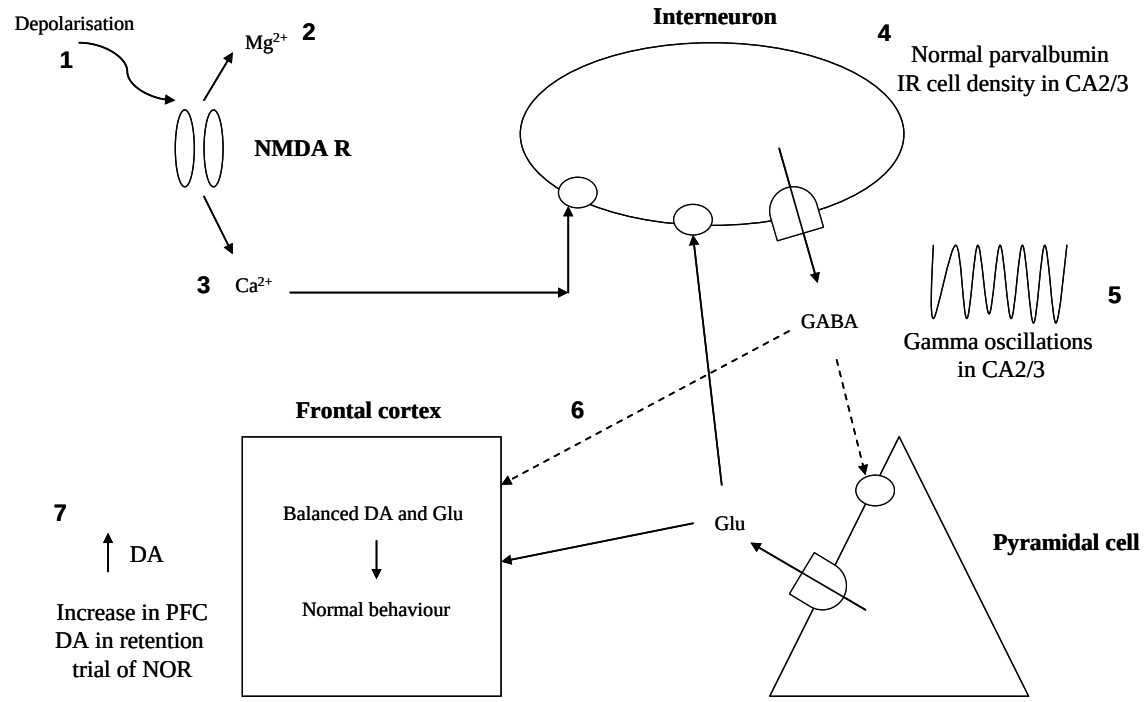
in our animal model are apparant up to 6 months post PCP treatment (Grayson and Idris, personal communication). However, the gamma oscillations at 6-8 weeks post PCP treatment were increased compared to the vehicle-treated group, and this was accompanied by no change in parvalbumin IR cell density at 8 weeks post PCP treatment. This suggests that a time-dependent compensatory mechanism is possibly recovering the gamma oscillations and parvalbumin IR cell density. It is suggested that blocking NMDA receptors on interneurons changes the phenotype of the neurons. If the PV interneurons are still present but are not expressing parvalbumin, this may allow PV expression to be increased with time due to compensatory mechanisms, such a mechanism may involve the increase in NMDA receptors or NMDA receptor subunits. It is also not possible to say whether the levels of expression return to normal because we can only measure whether expression is there or not, rather than quantifying the levels.

Though gamma oscillations were found to increase over time, this may still cause cognitive deficits, as it still represents an unsynchronised network of GABA interneurons and pyramidal cells. Indeed, it has been shown that both decreases and increases in gamma oscillations are found in patients with schizophrenia (Light *et al.*, 2006). In this thesis it was shown that there was a reduction in parvalbumin IR cell density in the CA2/3 region of the hippocampus at 2 weeks following PCP treatment; however cell density was unchanged at 8 weeks following PCP. Immunohistochemistry does not allow us to determine whether these reductions are due to a diminished number of PV-containing interneurons or a down-regulation of parvalbumin expression, i.e. rendering the neurons in a less excitable state (Kohr *et al.*, 1991). If the latter case was true it may be possible for this functionality to return over time.

If these deficits in GABAergic neurotransmission are underpinning the cognitive deficits observed in our model, then another mechanism must also be producing cognitive deficits in order for the deficits in cognition to be observed 6 months following PCP treatment. This could be the lack of prefrontal dopamine recruitment during the cognitive task.

Results from this thesis also suggest a role for dopamine in cognition. The D_1 receptor agonist SKF-38393 was shown to improve the PCP-induced deficit in reversal learning. Furthermore, it was found that in vehicle-treated rats performing the NOR task there was a significant increase in dopamine in the PFC which was selective for the retention phase of the task i.e. dopamine was only increased when the rats were exposed to the novel object. This increase in prefrontal dopamine during the retention trial was absent in the PCP-treated rats. Therefore, this data may suggest a role for dopamine in NOR memory. This lack of dopamine recruitment may be due to disinhibition of the GABAergic system, which initially increases dopamine and glutamate, but sub-chronic PCP induces compensatory reduction in glutamate and dopamine in the frontal cortex. The GABAergic deficits in this thesis were found to recover after time, but it was not determined whether the dopaminergic deficit in the PFC recovered. It would be interesting to determine whether these deficits persist along with the behavioural impairments.

Figure 7.1


Depolarisation
1
Mg2+
2
NMDA R
3
Ca2+
Interneuron
4
Normal parvalbumin
IR cell density in CA2/3
5
Gamma oscillations
in CA2/3
GABA
Frontal cortex
6
Balanced DA and Glu
Normal behaviour
Glu
Pyramidal cell
7
DA
Increase in PFC
DA in retention
trial of NOR

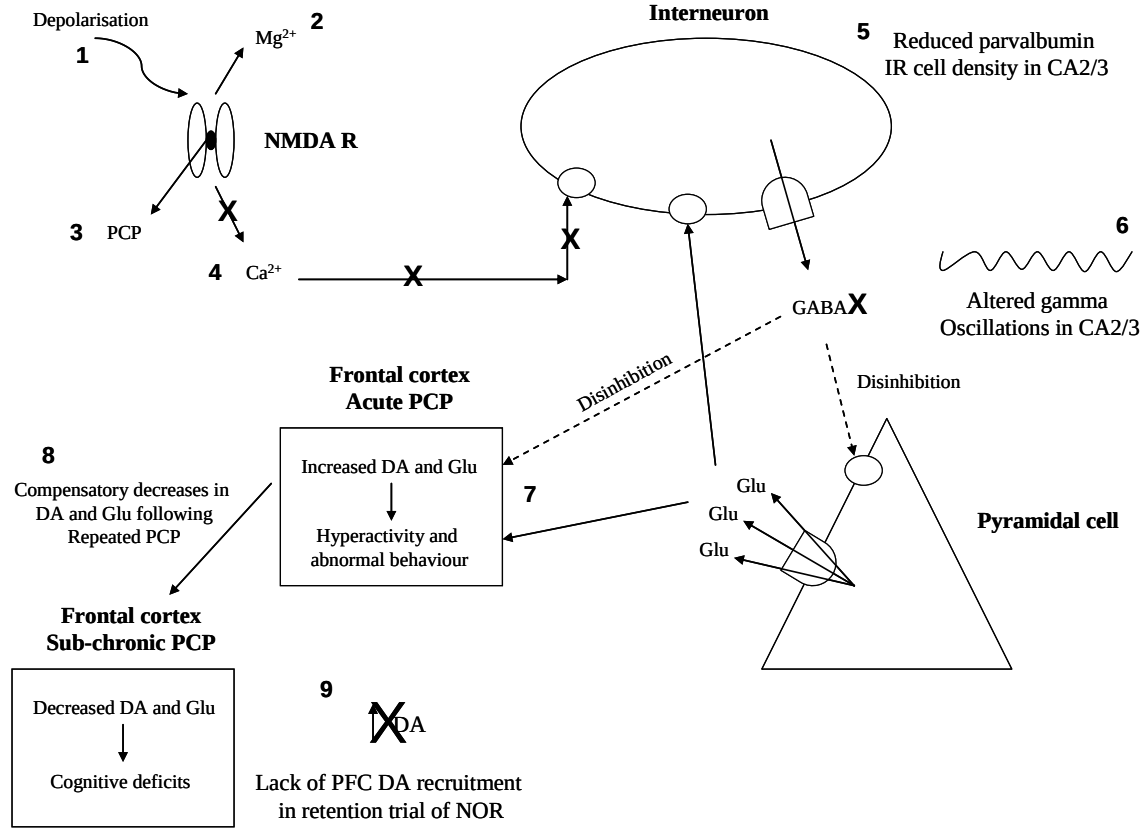

Depolarisation
1
Mg2+
2
NMDA R
3
PCP
4
Ca2+
Interneuron
5
Reduced parvalbumin
IR cell density in CA2/3
6
Altered gamma
Oscillations in CA2/3
GABA
Disinhibition
Disinhibition
Frontal cortex
Acute PCP
Increased DA and Glu
Hyperactivity and
abnormal behaviour
7
Glu
Glu
Glu
Pyramidal cell
8
Compensatory decreases in
DA and Glu following
Repeated PCP
Frontal cortex
Sub-chronic PCP
Decreased DA and Glu
Cognitive deficits
9
DA
Lack of PFC DA recruitment
in retention trial of NOR

