VALIDATION OF AN ANIMAL MODEL OF COGNITIVE DYSFUNCTION ASSOCIATED WITH SCHIZOPHRENIA

B. GRAYSON

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VALIDATION OF AN ANIMAL MODEL OF COGNITIVE DYSFUNCTION ASSOCIATED WITH SCHIZOPHRENIA

Development and validation of the novel object recognition task using behavioural manipulations and psychotomimetic dosing regimens to induce cognitive deficits of relevance to schizophrenia in hooded-Lister rats

Ben GRAYSON

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Abstract

Keywords: Rat, Phencyclidine, Antipsychotics, Cognition, NOR, 16-holeboard maze

Phencyclidine (PCP) is a non-competitive NMDA receptor antagonist that has been shown to induce schizophrenia-like psychotic symptoms that are clinically indistinguishable from schizophrenia in patients. When administered to rodents, PCP produces an array of behaviours that are characteristic of schizophrenia. Schizophrenia is associated with continual and treatment resistant cognitive deficits which are now recognised as a core feature of the disease.

The aim of the studies reported in chapter 3 were to establish a set of objects with equal preference in the NOR (novel object recognition) test. Furthermore, the inter-trial-interval (ITI) of the NOR test was investigated in an attempt to elucidate the effects of time and location of the rats during the ITI on the cognitive impairments following sub-chronic PCP treatment. The experiments in chapter 4 were designed to compare the performance of male and female rats in the NOR test following treatment with acute d-amphetamine (d-amph), PCP and sub-chronic PCP treatment. In chapter 5, validation of the cognitive deficits induced by sub-chronic PCP treatment was assessed using carefully selected pharmacological agents. The aim of the studies in chapter 6 was to determine the effects of isolation rearing on cognitive performance in the NOR test following increasing ITIs. Additionally, the sensitivity of isolation reared rats compared to social controls following acute administration of PCP and d-amph was assessed using the NOR test. Studies in chapter 8 utilised the 16-holeboard maze to determine the effects of acute treatment with d-amphetamine, PCP and scopolamine on working memory in the rat.

NOR is a visual learning and memory test that measures recognition memory which is impaired in patients with schizophrenia. Studies presented in this thesis demonstrate the importance of careful pilot studies when selecting objects for use in the NOR test. Initial studies in sub-chronic PCP (2 mg/kg for 7 days followed by 7 days drug free) treated female hooded-Lister rats revealed a preference of the rats for the wooden cone object; subsequently this object was eliminated from further NOR experiments.

Sub-chronic PCP treated rats were found to be highly susceptible to the disruptive influence of distraction during the short 1 min inter-trial-interval (ITI) in the NOR test. These results are consistent with clinical findings of the effects of distraction on cognition in schizophrenia patients. Following the initial validation experiments, a 1 min ITI in the home cage was selected for all subsequent NOR studies. Further experiments provided evidence to confirm that information presented in the acquisition trial is encoded but not retained in the retention trial of the NOR test by

PCP-treated rats. Male rats were less sensitive to the recognition memory deficits induced by acute treatment with PCP and d-amphetamine compared with females. Following sub-chronic PCP treatment, both males and females showed object recognition deficits, however, the impairments were more robust in female rats. Female rats were therefore selected for all subsequent experiments.

Pharmacological validation was carried out using carefully selected agents which were assessed for their ability to restore the sub-chronic PCP induced cognitive deficit in the object recognition test. It was found that the classical antipsychotic agents haloperidol and fluphenazine, the benzodiazepine anxiolytic chlordiazepoxide and the SSRI antidepressant fluoxetine were ineffective. Further studies showed that the atypical antipsychotic agents, clozapine and risperidone, the analeptic agent modafinil, the nAChR full agonist nicotine, and full agonist and positive allosteric modulator of the α7 nAChR (PNU-282987 and PNU120596 respectively) reversed the recognition memory deficit induced by sub-chronic PCP treatment in the NOR test.

Isolation rearing of rats at weaning is an environmental stressor that has relevance for modelling the symptomatology and pathology of schizophrenia. Isolates had a significantly increased locomotor activity (LMA) response to a novel environment and enhanced sensitivity to time delay-induced recognition memory deficits, compared with their socially reared counterparts. Isolates were less sensitive to an acute PCP-induced recognition memory deficit but more sensitive to an acute d-amphetamine induced recognition memory deficit in the NOR test compared to social controls.

Preliminary results from the 16-holeboard maze experiments reveal that acute administration of the mAChR antagonist scopolamine, d-amphetamine, PCP and sub-chronic PCP treatment reduced working memory scores compared to vehicle treated controls.

Taken together, these findings suggest that sub-chronic treatment with PCP induces cognitive deficits in behavioural tests of relevance to cognition associated with schizophrenia. This may allow the detection of novel pharmacotherapies to alleviate these cognitive deficits and exploration of the nature of cognitive disturbances in these patients.

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List of papers and refereed conference proceedings arising from work presented in this thesis

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Grayson B, Neill JC (2004). The effect of PCP on novel object recognition in the rat. Journal of Psychopharmacology, 18 (3), Suppl, A55.

Grayson B, Leviers N, Neill JC (2005). Acute PCP does not affect novel object recognition in male hooded-Lister rats. Journal of Psychopharmacology, (19), Suppl, A13.

Grayson B, Kirun A, Neill JC (2008). PCP impairs recall in an episodic memory task: influence of distraction. Journal of Psychopharmacology, 22 (5), Suppl, A16.

Grayson B, Gendle D, Neill JC (2009). Acute treatment with phencyclidine, d-amphetamine and scopolamine induce differential spatial working memory impairments in the 16-holeboard maze. Journal of Psychopharmacology, 23 (6), Suppl, A64.

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Grayson B, Idris NF, Neill JC (2007). Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the NOR task in the rat. Behavioural Brain Research 184: 31–38.

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List of Abbreviations

Acronym	Definition
5-CSRTT	5-choice serial reaction time test
5-HT	5-hydroxytryptamine or serotonin
$5\text{-HT}_{1\text{-}7}$	5-hydroxytryptamine receptor subtypes 1-7
Acb	Nucleus accumbens
AMPA	Alpha amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AOB	Accessory olfactory bulb
ANOVA	Analysis of variance
BDNF	Brain-derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
CGP39551	(E)-(±)-2-Amino-4-methyl-5-phosphono-3-pentenoic acid ethyl ester
CNS	Central nervous system
COMT	Catechol-O-methyl transferase
CPu	Caudate putamen
CT	Computed tomography
D_1	Dopamine receptor subtype 1
D_2	Dopamine receptor subtype 2
D_3	Dopamine receptor subtype 3
D_4	Dopamine receptor subtype 4
D_5	Dopamine receptor subtype 5
DA	Dopamine
DAO	D-amino acid oxidase
DAT	Dopamine transporter
DI	Discrimination index
DISC	Disrupted in schizophrenia
DNA	Deoxyribonucleic acid
EDS	Extradimensional shift
EPS	Extrapyramidal symptoms
FC	Frontal cortex
G-Proteins	Guanine nucleotide binding proteins
GABA	Gamma-aminobutyric acid
GluR1-4	Glutamate receptor subunits 1-4
HPA	Hypothalamus-pituatory-adrenal axis
IEG	Immediate early genes
ITI	Inter-trial interval
$_{L}$-DOPA	$_{L}$-3,4Dihydroxyophenylalanine
LMA	Locomotor activity
LSD	Lysergic acid diethylamide
LTP	Long-term potentiation
$M_{1\text{-}4}$	Muscarinic receptor subtypes 1-4
MAM	Methylmethazoxymethyl
MATRICS	Measurement and Treatment Research to Improve Cognition in Schizophrenia
mGLuR	Metabotropic glutamate receptor
MK-801	(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-monamine maleate (or) Dizocilpine
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid

NAA	N-acetylaspartate
NAAG	N-acetylaspartylglutamate
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate
NOR	Novel object recognition
NR1-4	NMDA receptor subunits 1-4
NRG	Neuregulin
NRHypo	NMDA receptor hypofunction
NS	Non significant
PAM	Positive allosteric modulator
PCP	Phencyclidine
PET	Positron emission tomography
PFC	Prefrontal cortex
PND	Post natal day
PPI	Pre-pulse inhibition
Prh	Perirhinal cortex
RELN	Reelin
RGS4	Regulator of G protein signalling
SEM	Standard error of the mean
SI	Social interaction
SN	Substantia nigra
TURNS	Treatment Units for Research in Neurocognition in Schizophrenia
VTA	Ventral tegmental area
WCST	Wisconsin Card Sorting Test
WMS	Working memory score

CHAPTER 1 - Introduction

1.1 General Introduction

1.1.1 Symptomology

Schizophrenia is a psychiatric disease that is chronic, severe and highly debilitating to the patients that are inflicted. It is a highly complex clinical disorder, displaying a huge variety of incapacitating symptoms. The current worldwide morbidity of schizophrenia is almost 1% of the population (Trivedi & Jarbe, 2011) and reports have suggested that schizophrenia costs the NHS more than any other mental illness, consuming more than 5% of the total budget (Hargreaves, 2003). Schizophrenia was termed demense precose (dementia praecox), a Latin phrase meaning "mental deterioration at an early age", a syndrome affecting teenagers and young adults by Benedict Morel in 1853. In 1887, Dr. Emile Kraepelin classified the symptoms of dementia praecox, with emphasis on the early age of onset and the deteriorating course of the illness as defining criteria.

In 1908, Eugen Bleuler was the first to use the term schizophrenia “the breaking up or splitting of psychic functioning”,; he rejected Kraepelins emphasis on the early onset and deteriorating course of the disease. Bleuler went on to describe schizophrenia as a group of disorders, patients with mild and severe forms, acute and chronic with both poor and encouraging outcomes.

Schizophrenia symptoms are currently divided into positive, negative and cognitive symptoms.

1.1.1.1 Positive Symptoms

The diagnostic and statistical manual of mental disorders, 4th edition text revision (DSM–IV TR) is currently used by clinicians and psychiatrists in the US to diagnose psychiatric illnesses. For schizophrenia to be diagnosed, patients need to exhibit at least 1-month duration of two or more of the positive or negative symptoms. The positive symptoms are usually satisfactorily treated with antipsychotic medication (Feldman et al, 1997). The symptoms include:

Delusions: False beliefs that are strongly held despite evidence to suggest otherwise. The delusions can be subdivided into paranoid delusions, delusions of reference, somatic delusions and delusions of grandeur.

Hallucinations: Mainly auditory, visual, tactile, olfactory and gustatory.

Disorganised speech: Moving swiftly through multiple topics, confused and repetitive speech and use of unrelated words without meaning.

Catatonic behaviour: Physical immobility, excessive mobility, extreme resistance and peculiar movements (DSM–IV TR).

1.1.1.2 Negative Symptoms

The negative symptoms are feelings, thoughts and behaviours that are normally present that are absent or diminished in patients suffering from schizophrenia. The negative symptoms are often more difficult to diagnose than the positive symptoms because they represent a lesser degree of normal, rather than the presence of undesirable or bizarre behaviours. The negative symptoms not only arise from the pathology of the disease but can also develop as secondary negative symptoms

related to antipsychotic medication. It has been reported that both classical and atypical antipsychotic agents have been shown to induce negative symptoms in healthy controls (Artaloytia et al, 2006) and exacerbate the primary negative symptoms in patients with schizophrenia. Conversely, atypical antipsychotics have also been shown to improve the negative symptoms of schizophrenia in certain patients (Meltzer, 1999). The negative symptoms include**:**

Affective flattening: Reduction in the range and intensity of emotional expression, including facial expression, voice tone, eye contact, and body language.

Alogia: Lessening of speech fluency and productivity, thought to reflect slowing or blocked thoughts, and often manifested as laconic, empty replies to questions.

Avolition: Reduction, difficulty, or inability to initiate and persist in goal-directed behaviour; it is often mistaken for apparent disinterest. (Source DSM IV and ICD-10, 2010).

1.1.1.3 Cognitive Symptoms

Since 1851 when the term 'dementia praecox' was used, impairments in cognitive function have been recognised and described as a core feature of the disease. Patients with schizophrenia show deficits in a variety of cognitive domains. The cognitive symptoms refer to difficulties with concentration and memory. These may include the following; disorganised thinking, slow thinking, difficulty understanding, poor concentration, poor memory, difficulty expressing thoughts, difficulty integrating thoughts, feelings and behaviour.

There is increasing evidence suggesting that cognitive deficits, particularly attentional deficits (Byrne et al, 1999; Lewis, 2004), verbal memory impairments

(Whyte et al, 2006) and other cognitive impairments (Niemi et al, 2003) are already present and detectable in adolescents, prior to the onset of illness, in subjects with high susceptibility to schizophrenia and in first-episode schizophrenia patients. Furthermore, many studies over the past 25 years have demonstrated that first degree relatives compared to second degree relatives of patients with schizophrenia exhibit greater cognitive deficits (see review by Snitz et al, 2006). Schizophrenia patients demonstrate cognitive impairments within various cognitive domains, which include memory and language, executive function and attention.

Working memory has been described as the temporary "online" storage and the subsequent retrieval and manipulation of information (Baddelay & Hitch, 1974). This domain of memory has an important functional role in briefly retaining information and allowing it to be used rapidly. Working memory dysfunction is increasingly recognised as a core feature in patients with schizophrenia (Thormodsen et al, 2011)

The cognitive aspect of schizophrenia has huge implications on lifestyle and impacts on ability to function within the community, at school, work and to form and maintain relationships. The cognitive impairments observed in schizophrenia are shown to be associated with functional outcome and studies have shown that cognitive deficits are the most reliable predictor of this outcome (Green et al, 2000). Patients vary in the types of cognitive dysfunctions they exhibit; however, the level of cognitive impairment generally remains stable over time and throughout the course of the disease.

Cognition is becoming the main target for schizophrenia therapy and recently the National Institute of Mental Health (NIMH) introduced the Measurement and

Treatment Research to Improve Cognition in Schizophrenia, known as the MATRICS Initiative. The MATRICS initiative's aim was to identify the worst affected cognitive domains and develop a clinical test battery targeting these domains. The MATRICS Consensus Cognitive Battery (MCCB) is intended to provide a relatively brief evaluation of the key cognitive domains (see table 1) relevant to schizophrenia and related disorders, and was designed to facilitate the research and development of novel pharmacological approaches for treating the cognitive impairments associated with schizophrenia (http://www.matrics.ucla.edu).

Tests used to measure Cognitive Performance in adults with Schizophrenia and related disorders		
Cognitive Domain	**Test**	**Description**
Speed of Processing	Brief Assessment of Cognition in Schizophrenia (BACS): Symbol-Coding	Timed paper and pencil test in which respondent uses a key to write digits that correspond to nonsense symbols
	Category Fluency: Animal Naming	Oral test in which respondent names as many animals as he/she can in one minute.
	Trail Making Test: Part A	Timed paper and pencil test in which the respondent draws a line to connect consecutively numbered circle placed irregularly on a sheet of paper.
Attention / Vigilance	Continuous Performance Test - Identical Pairs (CPT-IP)	Computer administered measure of sustained attention in which respondent presses a response button to consecutive matching numbers.
Working Memory (Nonverbal) (verbal)	Wechsler Memory Scale - 3rd Ed.	Using a board on which ten cubes are irregularly spaced, respondent taps cubes in same (or reverse) sequence as test administrator.
	(WMS-III): Spatial Span	
	Letter-Number Span	Orally administered test in which respondent mentally reorders strings of numbers and letters and repeats them to administrator.
Verbal Learning	Hopkins Verbal Learning Test - Revised (HVLT-R)	Orally administered test in which a list of twelve words from three taxonomic categories is presented and the respondent is asked to recall as many as possible after each of three learning trials.
Visual Learning	Brief Visuospatial Memory Test - Revised (BVMT-R)	A test that involves reproducing six geometric figures from memory.
Reasoning and Problem Solving	Neuropsychological Assessment Battery (NAB): Mazes	Seven timed paper and pencil mazes of increasing difficulty that measure foresight and planning.
Social Cognition	Mayer-Solovey-Caruso Emotional Intelligence Test (MSCEIT): Managing Emotions	Paper and pencil multiple choice test that assesses how people manage their emotions

Table 1 The key cognitive domains as outlined by the MATRICS initiative to test and assess performance (www.matrics.ucla.edu).

1.1.2 Aetiology of Schizophrenia

Schizophrenia is a highly complex disorder and likely to be derived from an interaction between genetic, behavioural and developmental components.

1.1.2.1 Genetics

There have been numerous epidemiological studies into schizophrenia, looking at family, twin and adoption studies, showing overwhelming evidence to suggest that pre-disposition to developing the illness is significantly increased in relatives of individuals who are already suffering from schizophrenia, thus depicting a strong genetic link (Pedersen & Mortensen, 2001; Owen et al, 2005; Mortensen et al, 2010).

The data from monozygotic twin studies have shown a 41-65% presence of schizophrenia in both pairs of twins compared to 0-28% in dizygotic twins, with heritability estimates at approximately 85%, which means schizophrenia is one of the most hereditable medical disorders (Craddock et al, 2005). Furthermore, the risk of schizophrenia in progeny of both the affected and unaffected monozygotic twins is similar, which means that the unaffected twin carries the hereditable genetic material for developing the illness without expressing schizophrenia themselves (Kety et al, 1994).

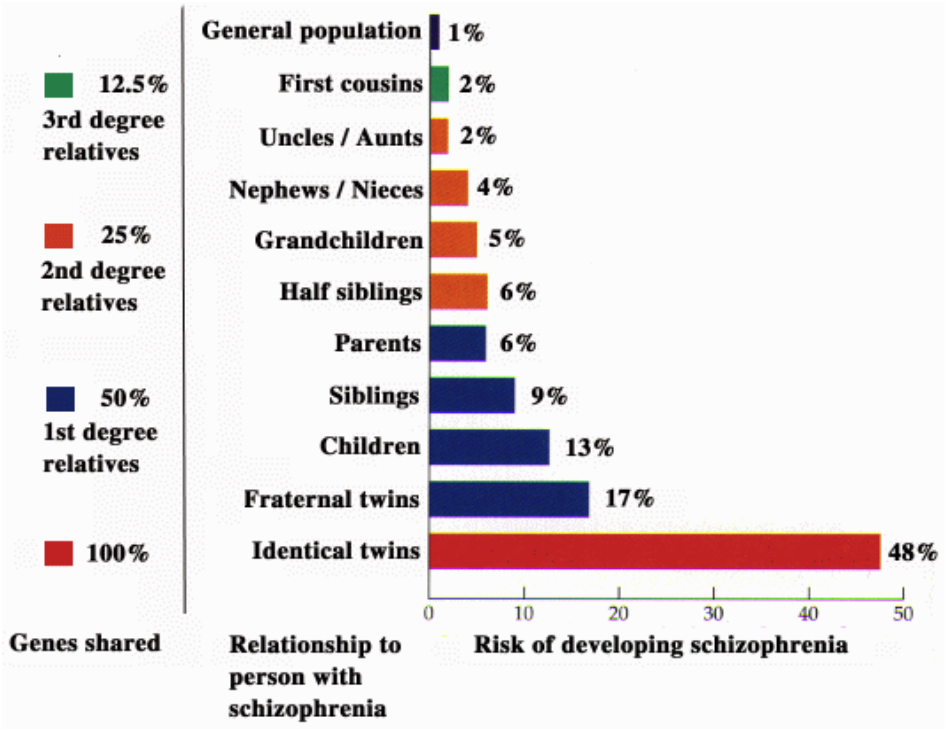


Figure 1 Percentage rates of developing schizophrenia when there is a family history of the disease (taken from Gottesman, 1991).

Experiments using linkage analysis and quantitative trait locus analysis have shown numerous chromosomal regions with nucleotide sequences that relate to phenotypes. These findings have led to detailed mapping studies of linked regions with the association of specific predisposing genes for schizophrenia. In the review by Owen (2005), the genes with the most information available and implicated in schizophrenia are those that encode dysbindin (DTNBP1), neuregulin1 (NRG1), D-amino-acid oxidase activator (DAOA; which was known as G72) and regulator of G-protein signalling 4 (RGS4), Dysbindin, catechol-O-methyltransferase (COMT), proline dehydrogenase (PRODH), Asp-His-His-Cys (DHHC), disrupted in schizophrenia DISC1 and DISC2, metabotropic glutamate receptor-3 (GRM3; mGluR3). Many of the susceptibility genes that have been identified for

schizophrenia are known to regulate neuronal connectivity, synaptogenesis, and *N*-methyl-D-aspartate (NMDA) glutamate receptor functions. Hypothetically, converging molecular abnormalities expressed by defective versions of these genes could cause dysregulation of NMDA receptors and NMDA synapses, leading to vulnerability for schizophrenia due to inefficient information processing at glutamate synapses (Stahl, 2007).

1.1.2.2 Neurodevelopment

Clearly genetic risk factors play an important role in the aetiology of schizophrenia, however there is a vast amount of literature available describing a non-genetic basis for schizophrenia.

Studies have shown that the possibility of developing schizophrenia seems to begin as early as the first trimester (0-90 days) of pregnancy, with a 7-fold increase in the risk of developing schizophrenia and schizophrenia spectrum disorders following the exposure to influenza (Brown et al, 2004), whereas the second and third trimester of pregnancy showed no increased susceptibility (Opler & Susser, 2005). Other researchers have indicated that the second trimester of pregnancy confirms the greatest risk for schizophrenia (Sullivan et al, 2006). Brown and colleagues (2001) also demonstrated a 20.4% increase in the susceptibility to schizophrenia following early prenatal exposure to the rubella virus. However the rubella virus has been shown to be active up to 18 months following birth, which could mean that the pro-schizophrenia risk caused by the rubella virus could actually be a postnatal effect (South & Sever, 1985). Subsequent studies have shown a large increase in subjects which were recently diagnosed with schizophrenia, 28.6% compared to 5% of chronic patients that test positively for the retroviral polymerase genes in the cerebral

spinal fluid (Karlsson et al, 2001). The presence of maternal antibodies to the parasitic protozoa Toxoplasma gondii, which can be transmitted to the mother from contact with cat faeces or from eating undercooked meat, has been shown to increase the risk of developing schizophrenia by 2.5% (Torrey & Yolken, 2003).

A study in 2008 by Khashan and colleagues suggests that severe stress to a mother during the first trimester of pregnancy may alter the risk of schizophrenia in offspring. Results from this study showed that the stress of a first relative dying or a relative being diagnosed with cancer, acute myocardial infarction, or stroke syndrome up to 6 months before conception or during pregnancy increased the risk of child developing schizophrenia (adjusted relative risk, 1.67 [95% confidence interval, 1.02-2.73]). The season of birth has also been implicated in schizophrenia; patients are more likely to be born during the winter months. The increased risk is approximately 10% for those born in winter compared to summer births (Torrey, 1997). The relationship between the increase in the incidence of schizophrenia and winter births may be explained by the timing of the peak flu season during the early stages of pregnancy. It is widely recognised that individuals with schizophrenia were more likely to have experienced a cluster of obstetric complications involving hypoxia than were controls (Clarke et al, 2006). In addition, interesting interactions have been described between foetal hypoxia and genetic risk for schizophrenia on brain structure. One particular study found that a history of foetal hypoxia is associated with greater structural brain abnormalities in groups with schizophrenia than among controls (Cannon et al, 2002). Schizophrenia patients have been shown to have lower mean birth weights compared to unaffected siblings (McNeil et al, 1993). Furthermore, in a monozygotic twin study, the patient with schizophrenia was

significantly more likely to have a lighter birth weight than their co-twins (Torrey, 1977).

1.1.3 Epidemiology of Schizophrenia

Socioeconomic class has been shown to influence the susceptibility to developing schizophrenia. People belonging to the "working class" social group are approximately five times more likely to develop schizophrenia than people in the other social classes (Mulvany et al, 2001; Muntaner et al, 2004).

Ethnic status has frequently been related to differential predisposition to schizophrenia. Immigrants to the UK from the Caribbean and Africa have ten times the prevalence of schizophrenia compared to other members of the population (Harrison et al, 1988; Wessley et al, 1991), whereas other non-black immigrants do not show such an increased risk (Cooper, 2005). The increased risk of schizophrenia in the Caribbean and African immigrants to the UK cannot be solely attributed to genetics since the origin of birth countries do not show an increased risk of developing schizophrenia.

Gender has been known to influence risk of developing schizophrenia since as early as 1919-1971, when Kraepelin described dementia praecox as a disorder that mostly affected young men. The age of onset of schizophrenia in men is earlier compared to women, with most studies reporting onset in the early 20s for men and late 20s in women (for review see Salem & Kring, 1998). Men are at increased risk of developing schizophrenia with male:female incidence of approximately 1.4:1 (Able et al, 2010). Men also have worse symptomatology, express more negative symptoms and show poorer response to certain medication (Leung and Chue 2000;

Moriarty et al, 2001; Canuso & Pandina, 2007). These differences in the onset of schizophrenia in men and women could be attributed to the putative anti-dopaminergic effects of oestrogens (Lindamer et al, 1997; Cutter et al, 2003).

Cannabis abuse has been shown to significantly reduce the age of onset of schizophrenia in male patients (Veen et al, 2004; Large et al, 2011) and also increased likelihood of developing schizophrenia in psychosis free people (van Os et al, 2010). In addition, it has been suggested that heavy cannabis use in adolescence can increase the risk of developing schizophrenia later in life (Zammit et al, 2002). Further studies have demonstrated that schizophrenia subjects who previously exhibited psychotic symptomatology have poorer prognosis when using cannabis (Martinez-Arevalo et al, 1994).

1.1.4 Neurochemistry and Schizophrenia

1.1.4.1 Dopamine

Dopamine is transmitted via four main pathways. The first is the mesolimbic pathway, and extends mostly from the ventral tegmental area (VTA; A10) and to a lesser extent from the retrorubal field (A8) and substantia nigra (A9) to the nucleus accumbens. The positive symptomatology of schizophrenia is likely to be modulated by the mesolimbic dopamine pathway.

The second, mesocortical pathway, transmits dopamine from the VTA to the frontal cortex and is linked to negative symptoms and cognition. The third, the nigrostriatal pathway, transmits dopamine from the pars compacta of substantia nigra (A9), ascends via the medial forebrain bundle and innervates the striatum. This pathway is vital for motor coordination, movement and initiation of movement. Blockade of this

dopaminergic system, required for reducing psychosis, particularly using the classical antipsychotics, induces extrapyramidal side effects.

The final main pathway is the tuberoinfundibular pathway, which transmits dopamine from the hypothalamus to the pituitary gland. Blockade of dopamine in this pathway prevents the inhibitory effect of dopamine on prolactin release which causes an increase in blood prolactin levels (hyperprolactinemia). Elevated prolactin levels can cause abnormal lactation (galactorrheoa) in men and women, disruptions to the menstrual cycle in women, headache, visual problems and sexual dysfunction (Stahl, 2000; Serri et al, 2003).

In June 1974, [^{3}H] haloperidol was prepared by Dr. P.A.J. Janssen (of Janssen Pharmaceutica, Beerse, Belgium) for Dr. Paul Seeman to detect the specific binding to striatal brain tissue (Seeman et al, 1975). The results confirmed that the "antipsychotic receptor" was in fact a dopamine receptor since dopamine was the most effective at inhibiting the binding of [^{3}H] haloperidol. Subsequently, five dopamine receptors subtypes have been identified, all members of the super family of transmembrane domain, G-protein coupled receptors and cluster into D_1-like and D_2-like families of receptors. Two D_1-like receptor subtypes (D_1 and D_5) couple to G-protein receptors Gq and Gs and activate adenlyl cyclase, whereas the D_2-like receptor subtypes (D_2, D_3 and D_4) couple to G-protein receptors Gi and Go and inhibit adenlyl cyclase.

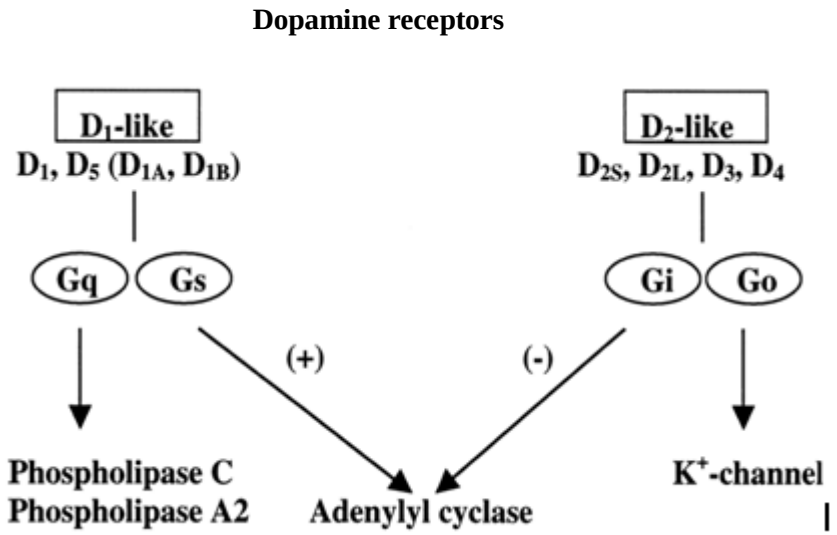


Figure 2 Dopamine receptor subtypes and their second messengers;
(+), stimulation; (−), inhibition (taken from Hussain & Lokhandwala, 2003)

The classical dopamine hypothesis of schizophrenia is the most widely considered neurochemical hypothesis and was first proposed by Snyder (1973). This hypothesis postulates that hyperactive dopaminergic neurotransmission resulting from an excess of DA in the brain, activating D_2 receptors account for the phenomenon of schizophrenia (Seeman, 1984). This hypothesis was formulated from three main sources:

Post mortem studies on schizophrenic brains have shown unusually high levels of dopamine and its metabolites (e.g. homovanillic acid) especially in the limbic regions (Iverson, 1979; Mackay et al, 1982). Subsequently, there is a large amount of consistent data showing increased density of striatal D_2 receptors in the post mortem brains of schizophrenics (Reynolds et al, 1987; Knable et al, 1997; Sumiyoshi et al, 1995). However, it has been suggested that the increase in D_2 receptor density may be the result of adaptation to antipsychotic drug treatment (i.e. up regulation of D_2 receptors) rather than a biochemical abnormality intrinsic to schizophrenia.

Furthermore, some PET studies show no significant difference in D_2 receptor densities between neuroleptic-naive schizophrenics and healthy controls (Farde et al, 1990). D_1-like receptors are predominantly found in the PFC, whereas D_2-like receptors are found in sub-cortical regions (see Guillin et al, 2007), although D_4 receptors are found in both PFC and hippocampus (Lahti et al, 1998).

1.1.4.1.1 D_2 antagonists

There is a strong relationship between clinical efficacy of classical antipsychotic drugs and potent affinity for D_2 receptors. All drugs that antagonise the D_2 receptor tend to improve the positive symptoms of schizophrenia (Creese et al, 1976; Seeman, 1986). As the classical antipsychotic drugs are D_2 receptor antagonists, an alteration of dopamine transmission at this receptor has long been suspected to play a role in the pathophysiology of schizophrenia. Specifically, excess D_2 transmission has been proposed to underlie positive symptomatology since these symptoms improve following D_2 receptor blockade compared with negative or cognitive symptoms (Snyder, 2008).

1.1.4.1.2 Dopamine agonists

Additionally, it has been shown that the dopamine agonist amphetamine and the dopamine pre-cursor $_L$-DOPA, enhance the activity of dopamine and produce psychotic symptoms when administered to healthy adults (for review see Angrist & van Kammen, 1984; Lieberman et al, 1987). These dopamine agonist-induced psychotic symptoms can be abolished with the administration of dopamine antagonists, providing further evidence of dopamine's involvement in schizophrenia. Additionally, these dopamine agonists have been shown to exacerbate the psychotic

symptoms in schizophrenia patients. A study demonstrating enhanced amphetamine-induced dopamine release in schizophrenia patients using PET (photon emission tomography), suggested the existence of a dysregulation of dopamine neurones leading to an increased dopamine transmission in response to amphetamine (Laruelle et al 1996).

Despite decades of extensive research, the causes and exact sites of the presumed dopamine-mediated hyperactivity remain elusive; therefore providing evidence to suggest that dopamine alone may be insufficient to explain all aspects of schizophrenia. Although the administration of the dopamine antagonist medications modify brain dopamine levels within minutes, the concomitant improvement in patient symptoms are usually not visible for at least several days, suggesting that dopamine may not be directly responsible for the illness (Thompson, 2000). Furthermore, dopamine antagonists demonstrate limited ability to treat the negative symptoms of schizophrenia, in addition the inability of dopamine agonists to fully recapitulate all the symptoms of the disease in humans suggests the involvement of other neurotransmitters.

1.1.4.2 Serotonin hypothesis

Serotonin (5-hydroxtryptamine; 5-HT) is a monoamine neurotransmitter, synthesised from dietary tryptophan. Tryptophan is converted to 5-hydroxytrptophan by the enzyme tryptophan hydroxylase and then decarboxylated to 5-HT by the action of amino acid decarboxylase (Figure 3).

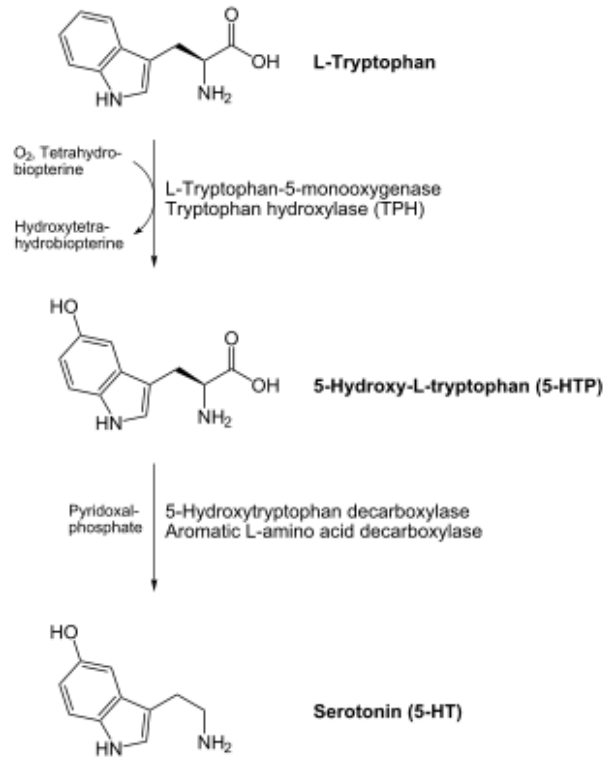


Figure 3 The synthesis of 5-HT (Taken from Goridis & Rohrer, 2002)

The neurones of the raphe nuclei are the main source of 5-HT in the brain, axons from these neurones form a neurotransmitter system that reaches almost every part of the CNS. Axons of neurons in the rostrally located nuclei terminate in parts of the cortex, hippocampus, basal ganglia, limbic system and hypothalamus whereas the caudally situated nuclei project to the cerebellum, medulla and spinal cord (figure 4).

Molecular biological techniques have allowed the identification of at least 15 different 5-HT receptor subtypes which have been classified and divided into seven groups (5-HT_{1-7}) and further subdivided into 5-HT_{2A-C}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}.

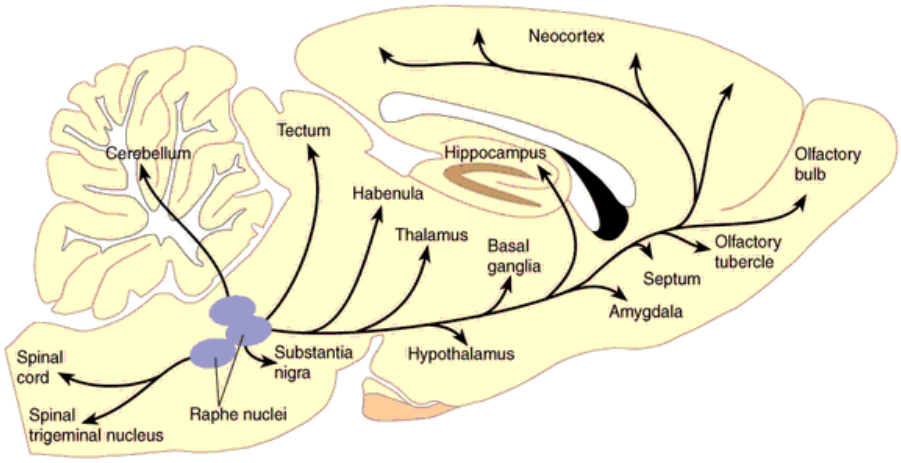


Figure 4 5-HT pathways in the rat brain (taken from Rang et al, 1995)

The role of the 5-HT system in schizophrenia was first suggested in 1954 by Wooley and Shaw, they observed that lysergic acid diethylamide (LSD), acting upon the 5-HT system (5-HT agonist) induced symptomatology of schizophrenia in man, such as hallucinations, delusions and other abnormal behavioural changes. Later it was identified that LSD exerts its action through the $5\text{-}HT_{2A/2c}$ receptor subtypes (McKenna et al, 1989).

The discovery of the atypical antipsychotic agent clozapine in 1959 with low D_2 and high $5\text{-}HT_{2A}$ affinity (Deutsch et al, 1991), and lower incidence of extrapyramidal side effects (EPSE's) compared to classical antipsychotics provoked the re-surge in interest in the 1980s in the serotonin system as a potential therapeutic target for schizophrenia (Busatto & Kerwin, 1997). Many of the atypical antipsychotics have significant affinity for the $5\text{-}HT_{2A}$ receptor, which has directed interest in this receptor subtype with regard to therapeutic antipsychotic development. The relationship of serotonergic and dopaminergic function has been extensively studied with the main focus on the mechanism of action by which clozapine in addition to

other atypical antipsychotics exert superior therapeutic efficacy. The serotonin-dopamine hypothesis of schizophrenia was suggested by Meltzer (1989) and implicated enhanced dopaminergic and serotonergic transmission in subcortical areas, leading to the manifestation of positive symptoms and decreased dopaminergic and serotonergic transmission in the prefrontal cortex leading to the negative and cognitive symptoms.

Many of the atypical antipsychotics have affinities not only for 5-HT_{2A} receptors but also for other 5-HT receptor subtypes including 5-HT_{1A}, 5-HT_{2C}, 5-HT_6 and 5-HT_7 (Schotte et al, 1996; Kuroki et al, 2008). It is likely that these 5-HT receptor subtypes may also be involved in the mechanism of action of atypical antipsychotics. Although physiological roles of the central 5-HT receptor subtypes have yet to be fully understood, the serotonin–dopamine interaction via 5-HT receptor subtypes has been thought to play an important role in the production of clinical effects of atypical antipsychotics (Kuroki et al, 2008).

Post-mortem studies in un-medicated patients with schizophrenia have revealed a reduced number of 5-HT_{2A} receptors (Arora & Meltzer et al, 1991) and an increase in the number of 5-HT_{1A} receptors in the frontal cortex (Hashimoto et al, 1991). Other post-mortem studies have reported confounding results with respect to the hippocampus. Joyce and colleagues (1993) also reported an increase in hippocampal 5-HT_{1A} receptors, whereas another study did not (Hashimoto et al, 1991).

1.1.4.3 Glutamate hypothesis

The glutamate hypothesis of schizophrenia originated with the observation that the dissociative anaesthetic PCP (Sernyl) produced psychotomimetic symptoms in

surgical patients (Luby et al, 1959). Glutamate is the major excitatory amino acid neurotransmitter in the central nervous system (CNS) and was first implicated in schizophrenia when decreased concentrations, up to 50% reductions compared to healthy controls, were found in the cerebrospinal fluid of schizophrenic patients alongside an increase in the density of NMDA receptors in the striatum (Kim et al, 1980). However, attempts to replicate these findings have been unsuccessful (Gattaz et al, 1982; Perry, 1982). Glutamate acts at two classes of receptors, ligated gated ion channels (ionotropic receptors; N-methyl-D-aspartate (NMDA-), AMPA-, kainite- and G-protein coupled metabotropic receptors.

1.1.4.3.1 Ionotropic glutamate receptors

The ionotropic glutamate receptors initiate rapid depolarization by facilitating sodium or calcium entry into neurons through channels made by the receptor itself. The NMDA receptor channels are permeable to calcium and are distinctive because they are "doubly gated" by voltage and ligands, which means they are activated only when glutamate binds at the same time as a depolarizing shift in the membrane potential.

1.1.4.3.2 Metabotropic glutamate receptors

To date, eight metabotropic glutamate receptors (mGlu1-mGlu8) have been identified by molecular cloning studies (Pin & Duvoisin, 1995) and control neurotransmission by activating G-protein coupled synaptic transduction mechanisms. Some mGlu receptors, in particular the mGlu5 subtype have been shown to interact closely with NMDA receptors and may directly modulate the function of the NMDA receptor channel (see review by Moghaddam, 2003).

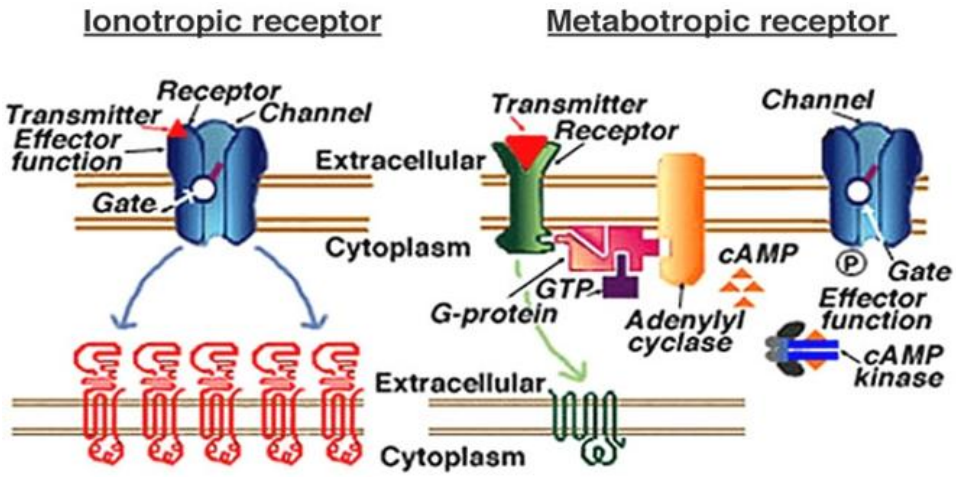


Figure 5 The ionotropic receptors and associated ion channels form one complex from co-assembly of multiple subunits. The metabotropic receptors are coupled to their associated ion channels by a second messenger cascade. Each mGluR is composed of one polypeptide, which is coupled to a G-protein (taken from Kandel et al, 1991).

1.1.4.3.3 NMDA receptors

Autoradiography studies in the rat have shown that NMDA receptors are widely distributed throughout the brain, the largest density is found in the nucleus accumbens, hippocampus and prefrontal cortex (Monaghan & Cotman, 1985; Maragos et al, 1988). The NMDA receptor is a tetrameric-transmembrane channel consisting of combinations of the necessary NR1 subunit with NR2 and/or NR3 subunits (see review by Henson et al, 2010). The function of the NMDA receptor is controlled by its subunit composition (Monyer et al, 1992), the most common NMDA receptors consist of 2 NR1 and 2 NR2 subunits of the identical subtype (Dingledine et al, 2000).

Since the classical antipsychotics are unable to treat the cognitive and negative symptoms of schizophrenia another theory other than the dopamine hypothesis came to light. One of these theories, the ‘glutamate hypothesis of schizophrenia’, emerged

in the early 1980s as an alternative to the popular theory of altered dopamine neurotransmission. Olney and Farber (1995) proposed a mechanism, which could help to explain the symtoms of schizophrenia based upon NMDA receptor hypofunction. The hypofunction of the NMDA receptor could be reproduced by pharmacological antagonism of the NMDA receptors. The glutamate hypothesis is based on studies showing that non-competitive NMDA receptor antagonists such as MK801, ketamine and phencyclidine can induce symptomatic, neurocognitive and neurochemical aspects of the disorder.

Results from genetic studies have reported that the majority of genes that have recently been implicated in the increased risk for schizophrenia can influence the function of glutamate receptors (Harrison et al, 2003; Moghaddam, 2003). Furthermore, post-mortem studies have reported alterations in the marker of viable neuronal tissue, the neuropeptide N-acethylaspartate (NAA) and the neurotransmitter *N*-acetylaspartylglutamate (NAAG) (Tsai et al, 2005). In addition, changes in glutamate receptor binding, transcription and subunit protein expression in the prefrontal cortex, thalamus, and hippocampus have been reported in subjects with schizophrenia (Clinton & Meader-Woodruff, 2004).

The growing evidence supporting the glutamate hypothesis of schizophrenia has led to interest in the modulation of glutamatergic neurotransmission as a therapeutic target. The glycine modulatory site of the NMDA receptor is currently a favoured therapeutic target, with several modulatory agents currently undergoing clinical development. For example the full agonists' glycine and D-serine have both been shown to significantly reduce negative and cognitive symptoms when added to classical and atypical antipsychotics in clinical studies (Javitt, 2004). However,

glycine has limited permeability of the blood-brain barrier and therefore large doses need to be administered (Javitt et al, 2000), whereas d-serine has been shown to produce necrosis of the kidney tubules in rats (Krug et al, 2007).

1.1.4.4 GABA

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter, synthesized from glutamate by glutamic acid decarboxylase (GAD), in the vertebrate central nervous system. There are two classes of GABA receptors; $GABA_A$ and $GABA_B$. $GABA_A$ receptors are divided into six subunits ($GABA_{A1-6}$) and are ligand-gated ion channels (ionotropic receptors), and $GABA_B$ receptors are divided into three sub-units ($GABA_{B1-3}$) and are G protein-coupled receptors (metabotropic receptors).

GABA acts post-synaptically (Kmjevic, 1976) by increasing membrane conductance to chloride ions. The main effect is to inhibit the production of an action potential in the postsynaptic cell. The inhibitory action of GABA led to the belief of a deficiency of GABA in schizophrenia with subsequent dysfunction in brain functioning (Roberts, 1972).

The GABAergic neurones are the second most prevalent cell population in the brain and most neuronal circuitry involves GABA. GABAergic cells are exclusively interneurones that are important for regulating the activity of the glutamatergic pyramidal cells (Benes & Berretta, 2001). Subsequently, the activities of the GABAergic interneurons are controlled by the glutamatergic afferents from projecting neurones (Coyle, 2004). GABAergic interneurons are known to express calcium binding proteins which include calbinin, calretinin and parvalbumin and are

important for modulation of intracellular calcium by acting as calcium buffers. These calcium binding proteins are the major determinants of the kinetics of fluctuations of intracellular calcium (Mojumder et al, 2008) and are predominantly expressed by chandelier and basket cells in the cortex (Mikkonen et al, 1997).

Parvalbumin modulates the activity of calcium-dependent K^+ channels and restores intracellular calcium (McPhalen et al, 1994), thereby protecting neurons from hyperexcitability (Rewal et al, 2005). Late expression of parvalbumin has been hypothesized as a possible mechanism for schizophrenia, as without parvalbumin, neurons are vulnerable to calcium mediated damage (Reynolds et al, 2004). Studies have shown that parvalbumin expression in the prefrontal cortex (Beasley & Reynolds, 1997; Hashimoto et al, 2003) and hippocampus (Zhang & Zeynolds, 2002) is reduced in schizophrenia. Similarly, reduced density of calbindin-containing interneurons in the prefrontal cortex (Beasley et al, 2002) and hippocampus without any change in calretinin expression (Zhang et al, 2002; Hashimoto et al, 2003) has been observed in post-mortem schizophrenia brains. These results suggest that the calretinin containing GABAergic neurones are preserved in schizophrenia.

The rate limiting GABA-synthesizing enzyme: glutamic acid decarboxylase (GAD), exists in both 65 kD (GAD65) and 67 kD (GAD67) forms, catalyzes the conversion of glutamate into GABA, and is a marker of GABAergic neurons (Olsen et al, 1999). Decreased GAD65 mRNA expression in the hippocampus is consistent with findings of reduced GAD activity and GABA concentrations in schizophrenia patients that had been free from antipsychotic treatment for at least 12 months (Todtenkopf & Benes, 1998).

Studies have reported alterations of $GABA_A$ receptors in schizophrenia patients (Benes et al, 1992; Impagnatiello et al, 1998; Lewis et al, 2004; Ichikawa et al, 2004). However, there are few studies indicating that the $GABA_B$ receptor is downregulated in the hippocampus or in the entorinal and temporal cortices in schizophrenia (see review by Wassef et al, 2003). Although there is some evidence for a role of $GABA_B$ receptor in schizophrenia, studies investigating the potential beneficial effects of using selective $GABA_B$ receptor agonists in schizophrenia have been mostly negative (see review by Guidotti et al, 2005).

The growing evidence GABA dysfunction in schizophrenia strongly suggests a role for GABA in the pathophysiology of schizophrenia and supports the development of novel antipsychotic agents targeting this system. Recently, development of compounds with selective efficacy for different α subunits at the benzodiazepine site of the $GABA_A$ receptor has renewed interest for the therapeutic potential of GABAergic drugs (Gray & Roth, 2007). One of these compounds is known as α5IA is a triazolophthalazine that selectively attenuates the effects of GABA at $GABA_A$ receptors containing an α5 subunit. It has been shown to enhance long-term potentiation in an in vitro model of mouse hippocampal synaptic plasticity, gives good in vivo receptor occupancy and improves cognitive performance in normal rats as measured using the delayed-matching-to-place version of the Morris water maze yet, importantly, it is without anxiogenic or proconvulsant liabilities (Atack et al, 2009).

1.1.5 Animal models

1.1.5.1 Genetic mouse models

The rapid growth in knowledge of the neurobiology and genetics of schizophrenia will allow more models of the disease to be developed in an attempt to understand the molecular mechanisms and pathophysiological changes in schizophrenia and design more effective therapies. Recent advances in molecular technologies to manipulate the mouse genome have made genetic mouse models the first choice for most human genetic diseases (Chen et al, 2006). The development of mice with specific mutations via gene knockout or transgenesis has allowed scientists to identify the functional significance of a targeted gene and encoded protein (Tecott & Wehner, 2001). Several schizophrenia susceptibility genes have been identified and led to the development of mouse models based on mutations of these genes (Harrison & Weinberger, 2005; Carter, 2009).

1.1.5.1.1 DISC1

The disrupted in schizophrenia (DISC1) gene which encodes for a synaptic protein, was first identified in a Scottish family (Millar et al, 2000). Transgenic mice have been developed based on the original findings that the DISC1 gene was truncated by a translocation that is associated with psychiatric diseases (Jaaro-Peled et al, 2010). The development of mouse models with truncated DISC1 have yielded interesting phenotypical information, such as enlarged lateral ventricles, and in some studies, decreased parvalbumin immunoreactivity in the mPFC and hippocampus, both pathologies observed in schizophrenic brains (Hikida et al, 2007; Shen et al, 2008; Brandon et al, 2009). Furthermore, mice with impaired DISC1 function have been

shown to have selective deficits in working memory which may relate to the working memory deficits observed in patients with schizophrenia (Kvajo et al, 2008). However, the behavioural data surrounding DISC1 mutant mice are rather variable (Jaaro-Peled et al, 2010).

1.1.5.1.2 NRG1

Neuregulin1 (NRG1) has been identified as a susceptibility gene for schizophrenia in an Icelandic population (Chen et al, 2006; Li et al, 2004) and plays an important role in the developing brain. NRG1 controls neurite outgrowth and neuronal migration, proliferation of oligidendrocytes and glia cells (Chen et al, 2006). NRG1 is also involved in excitatory and inhibitory neurotransmission in the mature brain via an interaction with receptor expression including GABA and glutamate receptors (Austin et al, 2005). Hypomorphic and hypermorphic transgenic NRG1 mice have been shown to exhibit behaviours that are associated with schizophrenia such as hyperactivity in an open field, decreased prepulse inhibition and decreased social interaction (Kato et al, 2010). On the contrary, these mice demonstrate an increase in paravalbumin immunoreactivity in the prefrontal cortex and decreased, rather than increased, dopamine levels in the hippocampus and prefrontal cortex (Kato et al, 2010).

1.1.5.1.3 COMT

The catechol-O-methyltransferase (COMT) gene has a known functional mutation in the encoding sequence that is linked with an increased risk of schizophrenia (Chen et al, 2006) and is thought to lead to a decrease in prefrontal cortex dopamine levels (Stefansson et al, 2004). Mice with COMT-knockout could be viewed as the

treatment model for schizophrenia (Chen et al, 2006). Studies have shown that transgenic mice (Val-tg) that over express COMT show disrupted attentional set-shifting abilities, impaired working memory in a T-maze task and disruption of novel object recognition memory (Papaleo et al, 2008). The brain pathology of the transgenic Val-tg mice has not yet been elucidated.

Most of the susceptibility genes have been linked with the cognitive dysfunctions associated with schizophrenia (Egan et al, 2001). Investigation of the roles of susceptibility genes in cognitive function using the susceptibility based genetic models could provide valuable information on novel molecular mechanisms associated with schizophrenia. However, it is very difficult to model the symptoms of schizophrenia in mice. Furthermore, schizophrenia is a polygenic disease, most likely related to gene-gene and gene-environment interactions that cannot be assessed using mice with single gene mutations (see review by Chen et al, 2006).

1.1.5.2 Prenatal models

As already described, research into schizophrenia has reported the risk for developing schizophrenia is significantly increased by exposure to prenatal virus (see review by Jones & Cannon, 1998), hypoxia (Verdoux et al, 1998), maternal stress, and other maternal medical complications (Kinney et al, 2010) and the subsequent onset of schizophrenia does not emerge until after puberty or early adulthood. It is not yet fully understood how disruption of early brain development may eventually lead to brain malfunction. Evidence has suggested that pre-natal manipulation of the foetus could potentially lead to an animal model of schizophrenia.

So far, models attempted include the prenatal stress model of schizophrenia. This involves the rodent foetus being exposed to elevated levels of corticosterone (cortisol in humans) during the third week of gestation. Like the developing human brain, the emergent rat brain is vulnerable to environmental stress. Exposing pregnant female rats to stressful manipulations during the third week of pregnancy, the developmental equivalent to the second trimester of human pregnancy (Bayer et al, 1993), is thought to reprogram the hypothalamic-pituitary-adrenal (HPA) axis (Henry et al, 1994; Zuckerman et al, 2003; Lee et al, 2007). The effect is perceptible at 56 days old, whereby the rats show reduced PPI, reduced cognitive performance in spatial tasks and enhanced locomotor activity in response to amphetamine (Walker et al, 1997).

Another prenatal procedure in the rat, involves feeding the pregnant dam a low protein-diet (prenatal protein deprivation; PPD), which has been shown to decrease learning, memory and hippocampus morphology in the offspring. Furthermore, these animals also displayed increased dopamine receptor binding in striatum and increased NMDA receptor binding in both striatum and hippocampus (Palmer et al, 2004).

Experimental data show that prenatal exposure to immune-activating agents such as the bacterial endotoxin lipopolysaccharide (LPS) induces long-term cognitive disruptions in spatial working memory, reference memory and novel object recognition (Meyer et al, 2005; Samuelsson et al, 2006; Ozawa et al, 2006).

Pre-natal methylazoxymethanol acetate (MAM) treatment has been proposed as a suitable model for the neurodevelopmental aspects of schizophrenia since the morphological abnormalities it induces in the brain are subtle and consistent with the

neuropathology in schizophrenic brains. MAM is a short acting, alkylating agent that permeates the placenta and leads to the death of cells that are actively replicating DNA (Matsumoto et al, 1972). Treatment with MAM at differential gestational days has resulted in behavioural changes such as reduced social behaviour (Talamini et al, 1999) and deficits in both the acquisition and retention phases of the Morris water maze (Fiore et al, 2002). Furthermore, administration of MAM to pregnant rats on gestational days 5-17 has been shown to induce a dose-dependent disruption in the development of the ventral hippocampal regions and the surrounding perirhinal cortex when the brain was examined after the rat reached adulthood (Lodge & Grace, 2009).

1.1.5.3 Postnatal neurodevelopmental models

Neurodevelopmental models of schizophrenia test hypotheses that this disease is caused by a defect in cerebral development, which manifests in altered neural connectivity and pathology, brain neurochemistry and abnormal behaviour observed in adult life (Lehner et al, 2003). It is proposed that the first 2 weeks of postnatal life in the rat corresponds to the second trimester of pregnancy in humans, during which exposure to toxins (environmental and viral) increases the risk of subsequently developing schizophrenia as an adult (Clancy et al, 2001). Glutamate has been shown to promote neuronal development and the NMDA receptor has been implicated in the structure and plasticity of neuronal circuits (see review by Mouri et al, 2007), which has created interest in using NMDA receptor antagonist treatments during postnatal development as a potential model for schizophrenia. The postnatal administration of PCP to rats on postnatal days (PND) 7, 9 and 11 is proposed as a neurodevelopmental model of schizophrenia (Wang et al, 2001).

Behavioural studies using adult rats that had previously received PCP on PND 7, 9 and 11 have shown enhanced locomotor activity (LMA) following an acute low-dose PCP and d-amphetamine challenge (Depoortere et al, 2005), disruptions in sensorimotor gating in PPI (Takahashi et al, 2006), deficits in ability to shift attentional set (Broberg et al, 2008), and reduction in total brain weight (Brookes et al, 1997). Postnatal PCP treatment (PND 7, 9 and 11) has been shown to disrupt social novelty, whereby the rats fail to discriminate between a familiar and novel rat. The disruption in social novelty induced by postnatal PCP treatment has been reversed with clozapine, mGluR2/3 agonist and an mGluR2 poteniator (Depoortere et al, 2005; Harich et al, 2007).

1.1.5.4 Psychotomimetic Agents

1.1.5.4.1 Phencyclidine

Phencyclidine (1-(1-phenylcyclohexyl) piperidine) is a non- competitive *N*-methyl-*d*-aspartate (NMDA) receptor antagonist, synthesized in 1926 and developed in the 1950s as an intravenous dissociative anaesthetic. Phencyclidine is more commonly referred to as PCP or 'angel-dust' and was discontinued from use as an anaesthetic in the 1960's because PCP was found to induce psychosis in healthy humans that was indistinguishable to psychiatrists from schizophrenia (Collins et al, 1960). Furthermore, when acute PCP is administered to patients with schizophrenia, a persistent worsening of the pre-existing psychotic symptoms occurs (Luby et al, 1959). Unlike the effects of the psychotomimetic agent amphetamine, PCP is proficient in mimicking all the core symptoms of schizophrenia that include, the positive, negative and importantly the cognitive symptomology (Javitt & Zukin, 1991; Jentsch & Roth, 1999; for review see Svensson, 2000). PCP has been shown

to induce cognitive dysfunction such as impairments in executive functioning, verbal fluency and recognition memory (Malhotra et al, 1996). The psychotomimetic ability of PCP, its derivative ketamine and the more selective NMDA receptor antagonist MK-801, suggests a powerful relationship between human psychosis and NMDA receptor blockade.

PCP has a diverse pharmacology, the main action is to antagonise, non-competitively the NMDA class of glutamate receptor (Anis et al, 1983). The PCP binds to the PCP site within the channel pore, but can only bind to this PCP site when the channel is open and the antagonism is therefore 'use dependent', PCP has also been shown to inhibit other ion channels and membrane proteins, including sodium and potassium channels, nicotinic acetylcholine receptors, sigma receptors and the dopamine and nor-adrenaline transporters (Morris et al, 2005). All these effects of PCP are much lower than the affinity for the NMDA receptor but the activity of PCP at the variety of sites may add to its psychotomimetic potential.

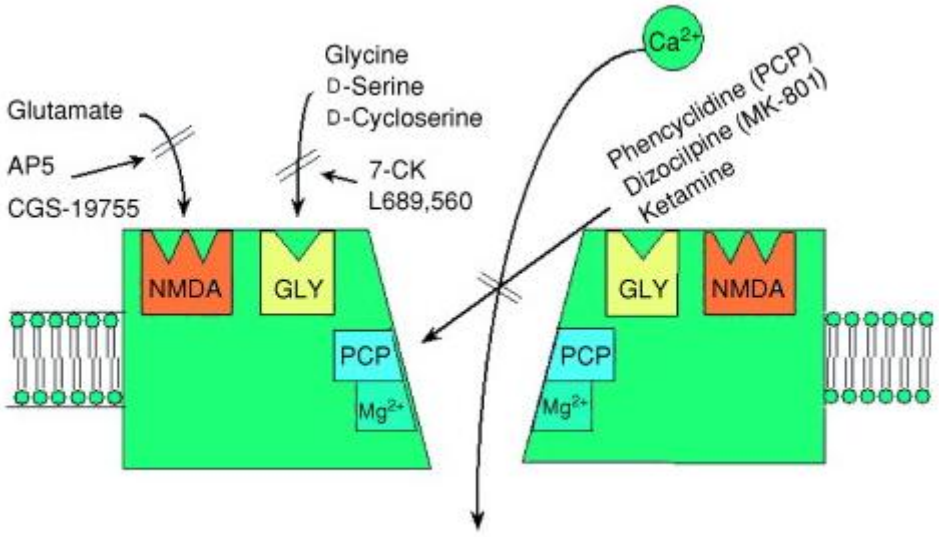


Figure 6 Schematic diagram of the NMDA receptor complex with PCP binding site (taken from Javitt, 2010).

1.1.5.4.1.1 Acute PCP

Acute administration of PCP has been shown to induce behavioural and neurochemical changes. Microdialysis studies have demonstrated an increase in extracellular dopamine, GABA, 5-HIAA in the dorsolateral striatum following acute treatment with PCP in conscious rats (Lillrank et al, 1994). Further microdialysis studies have demonstrated increased dopamine and glutamate release in the pre-frontal cortex and nucleus accumbens in rats treated with acute PCP (Hertel et al, 1995; Adams & Moghaddam, 1998). Optimum brain dopamine levels have long been implicated in cognitive processes. The relationships between cognitive performance and dopamine levels follow an "Inverted-U–shaped" function; where both too little and too much DA impairs performance (see review by Cools & D'Esposito, 2011). Additionally, acute PCP treatment has been shown to increase cerebral blood flow in the basal ganglia, thalamus, hippocampus and PFC in the rat (Gozzi et al, 2008). All these effects of acute PCP treatment most likely contribute to the disturbance of cognitive functions (Jentsch & Roth, 1999). There is extensive behavioural data following acute PCP exposure in the rat, which includes increased locomotor activity, stereotyped behaviours and ataxia (positive symptoms), social interaction/withdrawal (negative symptoms) and sensory information processing, selective attention and working memory (cognitive symptoms) (Geyer et al, 1984; Ogren & Goldstein, 1994; Sams-Dodd, 1995; Adams & Moghaddam, 1998; Jentsch & Anzivino, 2004; Klamer et al, 2004; Egerton et al, 2008; Palsson et al, 2005; Javitt, 2007). Furthermore, studies in our laboratory have demonstrated selective impairments in an operant serial reversal learning task following acute treatment with PCP (Abdul-Monim et al, 2003; Idris et al, 2005).

Limitations

The use of acute PCP treatment is not without limitations. The ability of acute PCP treatment to induce cognitive and negative symptomology associated with schizophrenia in animals is most certainly affected by the nonspecific alterations in general locomotor activity and arousal. In addition, studies have demonstrated that acute PCP treatment to animals, increases forebrain dopaminergic transmission, whereas long-term PCP treatment reduces frontal dopamine transmission which is associated with hypofrontality and some of the cognitive deficits observed in schizophrenia (Jentch & Roth, 1999). Furthermore, schizophrenia is an enduring disease with lasting symptomatology especially surrounding the negative and cognitive aspects. Long term administration of NMDA receptor antagonists have been shown to induce long-term neurochemical and behavioural changes that are more relevant to schizophrenia.

	Acute Exposure	Repeated Exposures
Psychosis	Intense (hours)	Intense (days to weeks)
Hallucinations	Visual illusions (hours)	Auditory and paranoid (days to weeks)
Delusions	Yes (hours)	Frequently religious (days to weeks)
Thought disorder	Yes (hours)	Yes (days to weeks)
Affect	Euphoric to catatonic (hours)	Anxious, labile or paranoid (days to weeks)
Cognition	Impaired (transiently)	Impaired (persistently)
Frontal blood flow	Increased (transiently)	Decreased (persistently)

Figure 7 The biological and psychiatric effects of acute versus repeated exposures to PCP/ketamine in humans (taken from review by Jentch & Roth, 1999).

1.1.5.4.1.2 Sub-chronic PCP treatment

Longer-term treatment with PCP may provide a more valid tool for modelling aspects of schizophrenia symptomatology in animals (see figure 7). Chronic PCP users are subjected to a huge array of side effects and describe the following symptoms: persistent problems with memory, speech and difficulty thinking. Recent memory capability appears to be primarily affected. Users complain of stuttering, inability to speak and difficulty with articulation. Speech and memory difficulties reported to last as long as 6 months to 1 year following prolonged daily use of large doses of phencyclidine (Cosgrove & Newell, 1991). They also complained of anxiety or nervousness during and following periods of regular PCP use. Depression and attempted suicide on repeated occasions after chronic exposure to PCP was also described. Users reported personality changes, social withdrawal and social isolation. In some cases, violent behaviour was one of the effects, as well as aggressive behaviour, paranoia, delusional thinking, and auditory hallucinations (Peterson & Stillman, 1978). These psychological and behavioural effects produced by chronic PCP use in humans have been shown to persist for several weeks after the cessation of PCP use, which provides support for the withdrawal/subchronic/repeated PCP administration regimen as a pharmacological model relevant to schizophrenia (Jentsch & Roth, 1999; Enomoto et al, 2007; Mouri et al, 2007; Seillier & Giuffrida, 2009; Neill et al, 2010).

Long-term PCP use has been shown to lead to a decrease in frontal blood flow in the human brain (Hertzman et al, 1990; Wu et al, 1991) and reduced glucose utilisation in the pre-frontal cortex of the rat, similar to that observed in man (Wolkine et al, 1992; Cochran et al, 2003). This provides support for its use to mimic schizophrenia

symptoms and support to clinical findings of hypofrontality in relation to cognitive dysfunction observed in schizophrenia (Weinberger & Berman, 1988; Goldman-Rakic, 1990).

It has been suggested that antagonism of the NMDA receptor results in an excessive release of the excitatory transmitter glutamate and subsequent overstimulation of postsynaptic neurones might explain the cognitive and behavioural disturbances associated with the NMDA receptor hypofunctionality in schizophrenia (Olney et al, 1999).

The behavioural effects of sub-chronic PCP treatment observed in animals are accompanied by pathological changes to the brain. Studies have shown reductions in the neurochemical marker, parvalbumin mRNA expression in GABAergic interneurones in areas of the prefrontal cortex and hippocampus (Abdul-Monim et al, 2006). Similarly, parvalbumin has also been shown to be altered in post-mortem tissue from schizophrenic brains (Ohnuma et al, 1999; Lewis, 2000; Zhang et al, 2002).

Cognitive effects of sub-chronic PCP treatment

Recent studies using the sub-chronic PCP treatment regimen (2 mg/kg, twice daily for seven days) and the chronic intermittent PCP treatment regimen (2.58 mg/kg, once daily on days 1-5, 8, 10, 12, 15, 17, 19, 22, 24 and 26) have been shown to produce cognitive deficits in an array of animal tests.

Sub-chronic administration of PCP has shown to consistently induce robust cognitive deficits in the established reversal learning paradigm that have been reversed by atypical antispychotics, novel agents but not classical antipsychotics

(Abdul-Monim et al, 2007; McLean et al, 2008; Idris et al, 2010). Studies in our laboratory have shown impairments in the 5CSRTT following sub-chronic PCP treatment which (Barnes et al, 2012). Other laboratories have shown impairments in 5CSRTT following intermittent repeated PCP treatment which was ameliorated following administration of the atypical antipsychotic clozapine (Amitai et al, 2007). The rodent model of attentional set-shifting which has already been shown to be sensitive to the effects of natural aging (Barense et al, 2002), prefrontal cortex lesions (Birrell & Brown, 2000) and muscarinic antagonism (Chen et al, 2004), has also ability to detect cognitive impairments in the extra-dimensional shift (EDS) stage of the task induced by sub-chronic PCP treatment (Rodefer et al, 2008; McLean et al, 2008). It has also been shown that the chronic intermittent PCP dosing regimen-induced impairments in attentional set-shifting in rats, which was subsequently reversed by treatment with modafinil (Dawson et al, 2010). Results from experiments using the NOR test in mice have demonstrated disruptions in recognition memory, specifically the ability of the mice to differentiate between novel and familiar objects in the retention trial following a sub-chronic PCP treatment dosing regimen (10 mg/kg, once daily for 10 days). This sub-chronic PCP-induced cognitive impairment was subsequently restored by sub-chronic (2 weeks) administration of clozapine (5 mg/kg), but not haloperidol (0.1 mg/kg) (Hashimoto et al, 2005). Sub-chronic PCP treatment has been shown to induce impairments in social behaviours in the social interaction test (Sams-Dodd, 1995; Bruins Slot et al, 2005). Furthermore, studies in our laboratory have shown that sub-chronic treatment with PCP induces deficits in social behaviour in the social interaction task, which are reversed by acute treatment with ziprasidone (Snigdha & Neill, 2008). The cognitive

deficits and other behavioural indices of positive and negative symptomatology induced by sub-chronic PCP treatment provide good predictive validity (see table 2).

	Animal models of psychiatric disorders can be classified as having construct, face or predictive validity
Construct Validity	Comparable underlying neurophysiological concept.
Face Validity	Comparable endpoint measurements in clinical and experimental models.
Predictive Validity	Comparable pharmacological profile in clinical and experimental studies.

Table 2 (adapted from van der Staay et al, 2009).

1.1.5.4.2 D-Amphetamine

The dopamine hypothesis of schizophrenia and the pharmacological demonstration of dopamine agonists producing and exacerbating symptoms gave rise to dopamine based models of schizophrenia. The administration of dopamine receptor agonists such as amphetamine has been shown to induce positive symptoms of schizophrenia such as hallucinations and delusions in normal humans and exacerbate symptoms in schizophrenia patients but is unable to induce the negative symptoms of schizophrenia (Ellinwood, 1967; Snyder, 1973; Meltzer, 1991). The dopamine hypothesis of schizophrenia suggests that symptoms may be due to hyperdopaminergia or increased sensitivity to the neurotransmitter, both of which can be simulated by administration of the dopamine agonist d-amphetamine.

In both humans and animals d-amphetamine has been seen to profoundly affect dopamine activity. It is thought to block the dopamine transporter (DAT) producing

elevated levels of extracellular dopamine and disruption of dopamine vesicular stores (Jones et al, 1998). D-amphetamine also exerts similar effects on serotonin (SERT) and noradrenaline transporters (NET) which is thought to attribute to its psychotropic effects (Kuczenski & Segal, 1997). In animals, d-amphetamine increases locomotor activity (LMA), stereotyped behavior and catalepsy (Gambill & Kornetsky, 1976). Furthermore, attention, sensory motor function and learning and memory are disrupted (Seiden et al 1993). Acute administration of d-amphetamine to model the symptomatology of schizophrenia is not without limitations since the effects are not chronic and self sustaining and it fails to mimic the negative symptoms (Sams-Dodd, 1998; Krystal et al, 2005).

1.1.5.5 Isolation rearing

Many years of research into the effects of post-weaning isolation rearing of rats from their normal social group have resulted in the identification of a number of abnormalities in behaviour, neurochemistry and physiology, symptoms of a condition known as the 'isolation syndrome' (Hatch et al, 1963; Sahakian, 1975; Gentsch et al, 1981; Heidbreder et al, 2000; Lapiz et al, 2003; Fone & Porkess, 2008).

Original early studies by Einon and Morgan (1977) into the effects of isolation rearing in the rat demonstrated a crucial timing of the start of isolation. Isolation of rats between (PND) 25 and postnatal day (PND) 45 produced irreversible increases in levels of object contact in an open field and were slower to emerge from a small enclosure into an unfamiliar environment. Isolation at PND 16-25 or after PND 45 had no long lasting effect upon behaviour. More recent studies have reported that isolation of the rats between PND 20-30, produces robust disruption in a variety of

behaviours (see review Fone & Porkess, 2008). Studies have demonstrated that to obtain persistent and reproducible behavioural effects with isolation rearing, it is very important to ensure strict control of the social environment, for example, minimal contact with the experimenter and no environmental enrichment in the home cages (Marsden et al, 2011).

The altered behavioural profile exhibited by these isolated rats include an enhanced level of spontaneous locomotor activity (Gentsch et al, 1963; Smith et al, 1997; Heidbreder et al, 2000), an enhanced reactivity to psychoactive agents such as d-amphetamine and cocaine (Jones et al, 1990; Jones, 1992), a heightened response to conditioned and unconditioned reinforcers (Schenk et al, 1987; Jones et al, 1990; Smith et al, 1997), a reduction in pain threshold (i.e. hyperalgesia) (Puglisi-Allegra & Oliverio, 1983) and an increase in food consumption and weight gain (Fiala et al, 1977). Furthermore, isolated rats have sensorimotor gating deficits, measured by reduced pre-pulse inhibition (PPI) of the startle response (Geyer et al, 1993; Cilia et al, 2001), recognition memory deficits, measured by NOR (Bianchi et al, 2006) and enhanced sensitivity to sub-chronic PCP induced hyperactivity (Lapiz et al, 2003). Many of these behavioural effects implicate alterations in dopamine function, particularly within the mesolimbic system. Further evidence suggesting that isolation rearing alters mesolimbic dopamine function has been gathered using *in-vivo* microdialysis techniques and suggests that isolation rearing produces an increase in extracellular levels of dopamine in the nucleus accumbens (NAc) and striatum, along with a reduction in 5-HT levels in the NAc (Jones, 1991; Fulford & Marsden, 1998). Isolation rearing has been shown to alter brain neurochemistry, however the results are often contradictory. Radioligand binding studies have identified that the

functional high affinity state D_2 receptor numbers in the striatum are increased by isolation rearing (King et al, 2009). However, other groups have observed no changes in density or affinity of D_1 or D_2 receptors in the nigrostriatal system (Bardo & Hammer, 1991). There are also studies showing that isolation rearing induces changes in pre-synaptic dopamine receptor function in addition to post synaptic dopamine changes including decreased D_2 receptors in the nucleus accumbens (Hall et al, 1998).

The link between the mesolimbic dopamine system and the serotonergic system led to investigations into the effects of isolation rearing on serotonergic function. Results to date suggest that isolated rats showed reduced 5-HT activity in the nucleus accumbens but not in the caudate putamen or prefrontal cortex (Jones et al, 1992). *In-vivo* microdialysis has been used to investigate 5-HT release in isolated rats compared to social controls. Bickerdike and colleagues (1993) demonstrated a KCl-stimulated and novelty-induced increase in 5-HT levels in the frontal cortex of socially reared rats compared to isolates, suggesting that isolation reared rats have reduced pre-synaptic neuronal function. The changes in pre-synaptic dopamine and 5-HT function in NAC are also associated with decreased pre-synaptic 5-HT function in the frontal cortex and hippocampus (Lapiz et al, 2003). Findings by Heidbreder et al, (2000) have shown decreases in the basal dopamine turnover in the infralimbic area of the medial prefrontal cortex, a region important for cognition (Jentsch et al, 1997).

1.1.6 Animal tests of cognition

The MATRICS initiative has highlighted pre-clinical tests of cognition that are relevant translational models to study the seven cognitive domains impaired in schizophrenia (see table 3).

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

Table 3 Pre-clinical tests of cognition highlighted by the MATRICS initiative.

1.1.6.1 Executive functioning

Executive function is a high-order cognitive capacity that utilises the domains of attention, planning, multi-tasking and the ability to switch between several tasks and sources of information (Chan et al, 2008). People suffering with schizophrenia exhibit executive functioning deficits, these impairments are noticeable against a background of generalized cognitive deficits, and affect 40% to 95% of individuals

with this disorder. Damage to the prefrontal cortex in humans results in a variety of functional impairments but especially impairments of executive function. The Wisconsin Card Sorting Task (WCST) is a neuropsychological test developed by Berg (Grant & Berg, 1948), thought to measure aspects of executive function such as abstract reasoning, cognitive flexibility and the ability to maintain and shift cognitive set according to changing schedules of reinforcement (Nagahama et al, 1996; Moore et al, 2009). It has been shown that lesions to the dorsolateral prefrontal cortex in the Rhesus monkey induced impairments in executive functioning measured by a primate analogue of the WCST (Moore et al, 2009).

The attentional set-shifting task was developed by Birrel and Brown (2000) as a rodent analogue test of the WCST and the CANTAB ID/ED task (Downes et al, 1989). It requires the subject to simultaneously discriminate in two dimensions to obtain food reinforcement. For example, the food may be buried in sand versus gravel, and contained in a round bowl versus a square box. When the rats attend to a category (e.g., bowl shape) of a stimulus, learning to discriminate novel complex stimuli is more rapid when the discrimination rule is based on the same perceptual dimension (an intra-dimensional shift; IDS). However, if the new discrimination requires that attention is directed to a different category of dimension (e.g., texture of substrate within the bowl) and the previously attended stimulus (bowl shape) must be disregarded (an extra-dimensional shift; EDS), the new discrimination is acquired less rapidly. It has been reported that selective ibotenic acid lesions of the medial prefrontal cortex in the rat induce impairments of the EDS phase of the attentional set-shifting task (Birrell & Brown, 2000).

The five-choice serial reaction time task (5-CSRTT) has been widely used to assess attention and executive function in the rat. This task supplies information on attentional capacity, as measured by the accuracy of reporting of stimuli, in addition to inhibitory response control or executive functioning. The 5-CSRTT is conducted in an automated operant chamber with five exposed apertures located opposite the food magazine dispenser. The rat is required to sustain and divide its attention across five spatial locations in order to detect a visual light cue that flashes randomly in one of five nose poke locations. Numerous measures are recorded such as; target accuracy (response accuracy), premature 'impulsive' responses, perseverative 'compulsive' responses and latency. A variety of lesions to distinct areas of the prefrontal cortex have been shown to selectively impair differential aspects of the 5-CSRTT (Dalley et al, 2004).

1.1.6.2 Working memory

Working memory is the most severely affected domain in schizophrenia patients; it is a core feature of the illness and persists throughout the course of the illness (Manoach, 2003). Interestingly, working memory deficits have been shown to be present in healthy relatives of schizophrenia patients, which could mean that working memory deficits are a behavioural marker of genetic liability to schizophrenia (Parks et al, 1995). Spatial working memory tests have provided consistent results whereas verbal working memory tests are rather less predictable in schizophrenia patients. In rats and mice, the radial arm maze is one test that can be used to measure spatial working memory. It was first developed by Olton and Samuelson in 1976 and consists of 8 arms, radiating out from a central platform, that have shallow cups for placement of food rewards. Rats are trained to locate food rewards in the selected

arms over a varying number of days, visits to un-baited arms or re-visits to previously visited baited arms are scored as errors. Working memory is then required to prevent re-visiting the baited arms while reference memory is required to avoid visiting arms that have never been baited (Kay et al, 2010).