Figure 7.1: Schematic diagram of the findings in this thesis in vehicle-treated rats (top) and PCP-treated rats (bottom): a proposed mechanism of action. *Top*: (1) Depolarisation of neuronal membrane which results in (2) Mg^{2+} blockade of the NMDA receptor being removed, allowing (3) Ca^{2+} efflux is required for GABA neurons to function. (4) Parvalbumin IR cell density is normal in the CA2/3 region of the hippocampus. (5) Synchronous firing between interneurons and pyramidal cells produces gamma oscillations. (6) GABA inhibits the release, therefore controlling the levels of DA and Glu in the frontal cortex, resulting in normal behaviour. (7) *In vivo* microdialysis revealed an increase in DA in the PFC during the retention trial of the NOR task. *Bottom*: (1) Depolarisation of neuronal membrane which results in (2) Mg^{2+} blockade of the NMDA receptor being removed, following which (3) PCP binds to its site, (4) preventing Ca^{2+} efflux required for GABA neurons to function. (5) Parvalbumin IR cell density was reduced in the CA2/3 region of the hippocampus. (6) Lack of synchronous firing between interneurons and pyramidal cells produced a time-dependent reduction, then increase in gamma oscillations. (7) Dysfunction of GABA transmission results in disinhibition and therefore increased excitatory transmission, thus increasing DA and Glu following acute PCP. (8) Reduced DA and Glu levels are thought to be due to compensatory changes leading to a hypofunctional state resulting in the cognitive deficits described in this thesis. (9) An example of this is the lack of prefrontal DA recruitment in the retention trial of the NOR task.

7.4 The mechanisms of action of antipsychotics

From the experiments in the chapter 3 it is evident that D_1 receptor agonists, 5-HT_{1A} receptor partial agonists, 5-HT_{2C} receptor antagonists and positive modulators of the alpha 7 nACh receptor are able to reverse the sub-chronic PCP-induced deficit in reversal learning. Although many antipsychotics have affinity for muscarinic M_1 and histamine H_1 receptors, selective agents at these receptors were not able to improve the PCP-induced deficit. From previous experiments in the literature it is possible that these agents may improve cognition via increasing dopamine or acetylcholine levels in the prefrontal cortex (see fig 7.2).

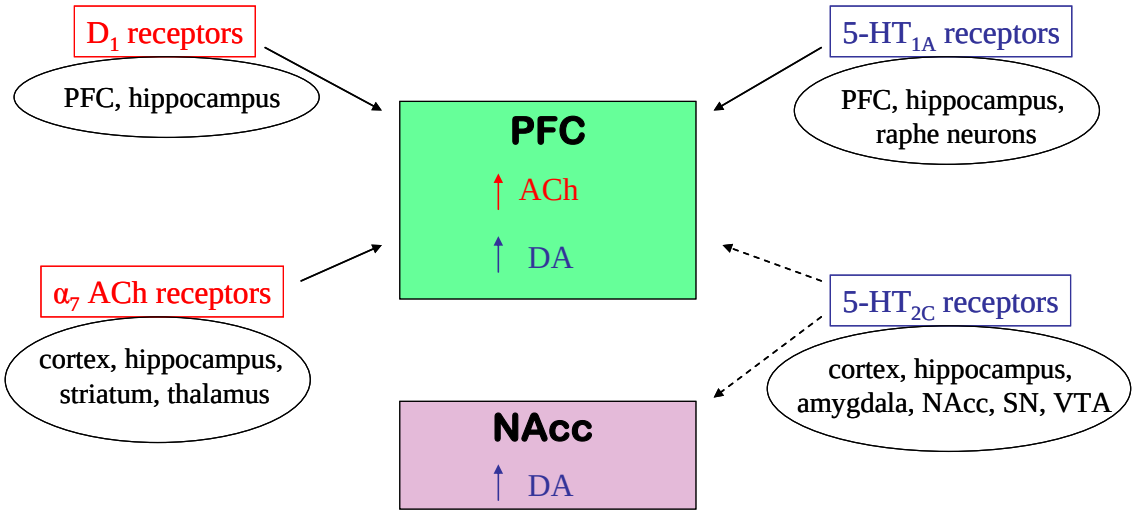


Figure 7.2: Summary diagram of the possible mechanisms involved in the attenuation of the sub-chronic PCP-induced deficit. Abbreviations: acetylcholine (ACh), dopamine (DA), prefrontal cortex (PFC); nucleus accumbens (NAcc), substantia nigra (SN), ventral tegmental area (VTA). Solid lines indicate agonism or activation, dotted lines indicate antagonism. Red highlights increases in ACh and blue highlights increases in DA.

Although the effects in the gamma oscillations and parvalbumin studies (chapter 5) appear to be time-dependent, the evidence here and in the literature does suggest a role of GABAergic neurotransmission in schizophrenia. It should be an aim of antipsychotic treatment to reverse the deficits in PV expression. In a rodent model of schizophrenia using chronic PCP treatment it was shown that clozapine reversed the PCP-induced deficits in PV expression (Cochran *et al.,* 2003). Furthermore, it was shown that co-administration of SSR180711, an α7 nAChR agonist, prevented the PV and behavioural deficits (Thomsen *et al.,* 2009). Indeed, the $GABA_A$ receptor could be a novel target for cognitive dysfunction associated with schizophrenia. For example the positive allosteric modulator of $GABA_A$ receptors, MK-0777, was shown to have high selectivity for the α2/α3 subunits and was found to improve working memory in schizophrenia patients; furthermore it was also shown to increase gamma power during the Preparing to Overcome Prepotency task (Lewis *et al.,* 2008). This cognitive task is a cued stimulus-response reversal paradigm that requires increases in cognitive control through the maintenance and use of context information to overcome prepotent response tendencies (Cho *et al.,* 2006).

7.5 Future Studies

The attentional set-shifting studies revealed that a single dose of the atypical antipsychotics reversed the PCP-induced deficit. However, if receptor selective compounds were to be used a dose-response would need to be carried out. This task is not ideal for carrying out multiple doses due to the time taken to carry out the experiments. Therefore, it would be advantageous to carry out a quicker experiment in, for example, the novel object recognition task to obtain an efficacious dose to carry forward to be tested in attentional set-shifting. Isolation rearing also produced a

significant deficit in the EDS phase of the attentional set-shifting task; however, the data were more consistent using the sub-chronic PCP model. It may be interesting to combine these two models in order to obtain a cognitive deficit that is more difficult to reverse. This would also combine a pharmacological model with a neurodevelopmental model, which may more closely mimic the brain disturbances observed in schizophrenia.

The attentional set-shifting task has been shown to be dependent on the medial PFC (Birrell and Brown, 2000). However, lesion studies have not been carried out in an operant reversal learning task. Therefore, it would be beneficial to carry out lesion studies (possibly of the OFC) in our operant reversal learning task to determine which region is involved in the task. From the results in chapter 4, it may be possible to distinguish between antipsychotics efficacies by using the extended reversal learning phase or the double reversal. Thus, the experiments in chapter 3 using selective compounds could be repeated with the aim that it could be determined how quickly the drugs begin to reverse the PCP-induced deficit. In addition, the use of the double reversal (or even multiple reversal) task could differentiate antipsychotics in that the more efficacious drugs would be able to continue reversing the deficit when the rule is repeatedly switched. All of the experiments carried out in the reversal learning task, tested compounds acutely. More studies utilising long-term treatment regimes with testing post-dosing could provide a better insight into the effects of drugs that are more likely to result in molecular/pathological changes and that could abolish cognitive dysfunction in the long-term and provide a real benefit for patients.