Another pre-clinical working memory task that has been selected and highlighted by the MATRICS initiative is the delayed nonmatch to sample task (DNMTS). The DNMST task requires the subject to compare a presented sample object with a previously presented comparison object and encourages the selection of a novel object with an object's second presentation. The DNMST is an operant task that has three behavioural components; the sample phase, retention phase (delay) and the choice phase. During the sample phase the rat is presented with a brief light flash above one of two levers (e.g. left light), to gain a food reward the rat must press the lever directly under light that flashed. During the retention phase (delay), which is usually 1-15s, the rats must turn around and move to the back of the box and remember the sample phase information. During the choice phase, both lights flash (left and right), and the rat must press a non-matching lever (e.g. non matching to the sample; right lever). A correct non-match to sample results in a small food or water reward. Lesions of the prefrontal cortex and hippocampus have been shown to impair the ability of experimental animals to perform DNMST (Hampson et al, 1999; Porter et al, 2000). Hippocampal lesions are delay dependent (i.e. rats perform at a lower level when the delay increases compared to controls), whereas prefrontal lesions are independent of delay (Porter et al, 2000).

1.1.6.3 Novel object recognition

The novel object recognition task (NOR) was first described in 1950 by Berlyn and was later utilised by Ennaceur and Delacour in 1987 for their studies on the nootropic effects of piracetam. The NOR test has been identified by the MATRICS initiative and Treatment Units for Research on Neurocognition and Schizophrenia (TURNS) as measuring visual learning and memory, a pre-clinical tool for identification of potential pro-cognitive co-treatments for cognitive deficits in schizophrenia. The NOR test measures recognition memory and is ethologically relevant, relies on exploratory behaviour and utilises the innate instinct of rodents to explore novel compared with familiar objects and importantly requires no training or administration of food rewards. The NOR test is relevant to the cognitive dysfunction associated with schizophrenia since recognition memory impairments have been extensively described in schizophrenia patients (Huron et al, 2003). Furthermore, face recognition memory and visual object memory performance impairments have been observed in schizophrenia patients compared to healthy controls (Calkins et al, 2005). The NOR test is relatively fast and easy for the experimenter and consists of 3 phases; acquisition trial, inter-trial interval (ITI) and retention trial. The experiment is carried out in an open field test box and during the acquisition trial two identical objects are presented to the rodent for a time period, usually 3-5 min and exploration of the objects is timed. The ITI can be varied (typically, 1 min-48 h), depending upon the aspect of memory and brain region of interest, during this time the rodents are placed back in to their home cage or a separate holding box. The third phase is the retention trial; rats are again presented with two objects, one familiar (a triplicate object from the acquisition trial) and one

novel object, exploration of the two objects is carefully timed. Lesion studies in the mouse have demonstrated that, when the hippocampus is inactivated, impaired object recognition is observed at relatively long ITIs (24 h) but not at short ITIs (5 min) whereas the perirhinal cortex/PFC are believed to be responsible for recognition memory following it is less than 5 min (Hammond et al, 2004; reviewed by Dere et al, 2007). Evidence to support the role of the PFC in the NOR test has been shown in neuroimaging studies of object recognition in normal subjects. PET scanning has shown activation of brain regions implicated in the pathogenesis of schizophrenia: the bilateral medial temporal lobes and prefrontal areas during the recognition of previously seen objects and the thalamus, prefrontal, and medial temporal lobe areas during the recognition of new objects (Schacter et al 1995), (Schacter et al 1997; Schacter et al 1999; Uecker et al 1997). In comparison with control subjects, patients with schizophrenia demonstrated abnormal thalamic and prefrontal cortical function during object recognition testing (Heckers et al, 2000).

1.1.6.4 16-Holeboard maze

The 16-holeboard maze was first described by Oades and Isaacson in 1978 and later by van der Staay et al, 1990, also known as the 'cognitive' holeboard and is described by the TURNS pre-clinical subcommittee as a test of visual learning and memory and working memory (visit http://www.turns.ucla.edu). The 16-holeboard maze requires the rats to be food deprived which encourages exploration and foraging behaviour on the maze. During the habituation procedure, rats are habituated to the box for 1h for 5 days with all 16 holes baited with a food reward. The training procedure, based on a description by Oades and Isaacson (1978) is divided into two sessions per day (morning and afternoon) consisting of 10 trials for

a total of 10 sessions whereby rats are trained to forage around the 16-holeboard maze to find and eat the 4 food rewards in the fixed set of 4 holes. A visit to a hole can be scored when the nose of a rat turned to the edge of a hole, moved over it or was placed in it (Oades & Isaacson, 1978) whereas others define a hole visit as head-dip into the hole (Wrubel et al, 2007). Rats use win-shift learning strategy to visit the food rewarded holes and avoid revisiting holes from which they already collected the food reward within a trial (Olton, 1987). Measures of cognition on the 16-holeboard range from working memory errors (number of re-visits to food rewarded holes), working memory ratio or working memory score (food rewarded hole-visits/total hole visits), reference memory ratio (total food hole rewarded visits/total non food rewarded visits). For comprehensive and detailed information regarding the 16-holeboard maze measures see review by van der Staay et al (2012). It has been reported that lesions of the hippocampus and ventral tegmental area (VTA) produce impairments of both reference memory and working memory in the 16-holeboard maze.

1.1.7 Antipsychotics

1.1.7.1 Typical antipsychotics

The first antipsychotic medication to be developed was in the 1950s with a drug belonging to the phenothiazine class, called chlorpromazine. This drug was synthesised by the chemist Paul Charpentier and went forward into clinical trials in 1952. Pierre Deniker performed a study in Paris and results from his study using 38 psychotic patients receiving daily injections of chlorpromazine showed dramatic improvements in thinking and emotional behaviour (Lopez-Munoz et al, 2005). By 1994, chlorpromazine was being used throughout the USA to treat schizophrenia,

mania, psychomotor excitement, and other psychotic disorders. The discovery of chlorpromazine was the beginning of the pharmacological era in psychiatry. Chlorpromazine was soon followed by other different classical antipsychotic drugs, such as haloperidol, fluphenazine, droperidol, pimozide, sulpiride, perphenazine, flupenthixol, zuclopenthixol and trifluoperazine. Their main benefit is in treating the positive symptoms of schizophrenia. However, these drugs are less effective against the negative symptoms or the cognitive symptoms associated with schizophrenia. This may be due to the sedative and anticholinergic properties or extrapyramidal symptoms (EPS) (Csernansky, 2002). The effect that is common to all classical antipsychotics is a high affinity for dopamine D_2 receptors, and there is a strong correlation between the therapeutic doses of these drugs and their binding affinity for the D_2 receptor (Creese et al, 1976).

As with all drugs, classical antipsychotic treatments can produce side effects which include; dry mouth, tremors, weight gain, muscle tremors, and stiffness. In addition, classical antipsychotics produce EPS caused by the blockade of D_2 receptors in the nigrostriatal dopamine pathway. These side effects include; motor disturbances, Parkinsonian effects, akathesia (a feeling of "inner restlessness", a constant urge to be moving), dystonia (sustained muscle contractions causing twisting and repetitive movements or abnormal postures), akinesia (impaired body movement; without any or much movement), tardive dyskinesia (involuntary movements of the tongue, lips, face, trunk, and extremities), and neuroleptic malignant syndrome (combination of hyperthermia, rigidity, and autonomic dysregulation). Patients exhibiting these side effects have described them to be worse than the actual symptoms of schizophrenia itself and therefore, EPS are a major cause of noncompliance to drug therapy.

1.1.7.2 Atypical antipsychotics

The original aim was to develop new drugs that would have antipsychotic efficacy without the extensive side effects of the classical agents. The aim was to selectively antagonise the D_2 receptors in the meso-limbic and meso-cortical pathways whilst sparing the nigro-striatal (EPS) and tuberoinfundibular (hyperprolactinaemia) D_2 receptors. The first atypical antipsychotic to be developed was clozapine in the 1960s, with the term "atypical" originally used to describe drugs that, in animal models predict antipsychotic efficacy, but do not produce catalepsy. Clozapine is claimed to have superior efficacy and to cause fewer motor adverse effects than typical drugs for people with treatment-resistant illnesses. Other atypical antipsychotic medications were introduced to the market in the 1990s, including olanzapine, risperidone, quetiapine and between 2001-2004 ziprasidone and aripiprazole. In 2009, asenapine, the latest atypical antipsychotic was released on to the market and is claimed to have a lesser propensity to cause metabolic disorders and weight gain (Balaraman & Gandhi, 2010).

Atypical antipsychotics have enhanced tolerability compared to typical antipsychotics and are efficacious in improving the positive symptoms of schizophrenia as well as providing some improvement in the negative and cognitive symptoms (Meltzer et al, 1999; Reynolds, 2000). They cause fewer EPS effects than classical antipsychotics and also have a different pharmacological profile (Essali et al, 2009). All the atypical antipsychotics have differential receptor binding profiles and demonstrate greater affinity for 5-HT compared with the dopamine receptors (see table 4).

Receptor	Aripiprazole	Olanzapine	Paliperidone	Risperidone	Quetiapine	Ziprasidone	Clozapine	Haloperidol	Molindone	Perphenazine
Pharmacodynamic receptor binding profile: receptor binding affinity expressed as equilibrium constant (Ki)[a]										
D_2	0.66[b,c]	20	2.8	3.77	770	2.6	210	2.6	120	1.4[c]
5-HT_{1A}	5.5[b,c]	610	480	190	300	1.9[b,c]	160	1,800	3,7997[c]	421
5-HT_{2A}	8.7[c]	1.5	1.2	0.15	31	0.12	2.59	61	5,000	5c
5-HT_{2C}	22[c]	4.1	48	32	3,500	0.9	4.8	4,700	>10,000[c]	132[c]
α_1	26[c]	44	10	2.7	8.1	2.6	6.8	17	2,500	10
H_1	30[c]	0.08	3.4	5.2	19	4.6	3.1	260	123,456	8
M_1	6,780[c]	2.5[c]	>10,000[c]	>10,000[c]	120[c]	300[c]	1.4[c]	>10,000[c]	384,000	1,500
M_2	3,510[c]	622[c]	>10,000[c]	>10,000[c]	630[c]	>3,000[c]	204[c]	>10,000[c]	N/A	N/A
M_3	4,680[c]	126[c]	>10,000[c]	>10,000[c]	1,320[c]	>1,300[c]	109[c]	>10,000[c]	>10,000[c]	1,848[c]
M_4	1,520[c]	350[c]	>10,000[c]	>10,000[c]	660[c]	>1,600[c]	27[c]	>10,000[c]	N/A	N/A
Pharmacokinetic profile: half-life										
$t_{1/2}$, h	72	30	20	3	7	7	16	20	1.5-3	8-12

Note: based exclusively on data from human brain receptors (49-56).

[a] Data represented as the equilibrium constant (Ki) (nM), i.e., nanomolar amount of the antipsychotic needed to block 50% of the receptors in vitro. Therefore, a lower number denotes stronger receptor affinity and binding.

[b] Partial agonism.

[c] Data form cloned human brain receptors.

Table 4 Receptor binding affinity for antipsychotic medications (adapted from Correll, 2008)

1.1.7.3 Typical antipsychotics and cognition

Despite being effective in treating the positive symptoms, there is evidence to suggest that classical antipsychotics, rather than improving cognitive deficits, actually add to the cognitive dysfunction associated with schizophrenia (Marsden, 1976; Meltzer & McGuirk, 1999; Kasper & Resinger, 2003).

Furthermore, the most frequent treatment of EPS is the co-administration of drugs with powerful anticholinergic properties, which are known to impair cognition in schizophrenia patients (Tune et al, 1982; Meltzer & McGurk, 1999). Interestingly, these anticholinergic agents have frequently been used in animal studies to model the memory impairments observed in Alzheimer's disease.

Importantly, the National Institute of Mental Health sponsored a study; Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE). This randomized, double blind study compared the cognitive effects of the typical antipsychotic perphenazine with three atypical antipsychotic agents; olanzapine, risperidone and ziprasidone in chronic schizophrenia patients. Results from this study showed there

was no difference between typical and atypical antipsychotics on cognitive performance (Keefe et al, 2007).

1.1.7.4 Pharmacology of atypicals

The effectiveness of the atypical antipsychotics has been linked to their 5-HT_2-D_2 antagonistic properties. The 5-HT_2 receptor antagonism and atypical antipsychotic profiles correlate but the exact mechanisms by which 5-HT_2 antagonism improves negative symptoms with fewer EPS remain unclear. The positive symptoms are associated with a hyperdopaminergic state in the limbic lobe, which is rich in dopaminergic innervation. Serotonin regulates DA release and the presence of 5-HT in the nigrostriatal pathway prevents the release of DA but has little effect in the mesolimbic DA pathway. When the 5-HT_{2A} receptors are blocked, dopamine is released in the nigrostriatal dopamine pathway which is thought to reverse some of the D_2 induced-EPS symptoms by a process called disinhibition. The 5-HT_{2A} antagonism results in elevated DA, which dis-inhibits the D_2 receptors and prevents the antagonism by the antipsychotic resulting in less EPS. Disinhibition of the D_2 receptors in the mesolimbic DA pathway does not affect the efficacy of the antipsychotic because of the low number of 5-HT_{2A} receptors located in this region (Stahl, 2000).

1.1.7.5 Atypicals antipsychotics and cognition

The atypical antipsychotics may help to improve cognition in patients with schizophrenia by reducing the EPS. Furthermore, schizophrenia patients are less likely to be prescribed the cognition impairing anticholinergic agents which are more likely required to reduce the EPS observed following treatment with the classical

antipsychotic agents. In addition, most of the atypical antipsychotic medications display a high affinity for 5-HT_2 receptors and it has been suggested that an interaction with the 5-HT_{2A} receptors are important for cognition (Williams et al, 2002; Kasper & Resinger, 2003; Nichols, 2009). The 5-HT_{2A} receptors have been shown to have a high density in the frontal cortex, an area of great importance to cognition and lower densities throughout the rest of the brain (Nichols & Nichols, 2008). Atypical antipsychotics have been shown to enhance cognition in a variety of tests in patients with schizophrenia (Meltzer & McGurk, 1999). A meta-analysis revealed that atypical antipsychotics are superior to classical agents in improving overall cognitive function (Woodward et al, 2005). However, the reported improvements in cognition following the treatment with atypical antipsychotic agents are not always replicated. A comparative study by Lieberman (2006) demonstrated no significant difference in the efficacy of atypical antipsychotics to improve the cognitive impairments when compared with the classical antipsychotic agents.

Despite the expansion of available antipsychotic medication over the last 50 years, the functional outcome of schizophrenia patients has not improved significantly (Floresco et al, 2005). The current antipsychotics are effective in treating the positive symptoms but are much less efficacious at ameliorating the negative symptoms and restoring the cognitive impairments. Since all currently marketed antipsychotic agents possess some degree of dopamine antagonist effects, the role of other neurotransmitters in the primary antipsychotic activity remains largely unclear. It is possible that different symptomatology of schizophrenia may benefit from different specific classes of medications. Animal models are essential in the initial stage of

discovery of novel compounds with potential to treat the cognitive impairments associated with schizophrenia. However, improved and refined animal models are needed that can predict efficacy in patients. Recently, the importance of predictive, *in-vivo* animal models has been recognized as an essential component of modern drug discovery if late stage failure for lack of clinical efficacy is to be avoided (Markou et al, 2009).

1.1.8 Cognitive enhancers

Since the cognitive dysfunctions associated with schizophrenia are now recognised as a core feature of the disease there is a cognitive 'revolution' in drug discovery. At present the available antipsychotics for the treatment of schizophrenia, are insufficient at alleviating the cognitive deficits.

The MATRICS initiative has highlighted potential targets and classes of drugs that may be useful in treating the cognitive impairments observed in schizophrenia. These include; dopaminergic agents, including D1 receptor agonists; glutamatergic agents and modulators acting on both ionotropic and metabotropic receptors, including ampakines; glycine uptake inhibitors, including GlyT-1 inhibitors; cholinergic agents, including alpha7 nicotinic acetylcholine (ACh) receptor (nAChR) agonists; M1 muscarinic ACh receptor (mAChR) agonists and alpha-2 adrenergic receptor agonists and agents acting on the GABA system and on various serotonin receptors (see review by Barak & Weiner, 2011).

1.1.9 Aims and objectives

The aim of this thesis is to study, validate and investigate the NOR test using behavioural manipulations and psychotomimetic dosing regimens in both male and female rats.

Specific objectives:

1. Carefully validate the NOR test by determining the object preference and inter-trial-interval; effect of time and location, using sub-chronic PCP treated and vehicle control rats.

2. Determine the effect of gender differences on the sensitivity to the cognitive impairments induced by acute d-amphetamine, acute PCP and sub-chronic PCP in the NOR test.

3. Evaluate the ability of classical and atypical antipsychotic agents, other pharmacological agents and potential novel cognitive enhancers to reverse the PCP-induced cognitive deficits in the NOR test.

4. To investigate the effects of isolation rearing on inter-trial-interval induced forgetting and sensitivity to the cognitive impairments induced by acute PCP and acute d-amphetamine in the NOR test.

5. Investigate the effects of acute treatment with d-amphetamine, scopolamine, PCP and sub-chronic PCP treatment on a measure of working memory in the 16-holeboard maze.

CHAPTER 2 - General Methods

2.1 Materials and methods

2.1.1 Experimental Animals

All studies used adult female (180-280g) or male (350-400g) hooded-Lister rats (Charles River or Harlan, UK). Rats were housed (4-5 per cage) in solid floored plastic cages (38 x 59 x 24 cm) containing sawdust, paper sizzle nest (Datesand, Ltd., Manchester, England) and fun tunnels (plastic environmental enrichment tubes., Datesand, Ltd., Manchester, England). Food (Special Diet Services Ltd., Essex, England) and drinking water was available *ad-libitum* in the home cage. The rats were disturbed only for cleaning, which consisted of changing the cage twice per week. Rats were housed in a single sex colony which was maintained under a constant temperature of approximately 21 ± 1 °C and humidity (40-50 %) under a 12h light:dark cycle (lights on at 0700 h). All experiments were conducted during the light phase and were carried out between 0900 h and 1630 h. All studies were compliant with the Animal Scientific Procedures act (1986) and approved by the University of Bradford Ethical Review Process.

Cohort of rats used and total n=	***Chapter (and experiment)***
1 (n=50) 1 (n=50)	3 (object preference and differential ITI; sub-chronic PCP)
2-6 (n=180)	4 (sex; acute PCP, acute d-amph and sub-chronic PCP in males and female rats)
7-13 (n=364)	5 (pharmacology; sub-chronic PCP)
14 & 15 (n=66)	6 (isolation rearing; acute PCP and d-amph)
16 & 17 (n=28)	7 (16-holeboard maze; acute PCP, d-amph, scopolamine and sub-chronic PCP)

Table 2.1 Cohorts of rats used throughout these studies

2.1.2 Drugs

Drug	Source	Dose (mg/kg)	Volume (ml/kg)	Route	Vehicle and pre-treatment time	Receptors
d-amphetamine	Sigma-Aldrich, UK	0.25, 0.5, 0.75, 1.0, 2.5	1	i.p.	0.9% saline (30 min)	DAT
Chlordiazepoxide hydrochloride	Sigma-Aldrich, UK	1.25, 2.5, 5.0	1	i.p.	0.9% saline (30 min)	$GABA_A$
Clozapine	Tocris, UK	1.0, 5.0	1	i.p.	Distilled H_2O + 10µl acetic acid and pH balanced with NaOH (1M) (30 min)	5-HT_{2A}, α_1 and α_2-adrenergic,H1, M1, D_1, D_2, D_4.
Fluoxetine hydrochloride	Sigma-Aldrich, UK	5.0	1		0.9% saline (30 min)	5-HT Transporter
Fluphenazine dihydrochloride	Sigma-Aldrich, UK	0.2	1	i.p.	0.9% saline (30 min)	5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_6, 5-HT_7, α_1 and α_2-adrenergic, H_1, M_1, D_1, D_2, D_3, D_4.
Haloperidol	Baker, UK	0.05, 0.075	1	i.p.	Distilled H_2O (30 min)	D_2, α_2-adrenergic
Modafinil	Tocris, UK	50	2	p.o.	1% methylcellulose (30 min)	DAT. NET
(-)-Nicotine hydrogen tartrate	Sigma-Aldrich, UK	0.2	1	i.p.	0.9% saline (30 min)	nAChRs agonist
Phencyclidine hydrochloride	Sigma-Aldrich, UK	2.0	1	i.p.	Distilled H_2O (Acute: 30 min)	NMDA antagonist
PNU-282987 hydtrate	Tocris, UK	5.0, 10, 20	2	s.c.	Isotonic H_2O (60 min)	Nicotinic Alpha 7 agonist
PNU-120596	Tocris, UK	5.0, 10, 20	2	p.o.	100% PEG400 (60 min)	Nicotinic alpha 7 PAM
Risperidone	GSK, UK	0.2	1	i.p.	Distilled water + 10µl acetic acid and pH balanced with NaOH (1M) (30 min)	5-HT_{2A}, 5-HT_7, α_1 and α_2-adrenergic, D_2
Scopolamine	Tocris, UK	0.75	1	i.p.	0.9% saline (30 min)	M_1 antagonist

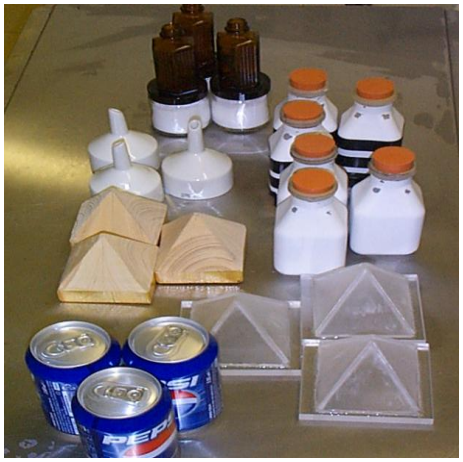
Table 2.2 Routes and volume of administration (intra-peritoneal (i.p.), sub-cutaneous (s.c), per os, (i.e. oral, p.o), vehicles and receptor affinity of the drugs used. DAT (dopamine transporter), NET (norepinephrine transporter), PAM (positive allosteric modulator).

2.1.3 Sub-chronic PCP dosing regimen

The sub-chronic PCP dosing regimen was adapted from earlier studies by Jentsch and Roth (1999) and has been shown to produce enduring behavioural, neurochemical and pathological changes associated with schizophrenia (Fletcher et al, 2005). Sub-chronic PCP was administered via the intraperitoneal route (i.p.) in a volume of 1 ml/kg, calculated as the base equivalent weight, twice per day for 7-days. Treatment with PCP or vehicle was followed by a 1-week washout period prior to NOR testing. The 1-week washout period following sub-chronic PCP treatment is necessary to prevent the behaviour of the rats being influenced either by direct drug effects or by drug withdrawal effects (Jentsch et al, 1998).

2.1.4 NOR apparatus

The apparatus consisted of an open box made of Plexiglas (52 cm L; 52 cm W; 31 cm H) and was positioned 27 cm above the floor on a moveable trolley. The walls of the box were black and the objects to be discriminated (in triplicate) were made of Plexiglas, metal, glass or wood. The height of the objects was approximately equal (10 cm ± 2 cm) and they were heavy enough not to be displaced by the animals, to achieve this, some objects were filled with NaCl e.g. bottles. Objects were positioned 6 cm away from the walls of the box, in opposite corners. After each trial, ethanol (10 % v/v) was used to clean the objects in an attempt to remove any lingering olfactory trails on the objects and in the box.



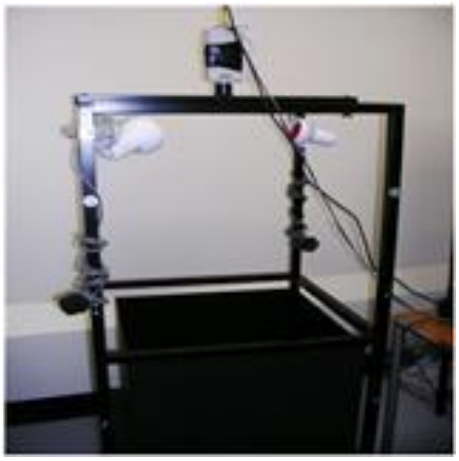


Figure 2.1(a) **Figure 2.1(b)**

Figure 2.1 A Selection of some of the objects used throughout the NOR experiments (a) and the moveable trolley with NOR arena with the position of the camera (b).

Figure 2.2(a) **Figure 2.2(b)**

Figure 2.2 The NOR arena showing a rat exploring during the habituation trial (a) and exploring the two identical objects during the acquisition trial (b).

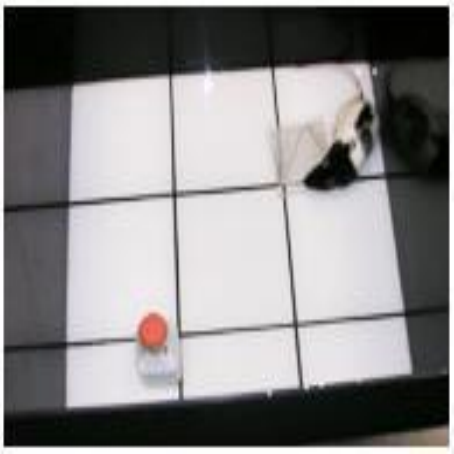


Figure 2.2 (c)

Figure 2.2(c) A rat exploring the novel object during the retention trial in the NOR test box.

2.1.5 NOR testing

2.1.5.1 Habituation

Rats were handled and habituated to the empty NOR apparatus and test room environment for 3 consecutive days for 20 min, always allowing one of the habituation days to precede the test day.

2.1.5.2 Behavioural testing

A further 3 min habituation session to the NOR box preceded the experimental trials on the day of testing.

2.1.5.2.1 Acquisition trial

In the acquisition trial the rats were exposed to two identical objects A1 and A2 for a period of 3 min.

2.1.5.2.2 Inter-trial-interval (ITI)

The rats were returned to their home cage for a 1 min ITI (or otherwise stated in section 3.2.4.3), during this period, both objects were removed, the entire box and objects were cleaned with an ethanol solution (10 % v/v) and dried with paper towels to remove any lingering olfactory trails. A novel object and a triplicate copy of the familiar object was replaced into the NOR test apparatus.

2.1.5.2.3 Retention trial

Following the 1 min ITI, rats were returned to the NOR apparatus to explore the familiar and novel objects for a period of 3 min. The location of the novel object in the retention trial was randomly assigned for each rat using a pseudorandom schedule. The pseudorandom sequences followed the criteria suggested by Gellerman, 1933, and thus reduced the effects of object and place preference.

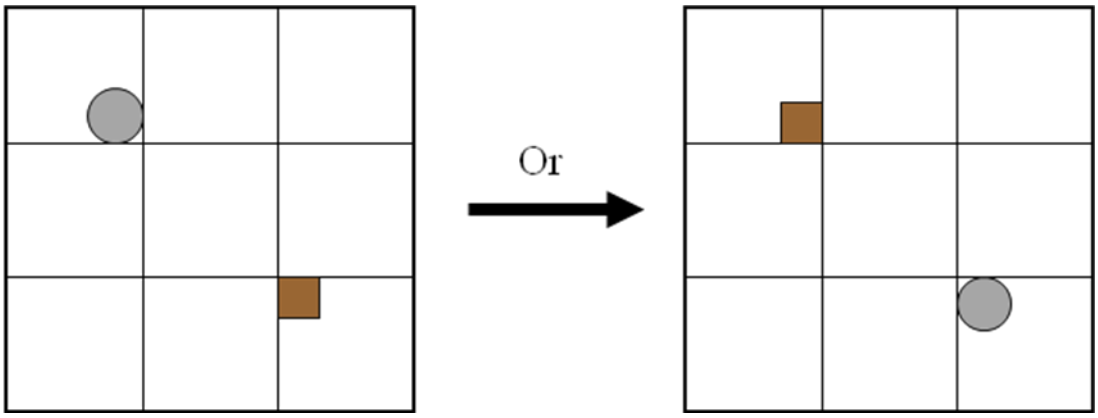


Figure 2.3 Schematic diagram showing the positioning (A or B) of the novel objects in the retention trial.

2.1.5.3 Behavioural assessment

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 (Grayson et al, 2007). The exploration time (s) of each object in each trial was recorded manually using two stopwatches by an experimenter blind to treatment and the following factors were calculated. The exclusion criterion for the NOR test was defined; If an animal failed to explore one or both of the objects (for less than 1 (s) in the acquisition or retention trial it was excluded from the final data analysis.

E1 = the total exploration time of both objects in the acquisition trial ($E_{A1} + E_{A2}$), E2 = the total exploration time of both objects in the retention trial ($E_A + E_B$). DI = discrimination index $(E_B-E_A)/(E_B+E_A)$, the DI represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects in the retention trial.

Line crossings were recorded by counting the total number of sectors (i.e. lines) crossed by the rats during the acquisition and retention trials, one line crossing was counted when the base of the rat's tail crossed over a line, there were 9 square sectors in the box measuring 17.3 cm x 17.3 cm.

2.1.5.4 Statistical analysis

All data are expressed as the mean ± S.E.M and were analysed by IBM SPSS version 18. Exploration data (including differential ITI) were analysed by a repeated measures two-way ANOVA. This detected the main effect of drug treatment, main effect of the task (exploration of both objects) and the interaction between drug

treatment and the two trials (acquisition and retention). Further analysis by Student's t-test was carried out to compare the time spent exploring the novel and familiar objects.

DI and line crossing data were analysed by a one-way ANOVA, followed by a post-hoc Dunnett's t-test or LSD test (chapter 5; DI).

2.1.6 Isolation rearing

The rats were randomly assigned to one of two housing conditions for a period of 12 weeks prior to experimentation. Isolation reared rats were housed in individual cages (38 x 24 x 18 cm; figure 2.4 (a)) such that they could hear, see and smell other rats, but were prevented from any physical contact. Group housed rats were housed in 4 per cage (38 x 59 x 24 cm; figure 2.4(b)). Food and drinking water were available *ad-libitum*. The rats were disturbed only for cleaning, which consisted of changing the cage once per week for isolates and twice per week for group housed rats. Both isolates and group housed animals were housed in a single sex colony in the same holding room which was maintained under a constant temperature of approximately 21 ± 1°C and humidity of 40 – 50 %, under a 12 h light: dark cycle (lights on at 0700 h). All experiments were conducted during the light phase and were carried out between 0900 h and 1600 h. No handling of the rats occurred during the period before experimentation except for cage cleaning.

Figure 2.4 (a) Isolation rearing cages

Figure 2.4 (b) Social housing cages.

2.1.7 Locomotor Activity (LMA)

Locomotor activity experiments were conducted in a behavioural laboratory maintained at 21 ± 1°C. The room contained no windows and was illuminated with white ceiling strip lights and included a radio to supply background noise. Following transportation to the laboratory, rats were allowed a 1h environmental acclimatisation period before LMA testing began. LMA was measured in individual

translucent Plexiglas boxes which were covered with a translucent Plexiglas lid, using AM1052 Activity Monitor, positioned on a four-tier shelf against a wall in a total bank of 16 boxes (figure 2.5 (b)). Each LMA box (figure 2.5 (a)) measured 16 x 26 x 19 cm, contained a small amount of wood shavings and was fitted with 11 infrared photocell units incorporating emitters and detectors. Movement of the rat within the LMA box produced multiple breaks in the light beams resulting in LMA counts. Counts were recorded using AmLogger software (Linton Instruments, UK), every 5 minutes over a total test period of 60 minutes.

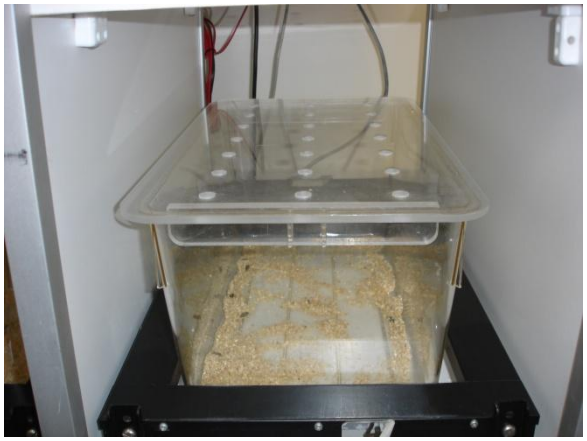


Figure 2.5 (a) An individual automated photocell cage.

Figure 2.5 (b) A bank of 16 photocell cages

2.1.7.1 Statistical analysis

Locomotor activity data was analysed by a one-way ANOVA, followed by a post-hoc Dunnett's t-test.

2.1.8 16-Holeboard maze

2.1.8.1 Apparatus

The apparatus (figure 2.6(a)) consisted of an open box made of Plexiglas (70 cm L; 70 cm W; 50 cm H) and was positioned 30 cm above the floor on a small table inside a designated behavioural test room, environmental conditions were identical to the animal holding room (19-23°C and 45-65% relative humidity). The walls of the box were clear and the floor was grey. There were 16 individual equally spaced bait holes on the floor of the box which were 4 cm in depth. A camera was positioned above the 16-holeboard maze and connected to a portable TV positioned outside the behavioural test room to allow observation and scoring of animal behaviour without any disturbance from the experimenter. Background radio music was constantly played within the behavioural test room.

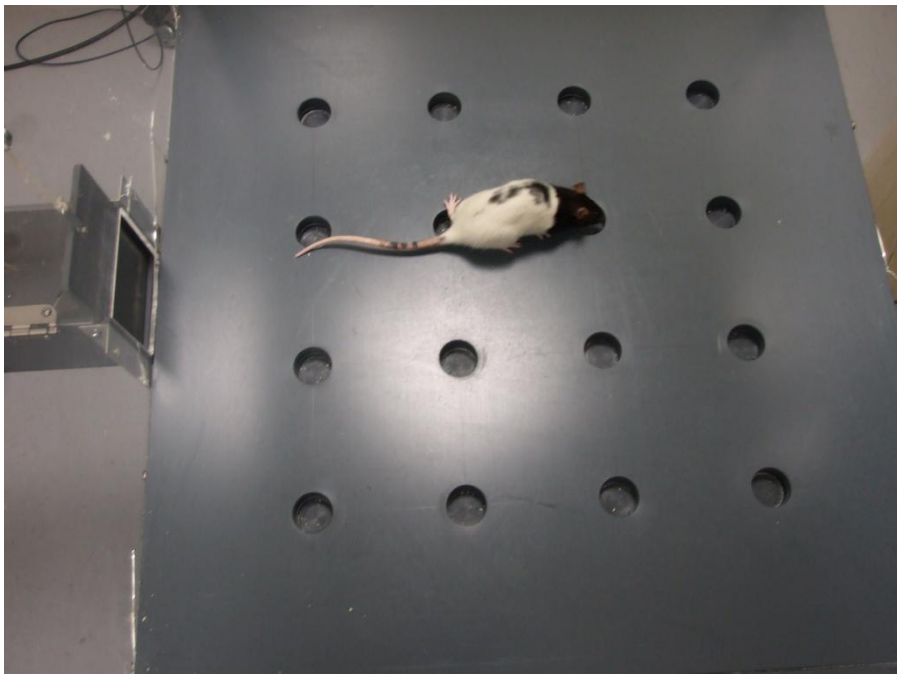


Figure 2.6 (a) Photograph of a rat exploring the 16-holeboard maze.

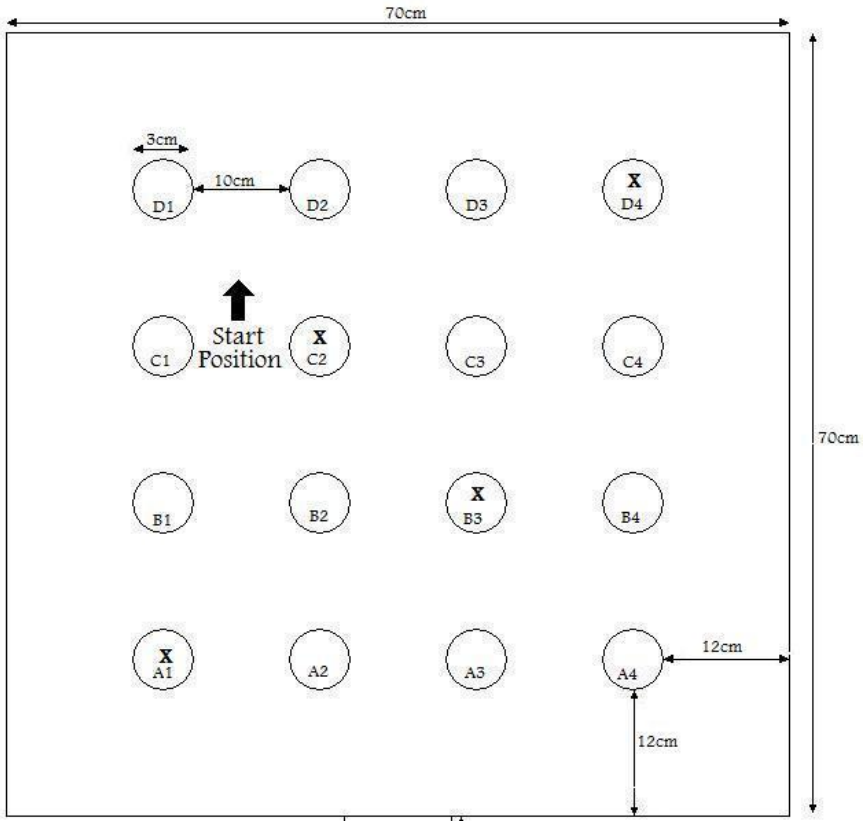


Figure 2.6 (b) Schematic diagram of the 16-holeboard maze with dimensions (baited holes are shown with an X).

2.1.8.2 Food restriction

Rats were food restricted to 90% of their free feeding body weight by daily administration of 12-15 g of standard rat chow (Special Diet Services Ltd., Essex, England). Rats were weighed daily and food quantity altered to ensure a healthy and stable bodyweight.

2.1.8.3 16-Holeboard maze protocol

2.1.8.3.1 Handling

Since the 16-holeboard maze paradigm requires a great deal of handling of the rats by the experimenter during the training phase (Oades, personal communication), it

was necessary to handle the rats daily for 1 week prior to training on the 16-holeboard maze; handling includes scruffing and firmly holding the rats until they appeared relaxed. This habituation process ensured that the rats were not stressed during the training protocol, allowing them to rapidly learn the task without fear of the experimenter (Oades, personal communication). Pre-handling may have prevented any of the rats from being excluded from the study.

2.1.8.3.2 Habituation

The 16-holeboard maze was thoroughly cleaned with ethanol (10% v/v) into which 2 g per 100 ml of TestDiet AIN-76A Rodent Tablet was dissolved, this was to remove lingering olfactory trails prior to habituation and also to prevent the rats working out the food reward distribution by olfactory stimuli. Approximately 20 food pellets (TestDiet AIN-76A Rodent Tablet) were placed in all 16-holes. Rats were habituated in cage groups (n=3 or 5 rats) and placed in the 16-holeboard maze apparatus twice per day (morning 09:00h and afternoon 14:00h) for a period of 30 min for 5 days.

2.1.8.3.3 Training

Training sessions were performed in the morning (09:00h) and again in the afternoon (02:00) for 4 days. On the 5^{th} day, a training session in the morning (09:00h) preceded a drug testing session in the afternoon (14:00h).

During training, individual rats underwent sessions of 10-trials with an ITI of 20 s, whereby the rats were gently held by the experimenter whilst the 16-holeboard maze was thoroughly cleaned with ethanol (10% v/v)/TestDiet AIN-76A Rodent Tablet solution.