The *in vitro* electrophysiology data revealed a time-dependent effect of sub-chronic PCP on gamma oscillations in the CA2/3 region of the hippocampus. There was no observed effect in the PFC; however, this was probably due to the small signal. Gamma oscillations are more prevalent in regions with recurring architecture, other

regions to be studied for gamma oscillations include the entrorhinal and perirhinal cortices, and it has been suggested that these parahippocampal regions are involved in object recognition memory (Aggleton *et al.*, 1997). Studies could also be carried out using antipsychotics or selective receptor compounds to investigate whether the PCP-induced reduction in gamma oscillations (observed in weeks 2-5) could be restored. Studies could also be carried out using cognitive enhancers such as α7 nACh receptor agonists or positive allosteric modulators to see if the gamma oscillations in vehicle or drug-naive rats can be enhanced. The role of specific $GABA_A$ receptor subunits could also be investigated as these are altered in schizophrenia (Hashimoto *et al.*, 2008).

In the *in vivo* microdialysis experiment, due to methodological problems, the final number of rats (5 vehicle and 5 PCP) was a limitation of the study. It would have been beneficial to include more rats; however, due to time constraints this was not possible. Future studies using *in vivo* microdialysis may also include the use of antipsychotics to elucidate whether the lack of prefrontal dopamine in PCP-treated rats could be restored; this would help to identify the mechanistic properties of antipsychotics. The NOR task was performed using 10 min trials and a 10 min inter-trial interval. This was to ensure that an adequate sample volume (20 µL) could be collected for detection by HPLC (15 µL injected onto the column). In our laboratory this task is usually carried out using 3 min trials with a 1 min inter-trial interval, as generally most object exploration occurs within the first 2 min. Although 1 min sampling has been carried out (Young *et al.*, 2004), it may also be interesting to utilise *in vivo* fast-cyclic voltametry to measure bursts of dopamine activity. Additional utilisation of *in vivo* microdialysis could be extended to the reversal learning task, although, this would require 5 min sampling, unless the extended reversal phase was used.

It is noteworthy that the sub-chronic PCP model in adults does not account for the developmental aspects of schizophrenia. This is perhaps a limitation of the sub-chronic PCP model. In chapter 2, an isolation rearing model was utilised to examine differences in attentional set-shifting ability; however, although deficits were induced in the relevant stage of the task, this deficit was not as robust as in the sub-chronic PCP model. It may be useful to produce a pharmacological early insult, such as a neo-natal PCP model to investigate whether the cognitive deficits observed in adults would differ and indeed to ascertain whether behavioural deficits emerge only after puberty. Studies have been carried out using a neonatal PCP regimen in attentional set-shifting (Broberg *et al.*, 2008; 2009).

7.6 Conclusions

Schizophrenia remains a disorder caused by largely unknown factors including susceptibility genes, dysfunction of multiple neurotransmitter systems, and environmental factors. One or several of these factors presents diverse clinical symptoms. The complexity of schizophrenia has presented the scientific research community with a complicated challenge to develop a valid animal model of the disorder.

The behavioural and neurobiological data presented in this thesis strongly argue for considerable validity of a sub-chronic PCP dosage regimen in female rats as an effective model of schizophrenia. This model may, therefore, provide an excellent research tool into the mechanisms underpinning cognitive dysfunction observed in schizophrenia. Furthermore, the model may potentially predict the clinical response and functional outcome of schizophrenic patients to novel antipsychotic pharmacological therapies.

Appendix 1 – Attentional Set-shifting score sheet

TRAINING

1. 5 min habituation to empty test box.

2. Shaping to dig
   a) bowls + reward □ □ □
   b) bowls + reward on 1 cm media □ □ □
   c) bowls + reward on 1cm media under thin layer □ □ □
   d) bowls + reward on 1cm media under thick layer □ □ □ □ □

3. SD - Medium

Trial	L	R	Correct	Trial	L	R	Correct
1	M2	**M1**		13	M2	**M1**	
2	**M1**	M2		14	**M1**	M2	
3	**M1**	M2		15	M2	**M1**	
4	M2	**M1**		16	**M1**	M2	
5	**M1**	M2		17	**M1**	M2	
6	M2	**M1**		18	**M1**	M2	
7	M2	**M1**		19	M2	**M1**	
8	**M1**	M2		20	M2	**M1**	
9	M2	**M1**		21	**M1**	M2	
10	M2	**M1**		22	**M1**	M2	
11	**M1**	M2		23	M2	**M1**	
12	**M1**	M2		24	**M1**	M2	

4. SD - Odour

Trial	L	R	Correct	Trial	L	R	Correct
1	O2	**O1**		13	O2	**O1**	
2	**O1**	O2		14	**O1**	O2	
3	**O1**	O2		15	O2	**O1**	
4	O2	**O1**		16	**O1**	O2	
5	**O1**	O2		17	**O1**	O2	
6	O2	**O1**		18	**O1**	O2	
7	O2	**O1**		19	O2	**O1**	
8	**O1**	O2		20	O2	**O1**	
9	O2	**O1**		21	**O1**	O2	
10	O2	**O1**		22	**O1**	O2	
11	**O1**	O2		23	O2	**O1**	
12	**O1**	O2		24	**O1**	O2	

SD

Relevant Dimension: Media **Correct Exemplar:** M1 **Rat** **Date** **Total Time**

Discovery trials		
L	**R**	**Done**
M1	M2	
M2	**M1**	
M2	**M1**	
M1	M2	

Set-shifting

Trial	**L**	**R**	**Correct**	**Both**
1	M2	**M1**		
2	**M1**	M2		
3	**M1**	M2		
4	M2	**M1**		
5	**M1**	M2		
6	M2	**M1**		
7	M2	**M1**		
8	**M1**	M2		
9	M2	**M1**		
10	M2	**M1**		
11	**M1**	M2		
12	**M1**	M2		
13	M2	**M1**		
14	**M1**	M2		
15	M2	**M1**		
16	**M1**	M2		

Trial	**L**	**R**	**Correct**	**Both**
17	**M1**	M2		
18	**M1**	M2		
19	M2	**M1**		
20	M2	**M1**		
21	**M1**	M2		
22	**M1**	M2		
23	M2	**M1**		
24	**M1**	M2		
25	M2	**M1**		
26	M2	**M1**		
27	**M1**	M2		
28	M2	**M1**		
29	M2	**M1**		
30	**M1**	M2		
31	**M1**	M2		
32	M2	**M1**		
33	**M1**	M2		
34	M2	**M1**		
35	**M1**	M2		
36	**M1**	M2		
37	**M1**	M2		
38	M2	**M1**		
39	M2	**M1**		

Trial	**L**	**R**	**Correct**	**Both**
40	**M1**	M2		
41	**M1**	M2		
42	M2	**M1**		
43	**M1**	M2		
44	M2	**M1**		
45	M2	**M1**		
46	**M1**	M2		
47	M2	**M1**		
48	M2	**M1**		
49	**M1**	M2		
50	**M1**	M2		
51	M2	**M1**		
52	**M1**	M2		
53	M2	**M1**		
54	**M1**	M2		
55	M2	**M1**		
56	M2	**M1**		
57	M2	**M1**		
58	**M1**	M2		
59	**M1**	M2		
60	M2	**M1**		

Abort if >60 trials needed = failed set

CD

Relevant Dimension: Media **Correct Exemplar: M1** +O1/O2 **Rat** **Date** **Total time**

Discovery trials		
L	**R**	**Done**
M2 ,O2	**M1** ,O1	
M1 ,O2	M2 ,O1	
M1 ,O1	M2 ,O2	
M2 ,O1	**M1** ,O2	

Set-shifting				
Trial	**L**	**R**	**Correct**	**Both**
1	M2 ,O2	**M1** ,O1		
2	**M1** ,O2	M2 ,O1		
3	**M1** ,O1	M2 ,O2		
4	M2 ,O2	**M1** ,O1		
5	**M1** ,O2	M2 ,O1		
6	M2 ,O1	**M1** ,O2		
7	M2 ,O2	**M1** ,O1		
8	**M1** ,O2	M2 ,O1		
9	M2 ,O2	**M1** ,O1		
10	M2 ,O1	**M1** ,O2		
11	**M1** ,O1	M2 ,O2		
12	**M1** ,O2	M2 ,O1		
13	M2 ,O2	**M1** ,O1		
14	**M1** ,O1	M2 ,O2		
15	M2 ,O1	**M1** ,O2		
16	**M1** ,O2	M2 ,O1		

Trial	**L**	**R**	**Correct**	**Both**
17	**M1** ,O1	M2 ,O2		
18	**M1** ,O2	M2 ,O1		
19	M2 ,O2	**M1** ,O1		
20	M2 ,O2	**M1** ,O1		
21	**M1** ,O2	M2 ,O1		
22	**M1** ,O1	M2 ,O2		
23	M2 ,O2	**M1** ,O1		
24	**M1** ,O2	M2 ,O1		
25	M2 ,O1	**M1** ,O2		
26	M2 ,O2	**M1** ,O1		
27	**M1** ,O2	M2 ,O1		
28	M2 ,O2	**M1** ,O1		
29	M2 ,O1	**M1** ,O2		
30	**M1** ,O1	M2 ,O2		
31	**M1** ,O2	M2 ,O1		
32	M2 ,O2	**M1** ,O1		
33	**M1** ,O1	M2 ,O2		
34	M2 ,O1	**M1** ,O2		
35	**M1** ,O2	M2 ,O1		
36	**M1** ,O1	M2 ,O2		
37	**M1** ,O2	M2 ,O1		
38	M2 ,O2	**M1** ,O1		
39	M2 ,O2	**M1** ,O1		

Trial	**L**	**R**	**Correct**	**Both**
40	**M1** ,O2	M2 ,O1		
41	**M1** ,O1	M2 ,O2		
42	M2 ,O2	**M1** ,O1		
43	**M1** ,O2	M2 ,O1		
44	M2 ,O1	**M1** ,O2		
45	M2 ,O2	**M1** ,O1		
46	**M1** ,O2	M2 ,O1		
47	M2 ,O2	**M1** ,O1		
48	M2 ,O1	**M1** ,O2		
49	**M1** ,O1	M2 ,O2		
50	**M1** ,O2	M2 ,O1		
51	M2 ,O2	**M1** ,O1		
52	**M1** ,O1	M2 ,O2		
53	M2 ,O1	**M1** ,O2		
54	**M1** ,O2	M2 ,O1		
55	**M1** ,O1	M2 ,O2		
56	**M1** ,O2	M2 ,O1		
57	M2 ,O2	**M1** ,O1		
58	M2 ,O2	**M1** ,O1		
59	**M1** ,O2	M2 ,O1		
60	**M1** ,O1	M2 ,O2		

Abort if >60 trials needed = failed set

R1 **Relevant Dimension**: Media **Correct Exemplar:** **M2** +O1/O2 **Rat** **Date** **Total time**

Discovery trials		
L	**R**	**Done**
M2 ,O2	M1 ,O1	
M1 ,O2	**M2** ,O1	
M1 ,O1	**M2** ,O2	
M2 ,O1	M1 ,O2	

Set-shifting

Trial	**L**	**R**	**Correct**	**Both**
1	**M2** ,O2	M1 ,O1		
2	M1 ,O2	**M2** ,O1		
3	M1 ,O1	**M2** ,O2		
4	**M2** ,O2	M1 ,O1		
5	M1 ,O2	**M2** ,O1		
6	**M2** ,O1	M1 ,O2		
7	**M2** ,O2	M1 ,O1		
8	M1 ,O2	**M2** ,O1		
9	**M2** ,O2	M1 ,O1		
10	**M2** ,O1	M1 ,O2		
11	M1 ,O1	**M2** ,O2		
12	M1 ,O2	**M2** ,O1		
13	**M2** ,O2	M1 ,O1		
14	M1 ,O1	**M2** ,O2		
15	**M2** ,O1	M1 ,O2		
16	M1 ,O2	**M2** ,O1		

Trial	**L**	**R**	**Correct**	**Both**
17	M1 ,O1	**M2** ,O2		
18	M1 ,O2	**M2** ,O1		
19	**M2** ,O2	M1 ,O1		
20	**M2** ,O2	M1 ,O1		
21	M1 ,O2	**M2** ,O1		
22	M1 ,O1	**M2** ,O2		
23	**M2** ,O2	M1 ,O1		
24	M1 ,O2	**M2** ,O1		
25	**M2** ,O1	M1 ,O2		
26	**M2** ,O2	M1 ,O1		
27	M1 ,O2	**M2** ,O1		
28	**M2** ,O2	M1 ,O1		
29	**M2** ,O1	M1 ,O2		
30	M1 ,O1	**M2** ,O2		
31	M1 ,O2	**M2** ,O1		
32	**M2** ,O2	M1 ,O1		
33	M1 ,O1	**M2** ,O2		
34	**M2** ,O1	M1 ,O2		
35	M1 ,O2	**M2** ,O1		
36	M1 ,O1	**M2** ,O2		
37	M1 ,O2	**M2** ,O1		
38	**M2** ,O2	M1 ,O1		
39	**M2** ,O2	M1 ,O1		

Trial	**L**	**R**	**Correct**	**Both**
40	M1 ,O2	**M2** ,O1		
41	M1 ,O1	**M2** ,O2		
42	**M2** ,O2	M1 ,O1		
43	M1 ,O2	**M2** ,O1		
44	**M2** ,O1	M1 ,O2		
45	**M2** ,O2	M1 ,O1		
46	M1 ,O2	**M2** ,O1		
47	**M2** ,O2	M1 ,O1		
48	**M2** ,O1	M1 ,O2		
49	M1 ,O1	**M2** ,O2		
50	M1 ,O2	**M2** ,O1		
51	**M2** ,O2	M1 ,O1		
52	M1 ,O1	**M2** ,O2		
53	**M2** ,O1	M1 ,O2		
54	M1 ,O2	**M2** ,O1		
55	M1 ,O1	**M2** ,O2		
56	M1 ,O2	**M2** ,O1		
57	**M2** ,O2	M1 ,O1		
58	**M2** ,O2	M1 ,O1		
59	M1 ,O2	**M2** ,O1		
60	M1 ,O1	**M2** ,O2		

Abort if >60 trials needed = failed set

IDS

Relevant Dimension: Media **Correct Exemplar:** **M3**+O3/O4 **Rat** **Date** **Total time**

Discovery trials		
L	**R**	**Done**
M3,O3	M4,O4	
M4,O3	**M3,**O4	
M4,O4	**M3**,O3	
M3,O4	M4,O3	

Set-shifting

Trial	**L**	**R**	**Correct**	**Both**
1	**M3**,O3	M4,O4		
2	M4,O3	**M3,**O4		
3	M4,O4	**M3**,O3		
4	**M3**,O3	M4,O4		
5	M4,O3	**M3,**O4		
6	**M3,**O4	M4,O3		
7	**M3**,O3	M4,O4		
8	M4,O3	**M3,**O4		
9	**M3**,O3	M4,O4		
10	**M3,**O4	M4,O3		
11	M4,O4	**M3**,O3		
12	M4,O3	**M3,**O4		
13	**M3**,O3	M4,O4		
14	M4,O4	**M3**,O3		
15	**M3,**O4	M4,O3		
16	M4,O3	**M3,**O4		

Trial	**L**	**R**	**Correct**	**Both**
17	M4,O4	**M3**,O3		
18	M4,O3	**M3,**O4		
19	**M3**,O3	M4,O4		
20	**M3**,O3	M4,O4		
21	M4,O3	**M3,**O4		
22	M4,O4	**M3**,O3		
23	**M3**,O3	M4,O4		
24	M4,O3	**M3,**O4		
25	**M3,**O4	M4,O3		
26	**M3**,O3	M4,O4		
27	M4,O3	**M3,**O4		
28	**M3**,O3	M4,O4		
29	**M3,**O4	M4,O3		
30	M4,O4	**M3**,O3		
31	M4,O3	**M3**,O4		
32	**M3**,O3	M4,O4		
33	M4,O4	**M3**,O3		
34	**M3**,O4	M4,O3		
35	M4,O3	**M3,**O4		
36	M4,O4	**M3**,O3		
37	M4,O3	**M3,**O4		
38	**M3**,O3	M4,O4		
39	**M3**,O3	M4,O4		