Each trial required 4 food pellets to be placed in the same 4 holes (A1, B3, C2 and D4; see figure 2.6(b) throughout the entire training procedure. The trial ended when all of the 4 food pellets had been found and eaten or after a period of 2 min whereby the rats had not been successful in finding the 4 food pellets.

2.1.8.3.4 Testing

The testing phase was identical to the training procedure and was adapted from methodology by Oades and Isaacson, 1978. On the afternoon (14:00h) of day 5, an acute injection of PCP (2.0 mg/kg, i.p., chapter 7), d-amphetamine (0.75 mg/kg, i.p., chapter 7), scopolamine (0.25 mg/kg, i.p.,) or vehicle (0.9% saline, i.p.) was administered 30 min prior to testing (see table 7.1). Rats used in the acute studies were given a 1-week washout period in-between experiments (see section 7.2.1). The rats used in the sub-chronic PCP study were also tested following a 1-week washout period.

2.1.8.4 Behavioural assessment

The number of visits to each hole and the time (latency) taken to find and eat all the food pellets (i.e. complete the task) were recorded manually by an experimenter blind to drug treatment. Rats that failed to complete the task in less than 2 min were removed from the 16-holeboard maze before being replaced back into the arena for the next trial.

2.1.8.4.1 Food hole and non-food hole visits

A visit to a hole was recorded when a rat placed its nose into a hole.

2.1.8.4.2 Working memory score (WMS)

This was adapted from Oades et al (1981) and calculated by the number of visits to the food holes that contain food (i.e. 4) / total number of hole visits (food holes + non food hole visits) with the maximum achievable score being 1 (i.e. 4/4 =1). In this study, for example, on day 9 of training (figure 7.1) the rats visited all the 4 food holes (ate the food pellets) and either re-visited the food holes or visited the non-food holes approximately a further 4 times to gain a total number of hole visits of 8. Therefore total number of hole visits = 8. 4/8 = WMS would be 0.5.

With further training sessions on the 16-holeboard maze a higher WMS may be obtained but it was observed that even when rats had learnt the pattern of food holes (i.e. A1, B3, C2 and D4; figure 2.6(b) they were likely to explore a hole as they passed by to complete the task.

2.1.8.4.3 Latency

Time (s) taken to complete the task.

2.1.8.5 Statistical Analysis

All data are expressed as the mean ± S.E.M. The training data (WMS and latency) was analysed by a one-way ANOVA followed by post-hoc Dunnett's t-test. Following drug treatments, the WMS and latency was analysed by a repeated measures two-way ANOVA. This detected the main effect of drug treatment, main effect of the trial and the interaction between drug treatment and the trial (1-10). Planned further analysis by unpaired Student's t-test was carried out to compare differences in WMS or latency between drug groups and vehicle control over the 10 trials.

CHAPTER 3 - Initial validation of the NOR test: Investigation of object preference and varying inter-trial intervals

3.1 Introduction

Cognitive deficits from many domains are observed in schizophrenia patients, and appear to be the main determinants of the patients' functional outcome (Bowie & Harvey, 2006). Furthermore, there are a number of studies demonstrating deficits in recognition memory in schizophrenia patients (Pelletier et al, 2005; Calkins et al, 2005; Gruzelier, 1999). Additionally, schizophrenia patients are more susceptible to distraction during visual memory tests when compared to control subjects (Anticevic et al, 2011).

The MATRICS initiative has highlighted seven key cognitive domains that are disrupted in schizophrenia, and recommended a battery of neuropsychological tests (Green et al, 2004). Given that rodents are primarily selected as subjects for pre-clinical tests, paradigms are required to assess the learning and memory deficits associated with schizophrenia in these animals.

The need to design and develop paradigms to assess the various cognitive domains that are disrupted in schizophrenia is vitally important since as yet, no medication has been licensed to treat these symptoms. The NOR test was selected and mapped on to the MATRICS test battery and described as measuring visual learning and memory. The NOR test is ethologically relevant and uses rodents' natural affinity for novelty, i.e. rats explore a novel object compared with a familiar one (see chapter 1). This spontaneous preference for novelty ensures no training is needed which manifests in the speed of the test to generate results. The NOR test is non-aversive and does not require the animals to be food restricted; therefore no confounding effects of food deprivation induced-stress on the data are produced. The ITI period,

between the acquisition and retention trial, can be manipulated to modify the cognitive load and determine the specific brain region of interest (see review by Dere et al, 2007).

Previous research in our laboratory (Abdul-Monim et al, 2006) and others (Hashimoto et al, 2005; Rodefer et al, 2008; Egerton et al, 2008) have shown that the sub-chronic PCP dosing regimen induces impairments in cognitive function in rodents.

The main focus of the studies in this thesis have been to validate and characterize the NOR test and object recognition deficits induced by PCP following a 1 min ITI. Preliminary methododological validation studies are important when creating and developing animal tests to ensure results are meaningful and reproducible.

The aim of these studies was to establish a set of objects with equal preference in control and sub-chronic PCP treated rats that could be used hereafter to study NOR deficits in the NOR test. Furthermore the ITI was investigated in an attempt to determine the effects of manipulations (such as time and location of the rats) during the ITI period on the cognitive impairments following sub-chronic PCP treatment in the NOR test.

3.2 Materials and Methods

3.2.1 Experimental Animals and Design

The initial object preference study used a total of 50 adult female hooded-Lister rats (Charles River, UK) weighing 190-240 g at the start of the experiment (cohort 1; see table 2.1).

The differential ITI experiments used all the same rats as described above (cohort 1) and weighed 200-250 g at the start of this experiment. For housing conditions see section 2.1.1.

3.2.2 Sub-chronic PCP dosing regimen

Half of the rats (n=25) were treated with PCP hydrochloride and half (n=25) treated with vehicle (distilled water). For details of the sub-chronic PCP dosing regimen see section 2.1.3. Since the aim of these studies was to validate the sub-chronic PCP treatment regimen in NOR, both control and sub-chronic PCP treatment groups were used to determine object preference. For details of the drugs used in this study, see table 2.2

3.2.3 Apparatus

For information regarding the NOR apparatus see section 2.1.4.

3.2.4 Object preference and differential ITI testing

3.2.4.1 Habituation

For information regarding habituation procedure see section 2.1.5.1.

3.2.4.2 Object preference testing

A further 3-min habituation session in the NOR box preceded the experimental trials on the day of testing.

Ten different combinations of the objects were tested in the acquisition and retention trials of the NOR test (see table 3.1). Rats were habituated to the NOR test box and tested as described earlier.

Figure 3.1 Objects (available in triplicate) that were used in experimental procedure 1. From left to right: Frijj© bottle, Coke© can, wood cone, plastic cone and water bottle.

Each object was compared to each other, with 10 possible combinations of objects. The individual object preference experiments were separated by 3 days (i.e. tested on Monday and Friday), rats were tested twice (i.e. the rats tested on Monday were re-tested on Friday using different object combinations; n=5 per combination) and the study took 1 week to complete. Rats were placed into the NOR apparatus to explore the objects for a period of 3 min. The location of the objects within the NOR apparatus was randomly assigned for each rat using a pseudorandom schedule. The pseudorandom sequences followed the criteria suggested by Gellerman, 1933, and thus reduced any effects of object location (left or right) within the NOR box upon object preference. Following the 3 min test period, objects were removed; the entire box and objects was cleaned with an ethanol solution (10% v/v) and dried with paper towels to remove any lingering olfactory trails. All experiments were video recorded for subsequent behavioural analysis by an experimenter, blind to treatment.

Experiment order	*Objects*
1	Coke can *cf.* Wood cone
2	Coke can *cf.* Frijj bottle
3	Coke can *cf.* Plastic cone
4	Coke can *cf.* Water bottle
5	Wood cone *cf.* Frijj bottle
6	Wood cone *cf.* Plastic cone
7	Wood come *cf.* Water bottle
8	Frijj bottle *cf.* Plastic cone
9	Frijj bottle *cf.* Water bottle
10	Plastic cone *cf.* Water bottle

Table 3.1 Experimental order and object combinations used in the object preference study

3.2.4.3 Differential ITI testing

The differential ITI experiments were separated by 3 days (i.e tested on Monday and Friday), rats were tested twice and the study took 1 week to complete.

A further 3-min habituation session to the NOR box preceded the experimental trials on the day of testing. Testing consisted of a 3-min acquisition trial whereby rats explored two identical objects followed by a differential ITI period and location (NOR box or home cage). All combinations of objects (see table 3.1), except the wooden cone, were randomly selected as pairs for these experiments and all subsequent NOR experiments. The five ITI experiments were selected in an attempt to determine the effects of manipulations (such as time and location of the rats) during the ITI period as shown below (table 3.2), on the cognitive impairments following sub-chronic PCP treatment in the NOR test. In experiment 4 (0 min ITI in

the NOR box), following the acquisition trial, the identical objects were removed when the rat was facing the wall of the NOR box and the novel and familiar objects were replaced immediately for the retention trial. For experiment 5, the distracter object was placed in the centre of the NOR box during the 1 min ITI and was quietly removed prior to the retention trial. The distracter object was carefully selected on the basis that rats found it highly interesting, based on previous studies showing that time spent exploring the wooden cone was significantly greater compared to some of the other objects (see summary table 3.4). Following the differential ITIs, the objects were cleaned with an ethanol solution (10% v/v) and dried with paper towels to remove any lingering olfactory trails. For details of the retention trial see section 2.1.5.2.3 and for details of animal batches used see table 2.1.

Experiment order	***Initial number of rats***	***Number of rats excluded***	***ITI and location***
1	5	0	1 min ITI in the home cage (normal conditions)
2	5	0	10 s ITI in the home cage
3	5	0	1 min ITI in the NOR test box
4	5	0	0 min ITI in the NOR test box
5	5	0	1 min ITI in the NOR test box with a distracter

Table 3.2 Experimental order and the selected ITIs used for to assess the role of distraction in vehicle and sub-chronic PCP treated rats.

3.2.5 Statistical analysis

Object preference data are expressed as the mean ± S.E.M. (Veh; n=5 and PCP; n=5). Student's paired t-test was used to compare the time spent exploring the different objects. For statistical analysis of the differential ITI see 2.1.5.4.

3.3 Results

3.3.1 Determination of object preference for the NOR test

Student's paired t-test revealed a significant ($t(5)=2.9$; $P<0.05$; figure 3.2) increase in object preference when comparing the time spent exploring the wood cone compared to the coke can in the sub-chronic PCP-treated rats and the time spent exploring the wood cone compared with the water bottle in the vehicle treated rats, ($t(5)=-3.06$; $P<0.05$; figure 3.8). There were no significant object preferences in the other pairs of objects tested in vehicle or sub-chronic PCP treated rats.

A one way ANOVA on the overall total object exploration times totalled throughout the series of object preference experiments in the vehicle treated rats revealed a significant effect ($F_{4, 99}=3.1$, $P<0.05$, table 3.3). However, post-hoc analysis revealed no significant individual effects.

A one-way ANOVA on the overall total object exploration times totalled throughout the series of object preference experiments in sub-chronic PCP treated rats revealed a significant effect ($F_{4, 99}=3.7$, $P<0.01$). Post-hoc analysis revealed a significant ($P<0.01$) increase in the total exploration of the wood cone when compared to the water bottle (table 3.3).

Independent Student's t-test comparisons revealed no significant differences in overall total exploration times throughout the series of object preference experiments when comparing the vehicle and sub-chronic PCP treated rats (table 3.3).

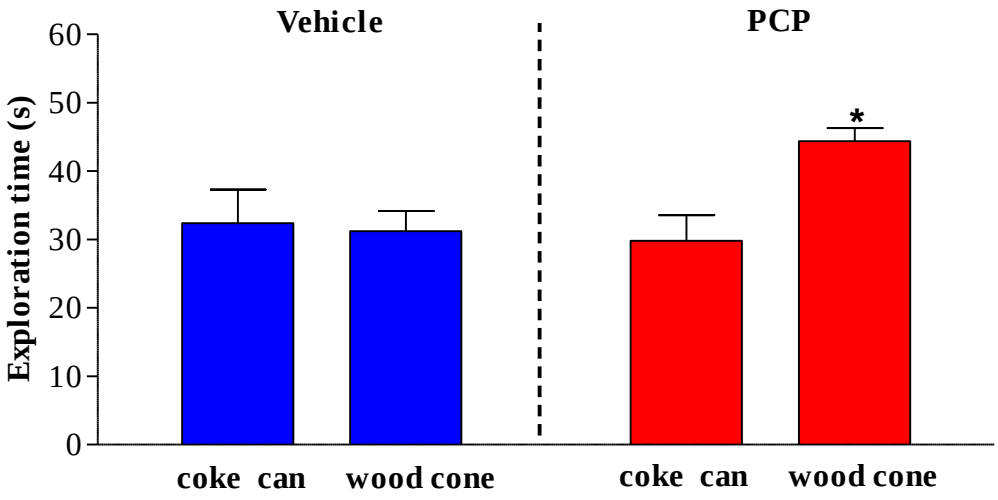


Figure 3.2 Object preference comparing the time (s) spent exploring two novel objects (coke can and wood cone) in female rats treated with PCP (2mg/kg, i.p.) or 0.9% saline (veh, i.p.) twice daily for 7 days. Data are expressed as the mean ± S.E.M. (n=5 per group). *P<0.05; significant increase in object exploration time.

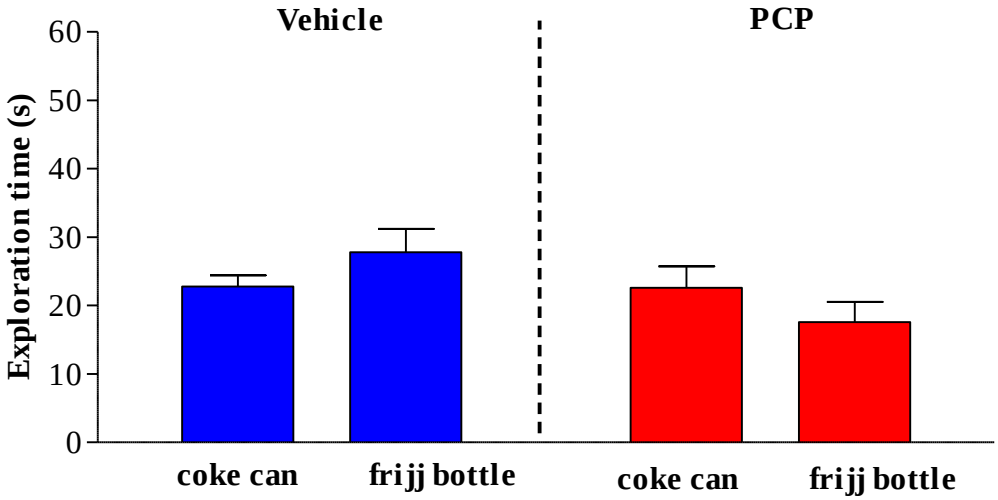


Figure 3.3 Object preference comparing the time (s) spent exploring two novel objects (coke can and Frijj bottle) in female rats treated with PCP (2mg/kg, i.p.) or 0.9% saline (veh, i.p.) twice daily for 7 days. Data are expressed as the mean ± S.E.M. (n=5 per group).

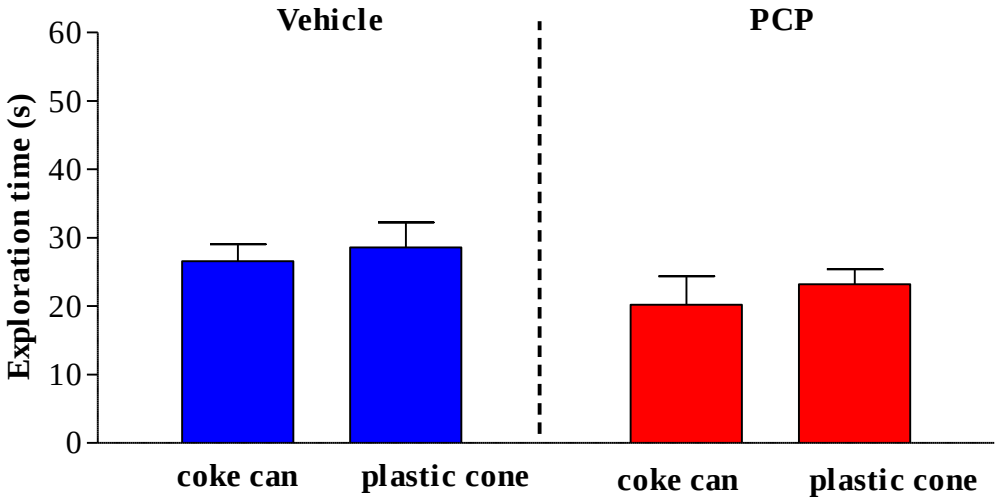


Figure 3.4 Object preference comparing the time (s) spent exploring two novel objects (coke can and plastic cone) in female rats treated with PCP (2mg/kg, i.p.) or 0.9% saline (veh, i.p.) twice daily for 7 days. Data are expressed as the mean ± S.E.M. (n=5 per group).

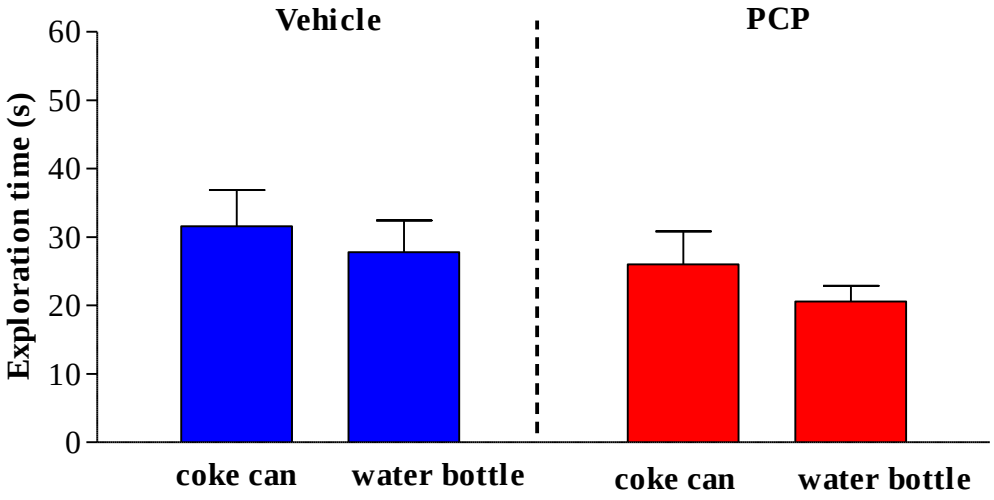


Figure 3.5 Object preference comparing the time (s) spent exploring two novel objects (coke can and water bottle) in female rats treated with PCP (2mg/kg, i.p.) or 0.9% saline (veh, i.p.) twice daily for 7 days. Data are expressed as the mean ± S.E.M. (n=5 per group).

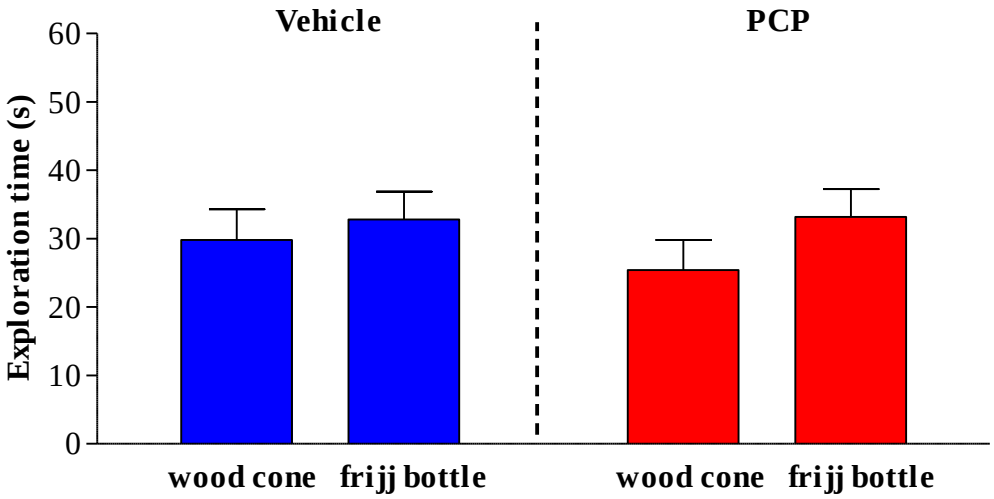


Figure 3.6 Object preference comparing the time (s) spent exploring two novel objects (wood cone and Frijj bottle) in female rats treated with PCP (2mg/kg, i.p.) or 0.9% saline (veh, i.p.) twice daily for 7 days. Data are expressed as the mean ± S.E.M. (n=5 per group).

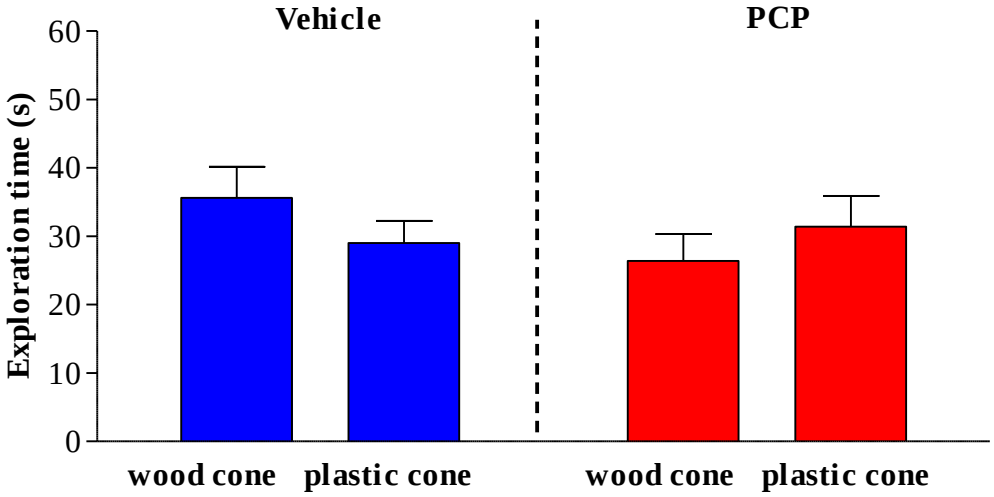


Figure 3.7 Object preference comparing the time (s) spent exploring two novel objects (wood cone and plastic cone) in female rats treated with PCP (2mg/kg, i.p.) or 0.9% saline (veh, i.p.) twice daily for 7 days. Data are expressed as the mean ± S.E.M. (n=5 per group).

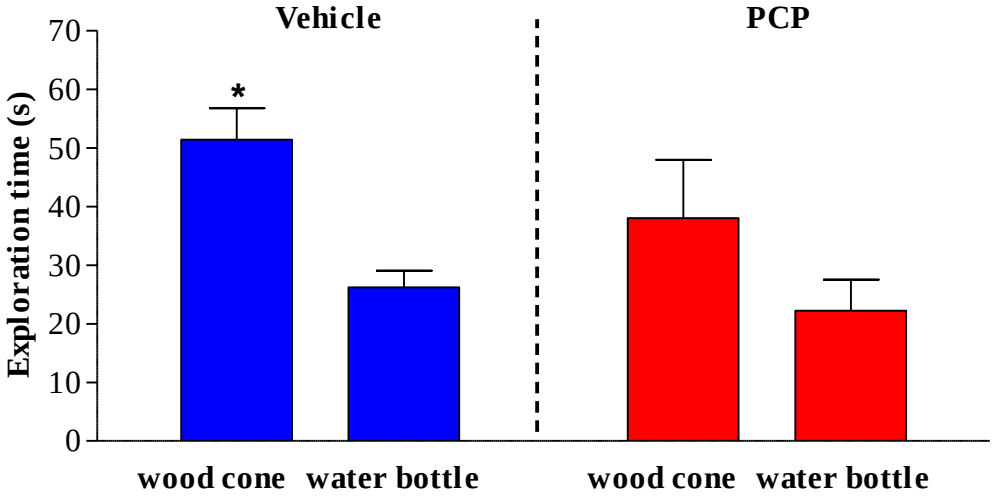


Figure 3.8 Object preference comparing the time (s) spent exploring two novel objects (wood cone and water bottle) in female rats treated with PCP (2mg/kg, i.p.) or 0.9% saline (veh, i.p.) twice daily for 7 days. Data are expressed as the mean ± S.E.M. (n=5 per group). *P<0.05; significant increase in object exploration.

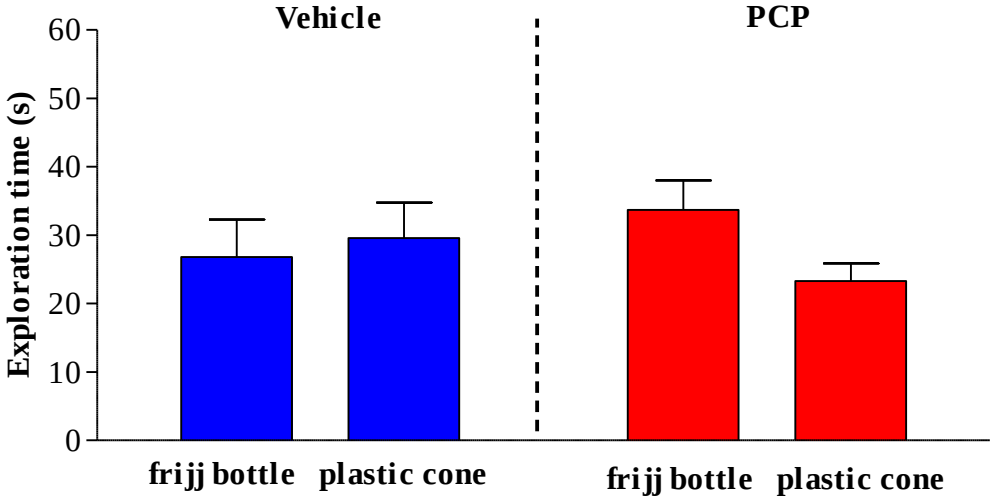


Figure 3.9 Object preference comparing the time (s) spent exploring two novel objects (frijj bottle and plastic cone) in female rats treated with PCP (2mg/kg, i.p.) or 0.9% saline (veh, i.p.) twice daily for 7 days. Data are expressed as the mean ± S.E.M. (n=5 per group).

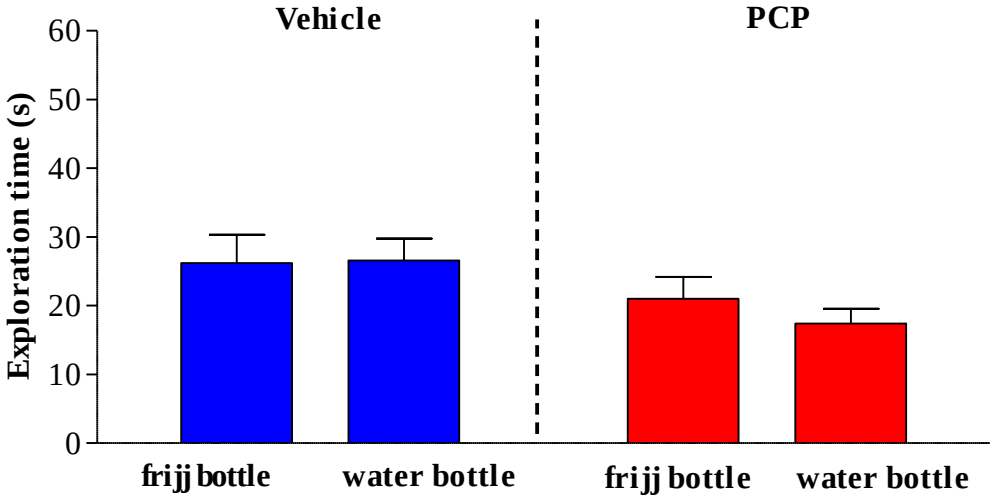


Figure 3.10 Object preference comparing the time (s) spent exploring two novel objects (frijj bottle and water bottle) in female rats treated with PCP (2mg/kg, i.p.) or 0.9% saline (veh, i.p.) twice daily for 7 days. Data are expressed as the mean ± S.E.M. (n=5 per group).

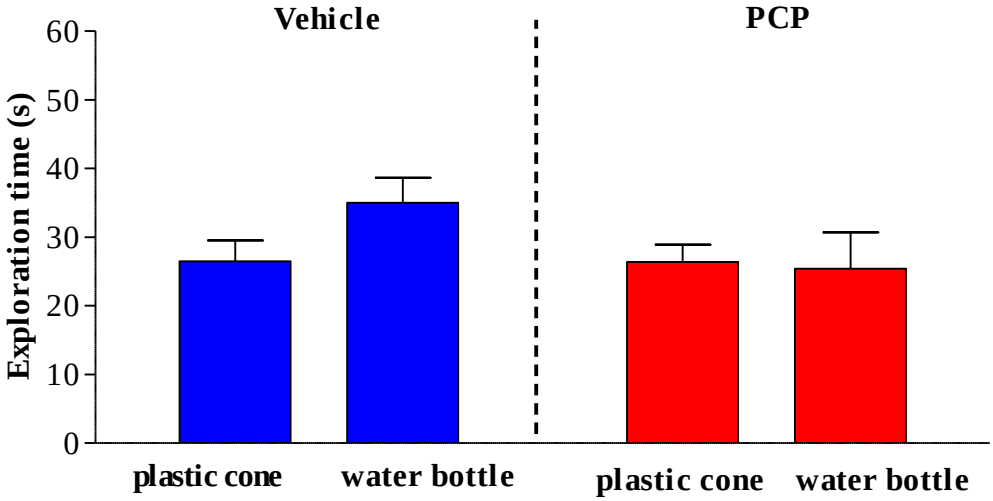


Figure 3.11 Object preference comparing the time (s) spent exploring two novel objects (plastic cone and water bottle) in female rats treated with PCP (2mg/kg, i.p.) or 0.9% saline (veh, i.p.) twice daily for 7 days. Data are expressed as the mean ± S.E.M. (n=5 per group).

Comparisons between objects	*Vehicle Exploration time (s)*	*Sub-chronic PCP Exploration time (s)*
Coke can	28.4 ± 1.9	24.6 ± 2.0
Wood cone	37.0 ± 2.8	33.6 ± 3.2**
Frijj bottle	28.4 ± 2.1	27.0 ± 2.6
Plastic cone	29 ± 1.6	25.8 ± 1.6
Water bottle	28.7 ± 2.0	25.4 ± 2.4

Table 3.3 The effect of treatment with PCP (2 mg/kg, i.p., twice daily for 7 days) or 0.9% saline (veh, i.p.) on the total (mean of 4 object preference experiments) object exploration time throughout the series of object preference experiments (n=20). Data were expressed as the mean ± S.E.M. and analysed by one-way ANOVA followed by Dunnett's t-test. **P<0.01; significant increase in total exploration time compared to the water bottle.

Object Comparisons	*Object preference: Vehicle*	*Object preference: Sub-chronic PCP*
Coke can cf. Wood cone	**No**	**Yes ***
Coke can cf. Frijj bottle	**No**	**No**
Coke can cf. Water bottle	**No**	**No**
Wood cone cf. Frijj bottle	**No**	**No**
Wood cone cf. Plastic cone	**No**	**No**
Wood cone cf. Water bottle	**Yes ***	**No**
Frijj bottle cf. Plastic cone	**No**	**No**
Frijj bottle cf. Water bottle	**No**	**No**

Table 3.4 Summary table showing the effect of treatment with 0.9% saline (veh, i.p.) or sub-chronic PCP (2 mg/kg, i.p., twice daily for 7 days) on the object preference throughout the series of experiments. Significant increase (*P<0.05) in object preference for the wood cone compared to the comparator object.

3.3.2 Effect of sub-chronic PCP treatment in the acquisition trial in female rats in the 1 min ITI in the home cage

A two-way ANOVA revealed that treatment with sub-chronic PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{1, 18}$=2.6, NS; figure 3.12). Rats from both treatment groups spent similar times exploring both objects. An unpaired Student's t-test on the total exploration time in the acquisition trial revealed no significant effect of drug treatment (table 3.5).

3.3.3 Effect of sub-chronic PCP treatment in the retention trial in female rats following a 1 min ITI in the home cage

The two way ANOVA revealed that treatment with sub-chronic PCP did not produce a significant effect on object exploration time in the retention trial of the NOR test ($F_{1, 18}$=2.2, NS; figure 3.13). Further, planned post-hoc comparisons revealed that vehicle control rats spent significantly ($P<0.001$) more time exploring the novel object during the retention trial following the 1 min ITI within the home cage. This significant preference in the vehicle treated rats for the novel object was not observed in the rats treated with sub-chronic PCP, i.e. these rats spent a similar amount of time exploring both objects. Analysis of the total exploration times in the retention trial by an unpaired Student's t-test revealed no significant effect of drug treatment (table 3.5).

3.3.4 Effect of sub-chronic PCP treatment on the DI in female rats following a 1 min ITI in the home cage

An unpaired Student's t-test on the DI data showed a significant ($P<0.05$) effect of sub-chronic PCP treatment compared to vehicle control on the rats' ability to discriminate between the familiar and the novel objects following a 1- min ITI within the home cage (figure 3.14).

3.3.5 Effect of sub-chronic PCP treatment on the number of line crossings in female rats in the 1 min ITI in the home cage

Figure 3.15 shows the effect of sub-chronic PCP treatment on the total number of line crossings of the rats during the acquisition and retention trials in the NOR test. An unpaired Student's t-test revealed no significant effect.

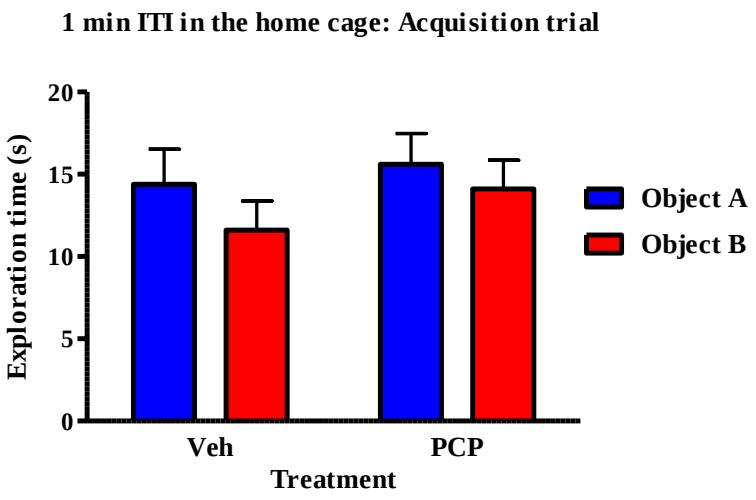


Figure 3.12 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on exploration times (s) of two identical objects in the 3 min acquisition trial of the NOR test in female rats. Data are shown as the mean ± S.E.M. (n=10 per group).

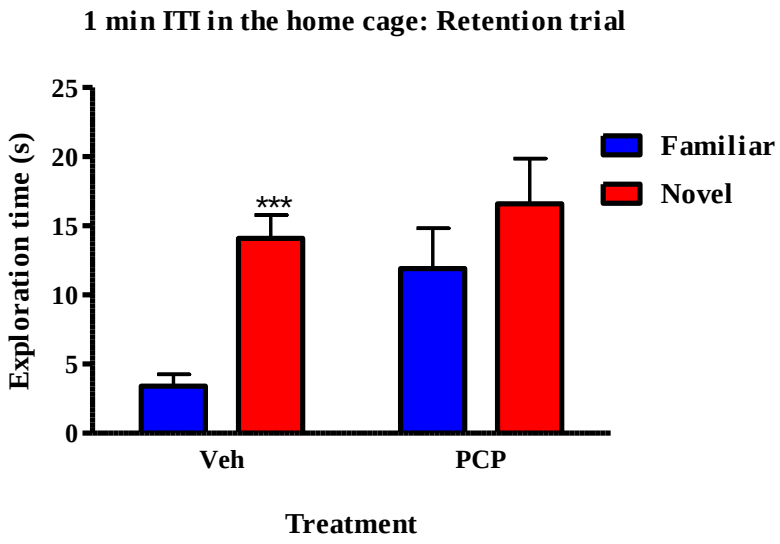


Figure 3.13 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on exploration time (s) of a familiar object and a novel object in the 3 min retention trial of the NOR test in female rats following a 1 min ITI in the home cage. Data are shown as the mean ± S.E.M. (n=10 per group). ***P<0.01; significant increase in the time spent exploring the novel compared with the familiar object.

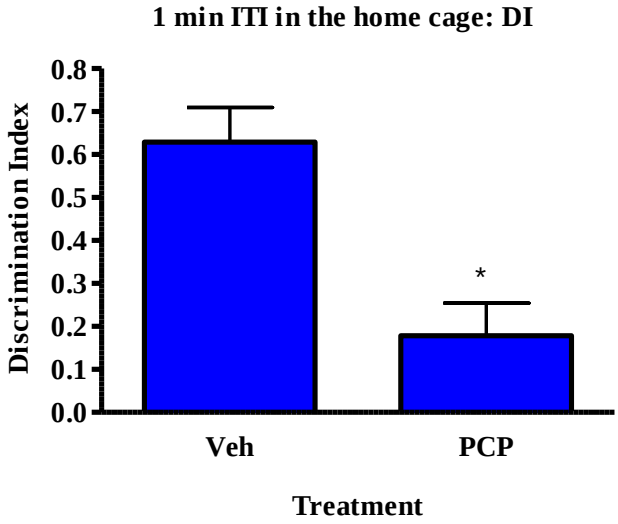


Figure 3.14 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the DI following a 1 min ITI in the home cage of the NOR test in female rats. Data are shown as the mean ± S.E.M. (n=10 per group) *P<0.05; significant reduction in DI when compared to vehicle.

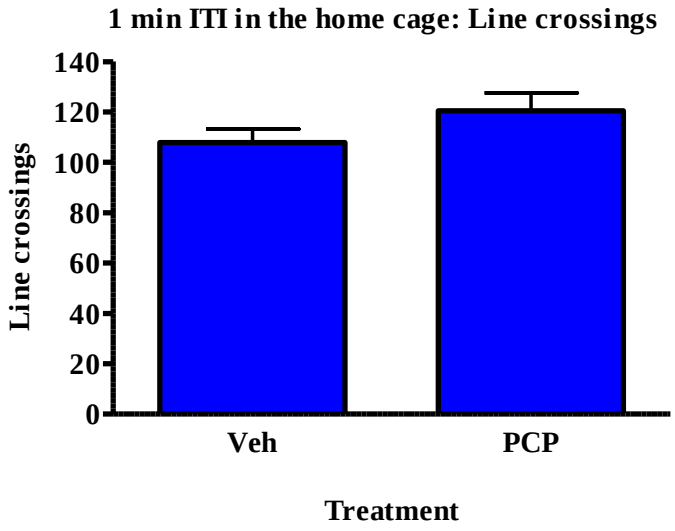


Figure 3.15 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats following a 1 min ITI in the home cage. Data are shown as the mean ± S.E.M. (n=10 per group).

Treatment	**Total exploration time (s)**	
	Acquisition trial	**Retention trial**
Vehicle	26.0 ± 3.3	17.5 ± 2.0
PCP	29.7 ± 3.2	28.5 ± 5.2

Table 3.5 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the total exploration time (s) in the acquisition and retention trials of the NOR test in female rats following a 1 min ITI in the home cage. Data are shown as the mean ± S.E.M. (n=10 per group).

3.3.6 Effect of sub-chronic PCP treatment in the acquisition trial in female rats in the 10 s ITI in home cage

A two-way ANOVA revealed that treatment with sub-chronic PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{1, 18}$=1.7, NS; figure 3.16). Rats from both treatment groups spent similar times exploring both the objects. An unpaired Student's t-test on the total exploration times in the acquisition trial revealed no significant effect of drug treatment (table 3.6).