Trial	**L**	**R**	**Correct**	**Both**
40	M4,O3	**M3,**O4		
41	M4,O4	**M3**,O3		
42	**M3**,O3	M4,O4		
43	M4,O3	**M3,**O4		
44	**M3,**O4	M4,O3		
45	**M3**,O3	M4,O4		
46	M4,O3	**M3,**O4		
47	**M3**,O3	M4,O4		
48	**M3,**O4	M4,O3		
49	M4,O4	**M3**,O3		
50	M4,O3	**M3,**O4		
51	**M3**,O3	M4,O4		
52	M4,O4	**M3**,O3		
53	**M3,**O4	M4,O3		
54	M4,O3	**M3**,O4		
55	M4,O4	**M3**,O3		
56	M4,O3	**M3,**O4		
57	**M3**,O3	M4,O4		
58	**M3**,O3	M4,O4		
59	M4,O3	**M3,**O4		
60	M4,O4	**M3**,O3		

Abort if >60 trials needed = failed set

R2

Relevant Dimension: Media **Correct Exemplar:** **M4**+O3/O4 **Rat** **Date** **Total time**

Discovery trials		
L	**R**	**Done**
M3,O3	**M4,**O4	
M4,O3	M3,O4	
M4,O4	M3,O3	
M3,O4	**M4**,O3	

Set-shifting				
Trial	**L**	**R**	**Correct**	**Both**
1	M3,O3	**M4,**O4		
2	**M4**,O3	M3,O4		
3	**M4,**O4	M3,O3		
4	M3,O3	**M4,**O4		
5	**M4**,O3	M3,O4		
6	M3,O4	**M4**,O3		
7	M3,O3	**M4**,O4		
8	**M4**,O3	M3,O4		
9	M3,O3	**M4,**O4		
10	M3,O4	**M4**,O3		
11	**M4,**O4	M3,O3		
12	**M4**,O3	M3,O4		
13	M3,O3	**M4,**O4		
14	**M4,**O4	M3,O3		
15	M3,O4	**M4**,O3		
16	**M4**,O3	M3,O4		

Trial	**L**	**R**	**Correct**	**Both**
17	**M4,**O4	M3,O3		
18	**M4**,O3	M3,O4		
19	M3,O3	**M4,**O4		
20	M3,O3	**M4,**O4		
21	**M4**,O3	M3,O4		
22	**M4,**O4	M3,O3		
23	M3,O3	**M4,**O4		
24	**M4**,O3	M3,O4		
25	M3,O4	**M4**,O3		
26	M3,O3	**M4,**O4		
27	**M4**,O3	M3,O4		
28	M3,O3	**M4,**O4		
29	M3,O4	**M4**,O3		
30	**M4,**O4	M3,O3		
31	**M4**,O3	M3,O4		
32	M3,O3	**M4,**O4		
33	**M4,**O4	M3,O3		
34	M3,O4	**M4**,O3		
35	**M4**,O3	M3,O4		
36	**M4,**O4	M3,O3		
37	**M4**,O3	M3,O4		
38	M3,O3	**M4,**O4		
39	M3,O3	**M4,**O4		

Trial	**L**	**R**	**Correct**	**Both**
40	**M4**,O3	M3,O4		
41	**M4,**O4	M3,O3		
42	M3,O3	**M4,**O4		
43	**M4**,O3	M3,O4		
44	M3,O4	**M4**,O3		
45	M3,O3	**M4,**O4		
46	**M4**,O3	M3,O4		
47	M3,O3	**M4,**O4		
48	M3,O4	**M4**,O3		
49	**M4,**O4	M3,O3		
50	**M4**,O3	M3,O4		
51	M3,O3	**M4,**O4		
52	**M4,**O4	M3,O3		
53	M3,O4	**M4**,O3		
54	**M4**,O3	M3,O4		
55	**M4,**O4	M3,O3		
56	**M4**,O3	M3,O4		
57	M3,O3	**M4,**O4		
58	M3,O3	**M4,**O4		
59	**M4**,O3	M3,O4		
60	**M4,**O4	M3,O3		

Abort if >60 trials needed = failed set

EDS

Relevant Dimension: Odour **Correct Exemplar:** **O5**+,M5/M6 **Rat** **Date** **Total time**

Discovery trials		
L	**R**	**Done**
O5,M5	O6,M6	
O6,M5	**O5,M6**	
O6,M6	**O5,M5**	
O5,M6	O6,M5	

Set-shifting

Trial	**L**	**R**	**Correct**	**Both**
1	**O5,**M5	O6,M6		
2	O6,M5	**O5,**M6		
3	O6,M6	**O5,**M5		
4	**O5,**M5	O6,M6		
5	O6,M5	**O5,**M6		
6	**O5,**M6	O6,M5		
7	**O5,**M5	O6,M6		
8	O6,M5	**O5,**M6		
9	**O5,**M5	O6,M6		
10	**O5,**M6	O6,M5		
11	O6,M6	**O5**,M5		
12	O6,M5	**O5,**M6		
13	**O5,**M5	O6,M6		
14	O6,M6	**O5,**M5		
15	**O5,**M6	O6,M5		
16	O6,M5	**O5,**M6		

Trial	**L**	**R**	**Correct**	**Both**
17	O6,M6	**O5,**M5		
18	O6,M5	**O5,**M6		
19	**O5,**M5	O6,M6		
20	**O5,**M5	O6,M6		
21	O6,M5	**O5,**M6		
22	O6,M6	**O5,**M5		
23	**O5,**M5	O6,M6		
24	O6,M5	**O5,**M6		
25	**O5,**M6	O6,M5		
26	**O5,**M5	O6,M6		
27	O6,M5	**O5,**M6		
28	**O5,**M5	O6,M6		
29	**O5,**M6	O6,M5		
30	O6,M6	**O5,**M5		
31	O6,M5	**O5,**M6		
32	**O5,**M5	O6,M6		
33	O6,M6	**O5**,M5		
34	**O5,**M6	O6,M5		
35	O6,M5	**O5,**M6		
36	O6,M6	**O5,**M5		
37	O6,M5	**O5,**M6		
38	**O5,**M5	O6,M6		
39	**O5,**M5	O6,M6		

Trial	**L**	**R**	**Correct**	**Both**
40	O6,M5	**O5,**M6		
41	O6,M6	**O5,**M5		
42	**O5,**M5	O6,M6		
43	O6,M5	**O5,**M6		
44	**O5,**M6	O6,M5		
45	**O5,**M5	O6,M6		
46	O6,M5	**O5,**M6		
47	**O5,**M5	O6,M6		
48	**O5,**M6	O6,M5		
49	O6,M6	**O5,**M5		
50	O6,M5	**O5,**M6		
51	**O5,**M5	O6,M6		
52	O6,M6	**O5,**M5		
53	**O5,**M6	O6,M5		
54	O6,M5	**O5,**M6		
55	O6,M6	**O5,**M5		
56	O6,M5	**O5,**M6		
57	**O5,**M5	O6,M6		
58	**O5,**M5	O6,M6		
59	O6,M5	**O5,**M6		
60	O6,M6	**O5,**M5		

Abort if >60 trials needed = failed set

R3

Relevant Dimension: Odour **Correct Exemplar: O6**+M5/M6 **Rat** **Date** **Total time**

Discovery trials		
L	**R**	**Done**
O5,M5	**O6,**M6	
O6,M5	O5,M6	
O6,M6	O5,M5	
O5,M6	**O6,**M5	

Set-shifting				
Trial	**L**	**R**	**Correct**	**Both**
1	O5,M5	**O6,**M6		
2	**O6,**M5	O5,M6		
3	**O6,**M6	O5,M5		
4	O5,M5	**O6,**M6		
5	**O6,**M5	O5,M6		
6	O5,M6	**O6,**M5		
7	O5,M5	**O6,**M6		
8	**O6,**M5	O5,M6		
9	O5,M5	**O6,**M6		
10	O5,M6	**O6,**M5		
11	**O6,**M6	O5,M5		
12	**O6,**M5	O5,M6		
13	O5,M5	**O6,**M6		
14	**O6,**M6	O5,M5		
15	O5,M6	**O6,**M5		
16	**O6,**M5	O5,M6		

Trial	**L**	**R**	**Correct**	**Both**
17	**O6,**M6	O5,M5		
18	**O6,**M5	O5,M6		
19	O5,M5	**O6,**M6		
20	O5,M5	**O6,**M6		
21	**O6,**M5	O5,M6		
22	**O6,**M6	O5,M5		
23	O5,M5	**O6,**M6		
24	**O6,**M5	O5,M6		
25	O5,M6	**O6,**M5		
26	O5,M5	**O6,**M6		
27	**O6,**M5	O5,M6		
28	O5,M5	**O6,**M6		
29	O5,M6	**O6,**M5		
30	**O6,**M6	O5,M5		
31	**O6,**M5	O5,M6		
32	O5,M5	**O6,**M6		
33	**O6,**M6	O5,M5		
34	O5,M6	**O6,**M5		
35	**O6,**M5	O5,M6		
36	**O6,**M6	O5,M5		
37	**O6,**M5	O5,M6		
38	O5,M5	**O6,**M6		
39	O5,M5	**O6,**M6		

Trial	**L**	**R**	**Correct**	**Both**
40	**O6,**M5	O5,M6		
41	**O6,**M6	O5,M5		
42	O5,M5	**O6,**M6		
43	**O6,**M5	O5,M6		
44	O5,M6	**O6,**M5		
45	O5,M5	**O6,**M6		
46	**O6,**M5	O5,M6		
47	O5,M5	**O6,**M6		
48	O5,M6	**O6,**M5		
49	**O6,**M6	O5,M5		
50	**O6,**M5	O5,M6		
51	O5,M5	**O6,**M6		
52	**O6,**M6	O5,M5		
53	O5,M6	**O6,**M5		
54	**O6,**M5	O5,M6		
55	**O6,**M6	O5,M5		
56	**O6,**M5	O5,M6		
57	O5,M5	**O6,**M6		
58	O5,M5	**O6,**M6		
59	**O6,**M5	O5,M6		
60	**O6,**M6	O5,M5		

Abort if >60 trials needed = failed set

Appendix 2: The order of microdialysis experiments

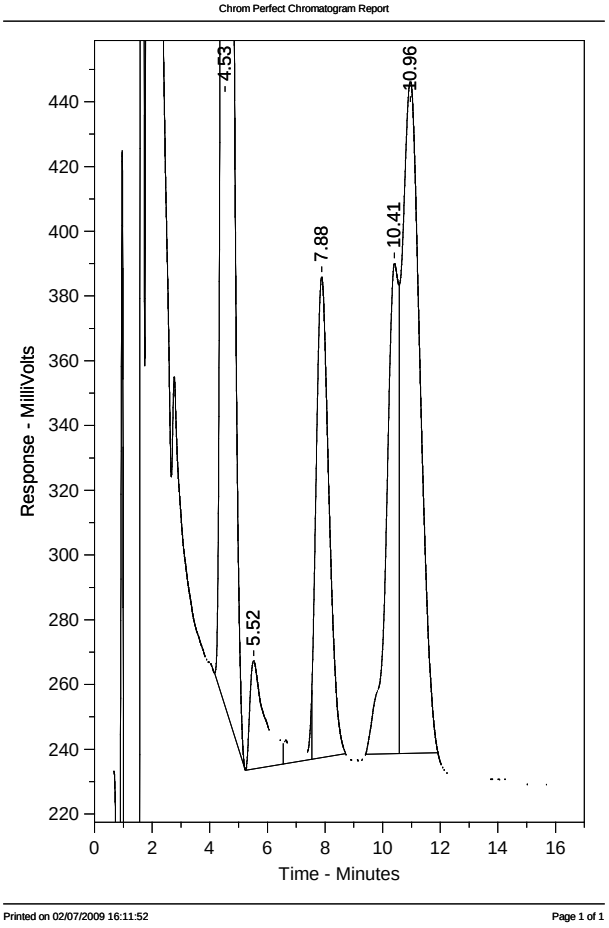
	Rat number					Rat number				
Week	1	2	3	4	5	6	7	8	9	10
1	2mg/kg PCP(1ml/kg): twice daily i.p.					1ml/kg saline : twice daily i.p.				
2	7-day washout					7-day washout				
3									**Surgery**	**Surgery**
										Habituation
									Habituation	Habituation
4	**Surgery**	**Surgery**	**Surgery**			**Surgery**			Habituation	Habituation
									Habituation	**Test**
									Test	
	Habituation									
	Habituation	Habituation								
5	Habituation	Habituation		**Surgery**		Habituation	**Surgery**			
	Test	Habituation	Habituation		**Surgery**	Habituation		**Surgery**		
		Test	Habituation			Habituation				
			Habituation	Habituation		**Test**				
			Test	Habituation			Habituation			
6				Habituation	Habituation		Habituation			
				Test	Habituation		Habituation	Habituation		
					Habituation		**Test**	Habituation		
					Test			Habituation		
								Test		
7	Analysis					Analysis				

Appendix 3 – Optimisation of dopamine detection using HPLC

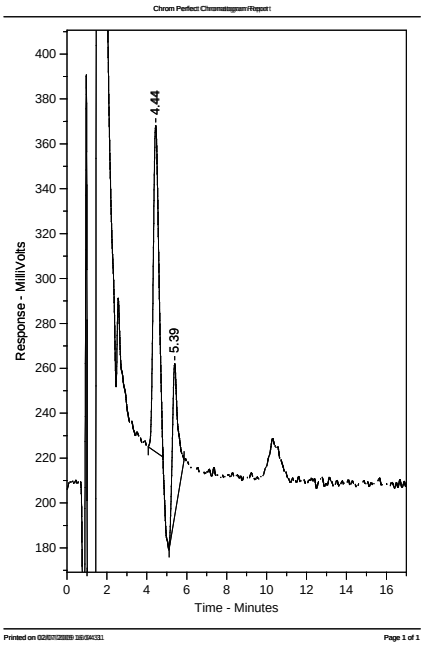
Results of using 1.2 mM OSA

Figure 1 shows the chromatograms of a 100 nM mixed standard (DA, DOPAC, HVA and 5-HIAA) and the individual standards at 100 nM. Figure 1A shows the following retention time (min): DOPAC, 4.53; 5-HIAA, 7.88; HVA 10.41; DA, 10.96. The identity of the peaks was determined following the individual standards shown in figures 1B-1E showing the respective retention times: DOPAC, 4.44; 5-HIAA, 7.77; HVA, 10.34; DA, 10.92. The separation of the peaks was insufficient to measure DA. Therefore, the OSA concentration was reduced from 1.2 mM to 1.0 mM.