3.3.7 Effect of sub-chronic PCP treatment in the retention trial in female rats following a 10 s ITI in the home cage

An overall two-way ANOVA revealed that treatment with sub-chronic PCP did not produce a significant effect on object exploration in the retention phase of the NOR test ($F_{1, 18}$=1.3, NS; figure 3.17). Planned post-hoc comparisons revealed that vehicle control rats spent significantly ($P<0.05$) more time exploring the novel objects during the retention trial following a 10 s ITI within the home cage. This significant preference in the vehicle treated rats for the novel object was not observed in the rats treated with sub-chronic PCP, i.e. these rats spent a similar amount of time exploring both objects. Analysis of the total exploration times in the retention trial by an unpaired Student's t-test revealed no significant effect of drug treatment (table 3.6).

3.3.8 Effect of sub-chronic PCP treatment on the DI in female rats following a 10 s ITI in the home cage

An unpaired Student's t-test on the DI data showed that although there was a decrease in the DI following sub-chronic PCP treatment, it did not reach statistical significance following a 10 s inter-trial interval in the home cage (figure 3.18).

3.3.9 Effect of sub-chronic PCP treatment on the number of line crossings in female rats (10 s ITI in the home cage)

Figure 3.19 shows the effect of sub-chronic PCP treatment on the total number of line crossings of the rats during the acquisition and retention trials in the NOR test. An unpaired Student's t-test revealed no significant effect.

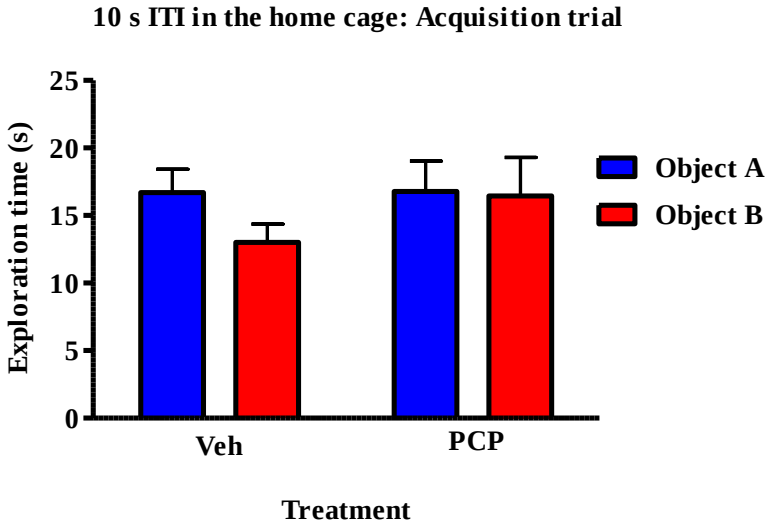


Figure 3.16 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on exploration times (s) of two identical objects in the 3 min acquisition trial of the NOR test in female rats. Data are shown as the mean ± S.E.M. (n=10 per group).

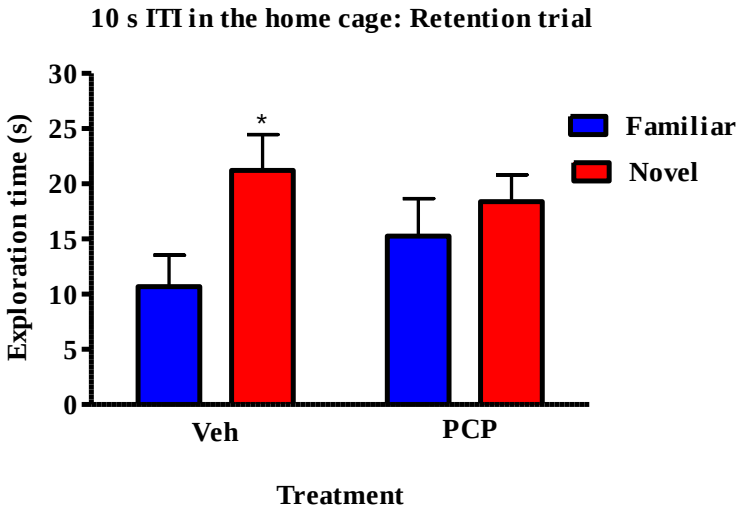


Figure 3.17 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on exploration time (s) of a familiar object and a novel object in the 3 min retention trial in the NOR test in female rats following a 10 s ITI in the home cage. Data are shown as the mean ± S.E.M. (n=10 per group). *P<0.05; significant increase in the time spent exploring the novel compared with the familiar object.

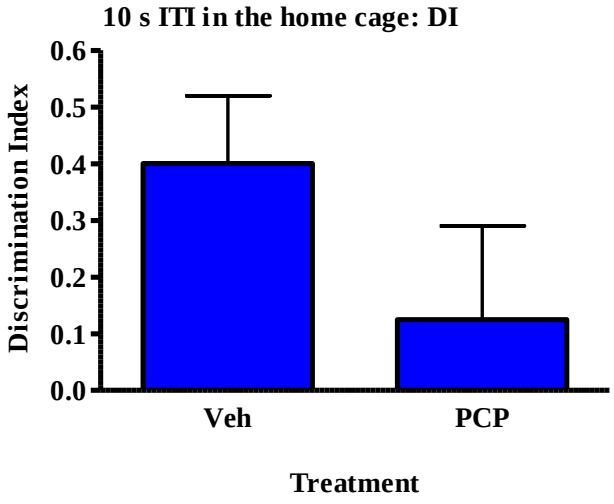


Figure 3.18 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the DI in the NOR test in female rats following a 10 s ITI in the home cage. Data are shown as the mean ± S.E.M. (n=10 per group).

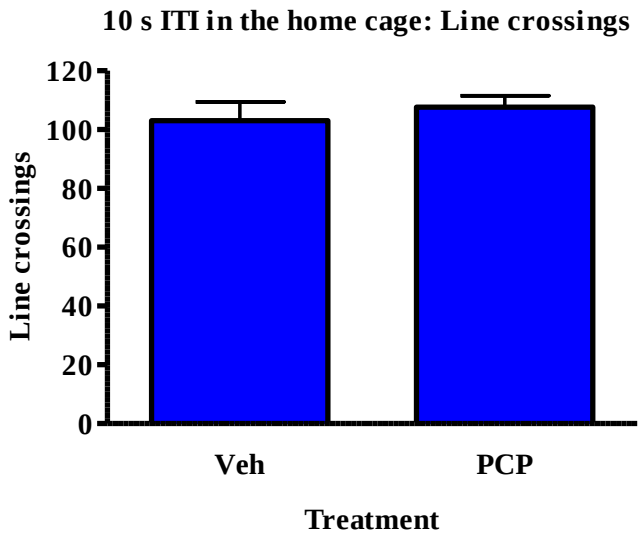


Figure 3.19 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats following a 10 s ITI in the home cage. Data are shown as the mean ± S.E.M. (n=10 per group).

Treatment	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
Vehicle	29.7 ± 2.8	31.9 ± 5.1
PCP	33.2 ± 4.6	33.1 ± 2.7

Table 3.6 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the total exploration time (s) in the acquisition and retention trials of the NOR test in female rats following a 10 s ITI in the home cage. Data are shown as the mean ± S.E.M. (n=10 per group).

3.3.10 Effect of sub-chronic PCP treatment in the acquisition trial in female rats in the 1 min ITI in the NOR test box

A two-way ANOVA revealed that treatment with sub-chronic PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{1, 18}$=0.3, NS; figure 3.20). Rats from both treatment groups spent similar times exploring both the objects. An unpaired Student's t-test on the total exploration times in the acquisition trial revealed no significant effect of drug treatment (table 3.7).

3.3.11 Effect of sub-chronic PCP treatment in the retention trial in female rats following a 1 min ITI in the NOR test box

A two-way ANOVA revealed that treatment with sub-chronic PCP did not produce a significant effect on object exploration in the retention trial of the NOR test ($F_{1, 18}$=0.9, NS; figure 3.21). However, planned post-hoc comparisons revealed that both treatment groups of rats spent significantly ($P<0.05$ - $P<0.01$) more time exploring the novel objects during the retention trial following a 1 min ITI within the NOR test box. Analysis of the total exploration times in the retention trial by an unpaired Student's t-test revealed no significant effect of drug treatment (table 3.7).

3.3.12 Effect of sub-chronic PCP treatment on the DI in female rats following a 1 min ITI in the NOR test box

An unpaired Student's t-test on the DI data showed no significant effect of sub-chronic PCP treatment on the rats' ability to discriminate between the familiar and novel objects following a 1 min ITI within the NOR test box (figure 3.22).

3.3.13 Effect of sub-chronic PCP treatment on the number of line crossings in female rats following a 1 min ITI in the NOR test box

Figure 3.23 shows the effect of sub-chronic PCP treatment on the total number of line crossings of the rats during the acquisition and retention trials in the NOR test. An unpaired Student's t-test revealed no significant effect.

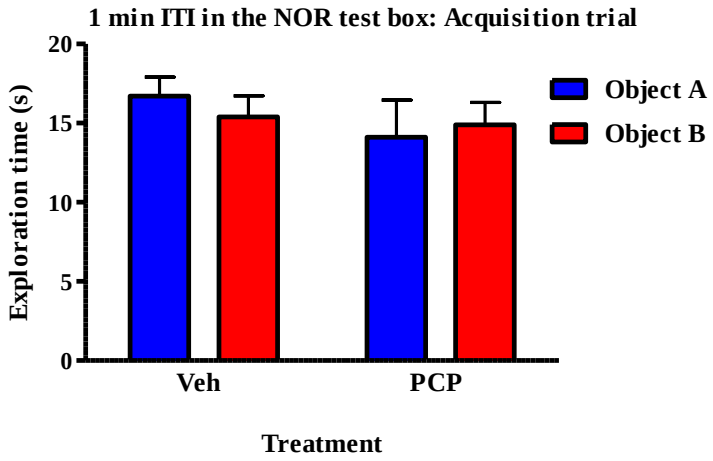


Figure 3.20 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on exploration times (s) of two identical objects in the 3 min acquisition trial of the NOR test in female rats. Data are shown as the mean ± S.E.M. (n=10 per group).

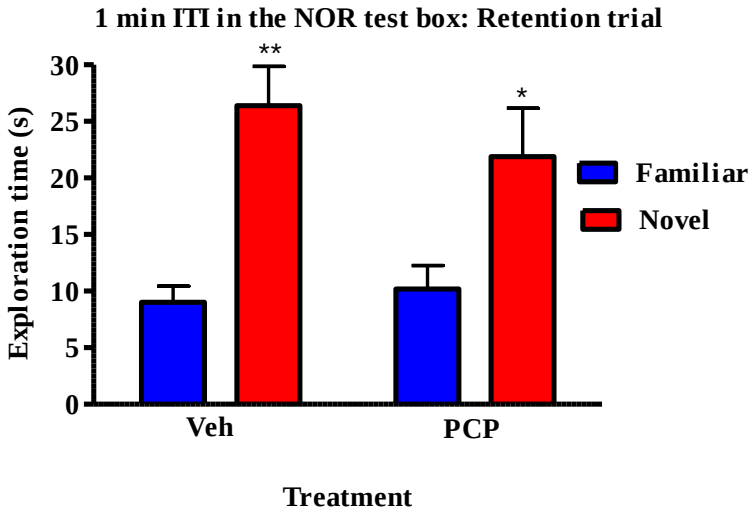


Figure 3.21 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on exploration time (s) of a familiar object and a novel object in the 3 min retention trial in the NOR test in female rats following 1 min ITI in the NOR test box. Data are shown as the mean ± S.E.M. (n=10 per group). *P<0.05-**P<0.01; significant increase in the time spent exploring the novel compared with the familiar object.

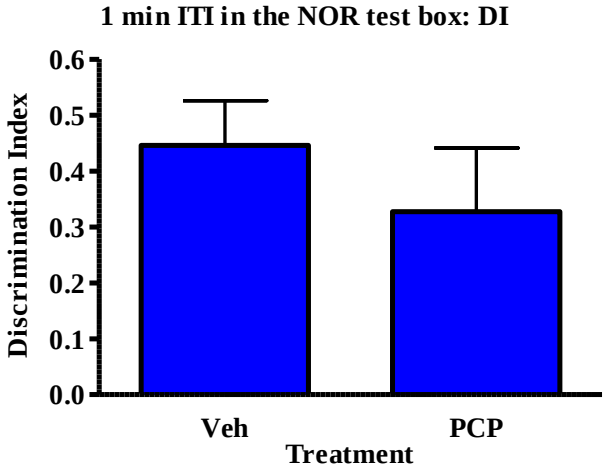


Figure 3.22 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the DI in the NOR test in female rats following a 1 min ITI in the test box. Data are shown as the mean ± S.E.M. (n=10 per group).

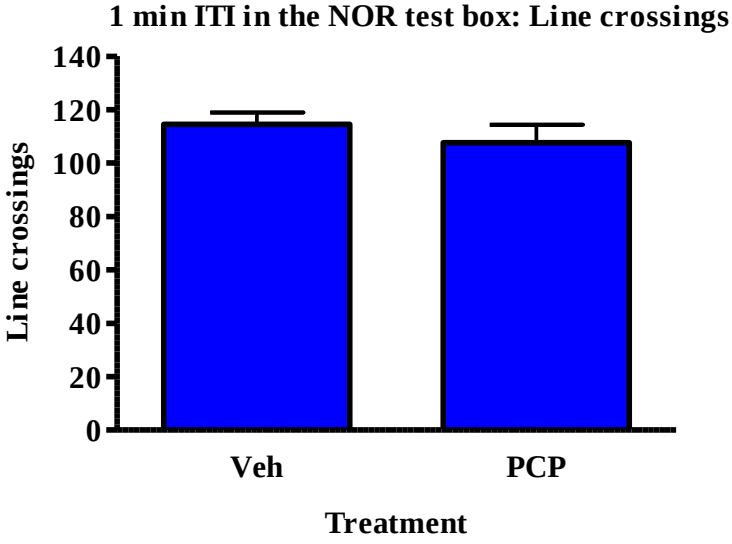


Figure 3.23 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats following a 1 min ITI in the test box. Data are shown as the mean ± S.E.M. (n=10 per group).

Treatment	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
Vehicle	32.1 ± 2.4	35.4 ± 4.5
PCP	29.7± 3.0	32.1± 4.3

Table 3.7 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the total exploration time (s) in the acquisition and retention trials of the NOR test in female rats following a 1 min ITI in the test box. Data are shown as the mean ± S.E.M. (n=10 per group).

3.3.14 Effect of sub-chronic PCP treatment in the acquisition trial in female rats in the 0 min ITI in the NOR test box study

A two-way ANOVA revealed that treatment with sub-chronic PCP did not produce a significant effect on object exploration in the acquisition trial of the NOR test ($F_{1, 14}$=1.6, NS). Rats from both treatment groups spent similar times exploring both the objects (figure 3.24). An unpaired Student's t-test on the total exploration times in the acquisition trial revealed no significant effect of drug treatment (table 3.8).

3.3.15 Effect of sub-chronic PCP treatment in the retention trial in female rats following a 0 min ITI in the NOR test box

A two-way ANOVA revealed that treatment with sub-chronic PCP did not produce a significant effect on object exploration in the retention trial of the NOR test ($F_{1, 14}$=3.9, P=0.07, NS; figure 3.25). However, planned post-hoc comparisons revealed that both treatment groups of rats spent significantly (P<0.001) more time exploring the novel objects during the retention trial following a 0 min ITI within the NOR test box. Analysis of the total exploration times in the retention trial by an unpaired Student's t-test revealed no significant effect of drug treatment (table 3.8).

3.3.16 Effect of sub-chronic PCP treatment on the DI in female rats following a 0 min ITI in the test box

An unpaired Student's t-test on the DI data showed no significant effect of sub-chronic PCP treatment on the rats' ability to discriminate between the familiar and novel objects following a 0 min ITI within the NOR test box (figure 3.26).

3.3.17 Effect of sub-chronic PCP treatment on the number of line crossings in female rats in the 0 min ITI in the NOR test box study

Figure 3.27 shows the effect of sub-chronic PCP treatment on the total number of line crossings of the rats during the acquisition and retention trials of the NOR test. An unpaired Student's t-test revealed no significant effect.

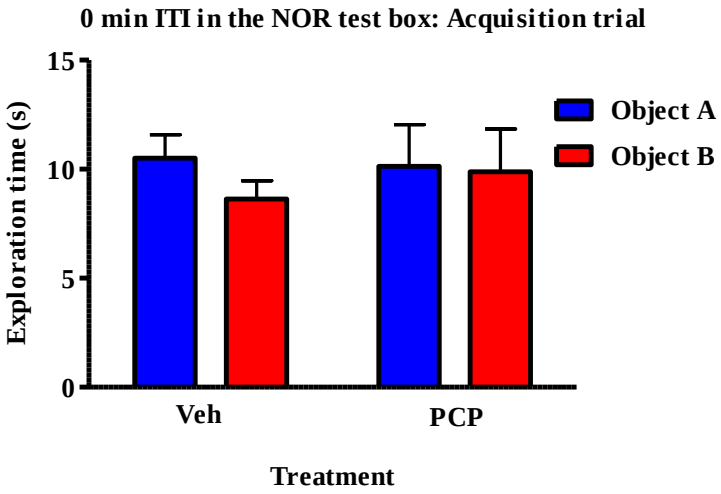


Figure 3.24 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on exploration times (s) of two identical objects in the 3 min acquisition trial of the NOR test in female rats. Data are shown as the mean ± S.E.M. (n=8 per group).

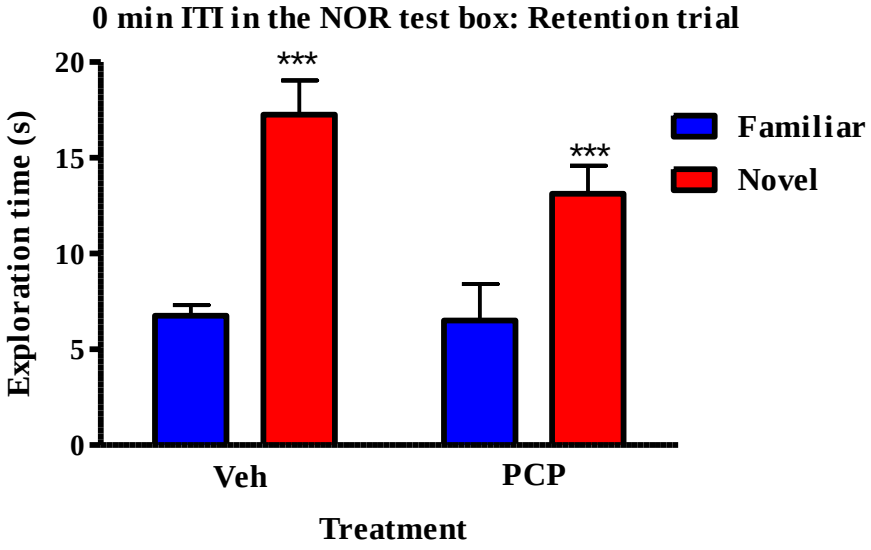


Figure 3.25 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on exploration of a familiar object and a novel object in the 3 min retention trial of the NOR test in female rats following a 0 min ITI in the NOR test box. Data are shown as the mean ± S.E.M. (n=8 per group). ***P<0.001; significant increase in the time spent exploring the novel compared with the familiar object.

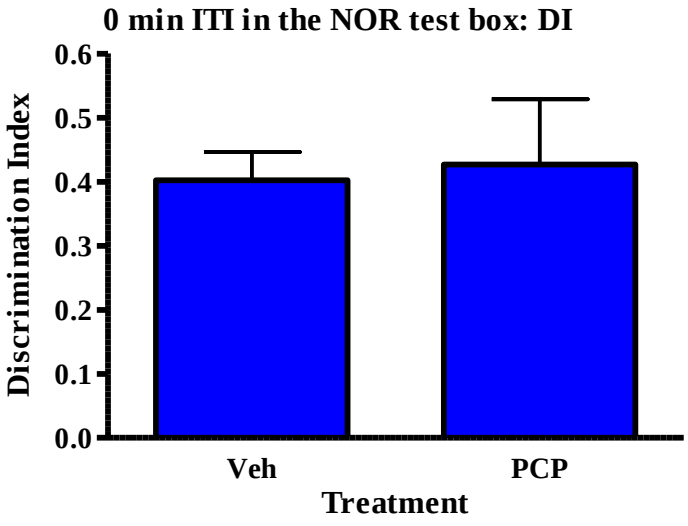


Figure 3.26 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the DI in the NOR test in female rats following a 0 min ITI in the NOR test box. Data are shown as the mean ± S.E.M. (n=8 per group).

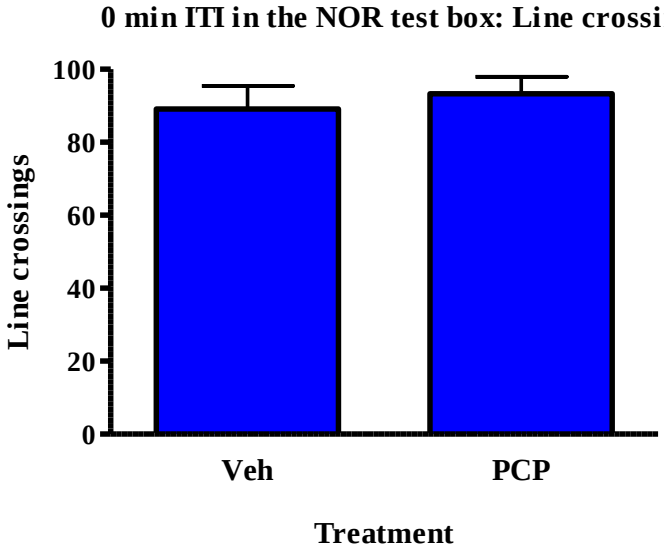


Figure 3.27 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats following a 0 min ITI in the NOR test box. Data are shown as the mean ± S.E.M. (n=8 per group).

Treatment	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
Vehicle	19.1 ± 1.6	24.0 ± 2.2
PCP	20.0 ± 3.7	19.6 ± 3.2

Table 3.8 The effect of sub-chronic (PCP treatment (2 mg/kg, i.p. for 7 days) or 0.9% saline (veh, i.p.) on the total exploration time (s) in the acquisition and retention trials of the NOR test in female rats following a 0 min ITI in the home cage. Data are shown as the mean ± S.E.M. (n=8 per group).

3.3.18 Effect of sub-chronic PCP treatment in the acquisition trial in female rats in the 1 min ITI in the NOR test box with a distracter object study

A two-way ANOVA revealed that treatment with sub-chronic PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{1, 18}$=2.2, NS; figure 3.28). Rats from both treatment groups spent similar times exploring both the objects. An unpaired Student's t-test on the total exploration times in the acquisition trial revealed no significant effect of drug treatment (table 3.9).

3.3.19 Effect of sub-chronic PCP treatment in the retention trial in female rats following a 1 min ITI in the NOR test box with a distracter object

An overall two-way ANOVA revealed that treatment with sub-chronic PCP did not produce a significant effect on object exploration in the retention trial of the NOR test ($F_{1, 18}$=2.6, NS; figure 3.29). Planned post-hoc comparisons revealed that vehicle control rats spent significantly ($P<0.001$) more time exploring the novel objects during the retention trial following a 1 min ITI within the NOR test box with a distracter object. This significant preference in the vehicle treated rats for the novel object was not observed in the rats treated with sub-chronic PCP, i.e. these rats spent a similar amount of time exploring both objects. Analysis of the total exploration times in the retention trial by an unpaired Student's t-test revealed no significant effect of drug treatment (table 3.9).

3.3.20 Effect of sub-chronic PCP treatment on the DI in female rats following a 1 min ITI in the NOR test box with a distracter object

An unpaired Student's t-test on the DI data showed a significant ($P<0.05$) effect of sub-chronic PCP treatment compared to vehicle control on the rats' ability to discriminate between the familiar and novel objects following a 1 min ITI within the NOR test box with a distracter object (figure 3.30).

3.3.21 Effect of sub-chronic PCP treatment on the number of line crossings in female rats in the 1 min ITI in the NOR test box with a distracter object

Figure 3.31 shows the effect of sub-chronic PCP treatment on the total number of line crossings of the rats during the acquisition and retention trial in the NOR test. An unpaired Student's t-test revealed no significant effect.

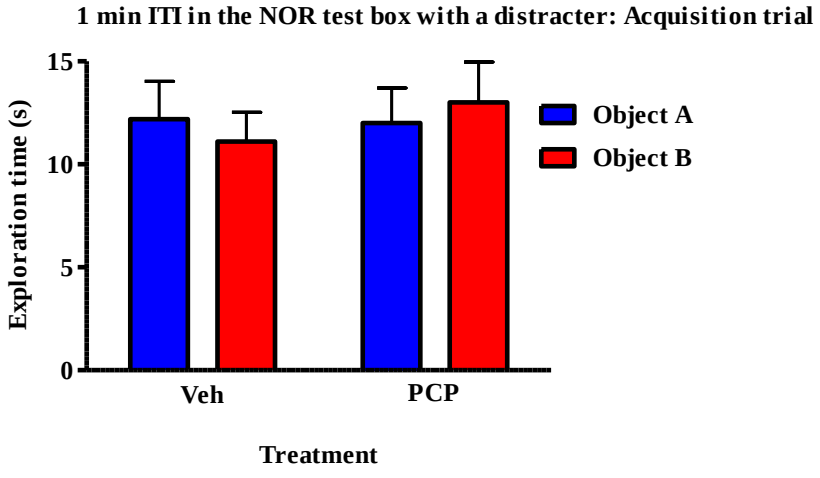


Figure 3.28 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days followed by 7 day washout period) or 0.9% saline (veh, i.p.) on exploration times (s) of identical objects in the 3 min acquisition trial in the NOR test in female rats. Data are shown as the mean ± S.E.M. (n=10 per group).

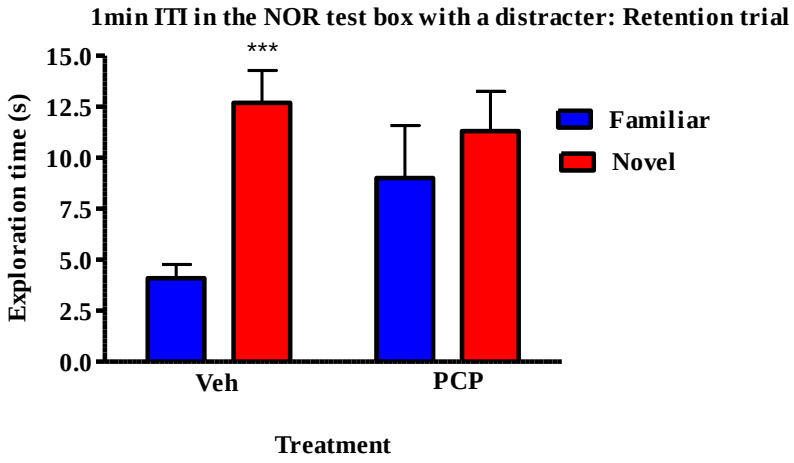


Figure 3.29 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days followed by 7 day washout period) or 0.9% saline (veh, i.p.) on exploration time (s) of a familiar object and a novel object in the 3 min retention trial in the NOR test in female rats following 1 min ITI in the test box with a distracter object. Data are shown as the mean ± S.E.M. (n=10 per group). ***P<0.001; significant difference between the time spent exploring the familiar compared with the novel object, Student's t-test.

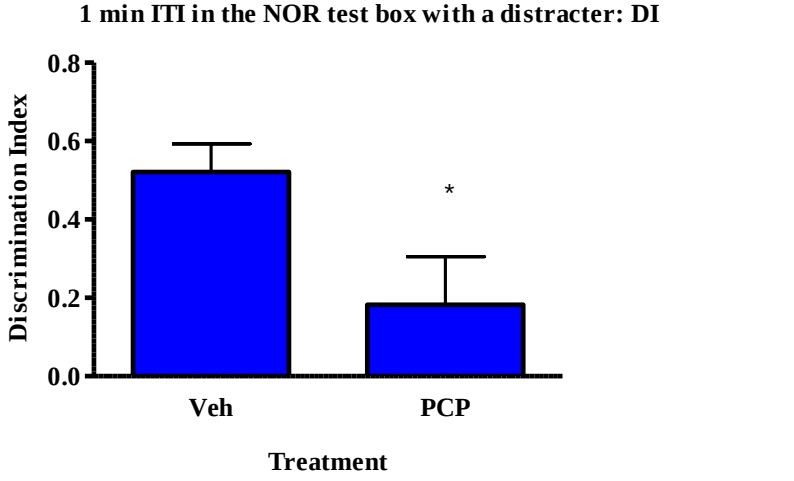


Figure 3.30 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days followed by 7 day washout period) or 0.9% saline (veh, i.p.) on the discrimination index in the NOR test in female rats following a 1 min ITI in the NOR test box with a distracter object. Data are shown as the mean ± S.E.M. (n=10 per group). *P<0.05; significant reduction in DI when compared to vehicle.

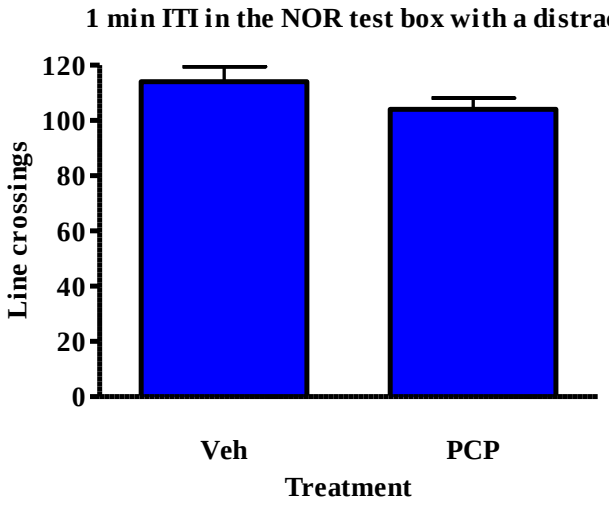


Figure 3.31 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days followed by 7 day washout period) or 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trial in the NOR test in female rats following a 1 min ITI in the NOR test box with a distracter object. Data are shown as the mean ± S.E.M. (n=10 per group).

Treatment	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
Vehicle	22.8 ± 3.8	16.4 ± 2.1
PCP	25.0 ± 3.5	20.3 ± 4.1

Table 3.9 The effect of sub-chronic PCP treatment (2 mg/kg, i.p. for 7 days followed by 7 day washout period) or 0.9% saline (veh, i.p.) on the total exploration time (s) in the acquisition and retention trial of the NOR test in female rats following a 1 min ITI in the NOR test box with a distracter object. Data are expressed as the mean ± S.E.M. (n=10 per group).

Treatments	*ITI and location*	*Deficit in the retention trial*	*Deficit in the DI*	*Effect on line crossings*	*Effect on total exploration time*
Sub-chronic PCP	1 min home cage	**Yes**	**Yes**	**No**	**No**
Sub-chronic PCP	10 s home cage	**Yes**	**Yes** (NS)	**No**	**No**
Sub-chronic PCP	1 min NOR box	**No**	**No**	**No**	**No**
Sub-chronic PCP	0 min ITI NOR box	**No**	**No**	**No**	**No**
Sub-chronic PCP	1 min ITI (with distracter)	**Yes**	**Yes**	**No**	**No**

Table 3.10 Summary table showing the effect of location (home cage or NOR test box) during differential ITIs in rats treated with sub-chronic PCP (2 mg/kg, i.p. twice daily for 7 days).

3.4 Discussion

3.4.1 Object Preference

During the initial object preference experiment, rats treated sub-chronically with PCP or vehicle, exhibited an increased exploration time of one of the potential NOR test objects compared with all the other selected objects. The object that induced increased exploration time was a wooden cone and subsequently discarded from further NOR experiments. The wooden cone object was explored more, possibly because it absorbed olfactory trails and was attractive because of its susceptibility to induce chewing. In contrast, the other objects were made from non-porous, less chewable materials which were more easily cleaned. This demonstrates the

importance of essential pilot studies before embarking on any behavioural experiments. A careful object selection process is required prior commencing NOR testing to avoid object bias confounding the subsequent data analysis.

3.4.2 Differential ITI

Results presented in this thesis show that rats treated sub-chronically with the NMDA receptor antagonist PCP, under certain conditions exhibit NOR deficits in visual recognition memory as assessed by the NOR test. It has been consistently shown that schizophrenia patients are impaired in recognition memory for familiar faces, these patients also show impairments in recognition memory tests for words and patterns (Conklin et al, 2002). Memory deficits observed in schizophrenia severely influence outcome (Ragland et al, 2009). When trying to remember information about prior experiences, internal selection processes are engaged to focus cognitive resources on the retrieval effort. Consequently, environmental stimulation that inundates our senses with information irrelevant to our memory goals can induce distraction during recall (Wais and Gazzaley, 2011).

The current studies have demonstrated the sub-chronic PCP treated rats' susceptibility to distraction during a short ITI period in the NOR test which is consistent with clinical findings in patients with schizophrenia (Cellard et al, 2007). Control rats were able to differentiate between the novel and familiar objects throughout all the differential ITI periods and their recognition of the previously encountered object was unaffected by the distraction of handling, ITI location and distracter object. However, the sub-chronic PCP treated rats were highly susceptible to the effect of distraction during the ITI of the NOR test. The 'normal' testing conditions following sub-chronic PCP treatment in the NOR test in our laboratory

utilises a short 1 min ITI, whereby the test rats are removed from the test box and placed back into the home cage with their cage mates. Following this 1 min ITI period, sub-chronic PCP treated rats are unable to discriminate between the novel and familiar object whereas vehicle treated rats can. A series of differential ITI experiments were conducted in this chapter to help provide an explanation for the sub-chronic PCP treated rats' inability to recognise the familiar object following such a short ITI. The results indicate that it is not only the ITI period that induces the deficits in object recognition observed in this test, since sub-chronic PCP treated rats were capable of discriminating between novel and familiar objects during a 1 min ITI period when they remained un-touched by the experimenter in the NOR test box. Furthermore, the sub-chronic PCP treated rats were still unable to differentiate between the novel and familiar objects when the ITI period was substantially reduced to 10 s in the home cage. The use of the highly 'interesting' wooden cone object as the distracter in the NOR test box during the 1 min ITI was important in providing further evidence for the role of distraction during the 1 min ITI. In summary, results suggest that distraction produced either by the experimenter handling the rats/home cage social interaction or interaction with the distracter object, produces distraction-induced object recognition deficits in sub-chronic PCP treated rats but not in control rats. This finding is in support of the clinical data showing that patients with schizophrenia are more susceptible to impairments in short term memory tests when subjects are presented with distracters during a computerised visual spatial task (Spring et al, 1991; Cellard et al, 2007). The distracter regimen selected for all subsequent studies in this thesis was a 1-min ITI in the home cage.

CHAPTER 4 - Effects of sex on deficits in recognition memory induced by acute d-amphetamine, PCP and sub-chronic PCP in the NOR test

4.1 Introduction

Gender differences in schizophrenia have provided much interest for researchers for many years. Schizophrenia affects both men and women, systematic independent reviews have concluded that the incidence of schizophrenia is significantly higher in men compared to women (Aleman et al, 2003; McGrath et al, 2004). A meta-analysis revealed that the average age of onset of schizophrenia peaks earlier in men, usually between 10-25 years, compared to women where the peak is observed at 25-35 years (Buchanan & Carpenter, 2005). Another peak occurs, particularly in women in mid life; with 23% of people with schizophrenia experiencing their first episode after the age of 40 (Salokangas et al, 2003). This second peak of incidence of schizophrenia coincides with the decrease in oestrogen observed during the menopause in women and supports the theory that oestrogen may provide some protection against schizophrenia in women (Seeman, 2012).

The literature regarding gender differences with respect to cognition in schizophrenia remains equivocal. Some researchers suggest that male schizophrenia patients are more impaired than female subjects (Goldstein et al, 1998; Seidman et al, 1997), and others report women to be more impaired than men (Goldberg et al, 1995; Lewine et al, 1996), whereas some report no significant sex differences on cognitive function (Bozikas et al, 2010; Hoff et al, 1998).

Sex differences have also been observed following administration of psychotomimetics to humans. Work presented in the literature suggests that female anaesthesia patients are more sensitive to the psychotomimetic effects of the NMDA receptor antagonist ketamine compared to male patients (Bovill et al, 1971).

Conversely, men showed a greater vulnerability to the amnesic effects of ketamine than women (Morgan et al, 2006).

As discussed earlier in chapter 1, it is well known that treatment with PCP or d-amphetamine can induce a model psychosis that mimics the positive symptoms of schizophrenia, but only PCP also mimics the negative symptoms (Sams-Dodd, 1998). There has been a surge of interest into the cognitive effects of these psychotomimetic agents. Research in our laboratory has demonstrated that acute and sub-chronic treatment with both PCP and acute d-amphetamine induces selective cognitive deficits in the reversal learning paradigm in female rats (Neill et al, 2010). In another study, acute treatment with d-amphetamine disrupted both reference and working memory in male rats on a baited 6-arm radial maze (Beatty et al, 1984). Furthermore, Jentsch & Taylor (2001) showed impairments when acquiring the reversal of a previously-learned stimulus-reward in male rats treated with acute PCP.

It is important when developing and validating a paradigm in the rat to model a human disease which affects both sexes, such as the cognitive impairments associated with schizophrenia, that the model can detect cognitive deficits in both male and female rats.

The principle aim of this work is to compare the performance of both male and female rats in the NOR test following treatment with acute d-amphetamine and both acute and sub-chronic treatment with PCP.

4.2 Materials and methods

4.2.1 Experimental Animals and Design

A total of 100 female and 82 male, adult, rats were used in these studies. See section 2.1.1 for housing conditions.

Treatment	**Doses (mg/kg)**	***Cohort***	***Male n= and weight (g)***	***Female n= and weight (g)***
Acute PCP	0.5, 1.0, 1.5, 2.0, 2.5, 5.0	2 (females) 3 (males)	30 (290-320)	40 (180-210)
Acute d-amph	0.1, 0.5, 1.0, 2.5	2 (females) 4 (males)	32 (260-290)	40 (180-215)
Sub-chronic PCP	2.0	5 (females) 6 (males)	20 (270-310)	20 (220-240)
Total n=			**82**	**100**

Table 4.1 showing the treatments, rat weights and cohorts used in this study.

4.2.2 Drugs

For drugs used in this study see tables 4.1 and 2.2 and for the number of rats used in each treatment group, see tables 4.2 - 4.5. Due to lack of efficacy of the lower dose range of PCP to produce a NOR deficit in male rats, a second experiment was carried in the male rats using a higher dose range (2.5-5.0 mg/kg) of PCP (see table 4.3).

Dose of PCP (mg/kg)	*Male*		*Female*	
	Initial number of rats	*Number of rats excluded*	*Initial number of rats*	*Number of rats excluded*
Veh	7	0	10	2
0.5			10	4
1.0	7	1	10	2
1.5	8	2	10	2
2.0	8	2	10	1

Table 4.2 The number of male and female rats excluded from the study following acute administration of PCP (0.5-2.0 mg/kg, i.p.) or vehicle (0.9% saline, i.p.).

Dose of PCP (mg/kg)	*Male*	
	Initial number of rats	*Number of rats excluded*
Veh	7	1
1.0	7	1
2.5	8	2
5.0	8	1

Table 4.3 The number of male rats excluded from the NOR study following acute administration of a higher dose range PCP (1.0-5.0 mg/kg, i.p.) or vehicle (0.9% saline, i.p.).

Dose of d-amph (mg/kg)	*Male*		*Female*	
	Initial number of rats	*Number of rats excluded*	*Initial number of rats*	*Number of rats excluded*
Veh	8	0	10	0
0.1	8	0	10	0
0.5	8	0	10	0
1.0	8	0	10	0
2.5	8	0		

Table 4.4 The number of male and female rats excluded from the NOR study following acute administration of d-amphetamine (0.1-2.5 mg/kg, i.p.) or vehicle (0.9% saline, i.p.).

Dose of PCP (mg/kg)	*Male*		*Female*	
	Initial number of rats	*Number of rats excluded*	*Initial number of rats*	*Number of rats excluded*
Veh	10	0	10	1
2.0	10	0	10	0

Table 4.5 The number of male and female rats excluded from the study following acute administration of PCP (2.0 mg/kg, i.p.) or vehicle (0.9% saline, i.p.).

4.2.3 NOR Apparatus

For details regarding the NOR apparatus see section 2.1.3.

4.2.4 NOR testing

4.2.4.1 Habituation

Rats were habituated to the NOR apparatus (see section 2.1.5.1).

4.2.4.2 Behavioural testing

For details regarding acquisition, ITI and retention trials see section 2.1.5.2.

4.2.4.3 Behavioural assessment

For details regarding behavioural assessment see section 2.1.5.3.

4.2.5 Statistical analysis

4.2.5.1 Acute PCP and d-amph

For details regarding statistical analysis see section 2.1.5.4.

4.2.5.2 Sub-chronic PCP

Male and female comparisons in total exploration time and DI were analysed by a repeated measures two-way ANOVA. This detected the main effect of drug treatment, main effect of sex (male and female) and the interaction between drug treatment and sex. Further planned analysis by unpaired Student's t-test was carried out to compare males with females on the total exploration time during the acquisition or retention trials. Paired Student's t-test was carried out to compare males with females on the DI.

4.3 Results

4.3.1 Effect of acute PCP treatment in the acquisition trial in female rats

A two-way ANOVA revealed that acute treatment with PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{4, 35}$=0.78, NS). Rats from all treatment groups spent similar times exploring both the objects (figure 4.1). A small reduction in total exploration of the identical objects was observed at the two highest doses of PCP (1.5 and 2.0 mg/kg), however a one-way ANOVA on the total exploration times revealed no significant effect ($F_{4, 39}$=0.67, NS; table 4.6).

4.3.2 Effect of acute PCP treatment in the retention trial in female rats

An overall two-way ANOVA revealed that treatment with acute PCP produced a significant effect on object exploration in the retention trial of the NOR test ($F_{4, 35}$=4.29, P<0.01; figure 4.2). Post-hoc analysis revealed that vehicle control rats had a significant preference for the novel compared with the familiar object (P<0.05).

This preference in the vehicle treated rats for the novel object was not observed in the acute PCP treated rats at any of the doses tested (0.5 – 2.0 mg/kg), i.e. these rats spent similar amount of time exploring both objects i.e. rats lost the ability to discriminate the familiar object from the novel object. Analysis of the total exploration time of the objects in the retention trial by a one-way ANOVA revealed no significant effect of drug treatment ($F_{4, 39}$=0.53, NS; table 4.6).

4.3.3 Effect of acute PCP treatment on Discrimination Index (DI) in female rats

One-way ANOVA on the DI data showed a significant effect of PCP treatment on the rats' ability to discriminate between the familiar and novel objects ($F_{4, 39}$=3.48, P<0.05). Further post-hoc analysis revealed that rats treated with 1.5 mg and 2.0 mg/kg of acute PCP showed a significant (P<0.05) reduction in DI when compared with the vehicle control group (Figure 4.3).

4.3.4 Effect of acute PCP treatment on the number of line crossings in the NOR test in female rats

Figure 4.4 shows the effect of acute PCP treatment on the total number of line crossings during the acquisition and retention trials of the NOR test. One-way ANOVA revealed no significant effect of acute PCP on line crossings ($F_{4, 39}$=0.59, NS).

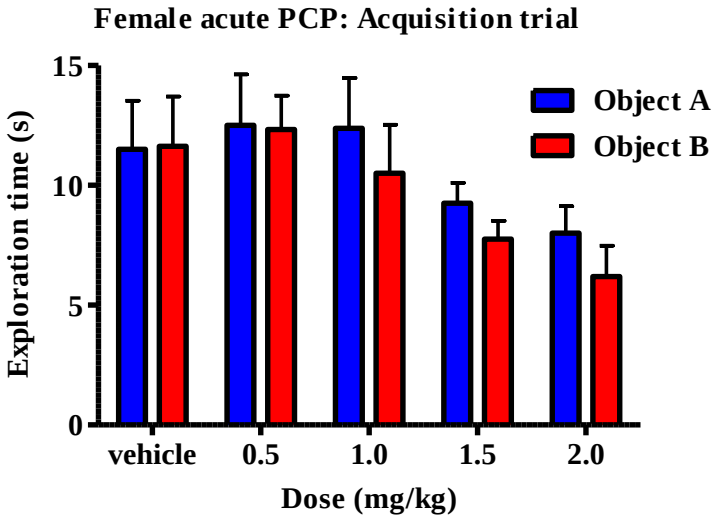


Figure 4.1 The effect of acute PCP (0.5-2.0 mg/kg, i.p.) on exploration times (s) of two identical objects in the 3 min acquisition trial in a NOR test in female rats. Data are shown as mean ± S.E.M. (n=6-10 per group).

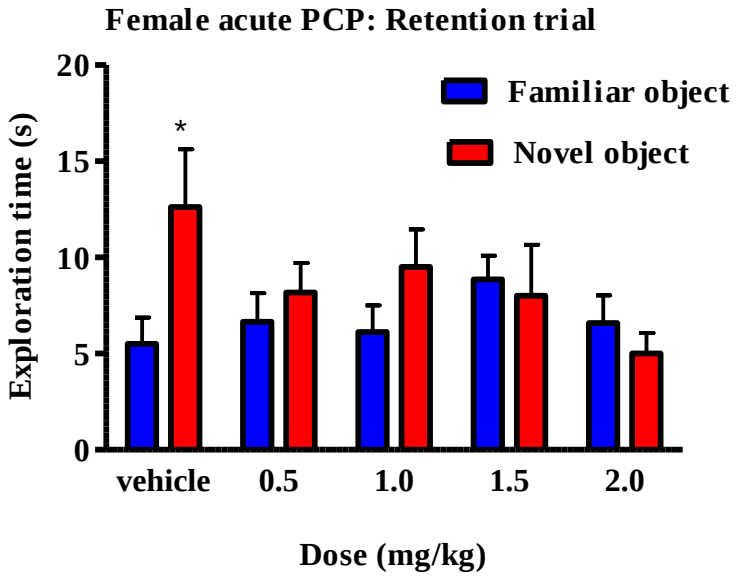


Figure 4.2 The effect of acute PCP (0.5-2.0 mg/kg, i.p.) on exploration times (s) of a familiar and a novel object in the 3 min retention trial in the NOR test in female rats. Data are shown as mean ± S.E.M. (n=6-10 per group) *P<0.05, significant increase in time spent exploring the novel compared with the familiar object.

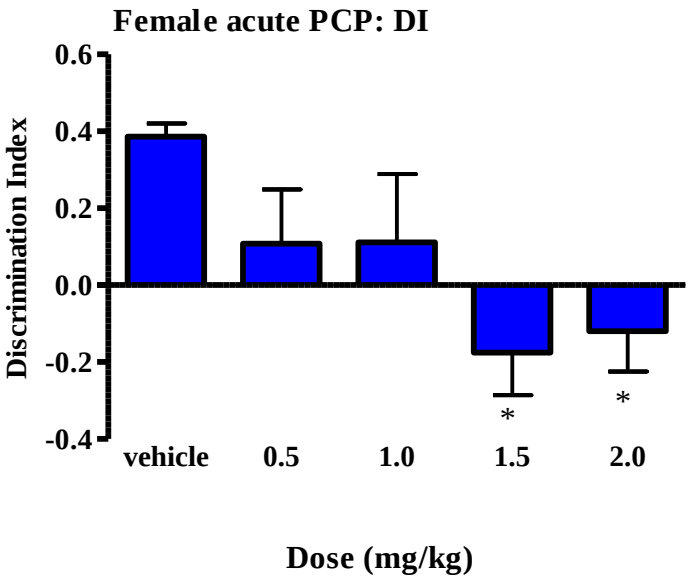


Figure 4.3 The effect of acute PCP (0.5-2.0 mg/kg, i.p.) on the DI in the NOR test in female rats. Data are expressed as mean ± S.E.M. (n=6-10 per group). *P<0.05 significant reduction compared to vehicle.

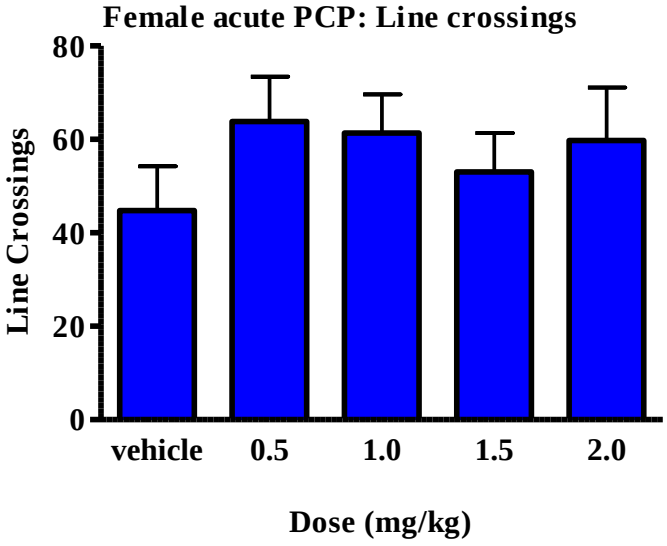


Figure 4.4 The effect of acute PCP (0.5-2.0 mg/kg, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats. Data are expressed as mean ± S.E.M. (n=6-10 per group).

PCP (mg/kg)	*Total exploration time (s)*	
	Acquisition trial	*Retention trial*
veh	23.1 ± 3.9	18.1 ± 4.3
0.5	24.8 ± 2.9	14.8 ± 1.7
1.0	22.9 ± 3.6	15.6 ± 2.9
1.5	16.3 ± 1.2	16.9 ± 3.6
2.0	14.2 ± 2.3	11.6 ± 2.3

Table 4.6 The effect of acute PCP (0.5-2.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on the total exploration time in the acquisition and retention trials of the NOR test in female rats. Data are expressed as the mean ± S.E.M. (n=6-10 per group).

4.3.5 Effect of acute PCP treatment (1.0-2.0 mg/kg) in the acquisition trial in male rats

A two-way ANOVA revealed that treatment with PCP (0.5-2.0 mg/kg) did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{3, 21}$=0.35, NS). Rats from all treatment groups spent similar times exploring both the objects (figure 4.5). Analysis of the total exploration of the objects in the acquisition trial by a one-way ANOVA revealed no significant effect of drug treatment ($F_{3, 24}$=0.75, NS; table 4.7).

4.3.6 Effect of acute PCP treatment (1.0-2.0 mg/kg) in the retention trial in male rats

An overall two-way ANOVA revealed that treatment with PCP (0.5-2.0 mg/kg) did not produce a significant effect on object exploration in the retention trial of the NOR test ($F_{3, 21}$=2.07, NS; figure 4.6). However, planned post-hoc analysis revealed

a significant preference for the novel object when compared with the familiar object following treatment with vehicle ($P<0.001$), 1.0 mg/kg of PCP ($P<0.001$) and 2.0 mg/kg PCP ($P<0.01$). Rats treated with the middle dose of PCP (1.5 mg/kg) failed to demonstrate a preference for the novel compared to familiar object. Acute treatment with PCP (1.0-2.0 mg/kg) did not produce any clear cognitive deficit in male rats; hence a higher dose range was selected (see section 4.3.10). Analysis of the total exploration time of the objects in the retention trial by a one-way ANOVA revealed no significant effect of drug treatment ($F_{3, 24}=1.57$, NS; table 4.7).

4.3.7 Effect of acute PCP treatment (1.0-2.0 mg/kg) on the Discrimination Index (DI) in male rats

One-way ANOVA on the DI data showed no significant effect of acute PCP treatment on the male rats' ability to discriminate between the familiar and novel objects ($F_{3, 24}=1.56$, NS; Figure 4.7).

4.3.8 Effect of acute PCP treatment (1.0-2.0 mg/kg) on the number of line crossings in the NOR test in male rats

Figure 4.8 shows the effect of acute PCP treatment on the total number of line crossings during the acquisition and retention trial of the NOR test. One-way ANOVA revealed no significant effect of PCP on line crossings ($F_{3, 24}=0.35$, NS).

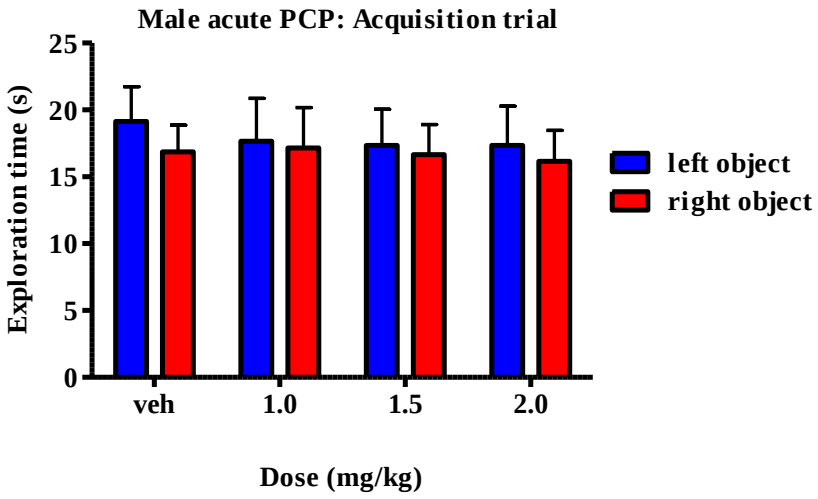


Figure 4.5 The effect of acute PCP (1.0-2.0 mg/kg, i.p.) on exploration times (s) of two identical objects in the 3 min acquisition trial in the NOR test in male rats. Data are shown as mean ± S.E.M. (n=6-7 per group).

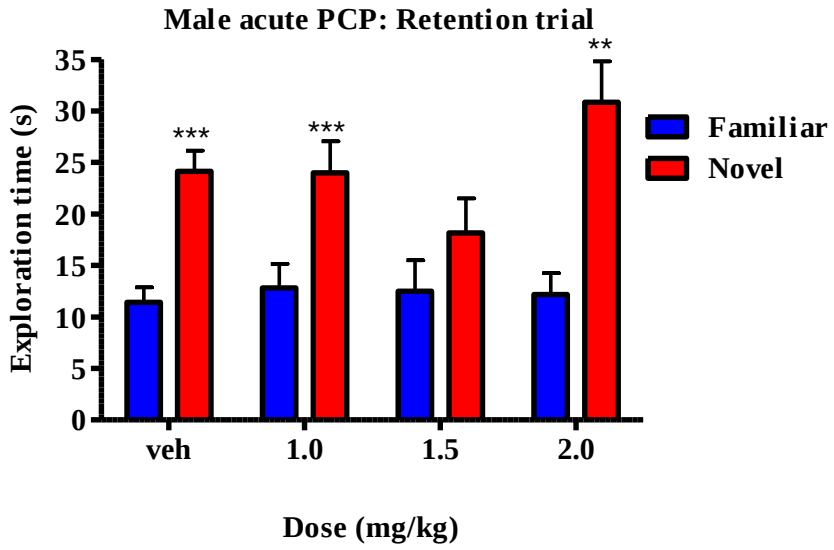


Figure 4.6 The effect of acute PCP (1.0-2.0 mg/kg, i.p.) on exploration times (s) of a familiar and a novel object in the 3 min retention trial of the NOR test in male rats. Data are shown as mean ± S.E.M. (n=6-7 per group) **P<0.01-***P<0.001, significant increase in time spent exploring the novel compared with the familiar objects.

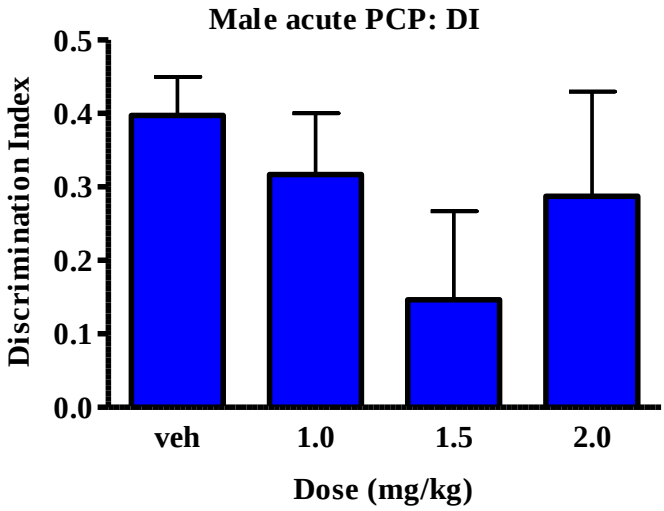


Figure 4.7 The effect of acute PCP (1.0-2.0 mg/kg, i.p.) on the DI in the NOR test in male rats. Data are expressed as mean ± S.E.M. (n=6-7 per group).

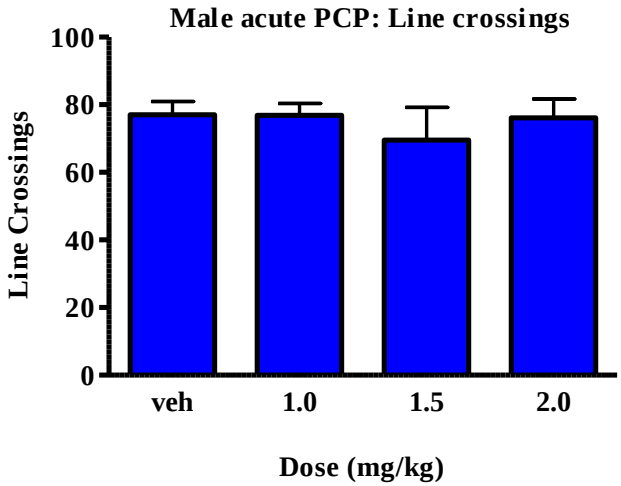


Figure 4.8 The effect of acute PCP (1.0-2.0 mg/kg, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in male rats. Data are expressed as mean ± S.E.M. (n=6-7 per group).

PCP (mg/kg)	*Total exploration time (s)*	
	Acquisition trial	*Retention trial*
Veh	36.0 ± 3.3	35.6 ± 3.2
1.0	34.8 ± 6.0	36.8 ± 4.6
1.5	33.6 ± 4.4	30.7 ± 5.1
2.0	33.5 ± 3.3	43.0 ± 2.9

Table 4.7 The effect of acute PCP (1.0-2.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on the total exploration time in the acquisition and retention trials of the NOR test in male rats. Data are expressed as the mean ± S.E.M. (n=6-7 per group).

4.3.9 Effect of acute PCP treatment (1.0-5.0 mg/kg) on the acquisition trial in male rats

A two-way ANOVA revealed that treatment with PCP (1.0-5.0 mg/kg) did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{3, 21}$=0.66, NS). Rats from all treatment groups spent similar times exploring both the objects (figure 4.9). Analysis of the total exploration of the objects in the acquisition trial by a one-way ANOVA showed a significant effect of drug treatment ($F_{3, 24}$=5.5, P<0.01). Further post-hoc analysis revealed that the highest dose of PCP (5.0 mg/kg) produced a significant (P<0.01) reduction in total object exploration time in the acquisition trial when compared with vehicle treated rats (table 4.8).

4.3.10 Effect of acute PCP treatment in the retention trial in male rats (higher dose range)

An overall two-way ANOVA revealed that treatment with PCP (1.0-5.0 mg/kg) did not produce any significant effect on object exploration in the retention trial of the

NOR test ($F_{3, 21}$=0.83, NS; figure 4.10). Planned post-hoc analysis revealed significant preferences for the novel object when compared with the familiar object following treatment with vehicle (P<0.05) and 5.0 mg/kg of PCP (P<0.05). Rats treated with the low dose of PCP (1.0 mg/kg) and middle dose of PCP (2.5 mg/kg) demonstrated a trend towards a preference for the novel compared to familiar object (P=0.08 and P=0.09 respectively). Analysis of the total exploration time of the objects in the retention trial by a one-way ANOVA revealed no significant effect of drug treatment ($F_{3, 24}$=1.44, NS; table 4.8).

4.3.11 Effect of acute PCP treatment on the DI in the NOR test in male rats (higher dose range)

One-way ANOVA on the DI data showed no significant effect of PCP treatment on the rats' ability to discriminate between the familiar and novel objects ($F_{3, 24}$=0.49, NS; figure 4.11).

4.3.12 Effect of acute PCP treatment on the total number of line crossings in the NOR test male rats (high dose range)

One-way ANOVA on the line crossings data showed a significant effect of PCP treatment ($F_{3, 24}$=5.22, P<0.01; figure 4.12). However, post-hoc analysis failed to demonstrate any individual treatment effects.

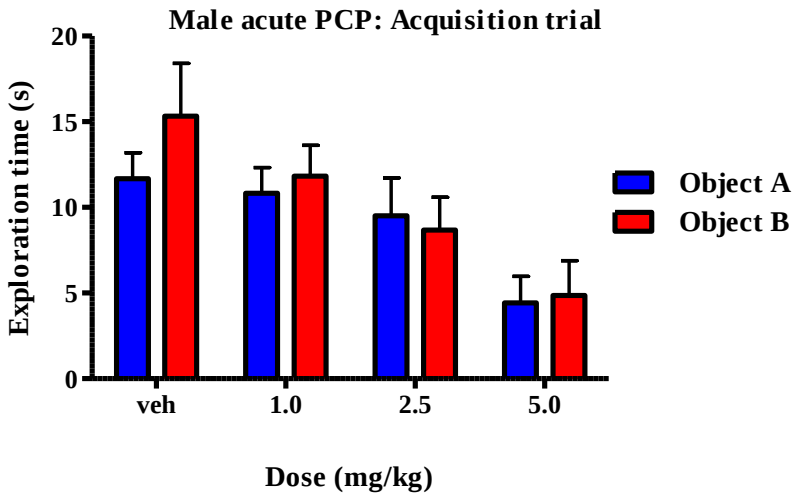


Figure 4.9 The effect of PCP (1.0-5.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on exploration times (s) of two identical objects in the 3 min acquisition trial of the NOR test in male rats. Data are shown as mean ± S.E.M. (n=6-7 per group).

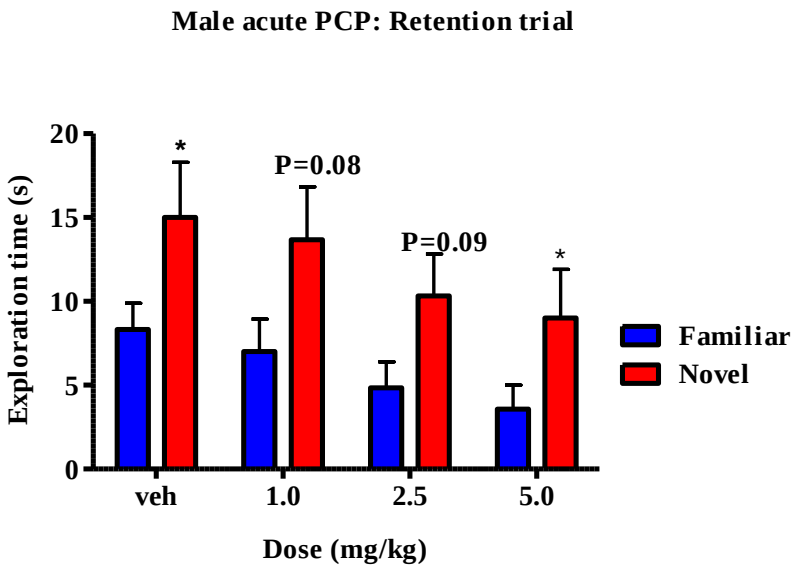


Figure 4.10 The effect of PCP (1.0-5.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on exploration times (s) of a novel and familiar objects in the 3 min retention trial of the NOR test in male rats. Data are shown as mean ± S.E.M. (n=6-7 per group) *P<0.05, significant increase in time spent exploring the novel compared with the familiar object, Student's t-test.

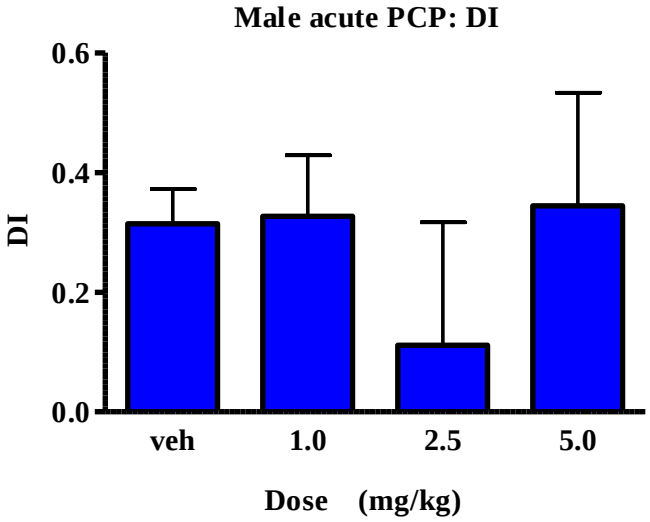


Figure 4.11 The effect of PCP (1.0-5.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on the DI of the NOR test in male rats. Data are expressed as mean ± S.E.M. (n=6-7 per group).

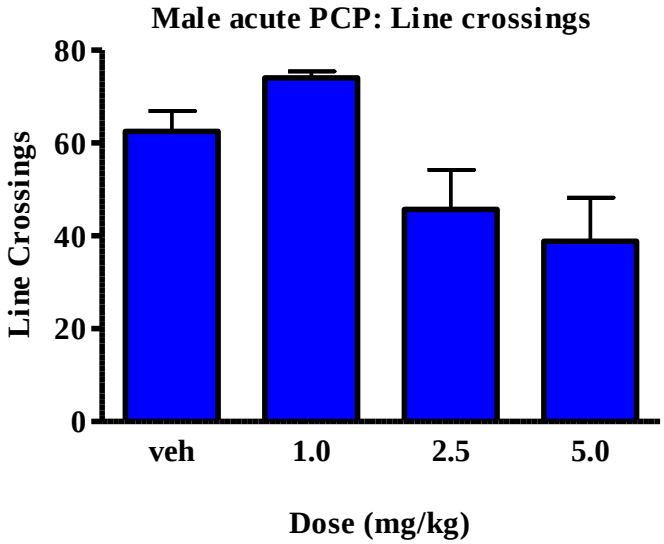


Figure 4.12 The effect of acute PCP (1.0-5.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in male rats. Data are expressed as mean ± S.E.M. (n=6-7 per group).

PCP (mg/kg)	*Total exploration time (s)*	
	Acquisition trial	*Retention trial*
Veh	27.0 ± 4.2	23.3 ±4.8
1.0	22.7 ± 2.8	20.7 ± 4.2
2.5	18.2 ± 3.1	15.2 ± 3.8
5.0	9.3 ± 2.9 **	12.6 ± 4.1 (P=0.17)

Table 4.8 The effect of acute PCP (1.0-5.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on the total exploration times in the acquisition and retention trial of the NOR test in male rats. Data are expressed as the mean ± S.E.M. (n=6-7 per group). **P<0.01; significant reduction in total exploration time when compared to vehicle control.

4.3.13 Effect of acute d-amph treatment in the acquisition trial in female rats

A two-way ANOVA revealed that treatment with d-amph did not produce any significant effect on object exploration in the acquisition trial of the NOR ($F_{3, 36}$=1.06, NS). Rats from all treatment groups spent similar times exploring both the objects (figure 4.13). A one-way ANOVA on the total exploration times in the acquisition trial revealed a significant effect ($F_{3, 39}$=2.97, P<0.05). Post-hoc analysis showed that the highest dose of d-amph (1.0 mg/kg) significantly (P<0.05) reduced the total exploration times of the identical objects when compare to vehicle control (table 4.9).

4.3.14 Effect of acute d-amph treatment in the retention trial in female rats

An overall two-way ANOVA revealed that treatment with d-amph did not produce a significant effect on object exploration in the retention trial of the NOR test ($F_{3,}$

$_{36}$=1.79, NS; figure 4.14). However, planned post-hoc comparisons revealed that rats spent significantly (P<0.05) more time exploring the novel objects during the retention trial in the vehicle control group and at the lowest dose of d-amph (0.1 mg/kg). This significant preference in the vehicle and lowest dose of d-amph (0.1 mg/kg) treated rats for the novel object was not observed in the rats treated with the higher doses of d-amph (0.5 – 1.0 mg/kg), i.e. these rats could not discriminate between the objects. Analysis of the total exploration time of the objects in the retention trial by a one-way ANOVA revealed no significant ($F_{3, 39}$=2.68, NS) effect of drug treatment. However, planned post-hoc analysis revealed that the highest dose of d-amph (1.0 mg/kg) significantly (P<0.05) reduced the total exploration time of the objects when compared to vehicle control (table 4.9).

4.3.15 Effect of acute d-amph treatment Discrimination Index (DI) in female rats

One-way ANOVA on the DI data showed no significant effect of d-amph treatment on the rats' ability to discriminate between the familiar and novel objects ($F_{3, 36}$=2.42, NS). Rats treated with the highest doses of d-amph (0.5-1.0 mg/kg) displayed a reduction in DI; however this failed to reach statistical significance (figure. 4.15).

4.3.16 Effect of acute d-amph treatment on line crossings in female rats

Figure 4.16 shows the effect of acute d-amph treatment on total line crossings of the rats during the acquisition and retention trials in the NOR test. One-way ANOVA revealed a significant effect of d-amph on line crossings ($F_{3, 39}$=8.46, P<0.001). Further post-hoc analysis of the total line crossings revealed that the two highest

doses of d-amph (0.5 and 1.0 mg/kg) induced a significant ($P<0.01$ and $P<0.05$ respectively) increase in line crossings.

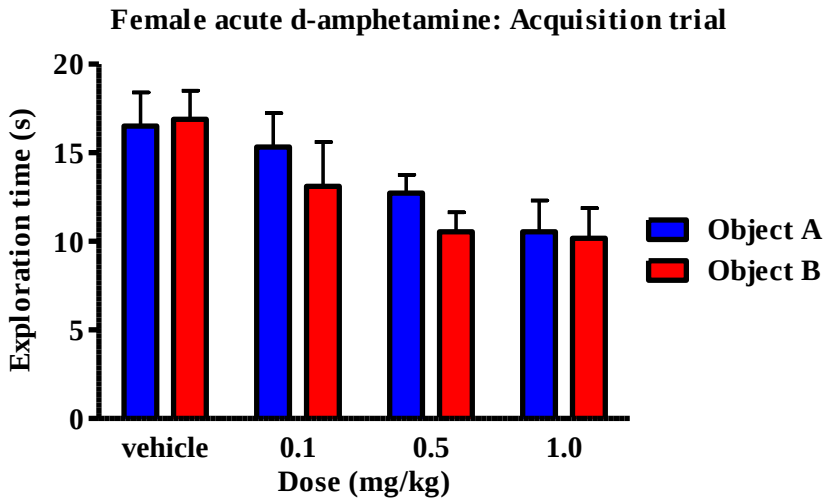


Figure 4.13 The effect of acute d-amph (0.1-1.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on exploration times (s) of two identical objects in the 3 min acquisition trial in the NOR test in female rats. Data are shown as mean ± S.E.M. (n=10 per group).

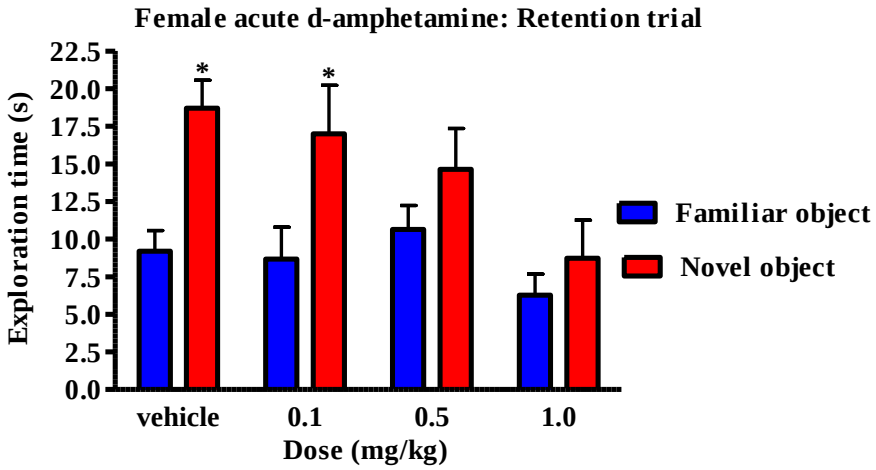


Figure 4.14 The effect of acute d-amph (0.1-1.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on exploration times (s) of a novel and familiar object in the 3 min retention trial in the NOR test in female rats. Data are shown as mean ± S.E.M. (n=10 per group). *$P<0.05$; significant increase in time spent exploring the novel compared with the familiar object.

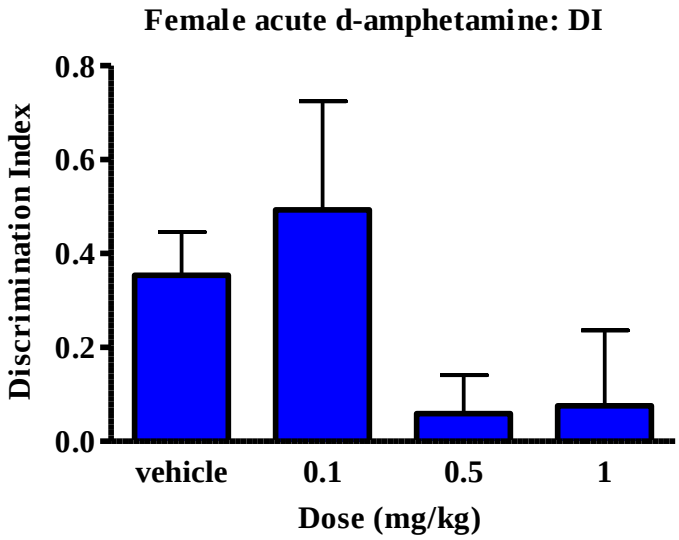


Figure 4.15 The effect of acute d-amph (0.1-1.0mg/kg, i.p.) or 0.9% saline (veh, i.p.) on the DI in female rats. Data are expressed as mean ± S.E.M. (n=10 per group).

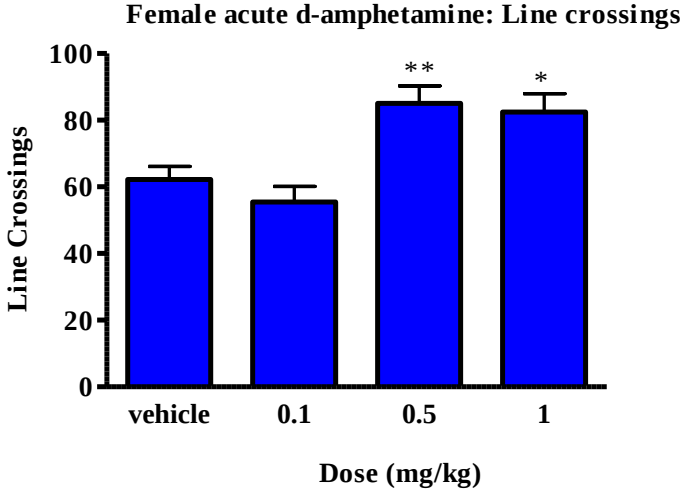


Figure 4.16 The effect of acute d-amph (0.1-1.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition + retention trial in the NOR task in female rats. Data are expressed as mean ± S.E.M. (n=10 per group). *P<0.05-**P<0.01; significant increase in line crossings compared to vehicle.

d-amph (mg/kg)	*Total exploration time (s)*	
	Acquisition trial	***Retention trial***
Vehicle	33.5 ± 3.3	27.9 ± 2.2
0.1	28.1 ± 4.2	26.6 ± 4.4
0.5	22.8 ± 2.0 (P=0.058)	24.5 ± 4.1
1.0	21.5 ± 3.3*	14.9 ± 3.6*

Table 4.9 The effect of acute treatment with d-amph (0.5-1.0 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the total exploration time in the acquisition and retention trial of the NOR test in female rats. Data are expressed as the mean ± S.E.M. (n=10 per group). *P<0.05, significant reduction in total exploration time when compared to vehicle control.

4.3.17 Effect of acute d-amph treatment in the acquisition trial in male rats

A two-way ANOVA revealed that treatment with d-amph did not produce any significant effect on object exploration in the acquisition trial of the NOR ($F_{3, 28}$=0.59, NS). Rats from all treatment groups spent similar times exploring both the objects (figure 4.17). A one-way ANOVA on the total exploration times in the acquisition trial revealed a significant effect ($F_{3, 31}$=3.3, P<0.05). Post-hoc analysis showed that the highest dose of d-amph (2.5 mg/kg) significantly (P<0.05) reduced the total exploration times of the identical objects when compare to vehicle control (table 4.10).

4.3.18 Effect of acute d-amph treatment in the retention trial in male rats

An overall two-way ANOVA revealed that treatment with d-amph produced a significant effect on object exploration in the retention trial of the NOR test ($F_{3,}$

$_{28}$=2.9, P<0.05; figure 4.18). Planned post-hoc comparisons revealed that rats spent significantly (P<0.05) more time exploring the novel objects during the retention trial in the vehicle control group and following treatment with the lowest dose of d-amph (0.5 mg/kg). This significant preference in the vehicle and lowest dose of d-amph (0.5 mg/kg) treated rats for the novel object was not observed in the rats treated with the higher doses of d-amph (1.0 – 2.5 mg/kg), i.e. these rats could not discriminate between the objects. Analysis of the total exploration time of the objects in the retention trial by a one-way ANOVA revealed no significant ($F_{3, 31}$=1.08, NS) effect of drug treatment (table 4.10).

4.3.19 Effect of acute d-amph treatment Discrimination Index (DI) in male rats

One-way ANOVA on the DI data showed a significant effect of d-amph treatment on the rats' ability to discriminate between the familiar and novel objects ($F_{3, 31}$=3.0, P<0.05). Rats treated with the highest dose of d-amph (2.5 mg/kg) displayed a significant reduction in DI (figure. 4.19).

4.3.20 Effect of acute d-amph treatment on the line crossings in male rats

Figure 4.20 shows the effect of acute d-amph treatment on total line crossings of the rats during the acquisition and retention trials in the NOR test. One-way ANOVA revealed a significant effect of d-amph on line crossings ($F_{3, 31}$=18.2, P<0.001). Further post-hoc analysis of the total line crossings revealed that the two highest doses of d-amph (1.0 and 2.5 mg/kg) induced a significant (P<0.01 and P<0.001 respectively) increase in line crossings.