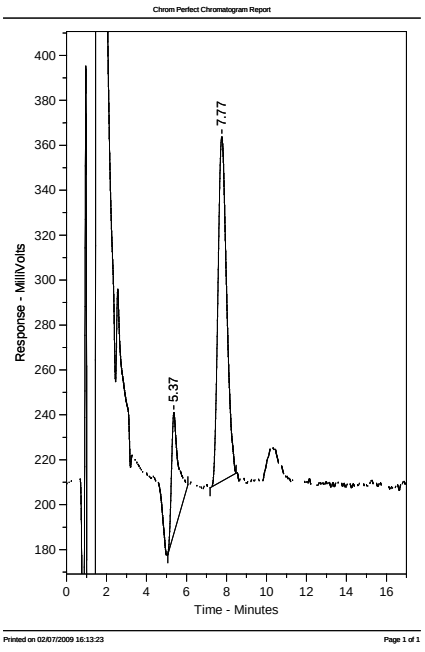
A Mixed standard



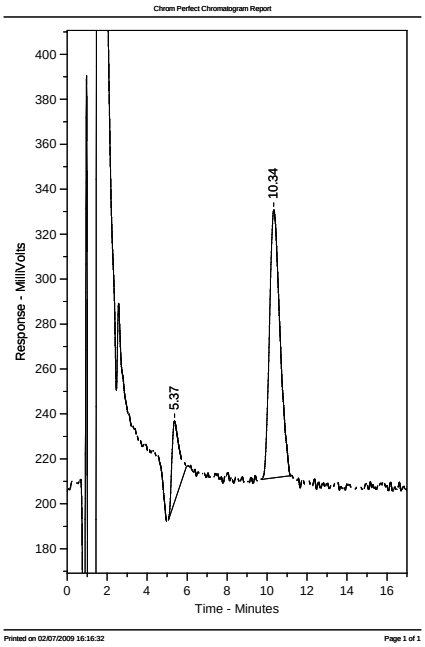
B DOPAC



C 5-HIAA



D HVA



E DA

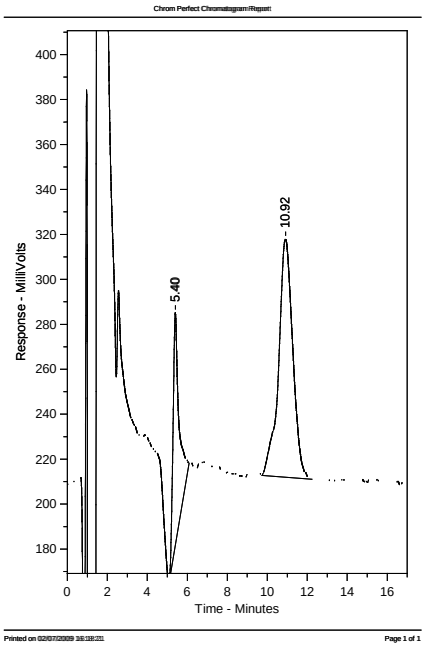
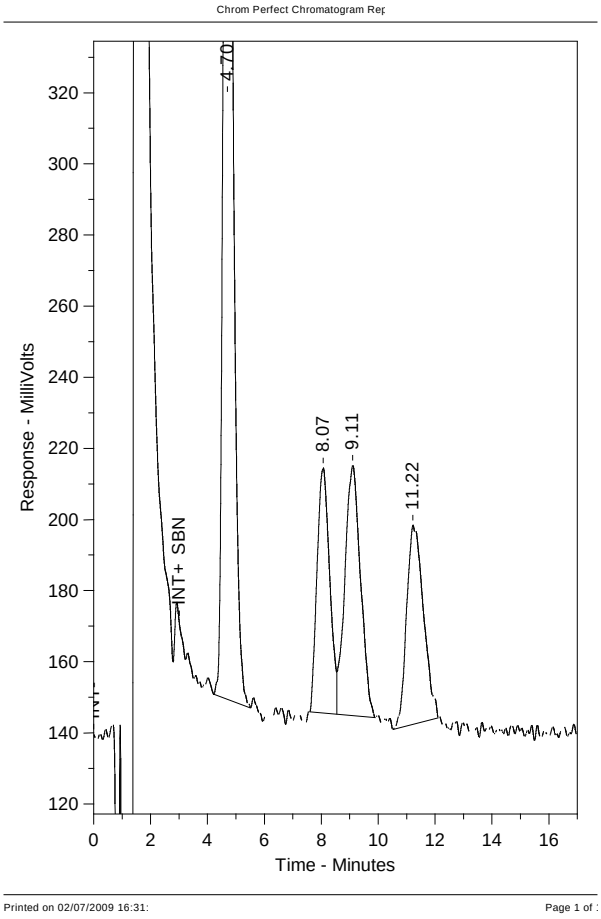


Figure 1: Chromotgrams at 1.2 mM OSA. (A) shows the following retention time (min): DOPAC, 4.53; 5-HIAA, 7.88; HVA 10.41; DA, 10.96. (B) shows the DOPAC peak at 4.44 min. (C) shows the 5-HIAA peak at 7.77 min. (D) shows the HVA peak at 10.34 min. (E) shows the DA peak at 10.92 min.

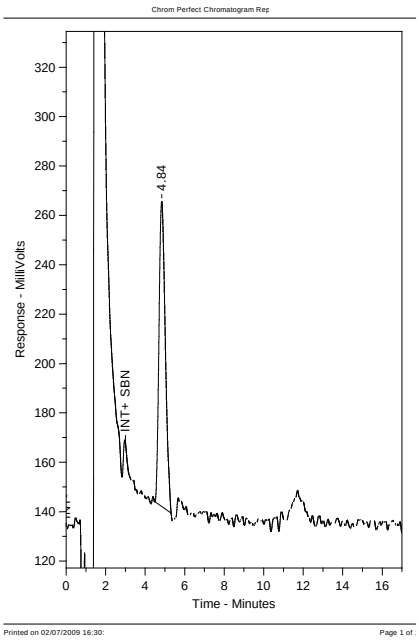
Results of using 1.0 mM OSA

Figure 2 shows the chromatograms of a 100 nM mixed standard (DA, DOPAC, HVA and 5-HIAA) and the individual standards at 100 nM. Figure 2A shows the following retention time (min): DOPAC, 4.70; 5-HIAA, 8.07; HVA 11.22; DA, 9.11. The identity of the peaks was determined following the individual standards shown in figures 2B-2E showing the respective retention times: DOPAC, 4.84; 5-HIAA, 8.48; HVA, 11.53; DA, 9.53. The separation of the peaks was again insufficient to measure DA. Therefore, the OSA concentration was increased from 1.0 mM to 1.1 mM.

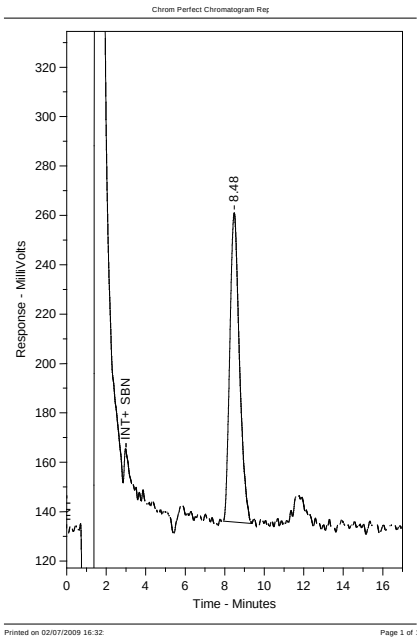
A Mixed standard 100 nM



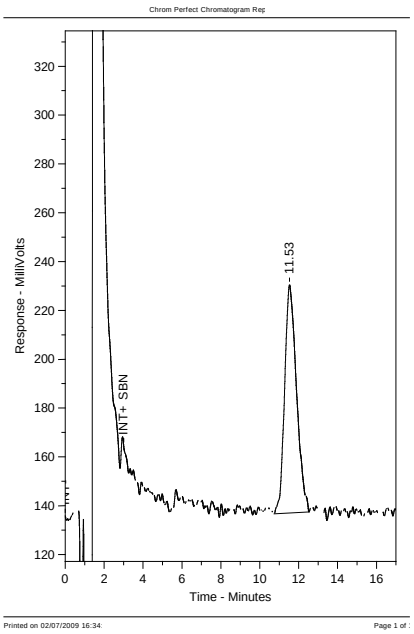
B DOPAC 100 nM



C 5-HIAA 100 nM



D HVA 100 nM



E DA 100 nM

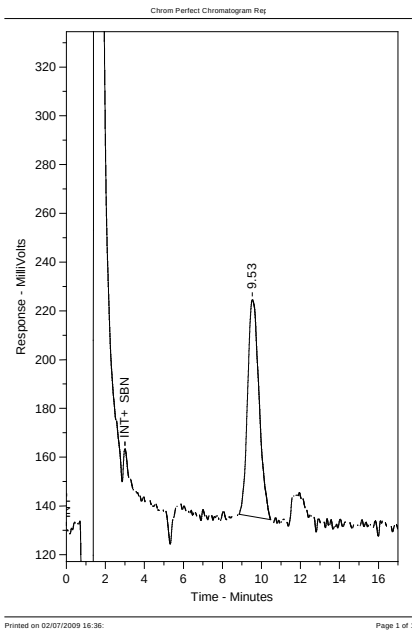
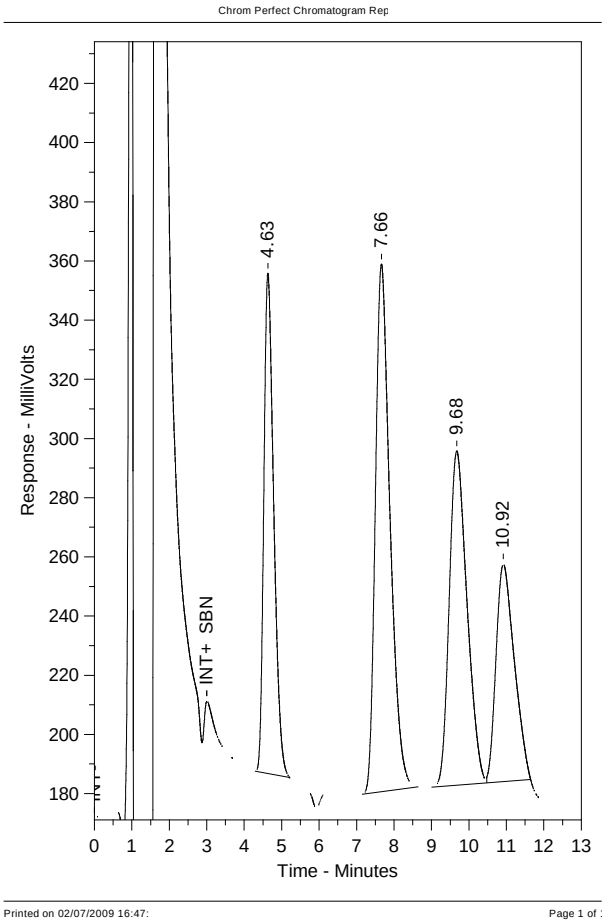


Figure 2: Chromotgrams at 1.0 mM OSA. (A) shows the following retention time (min): DOPAC, 4.70; 5-HIAA, 8.07; HVA 11.22; DA, 9.11. (B) shows the DOPAC peak at 4.84 min. (C) shows the 5-HIAA peak at 8.48 min. (D) shows the HVA peak at 11.53 min. (E) shows the DA peak at 9.53 min.