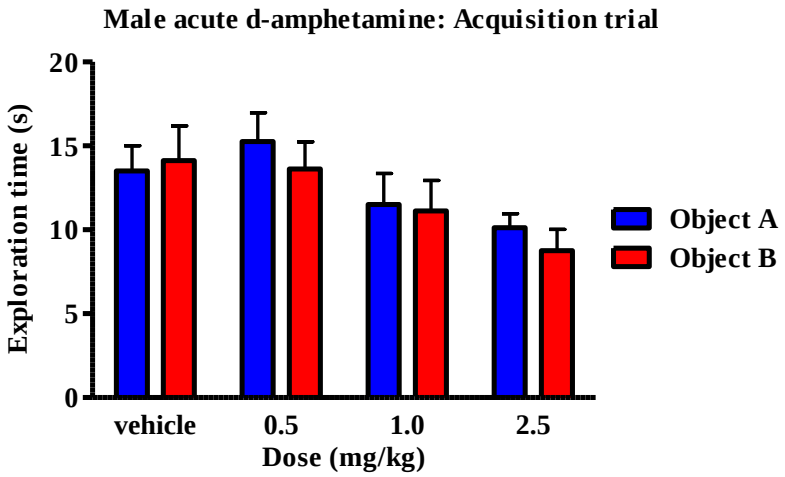


Figure 4.17 The effect of acute d-amph (0.5-1.0 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on exploration times (s) of two identical objects in the 3 min acquisition trial in the NOR test in male rats. Data are shown as mean ± S.E.M. (n=8 per group).

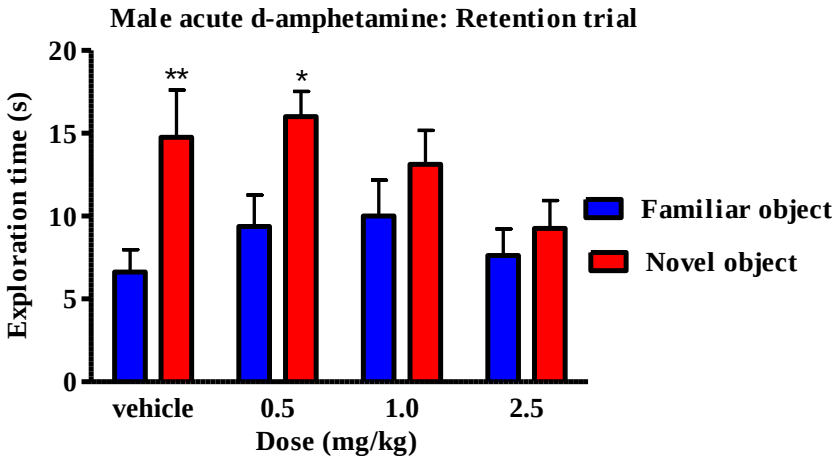


Figure 4.18 The effect of acute d-amph (0.5-2.5 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on exploration times (s) of a novel and familiar objects in the 3 min retention trial in the NOR test in male rats. Data are shown as mean ± S.E.M. (n=8 per group). *P<0.05-**P<0.01; significant increase in time spent exploring the novel compared with the familiar object.

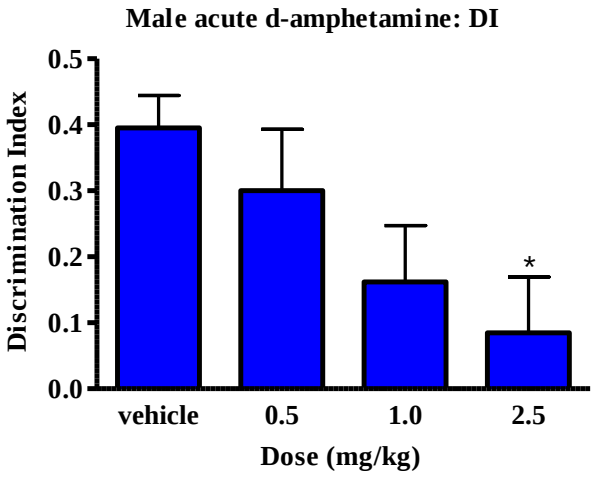


Figure 4.19 The effect of acute d-amph (0.5-2.5 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on the DI in male rats. Data are expressed as mean ± S.E.M. (n=8 per group). P<0.05; significant reduction in DI compared to vehicle.

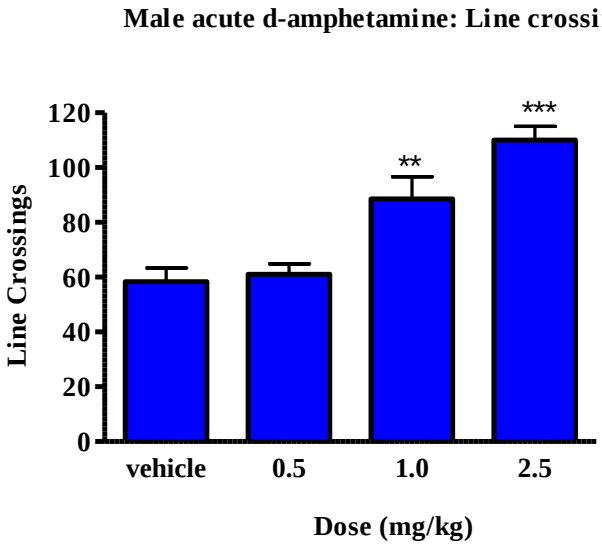


Figure 4.20 The effect of acute d-amph (0.5-2.5 mg/kg, i.p.) or 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition + retention trial in the NOR test in male rats. Data are expressed as mean ± S.E.M. (n=8 per group). **P<0.01-**P<0.001; significant increase in line crossings compared to vehicle.

d-amph (mg/kg)	*Total exploration time (s)*	
	Acquisition trial	*Retention trial*
veh	30.0 ± 2.8	21.4 ± 4.0
0.5	28.9 ± 3.2	25.4 ± 2.7
1.0	22.6 ± 3.5	23.1 ± 3.9
2.5	18.8 ± 1.8 *	16.9 ± 3.1

Table 4.10 The effect of acute treatment with d-amph (0.5-2.5 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trial of the NOR test in female rats. Data are expressed as the mean ± S.E.M. (n=8 per group). *P<0.05; significant reduction in total exploration time when compared to vehicle control.

4.3.21 Effect of sub-chronic PCP treatment in the acquisition trial in female rats

A two-way ANOVA revealed that sub-chronic treatment with PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{1, 17}$=0.89, NS). Rats from both treatment groups spent similar times exploring both the objects (figure 4.21). Post-hoc analysis of the total exploration of the objects in the acquisition trial by unpaired Student's t-test revealed no significant effect of drug treatment (table 4.11)

4.3.22 Effect of sub-chronic PCP treatment in the retention trial in female rats

An overall two-way ANOVA revealed that treatment with sub-chronic PCP produced a significant effect on object exploration in the retention trial of the NOR test ($F_{1, 17}$=17.94, P<0.001) (figure 4.22). Post-hoc analysis revealed that vehicle control rats had a significant preference for the novel compared with the familiar

object, P<0.05. This preference in the vehicle treated rats for the novel object was not observed in the sub-chronic PCP treated rats, i.e. these rats spent similar amount of time exploring both objects. Analysis of the total exploration of the objects in the retention trial by unpaired Student's t-test revealed a significant (P<0.05) increase in sub-chronic PCP treated rats compared with the vehicle control group (table 4.11).

4.3.23 Effect of sub-chronic PCP treatment on Discrimination Index (DI) in female rats

Following treatment with sub-chronic PCP, an unpaired Student's t-test revealed a significant (P<0.001) reduction in DI compared with the vehicle group. (figure 4.23).

4.3.24 Effect of sub-chronic PCP treatment on total number of line crossings in the NOR test in female rats

Figure 4.24 shows the effect of sub-chronic PCP treatment on the total number of line crossings during the acquisition and retention trials of the NOR test. Unpaired Student's t-test revealed no significant effect of sub-chronic PCP treatment on line crossings.

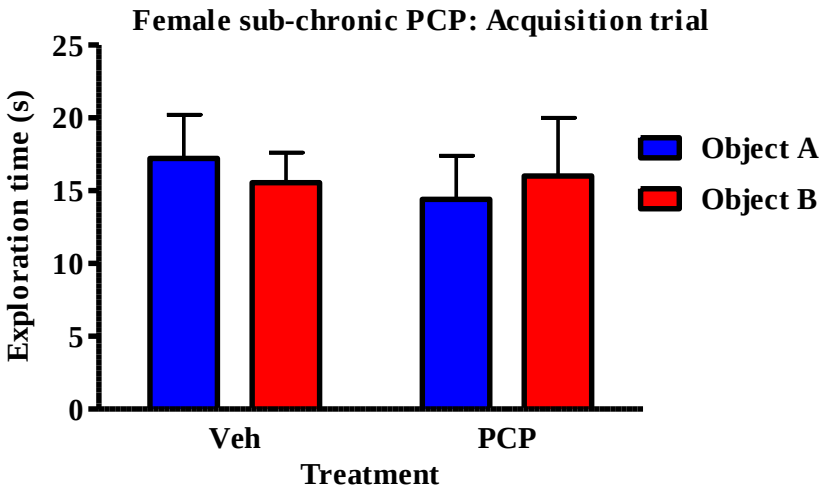


Figure 4.21 The effect sub-chronic PCP treatment (2 mg/kg i.p. twice daily for 7-days) or 0.9% saline (veh, i.p.) on exploration of two identical objects in the 3 min acquisition trial of the NOR test in female rats. Data are shown as mean ± S.E.M. (n=9-10 per group).

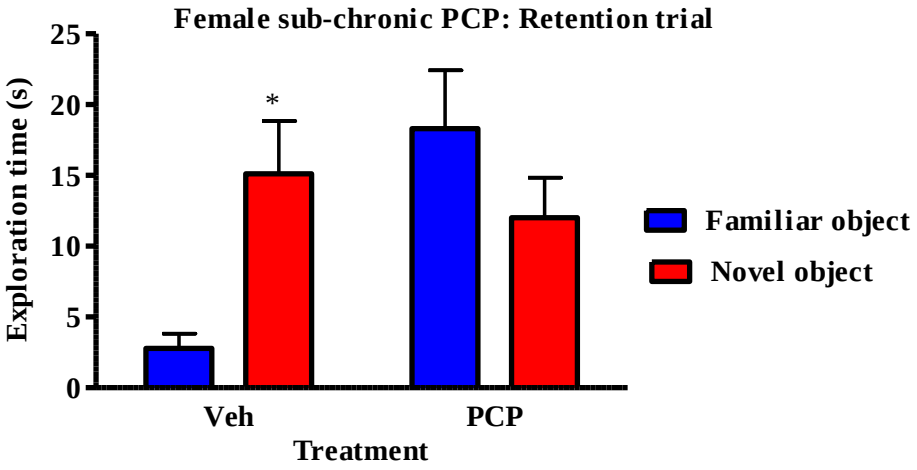


Figure 4.22 The effect sub-chronic PCP treatment (2 mg/kg i.p. twice daily for 7-days) or 0.9% saline (veh, i.p.) on exploration of a novel and a familiar object in the 3 min retention trial in a NOR test in female rats. Data are shown as mean ± S.E.M. (n=9-10 per group). *P<0.05; significant difference between the time spent exploring the familiar compared with the novel object.

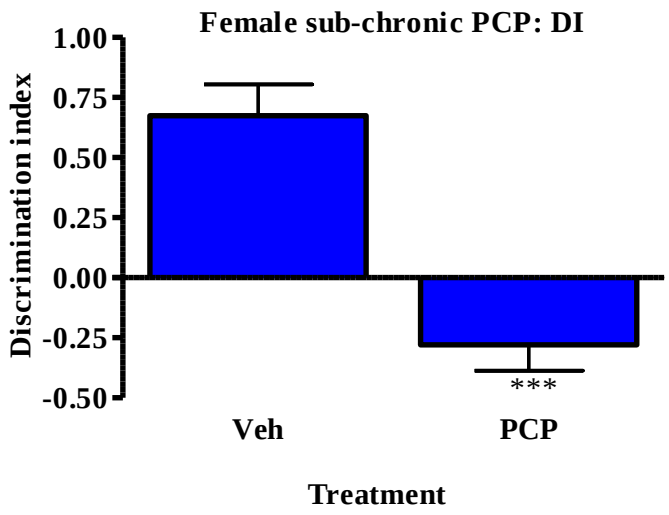


Figure 4.23 The effect of sub-chronic PCP treatment (2 mg/kg i.p. twice daily for 7-days) or 0.9% saline (veh, i.p.) on the DI in female rats. Data are expressed as the mean ± S.E.M. (n=9-10 per group). *** P<0.001; significant reduction in DI when compared with vehicle.

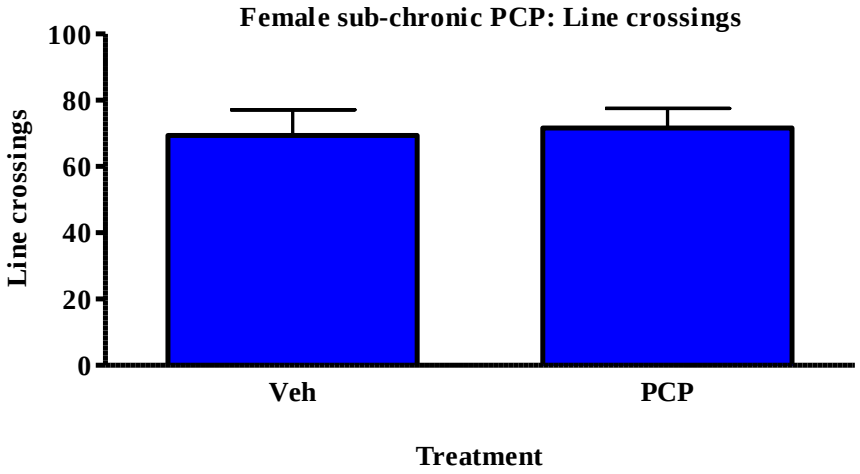


Figure 4.24 The effect sub-chronic PCP treatment (2 mg/kg i.p. twice daily for 7-days) or 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention phase in the NOR test in female rats. Data are expressed as the mean ± S.E.M. (n=9-10 per group).

Treatment	Total exploration time (s)	
	Acquisition trial	Retention trial
Veh	32.7 ± 4.5	17.8 ± 3.4
PCP	30.4 ± 6.7	30.3 ± 6.8*

Table 4.11 The effect of sub-chronic PCP treatment (2 mg/kg i.p. twice daily for 7-days) on the total exploration time in the acquisition and retention trial of the NOR test in female rats. Data are expressed as the mean ± S.E.M. (n=9-10 per group). *P<0.05; significant increase in total exploration time compared to vehicle.

4.3.25 Effect of sub-chronic PCP treatment in the acquisition trial in male rats

A two-way ANOVA revealed that sub-chronic treatment with PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{1, 18}$=2.42, NS). Rats from both treatment groups spent similar times exploring both the objects (figure 4.25). Post-hoc analysis of the total exploration of the objects in the acquisition trial by unpaired Student's t-test revealed no significant effect of drug treatment (table 4.12).

4.3.26 Effect of sub-chronic PCP treatment in the retention trial in male rats

An overall two-way ANOVA revealed that treatment with sub-chronic PCP produced no significant effect on object exploration in the retention trial of the NOR test ($F_{1, 18}$=1.00, NS) (figure 4.26). However, planned post-hoc analysis revealed that vehicle control rats had a significant preference for the novel compared with the familiar object, P<0.05. This significant preference in the vehicle treated rats for the

novel object was not observed in the sub-chronic PCP treated rats, i.e. these rats spent equivalent amount of time exploring both objects. Post-hoc analysis of the total exploration of the objects in the retention trial by unpaired Student's t-test revealed no significant effect of drug treatment (table 4.12).

4.3.27 Effect of sub-chronic PCP treatment on Discrimination Index (DI) in male rats

Following treatment with sub-chronic PCP, an unpaired Student's t-test revealed no significant difference in DI compared with the vehicle group (Figure 4.27).

4.3.28 Effect of sub-chronic PCP treatment on total number of line crossings in the NOR test in male rats

Figure 4.28 shows the effect of sub-chronic PCP treatment on the total number of line crossings during the acquisition and retention trials of the NOR test. Unpaired Student's t-test revealed no significant effect of sub-chronic PCP treatment on line crossings.

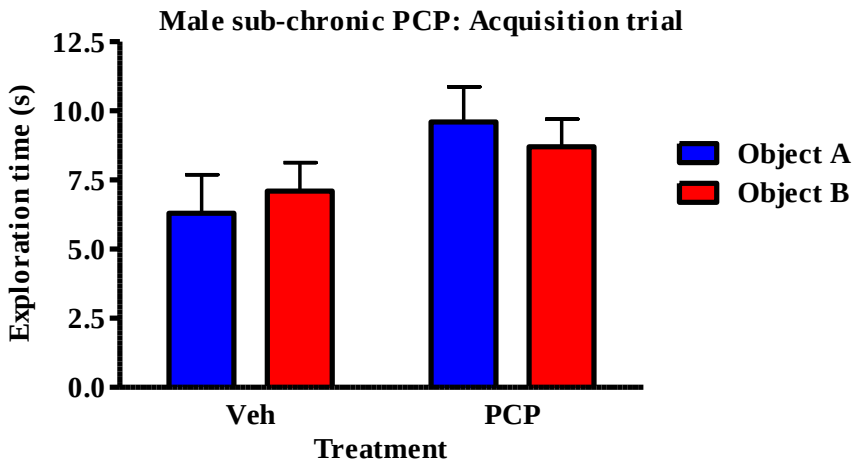


Figure 4.25 The effect sub-chronic PCP treatment (2mg/kg i.p. twice daily for 7-days) or 0.9% saline (veh, i.p.) on exploration of two identical objects in the 3 min acquisition trial in the NOR test in male rats. Data are shown as mean ± S.E.M. (n=10 per group).

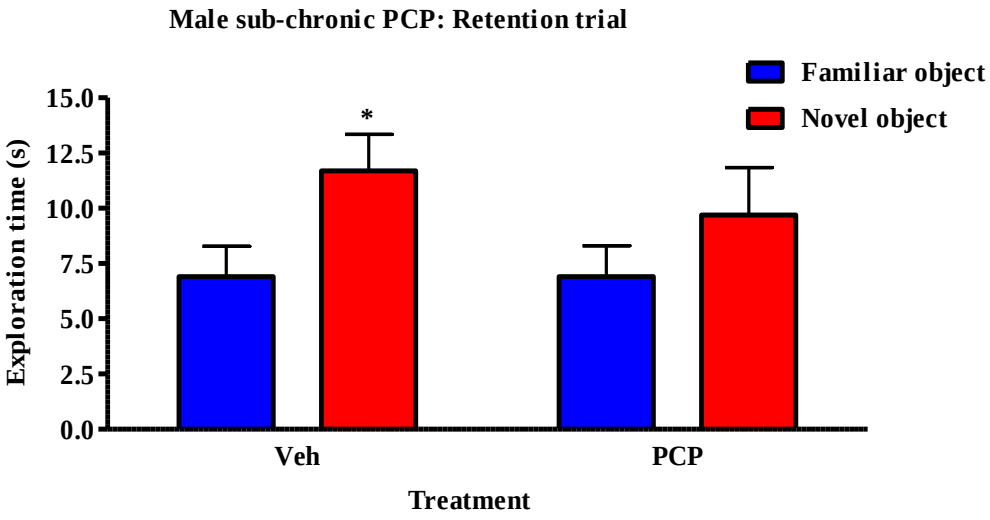


Figure 4.26 The effect sub-chronic PCP treatment (2mg/kg i.p. twice daily for 7-days) or 0.9% saline (veh, i.p.) on exploration of a novel and a familiar object in the 3 min retention trial in the NOR test in male rats. Data are shown as mean ± S.E.M. (n=10 per group). *P<0.05; significant increase in time spent exploring the novel compared with the familiar object.

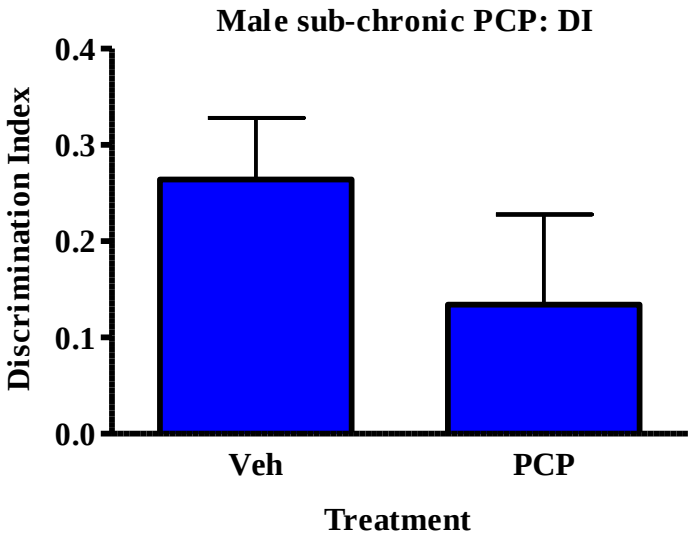


Figure 4.27 The effect sub-chronic PCP treatment (2mg/kg i.p. twice daily for 7-days) or 0.9% saline (veh, i.p.) on the DI in male rats. Data are expressed as the mean ± S.E.M. (n=10 per group).

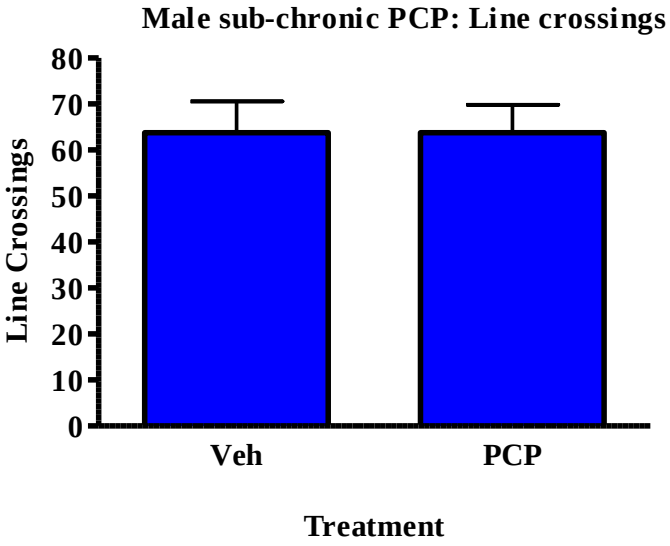


Figure 4.28 The effect sub-chronic PCP treatment (2mg/kg i.p. twice daily for 7-days) or 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials in the NOR test in male rats. Data are expressed as the mean ± S.E.M. (n=10 per group).

Treatment	*Total exploration time (s)*	
	Acquisition trial	*Retention trial*
Veh	13.4 ± 2.4	18.6 ± 2.8
PCP	18.3 ± 2.1	16.6 ± 3.3

Table 4.12 The effect of sub-chronic PCP treatment (2 mg/kg i.p. twice daily for 7-days) on the total exploration time in the acquisition and retention trial of the NOR test in male rats. Data are expressed as the mean ± S.E.M. (n=10 per group).

4.3.29 Male versus female comparisons

4.3.29.1 Acquisition trial (males v females; total exploration)

A two-way ANOVA revealed that sub-chronic treatment with PCP did not produce any significant effect when comparing males to females on total object exploration in the acquisition trial of the NOR test ($F_{1, 18}$=0.89, NS, table 4.12). However, planned post-hoc analysis using unpaired Student's t-test revealed a significant ($P<0.01$) decrease in total exploration time in the vehicle group of male rats when compared to female rats in the acquisition trial.

4.3.29.2 Retention trial (males v females; total exploration)

A two-way ANOVA revealed that sub-chronic treatment with PCP did not produce any significant effect when comparing males to females on total object exploration in the retention trial of the NOR test ($F_{1, 18}$=0.89, NS; table 4.12).

4.3.29.3 DI (males v females)

A two-way ANOVA revealed that sub-chronic treatment with PCP produced a significant effect when comparing males to females on DI in the NOR test ($F_{1, 18}$=25.0, P<0.001). Planned post-hoc analysis using unpaired Student's t-test on the vehicle treated rats revealed a significant reduction in DI in the male compared to female rats, suggesting reduced object discrimination (table 4.13).

Sub-chronic treatment	***Female Total exploration time (s)***		***Male Total exploration time (s)***	
	Acquisition trial	***Retention trial***	***Acquisition trial***	***Retention trial***
Veh	32.7 ± 4.5	17.8 ± 3.4	13.4 ± 2.4 ##	18.6 ± 2.8
PCP	30.4 ± 6.7	30.3 ± 6.8	18.3 ± 2.1	16.6 ± 3.3 (P= 0.08 male v female)

Table 4.13 The effect of sub-chronic PCP treatment (2 mg/kg i.p. twice daily for 7-days) on the total exploration time in the acquisition and retention trial of the NOR test in male and female rats. Data are expressed as the mean ± S.E.M. (n=10 per group). ##P<0.01; significant decrease in total exploration time in males when compared to female in the acquisition trial.

Sub-chronic treatment	***Female DI***	***Male DI***
Veh	0.67 ± 0.12	0.26 ± 0.08
PCP	-0.28 ± 0.12	0.13 ± 0.07

Table 4.14 The effect of sub-chronic PCP treatment (2 mg/kg i.p. twice daily for 7-days) on the DI of the NOR test in male and female rats. Data are expressed as the mean ± S.E.M. (n=10 per group).

Treatment	*Doses tested (mg/kg)*	*Deficit in the retention trial. Dose (mg/kg*	*Deficit in the DI. Dose (mg/kg)*	*Effect on line crossings. Dose (mg/kg)*	*Effect on total exploration. Dose (mg/kg)*
Females Acute PCP	0.5, 1.0, 1.5, 2.0	**Yes** 0.5, 1.0, 1.5, 2.0	**Yes** 1.5, 2.0	**No**	**No**
Males Acute PCP	0.5, 1.0, 1.5, 2.0	**Yes** 1.5	**No**	**No**	**No**
Males Acute (higher doses)	1.0, 2.5, 5.0	1.0 P=0.08, 2.5 P=0.09	**No**	**No**	#(acquisition;5.0, retention; 5.0, P=0.17)
Females Acute d-amph	0.1, 0.5, 1.0	**Yes** 0.5, 1.0	**No**	*0.5, 1.0	#(acquisition;1.0, retention; 1.0)
Males Acute d-amph	0.5, 1.0, 2.5	**Yes** 1.0, 2.5	**Yes** 2.5	*1.0, 2.5	#(acquisition;1.0)

Table 4.15 Summary table showing the effect of acute treatment with PCP (0.5-2.0 mg/kg; female and 0.5-5.0 mg/kg; male rats) and d-amph (0.1-1.0 mg/kg, i.p.; female and 0.5-2.5 mg/kg, i.p.; male rats) on performance in the NOR test. Significant increase* or decrease# (P<0.05-P<0.01) compared to the vehicle treated group.

Treatment	*Doses tested (mg/kg)*	*Deficit in the retention trial*	*Deficit in the DI*	*Effect on line crossings*	*Effect on total exploration*
Sub-chronic PCP: Females	2.0	**Yes**	**Yes**	**No**	**No**
Sub-chronic PCP: Males	2.0	**Yes**	**No**	**No**	**No**

Table 4.16 Summary table showing the effect of sub-chronic PCP treatment (2 mg/kg, i.p. twice daily for 7 days) on the on performance of males and female rats in the NOR test.

4.4 Discussion

4.4.1 Acute PCP (males v females)

Following acute treatment with PCP a cognitive impairment was observed in female rats at all of the doses tested in the NOR test, however the efficacy of acute PCP to impair recognition memory in male rats was reduced, such that a higher dose range of PCP was selected albeit with inconsistent results. The male rats appeared to be somewhat insensitive to the selective cognitive impairment observed in the female rats following acute PCP treatment. The acute treatment with PCP in female rats produced selective impairments in recognition memory as measured in the retention trial, without any non-specific effects in the acquisition trial or an effect on the number of line crossings or total exploration times. However, the male rats' lack of sensitivity to PCP was evident, even at 10x the effective dose of PCP needed to

produce a cognitive deficit in female rats; the male rats could still effectively discriminate between the novel and familiar objects in the retention trial. The increased sensitivity to PCP observed in female is in agreement with studies demonstrating that female rats are more sensitive to the neurotoxic and to the locomotor stimulation effect of acute PCP treatment (Johnson et al, 1998). These results may be explained in part by PCP metabolism studies comparing male and female rats. Shelnut and colleagues (1999) demonstrated that female rats from three different strains showed a significantly reduced ability to metabolise PCP compared to males. Furthermore, pharmacokinetic studies in Sprague-Dawley rats showed females to be significantly more sensitive to PCP (Nabeshima et al, 1984). Gartlon et al (2006) found higher levels of PCP in the plasma and brain tissue of female rats, following the same dose of PCP as male rats, which may explain the differential effects of acute PCP treatment in the NOR test. Because of the serious potential to produce adverse reactions to PCP in humans, clinical studies administering PCP at active pharmacological doses have not been conducted in 50 years (Domino, 1992). However, gender differences have been studied following the administration of ketamine (Lahti et al, 2001), which is an NMDA receptor antagonist like PCP with similar chemical structure and mechanism of action. The effects of ketamine are similar to PCP, but ketamine is less potent with effects of much shorter duration (Hevers et al, 2008). In agreement with the acute PCP studies, female rats have been shown to be more vulnerable to the neurotoxic effects of ketamine when compared to male rats (Jevtovic-Todorovic et al, 2001) and more susceptible to certain behavioural effects (Winters et al, 1986). The animal data is supported by an early clinical study in the 1970s which suggested that female surgical patients were more sensitive to the psychotomimetic effects of acute ketamine administration than males

(Bovill et al, 1971). However, clinical studies examining the effect of gender on the cognitive effects of ketamine have shown contradictory results to those of animal studies. A meta-analysis has shown that men are more sensitive to the memory impairing effects of ketamine than women (Morgan et al, 2006), particularly in a verbal recall test.

4.4.2 Acute d-amphetamine (males v females)

The effect of d-amphetamine in male and female rats in the NOR test has demonstrated an enhanced sensitivity to induce recognition deficits in females. A higher dose of amphetamine was required in male rats to induce the recognition memory impairment that was observed in the female rats. Furthermore, there was a lack of effect of d-amphetamine in male rats to induce a disruption in the DI which suggests a lack of effect of d-amphetamine in males. In both males and females, the lack of effect of d-amphetamine in the acquisition trial of the NOR test suggests selectivity for the retention trial, however the impairment in recognition memory induced by d-amphetamine was not observed without a significant increase in the total number of line crossings, which is a measure of general activity and therefore suggests a lack of specificity of d-amphetamine to disrupt recognition memory in this test. In another study, aimed at determining the effect of gender on locomotor activity response to amphetamine, it was shown that female rats were more sensitive to amphetamine-induced hyperactivity and stereotypic behaviours (Hallé-Milesi et al, 2007). Furthermore, ovariectomised rats treated with oestrogen demonstrate an enhanced amphetamine-induced release of dopamine from striatal tissue *in-vitro* (Becker et al, 2003) and *in-vivo* using microdialysis techniques (Castner et al, 1993).

In our laboratory, we have previously shown that administration of the same dose range of d-amphetamine to female rats induces selective impairments in reversal learning task, which can be successfully restored by haloperidol (Idris et al, 2005). The cognitive dysfunction induced by d-amphetamine is thought to be a result of an excessive release of dopamine in the prefrontal cortex (Mason et al, 1992; Ridley et al, 1998) and studies in our laboratory have previously shown d-amphetamine induced impairments in a version of the Wisconsin test modified for the marmoset (Smith et al, 1999).

4.4.3 Sub-chronic PCP (males v females)

These data demonstrate that sub-chronic treatment with PCP impairs recognition memory when comparing the time spent exploring the novel compared with familiar object in the NOR test in all rats (i.e. both male and female rats). However, the impairment appeared to be more effective in the female rats as demonstrated by the highly significant impairment in the female rats' ability to discriminate novel and familiar objects. There was a lack of effect of sub-chronic PCP treatment to induce a recognition deficit as measured by the discrimination index (DI). Interestingly, the discrimination index (DI) data in this study indicate that female control rats were superior at discriminating between the novel and familiar objects compared to the male control rats. These data provide support for the results by Sutcliffe et al (2007), whereby female rats compared to male rats, performed significantly better in the NOR test following increasing ITIs. The impairment in object recognition memory observed in both genders following treatment with sub-chronic PCP was selective for the retention trial only, and was observed without any non-specific effects in the acquisition trial, number of line crossings or the total exploration times. These

findings of improved performance in females and increased effect of sub-chronic PCP are in agreement with results of Snigdha and colleagues (2011), who reported that male rats were less sensitive to sub-chronic PCP-induced deficits in the extra-dimensional shift stage of the attentional set-shifting task compared to female rats.

The sub-chronic PCP induced recognition deficits were of similar magnitude to those cognitive deficits observed following acute PCP treatment in the female rats.

Acute PCP treatment has been shown to enhance dopamine in the PFC (hyperdopaminergia), whereas, sub-chronic PCP treatment, similar to the dosing regimen used in these studies, has been shown to lead to decreased DOPAC levels in the medial pre-frontal cortex, which may suggest a decreased dopamine utilisation in this area, cortical dopaminergic hypoactivity and impairments in cognition (Jentsch et al, 1998). Furthermore, recent work from our laboratory has shown that the PCP-induced object recognition deficit is accompanied by impaired dopamine neurotransmission in the PFC during the retention trial of the task (Snigdha et al, 2008). This result was also recently confirmed in another study in our laboratory (McLean, unpublished observations), suggesting a critical role for prefrontal dopamine in object recognition memory and lack of ability of sub-chronic PCP treated rats to recognise the familiar object in the retention trail.

Female gonadal steroid hormone levels such as oestrogen and progesterone and vaginal cytology of the female rats were not measured in this study, consequently it is not clear which stage of the oestrous cycle the rats were in during the NOR testing. However, it has recently been shown in our laboratory that the oestrous cycle has no effect on performance in the NOR test (Sutcliffe et al, 2007) or reversal learning (McLean et al, 2009).

CHAPTER 5 - Further validation of the NOR test: Pharmacological validation and effects of novel cognitive enhancers to reverse PCP-induced deficits.

5.1 Introduction

Neurochemical animal models of schizophrenia based on the dopamine hypothesis have been important in explaining some of the symptoms of schizophrenia, principally the positive symptoms and developing treatment considerations (Javitt, 2010). There are limitations surrounding the dopamine hypothesis and a major flaw is that only the positive symptoms of schizophrenia are treated with classical antipsychotics, the negative and cognitive symptoms appear to be untreated. Furthermore, there is no evidence to suggest that any intrinsic deficits have been observed within the dopamine system to account for the hyperdopaminergic state associated with schizophrenia (Javitt, 2007).

Sub-chronic treatment with PCP has been shown to produce cognitive impairments that mimic those observed in schizophrenia patients (Javitt & Zukin, 1991; Jentsch et al, 1997). People who repeatedly self-administer PCP or ketamine, demonstrate robust and long lasting cognitive deficits (Cosgrove & Newell, 1991; Curran & Morgan, 2000). Additionally, sub-chronic PCP administration has been shown to induce cognitive impairments in the extra-dimensional shift stage of the attentional set-shifting task in rats (McLean et al, 2008; Rodefer et al, 2008) and also selective impairments in the reversal phase of a reversal learning task (Abdul-Monim et al, 2006). Moreover, the cognitive impairments induced by sub-chronic PCP in the reversal learning and attentional set-shifting tasks can be reversed by atypical antipsychotics but not classical agents (Idris et al, 2010; McLean et al, 2010). The sub-chronic PCP model in the rat has also been shown to produce neurobiological changes of relevance to schizophrenia such as the decrease in parvalbumin

containing neurones found in the hippocampus (Abdul-Monim et al, 2007) and pre-frontal cortex (McKibben et al, 2010).

There is evidence from post mortem studies suggesting a decrease in the α-7 nicotinic receptors in the hippocampus of schizophrenia patients and further evidence substantiates the involvement by genetic linkage to the region containing the α-7 nicotinic receptor (Freedman et al, 1995; Freedman et al 1997; Breese et al, 2000). One therapeutic target identified by the MATRICS initiative is the α-7 nicotinic acetylcholine receptor (kucinski et al, 2011). Studies have demonstrated pro-cognitive findings of agonists of the α-7 nicotinic receptors, especially in the domain of working memory (see review by Leiser et al, 2009). The α-7 nicotinic receptor is less sensitive to nicotine than other nicotinic receptors, and therefore the heavy smoking displayed by schizophrenia patients may provide evidence that they are trying to activate this receptor, to perhaps compensate for its lower than normal expression in schizophrenia patients (Olincy et al, 1997). Research in our laboratory has demonstrated improvements in cognition following the administration of the α-7 nicotinic receptor full agonist PNU-282987 and the PAM (positive allosteric modulator) of the α-7 nicotinic receptor PNU-120596 in sub-chronic PCP-induced deficits in reversal learning (McLean et al, 2011). Additionally, clinical trials are taking place to determine the effect of compounds that activate or modulate the α-7 nicotinic receptor in schizophrenia patients (www.biospace.com). One of these compounds, EVP-6124 an α-7 nicotinic receptor agonist, has demonstrated positive effects on cognition and negative symptoms in schizophrenia patients in phase II clinical trials, and will be advancing into phase III in 2012.

During the development of potential animal models, pharmacological specificity must be determined so that only compounds that are clinically active in affecting memory should be active in the behavioural test, whereas compounds of other pharmacological classes such as antidepressants and anxiolytics should be inactive.

The main aim of the work presented in this chapter is to further validate the NOR test in rats treated with sub-chronic PCP, comparing the classical antipsychotics with the atypical agents, other pharmacological agents such as an anxiolytic, antidepressant, analeptic and potential novel pro-cognitive compounds.

5.2 Materials and methods

5.2.1 Experimental Animals and Design

All experiments (using cohorts 7-13) were conducted using adult (180-255g) female hooded-Lister rats (Harlan, UK), details are shown in the table 5.1. Experiment 6 was carried out over two separate experiments, and subsequently the data were pooled. For housing conditions see section 2.1.1. For further information regarding the drugs used in this study, see table 2.2.

Experiment number	*Cohort number*	*Total number of rats used*	*Weight range (g)*	*Compound tested*
1	7	**42** (n=10 veh; n=32 PCP)	190-235	Haloperidol
2	7	**42** (n=10 veh; n=32 PCP)	190-235	Clozapine
3	8	**50** (n=10 veh; n=40 PCP)	210-255	Risperidone
4	9	**50** (n=10 veh; n=40 PCP)	180-220	CDP
5	10	**60** (n=10 veh; n=50 PCP)	190-240	Fluphenazine Fluoxetine Risperidone Modafinil
6	11 & 12	**70** (n=20 veh; n=50 PCP)	190-220	PNU-282987 Nicotine
7	13	**50** (n=10 veh; n=40 PCP)	180-240	PNU-120596

Table 5.1 Details of the rats used in these studies.

Six cohorts of rats, totalling 322 were randomly assigned to receive either vehicle (n=70; 0.9% 0.9% saline twice a day, i.p.) or PCP (n=252; 2mg/kg twice a day, i.p.) in a volume of 1 ml/kg for 7 days. Subsequently, animals were given a 7-day drug free prior to NOR testing. For the sub-chronic PCP dosing regimen, see section 2.1.3.

In experiment 1, a total of 42 rats were used (n=10 veh; n=32 PCP, cohort 7). Ten sub-chronically vehicle treated rats received vehicle acutely and 32 sub-chronic PCP treated rats were randomly assigned to receive an acute injection of haloperidol (0.05 or 0.075 mg/kg, i.p.) or vehicle (distilled water) 30 min prior to NOR testing (see table 5.2). Haloperidol (Serenace liquid; 2 mg/ml, Baker Norton) was diluted in distilled water. The doses of haloperidol were selected on the basis of previous studies showing that haloperidol (0.05 mg/kg) significantly attenuated the d-amph-induced reversal learning impairment in female hooded-Lister rats (Idris et al, 2005). Furthermore, this dose of haloperidol has been shown to occupy 50% of dopamine

D_2 receptors (Kapur & Seeman, 2001, Kapur et al, 2003). For animal numbers in the treatment groups see table 5.2.