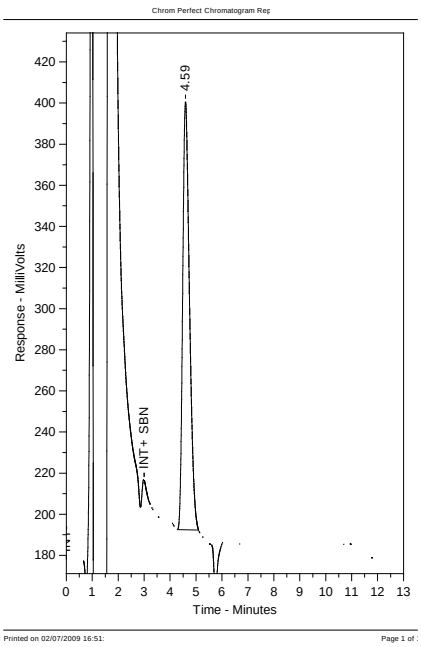
Results of using 1.1 mM OSA

Figure 3 shows the chromatograms of a 100 nM mixed standard (DA, DOPAC, HVA and 5-HIAA) and the individual standards at 100 nM. Figure 3A shows the following retention time (min): DOPAC, 4.63; 5-HIAA, 7.66; HVA 10.92; DA, 8.68. The identity of the peaks was determined following the individual standards shown in figures 3B-3E showing the respective retention times: DOPAC, 4.59; 5-HIAA, 7.55; HVA, 10.74; DA, 9.61. The separation of the peaks was now sufficient to measure DA. Therefore, the OSA concentration was maintained at 1.1 mM.

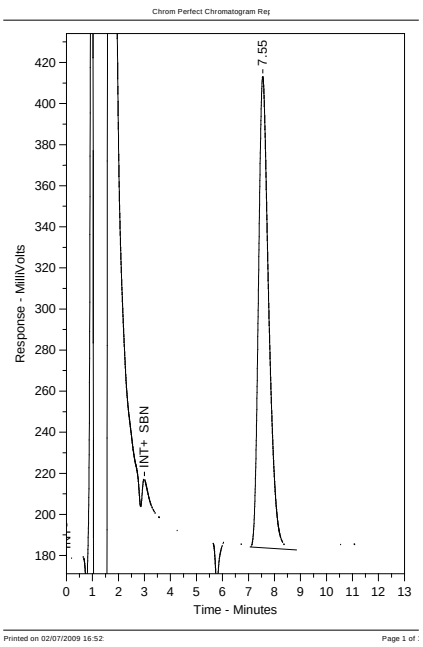
A Mixed standard 100 nM



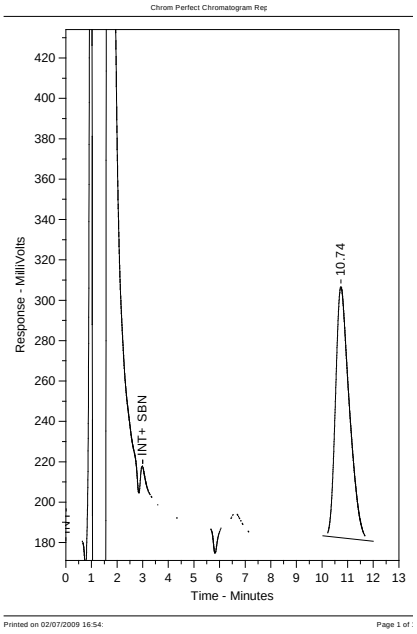
B DOPAC 100 nM



C 5-HIAA 100 nM



D HVA 100 nM



E DA 100 nM

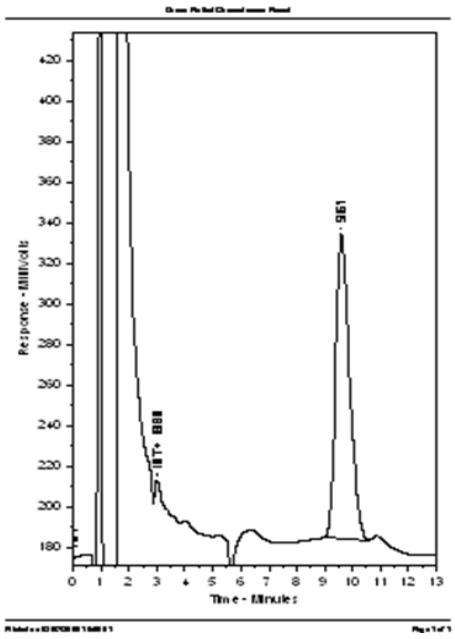
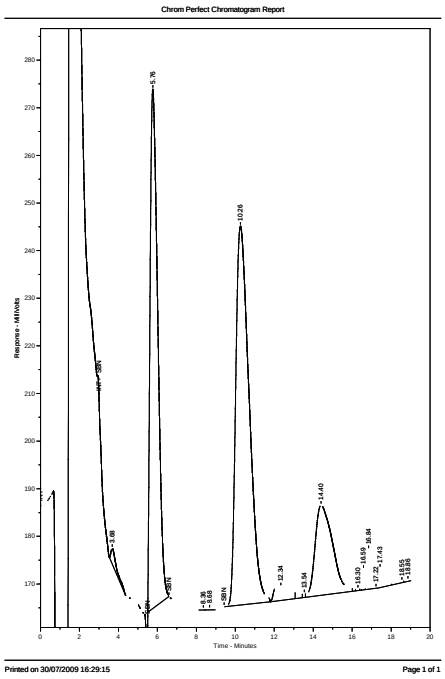


Figure 3: Chromotgrams at 1.1 mM OSA. (A) shows the following retention time (min): DOPAC, 4.63; 5-HIAA, 7.66; HVA 10.92; DA, 8.68. (B) shows the DOPAC peak at 4.59 min. (C) shows the 5-HIAA peak at 7.55 min. (D) shows the HVA peak at 10.74 min. (E) shows the DA peak at 9.61 min.

Appendix 4 – Example chromatograms from HPLC

Figure 1A shows an example chromatogram from a vehicle rat during the baseline phase. Figure 1B shows an example chromatogram from a PCP rat during the baseline phase.

1A Vehicle



1B PCP

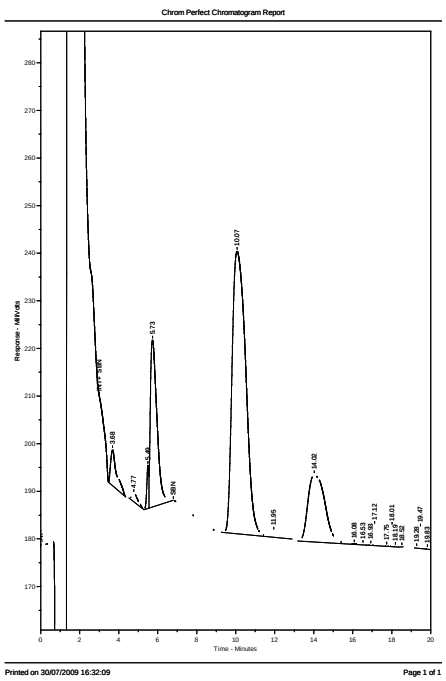


Figure 1: Example chromatograms from (A) a vehicle-treated rats and (B) a sub-chronic PCP-treated rat.

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