Dose of haloperidol (mg/kg)	***Initial number of rats***	***Number of rats excluded***
Veh + Veh	10	0
PCP + Veh	10	3
PCP + 0.05	12	0
PCP + 0.075	10	1

Table 5.2 The number of rats excluded from the NOR study following acute administration of haloperidol (0.05-0.075 mg/kg, i.p.) or vehicle (dH_2o, i.p.) to sub-chronically PCP treated rats (2mg/kg, i.p., twice daily for seven days).

In experiment 2, following a 1-week washout period from experiment 1, a total of 42 rats were used (n=10 veh; n=32 PCP, cohort 7), Ten sub-chronically vehicle treated rats received vehicle acutely (distilled water, i.p.), 30 min prior to testing. The 32 sub-chronically PCP treated rats were randomly assigned to receive an acute injection of clozapine (1 & 5 mg/kg, i.p.) or vehicle (distilled water, i.p.) 30 min prior to NOR testing. The doses of clozapine selected in this study were based upon previous work in the laboratory showing that 5 mg/kg of clozapine significantly attenuated a sub-chronic PCP-induced reversal learning impairment in female hooded-Lister rats (Abdul-Monim et al, 2006). For animal numbers in the treatment groups see table 5.3

Dose of clozapine (mg/kg)	*Initial number of rats*	*Number of rats excluded*
Veh + Veh	10	0
PCP + Veh	8	0
PCP + 1.0	12	0
PCP + 5.0	12	0

Table 5.3 The number of rats excluded from the NOR study following acute administration of clozapine (1.0-5.0 mg/kg, i.p.) or vehicle (dH_2o, i.p.) to sub-chronically PCP treated rats (2mg/kg, i.p., twice daily for seven days).

In experiment 3, a total of 50 rats were used (n=10 veh; n=40 PCP, cohort 8). Ten sub-chronically vehicle treated rats received vehicle acutely (saline 0.9%, i.p.), 30 min prior to testing. The sub-chronically PCP treated rats were randomly assigned to receive an acute treatment with risperidone (0.05-0.2 mg/kg, i.p.) or vehicle (saline 0.9%, i.p.), 30 min prior to testing. The dose range of risperidone (0.05-0.2 mg/kg) is slightly lower than the doses required for clinically comparable D_2 receptor occupancy (0.5-1.0 mg/kg; Kapur & Remington, 2001, Kapur et al, 2003). However, it has been demonstrated in operant studies that risperidone (0.2 mg/kg) is efficacious against sub-chronic PCP and acute PCP-induced cognitive deficit (McLean et al, 2010). For animal numbers in the treatment groups see table 5.4.

Dose of risperidone (mg/kg)	***Initial number of rats***	***Number of rats excluded***
Veh + Veh	9	1
PCP + Veh	11	0
PCP + 0.05	10	1
PCP + 0.1	10	0
PCP + 0.2	10	0

Table 5.4 The number of rats excluded from the NOR study following acute administration of risperidone (0.05-0.2 mg/kg, i.p.) or vehicle (dH_2o, i.p.) to sub-chronically PCP treated rats (2mg/kg, i.p., twice daily for seven days).

In experiment 4, a total of 50 rats were used (n=10 veh; n=40 PCP, cohort 9). Ten sub-chronically vehicle treated rats received vehicle acutely (saline 0.9%, i.p.), 30 min prior to testing. The sub-chronically PCP treated rats were randomly assigned to receive an acute treatment with CDP (1.25-5.0 mg/kg, i.p.) or vehicle (saline 0.9%, i.p.), 30 min prior to testing. The doses of CDP were selected from data showing anxiolytic-like behavioural effects in the open field test in rats at doses of 2.5-10 mg/kg (Angrini et al, 1998). For animal numbers in the treatment groups see table 5.5.

Dose of CDP (mg/kg)	***Initial number of rats***	***Number of rats excluded***
Veh + Veh	10	0
PCP + Veh	10	1
PCP + 1.25	10	0
PCP + 2.5	10	0
PCP + 5.0	10	1

Table 5.5 The number of rats excluded from the NOR study following acute administration of CDP (1.25-5.0 mg/kg, i.p.) or vehicle (saline 0.9%, i.p.) to sub-chronically PCP treated rats (2mg/kg, i.p., twice daily for seven days).

In experiment 5, a total of 60 rats were used (n=10 veh; n=50 PCP, cohort 10). Ten sub-chronically vehicle treated rats received vehicle acutely (saline 0.9%, i.p.), 30 min prior to testing. The sub-chronically PCP treated rats were randomly assigned to receive an acute treatment with fluphenazine (0.2 mg/kg, i.p.), fluoxetine (5.0 mg/kg, i.p.), risperidone (0.2 mg/kg, i.p.), modafinil (50 mg/kg, p.o.) or vehicle (saline 0.9%, i.p.), 30 min prior to testing. The dose of fluphenazine was chosen based on a study showing that 0.01-2.5 mg/kg fluphenazine inhibited the hyperactivity response to bilateral infusions of dopamine (25µg/24h for 13 days) into the nucleus accumbens of marmosets (Costall et al, 1987). The dose of fluoxetine is based upon a microdialysis study (Malagié et al, 2000) which demonstrated an increase in extracellular levels of serotonin (5-hydroxytryptamine, 5-HT) in the frontal cortex, ventral hippocampus and raphe nuclei in anaesthetised rats following treatment with fluoxetine (1-20 mg/kg). Furthermore, acute administration of fluoxetine (5 mg/kg), significantly reduced immobility in the tail-suspension test in wild-type mice (Svenningsson et al, 2002). For animal numbers in the treatment groups, see table 5.6. Risperidone (0.2 mg/kg) was included in this experiment as a positive control

comparator since we had previously demonstrated efficacy of risperidone to reverse the sub-chronic PCP-induced cognitive deficit in NOR. The dose of modafinil was selected based on data showing the ability of modafinil (64 mg/kg) to ameliorate the sub-chronic PCP-induced deficit in extra-dimensional shift in the attentional set-shifting task in the rat (Goetghebeur and Dias, 2009). Furthermore, acute modafinil treatment 64 mg/kg dramatically improved performance on a serial reversal discrimination task performed in a T-maze in mice (Béracochéa et al, 2003).

Treatment (mg/kg)	***Initial number of rats***	***Number of rats excluded***
Veh + Veh	10	0
PCP + Veh	10	2
PCP + Fluphenazine 0.2	10	2
PCP + Fluoxetine 5.0	10	2
PCP + Risperidone 0.2	10	0
PCP + Modafinil 50	10	2

Table 5.6 The number of rats excluded from the NOR study following acute administration of fluphenazine (0.25 mg/kg, i.p.), fluoxetine (5.0 mg/kg, i.p.), risperidone (0.2 mg/kg, i.p.), modafinil (50 mg/kg, p.o.) or vehicle (saline 0.9%, i.p.) to sub-chronically PCP treated rats (2mg/kg, i.p., twice daily for seven days).

In experiment 6, a total of 60 rats were used (n=10 veh; n=50 PCP, cohort 11 & 12). Twenty sub-chronically vehicle treated rats received vehicle acutely (isotonic H_2O s.c.), 60 min prior to testing. The sub-chronically PCP treated rats were randomly assigned to receive an acute treatment with PNU-282987 (10 and 20 mg/kg, s.c.), nicotine (0.2 mg/kg, s.c.) or vehicle (isotonic H_2O s.c.), 60 & 30 min respectively, prior to testing. The doses of PNU-282987 were selected based on operant reversal

learning data showing efficacy to reverse a sub-chronic PCP-induced cognitive deficit in female hooded-Lister rats (McLean et al, 2011). The dose of nicotine was selected from studies that demonstrated enhancement of acquisition, consolidation and restoration of the information in the NOR test, using a 24 h ITI, in male Wistar rats following treatment with nicotine at 0.2 mg/kg (Puma et al, 2009). For animal numbers in the treatment groups, see table 5.7.

Treatment (mg/kg)	***Initial number of rats***	***Number of rats excluded***
Veh + Veh	20	2
PCP + Veh	20	2
PCP + PNU-282987 10	10	0
PCP + PNU-282987 20	10	0
PCP + Nicotine 0.2	10	2

Table 5.7 The number of rats excluded from the NOR study following acute administration of PNU-282987 (10-20 mg/kg, s.c.), nicotine (0.2 mg/kg, i.p.) or vehicle (isotonic H_2o, s.c.) to sub-chronically PCP treated rats (2mg/kg, i.p., twice daily for seven days).

In experiment 7, a total of 50 rats were used (n=10 veh; n=40 PCP, cohort 13). Ten sub-chronically vehicle treated rats received vehicle acutely (100% PEG400), 60 min prior to testing. The sub-chronically PCP treated rats were randomly assigned to receive an acute treatment with PNU-120596 (5 - 10 mg/kg, p.o.) or vehicle (100% PEG400), 60 min, prior to testing. The doses for PNU-120596 were selected on the basis of previous work whereby administration of PNU-120596 at a dose of 3 mg/kg improved auditory gating deficits induced by d-amph in anaesthetised rats (Hurst et al, 2005). For animal numbers in the treatment groups, see table 5.8.

Dose of PNU-120596 (mg/kg)	*Initial number of rats*	*Number of rats excluded*
Veh + Veh	10	0
PCP + Veh	10	0
PCP + 5	10	0
PCP + 10	10	1
PCP + 20	10	1

Table 5.8 The number of rats excluded from the NOR study following acute administration of PNU-120596 (5-20 mg/kg, p.o.) or vehicle (100% PEG400, p.o.) to sub-chronically PCP treated rats (2mg/kg, i.p., twice daily for seven days).

5.2.2 NOR Apparatus

For the description of the NOR apparatus see section 2.1.4.

5.2.3 NOR testing

Rats were tested in the NOR task as described earlier see section 2.1.5.

5.2.4 Statistical analysis

Data were analysed as described earlier see section 2.1.5.4.

5.3 Results

5.3.1 Effect of acute haloperidol on sub-chronic PCP treatment in the acquisition trial in the NOR test in female rats

A two-way ANOVA revealed that administration of haloperidol (0.05 – 0.075 mg/kg) to rats treated with sub-chronic PCP did not produce any significant effect on object

exploration in the acquisition trial of the NOR test ($F_{3, 34}$=1.4, NS). Rats from both treatment groups spent similar times exploring both the objects (figure 5.1). A one way ANOVA on the total exploration times in the acquisition trial revealed no significant effect of drug treatment ($F_{3, 37}$=0.8, NS; table 5.9).

5.3.2 Effect of acute haloperidol on sub-chronic PCP treatment in the retention trial in the NOR test in female rats

A two-way ANOVA revealed that administration of haloperidol (0.05 – 0.075 mg/kg) to rats treated with sub-chronic PCP produced a significant effect on object exploration in the retention trial of the NOR test ($F_{3, 34}$=5.1, P<0.01; figure 5.2). Planned post-hoc comparisons showed that rats treated sub-chronically with vehicle spent significantly (P<0.05) more time exploring the novel object during the retention trial. However this significant preference for the novel compared to the familiar object was not observed in the sub-chronic PCP treated rats and the sub-chronic PCP treated rats which were administered acute haloperidol i.e. these rats spent equivalent amounts of time exploring both objects. A one way ANOVA on the total exploration times in the retention trial revealed no significant effect of drug treatment ($F_{3, 37}$=0.4, NS; table 5.9).

5.3.3 Effect of acute haloperidol on sub-chronic PCP treatment on the DI in the NOR test in female rats

One-way ANOVA on the DI data showed a significant effect of drug treatment on the rats ability to discriminate between the familiar and novel objects ($F_{3, 37}$=4.5, P<0.05; figure 5.3). Post-hoc analysis showed a significant reduction in the DI of the sub-chronic PCP treated group (P<0.05) compared to vehicle control. Sub-chronic

PCP rats treated with both doses of haloperidol also showed a significant ($P<0.01$) reduction in DI compared to vehicle.

5.3.4 Effect of acute haloperidol on sub-chronic PCP treatment on the number of line crossings in the NOR test in female rats

Figure 5.4 shows the effect of haloperidol treatment in sub-chronically PCP treated rats on the total number of line crossings during the acquisition and retention trial of the NOR test. One-way ANOVA revealed no significant effect of any treatment on the number of line crossings ($F_{3, 37}=1.80$, NS).

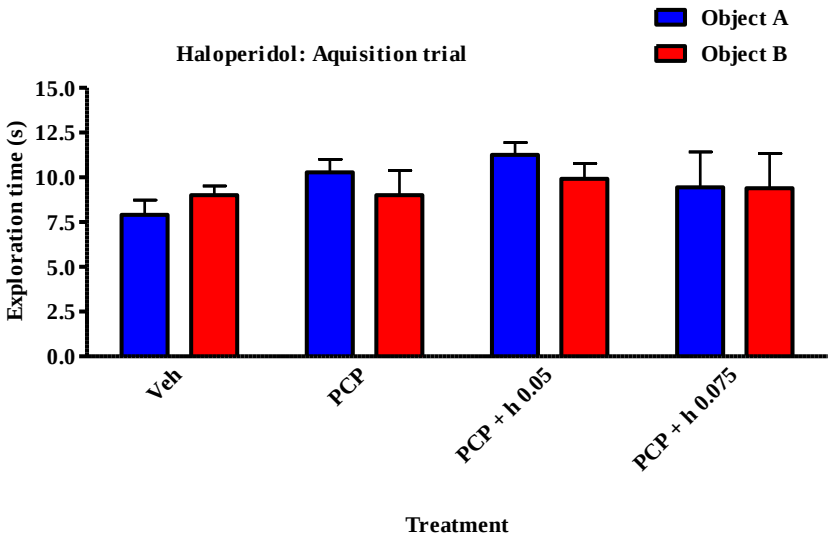


Figure 5.1 The effect of acute haloperidol (h; 0.05 & 0.075 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of two identical objects in the 3 min acquisition trial of the NOR test. Data are expressed as the mean ± S.E.M. (n=7-12 per group).

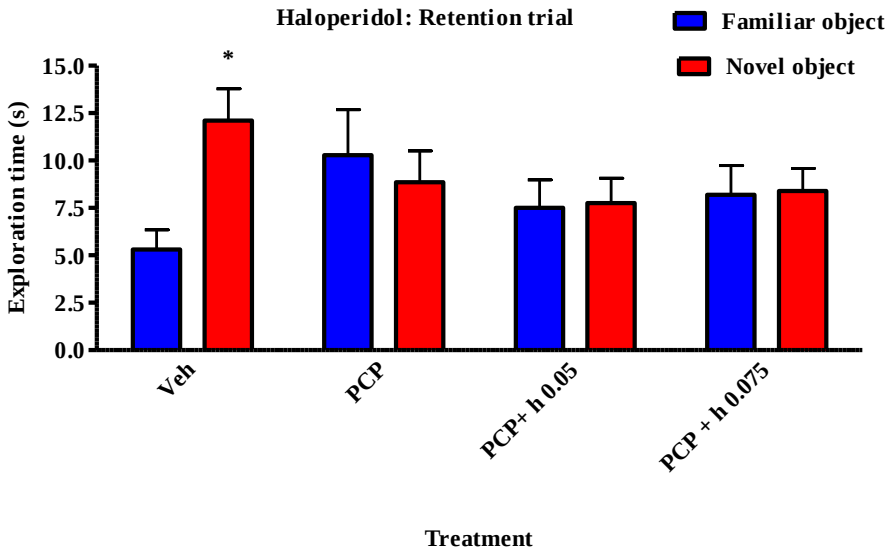


Figure 5.2 The effect of acute haloperidol (h; 0.05 & 0.075 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of a familiar object and a novel object in the 3 min retention trial. Data are expressed as the mean ± S.E.M. (n=7-12 per group). *P<0.05; significant increase in time spent exploring the novel compared with the familiar object, Student's t-test.

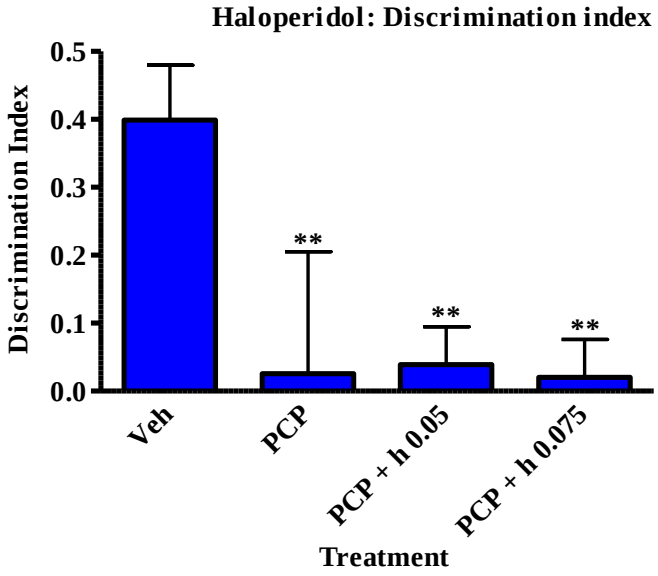


Figure 5.3 The effect of haloperidol (h; 0.05-0.075 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on the DI. Data are expressed as the mean ± S.E.M. (n=7-12 per group). **P<0.01; significant reduction in DI compared to vehicle.

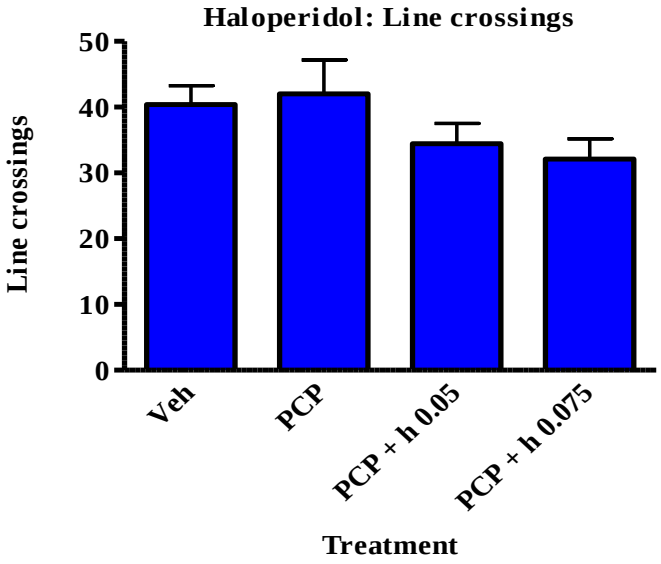


Figure 5.4 The effect of acute haloperidol (h; 0.05 & 0.075 mg/kg, i.p) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on line crossings in the acquisition and retention trial. Data are expressed as mean ± S.E.M. (n=7-12 per group).

Treatment (mg/kg)	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
Veh	16.9 ± 1.0	17.4 ± 2.2
PCP	19.3 ± 1.4	19.1 ± 2.4
PCP + h 0.05	21.2 ± 1.1	15.3 ± 2.7
PCP + h 0.075	17.9 ± 3.7	16.6 ± 2.5

Table 5.9 The effect of acute haloperidol (h; 0.05 & 0.075 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p. twice daily for seven days) on the total exploration time in the acquisition and retention trial of the NOR test in female hooded-Lister rats. Data are expressed as the mean ± S.E.M. (n=7-12 per group).

5.3.5 Effect of acute clozapine on sub-chronic PCP treatment in the acquisition trial in the NOR test in female rats

A two-way ANOVA revealed that administration of clozapine (1.0 – 5.0 mg/kg) to rats treated with sub-chronic PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{3, 38}$=0.9, NS). Rats from both treatment groups spent comparable times exploring both the objects (figure 5.5). A one-way ANOVA on the total exploration times in the acquisition trial of the NOR test revealed no significant effect of drug treatment (($F_{3, 37}$=0.8, NS; table 5.10).

5.3.6 Effect of acute clozapine on sub-chronic PCP treatment in the retention trial in the NOR test in female rats

A two-way ANOVA revealed that administration of clozapine (1.0 - 5.0 mg/kg) to rats treated with sub-chronic PCP produced a significant effect on object exploration in the retention trial of the NOR test ($F_{3, 38}$=4.1, P<0.05; figure 5.6). Planned post-hoc comparisons showed that rats treated sub-chronically with vehicle spent significantly (P<0.05) more time exploring the novel object compared with the familiar object during the retention trial. However this significant preference for the novel compared to the familiar object was not observed in the sub-chronic PCP treated rats i.e. these rats' spent similar amounts of time exploring both objects. Acute treatment with clozapine at both doses (1.0 & 5.0 mg/kg) significantly attenuated the sub-chronic PCP-induced impairment such that a significant (P<0.05) increase in the time spent exploring the novel compared with the familiar object was again observed. A one-way ANOVA on the total exploration times in the retention trial of the NOR test showed a significant effect of drug treatment ($F_{3, 41}$=4.6, P<0.01;

table 5.10.). Post-hoc comparisons revealed a significant increase in total exploration time in the sub-chronic PCP treatment group that received an acute treatment with vehicle ($P<0.01$) and in the sub-chronic PCP treated group that received an acute treatment with clozapine (1.0 mg/kg; $P<0.05$).

5.3.7 Effect of acute clozapine on sub-chronic PCP treatment on the DI in the NOR test in female rats

One-way ANOVA on the DI data showed no significant effect of drug treatment on the rats' ability to discriminate between the familiar and novel objects ($F_{3, 41}=0.28$, NS; figure 5.7).

5.3.8 Effect of acute clozapine on sub-chronic PCP treatment on the number of line crossings in the NOR test in female rats

Figure 5.8 shows the effect of acute clozapine treatment in sub-chronically PCP treated rats on the total number of line crossings during the acquisition and retention trials of the NOR test. One-way ANOVA revealed no significant effect of any treatment on the number of line crossings ($F_{3, 41}=0.68$, NS).

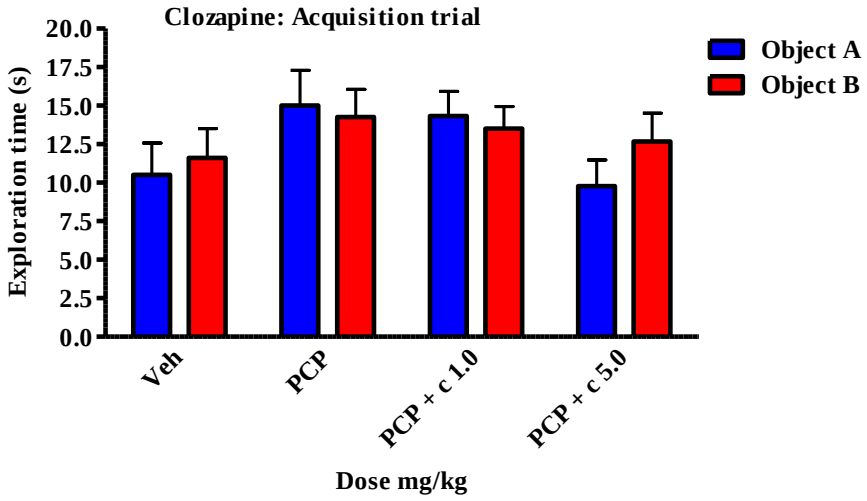


Figure 5.5 The effect of acute clozapine (c; 1.0 & 5.0 mg/kg, i.p) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of two identical objects in the 3 min acquisition trial. Data are expressed as the mean ± S.E.M. (n=8-12 per group).

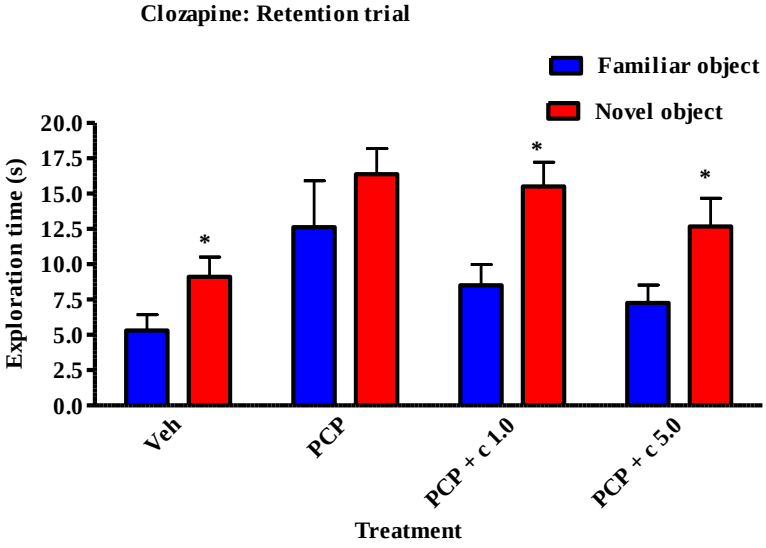


Figure 5.6 The effect of acute clozapine (c; 1.0 & 5.0 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p, twice daily for seven days) on exploration time (s) of a familiar object and a novel object in the 3 min retention trial. Data are expressed as the mean ± S.E.M. (n=8-12 per group) *P<0.05; significant increase in time spent exploring the novel compared with the familiar object.

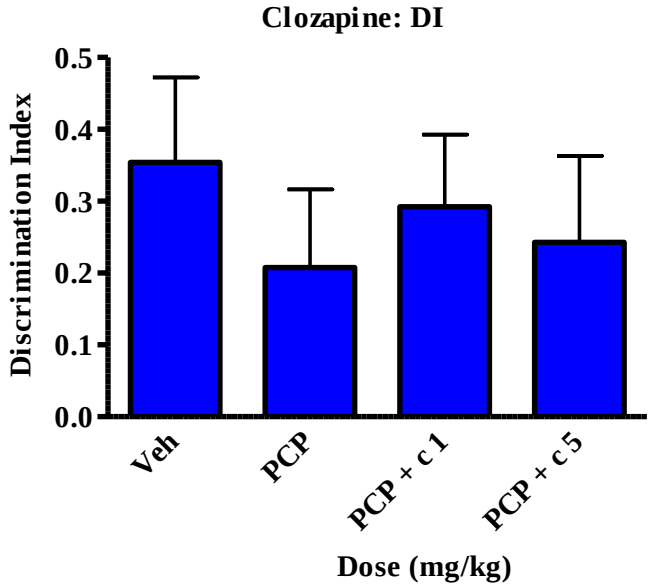


Figure 5.7 The effect of clozapine (c; 1.0-5.0 mg/kg, i.p) on sub-chronic PCP treatment on the DI in female rats. Data are expressed as the mean ± S.E.M. (n=8-12 per group).

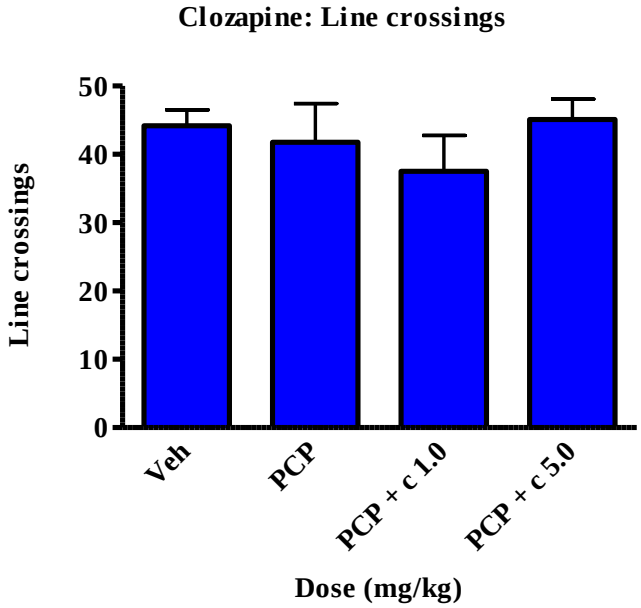


Figure 5.8 The effect of acute clozapine (c; 1.0 & 5.0 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p, twice daily for seven days) on the total number of line crossings in the acquisition and retention trial of the NOR test in female rats. Data are expressed as ± S.E.M. (n=8-12 per group).

Treatment (mg/kg)	*Total exploration time (s)*	
	Acquisition trial	*Retention trial*
Veh	22.1 ± 3.8	14.4 ± 2.0
PCP	29.3 ± 3.5	29.0 ± 4.4**
PCP + c 1.0	27.8 ± 3.0	25.2 ± 2.6*
PCP + c 5.0	22.4 ± 3.1	19.2 ± 2.9

Table 5.10 The effect of acute clozapine (c; 1.0 & 5.0 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p. twice daily for seven days) on the total exploration time in the acquisition and retention trial of the NOR test in female rats. Data are expressed as the mean ± S.E.M. (n=8-12 per group). *P<0.05-**P<0.01; significant increase in total exploration time compared to vehicle.

5.3.9 Effect of acute risperidone on sub-chronic PCP treatment in the acquisition trial in the NOR test in female rats

A two-way ANOVA revealed that administration of risperidone (0.05 – 0.2 mg/kg) to rats treated with sub-chronic PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{, 43}$=1.85, NS). Rats from both treatment groups spent similar times exploring both the objects (figure 5.9). A one-way ANOVA showed no significant ($F_{4, 47}$=1.9, NS) effect of drug treatment on the total exploration time in the acquisition trial of the NOR test (table 5.11).

5.3.10 Effect of acute risperidone on sub-chronic PCP treatment in the retention trial in the NOR test in female rats

A two-way ANOVA revealed that administration of risperidone (0.05 – 0.2 mg/kg) to rats treated with sub-chronic PCP produced a significant effect on object exploration in the retention trial of the NOR test ($F_{4, 43}$=4.1, P<0.01; figure 5.10).

Planned post-hoc comparisons showed that rats treated sub-chronically with vehicle spent significantly ($P<0.05$) more time exploring the novel objects during the retention trial. However, this significant preference for the novel compared to the familiar object was not observed in the sub-chronic PCP treated rats and also in the sub-chronic PCP treated rats administered with the two lowest doses of risperidone (0.05 & 0.1 mg/kg) i.e. these rats spent similar amounts of time exploring both objects. Treatment with risperidone at the highest dose (0.2 mg/kg) significantly attenuated the sub-chronic PCP-induced impairment such that a significant ($P<0.05$) increase in time spent exploring the novel compared with the familiar object was again observed. A one-way ANOVA showed no significant ($F_{4, 47}=0.5$) effect of drug treatment on the total exploration time in the retention trial of the NOR test (table 5.11).

5.3.11 Effect of acute risperidone on sub-chronic PCP treatment on the DI in the NOR test in female rats

One-way ANOVA on the DI data showed a significant effect of drug treatment on the rats' ability to discriminate between the familiar and novel objects ($F_{4,43}=6.8$, 0.001; figure 5.11). Post-hoc analysis showed a significant ($P<0.001$) reduction in the DI of the sub-chronic PCP treated group compared to vehicle control. Sub-chronic PCP rats treated with risperidone (0.05 & 0.1 mg/kg) also showed a significant ($P<0.01$) reduction in DI compared to vehicle control. Following treatment with risperidone (0.1 & 0.2 mg/kg) a significant ($P<0.05$-$P<0.001$, respectively) reversal of the sub-chronic PCP-induced reduction in DI was observed.

5.3.12 Effect of acute risperidone on sub-chronic PCP treatment on the number of line crossings in the NOR test in female rats

Figure 5.12 shows the effect of acute risperidone treatment in sub-chronically PCP treated rats on the total number of line crossings during the acquisition and retention trials of the NOR test. One-way ANOVA revealed no significant effect of any treatment on the number of line crossings ($F_{4, 47}$=0.58, NS).

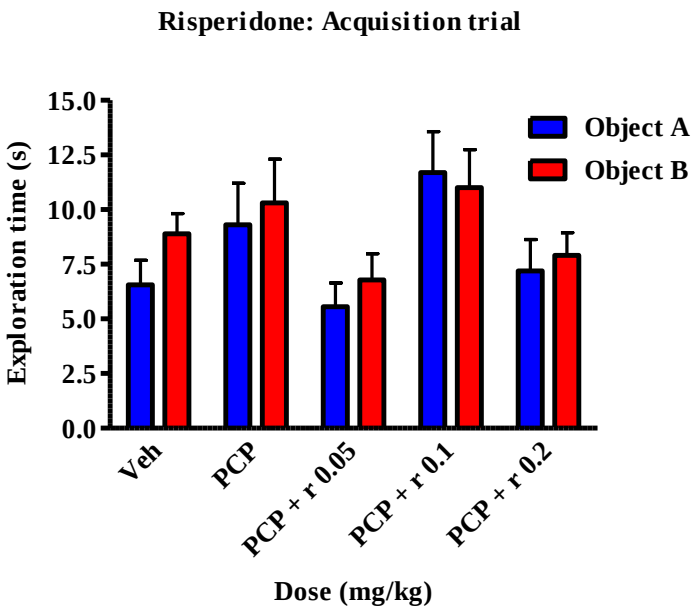


Figure 5.9 The effect of acute risperidone treatment (r; 0.05-0.2 mg/kg, i.p) and sub-chronic PCP (2.0 mg/kg, i.p, twice daily for seven days) on exploration time (s) of two identical objects in the 3 min retention trial. Data are expressed as the mean ± S.E.M. (n=8-11 per group).

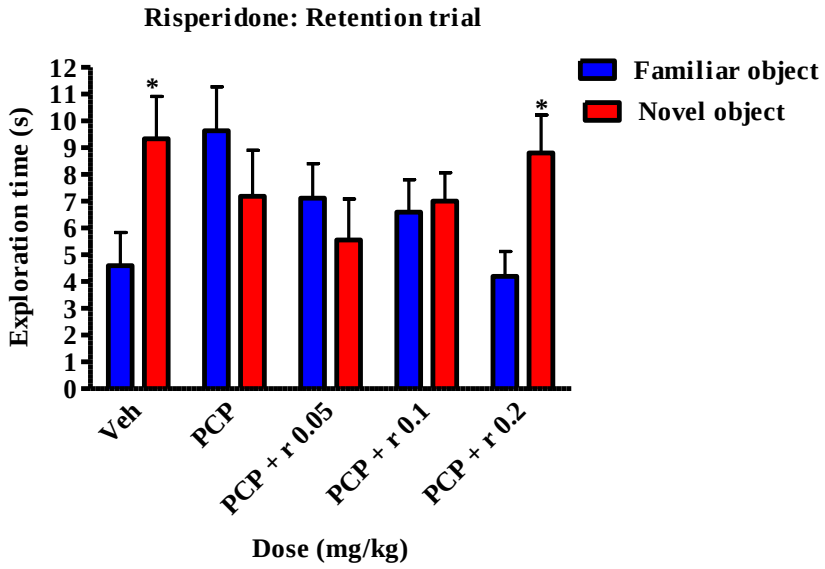


Figure 5.10 The effect of acute risperidone treatment (r; 0.05-0.2 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p, twice daily for seven days) on exploration time (s) of a familiar object and a novel object in the 3 min retention trial. Data are expressed as the mean ± S.E.M. (8-11 per group). P<0.05; significant increase in time spent exploring the novel compared with the familiar object.

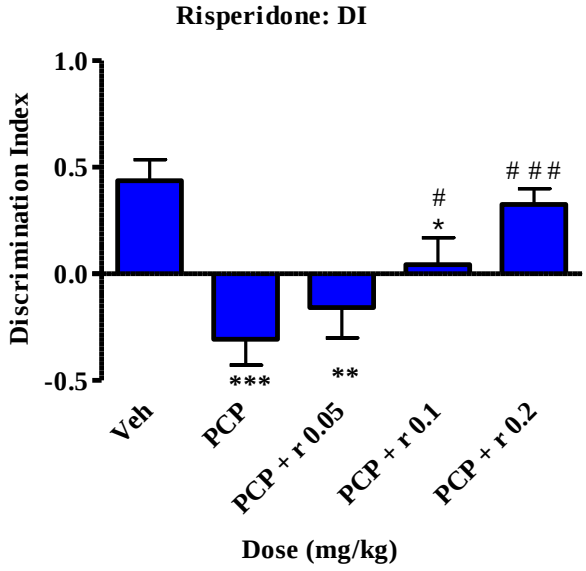


Figure 5.11 The effect of risperidone (r; 0.05-0.2 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on the DI. Data are expressed as the mean ± S.E.M. (n=8-11 per group). **P<0.01-***P<0.001; significant reduction in DI compared to vehicle. #P<0.05-P<0.001; significant reversal of the reduction in DI compared to sub-chronic PCP.

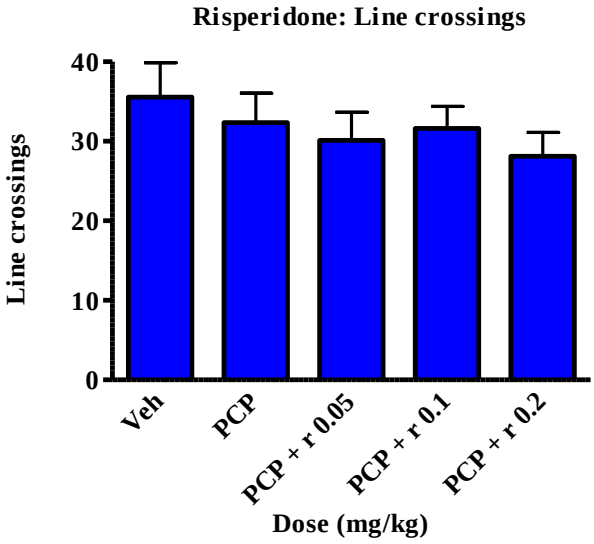


Figure 5.12 The effect of acute risperidone (r; 0.05-0.2 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p, twice daily for seven days) on the total number of line crossings in the acquisition and retention trial of the NOR test in female rats. Data are expressed as mean ± S.E.M. (n=8-11 per group).

Treatment (mg/kg)	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
Veh	15.4 ± 2.0	13.0 ± 2.7
PCP	19.6 ± 3.8	15.1 ± 2.7
PCP + r 0.05	12.3 ± 2.2	13.6 ± 2.3
PCP + r 0.1	22.7 ± 3.5	13.7 ± 2.0
PCP + r 0.2	15.1 ± 2.4	13.0 ± 2.1

Table 5.11 The effect of acute risperidone (r; 0.05-0.2 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p. twice daily for seven days) on the total exploration time in the acquisition and retention trial of the NOR test in female rats . Data are expressed as the mean ± S.E.M. (n=8-11 per group).

5.3.13 Effect of acute CDP on sub-chronic PCP treatment in the acquisition trial in the NOR test in female rats

A two-way ANOVA revealed that administration of CDP (1.25 – 5.0 mg/kg) to rats treated with sub-chronic PCP did not produce a significant effect on object exploration in the acquisition trial of the NOR test ($F_{4, 43}$=0.1, NS). Rats from both treatment groups spent similar times exploring both the objects (figure 5.13). A one way ANOVA on the total exploration times in the acquisition trial revealed no significant effect of drug treatment ($F_{4, 47}$= 0.56, NS; table 5.12).

5.3.14 Effect of acute CDP on sub-chronic PCP treatment in the retention trial in the NOR test in female rats

A two-way ANOVA revealed that administration of haloperidol (1.25 – 5.0 mg/kg) to rats treated with sub-chronic PCP produced a significant effect on object exploration in the retention trial of the NOR test ($F_{4, 43}$=0.65, NS; figure 5.14). Post-hoc comparisons showed that rats treated sub-chronically with vehicle spent significantly ($P<0.001$) more time exploring the novel objects during the retention trial. However this significant preference for the novel compared to the familiar object was not observed in the sub-chronic PCP treated rats and the sub-chronic PCP treated rats which were administered acute CDP i.e. these rats spent similar amounts of time exploring both objects. A one way ANOVA on the total exploration times in the retention trial revealed no significant effect of drug treatment ($F_{4, 47}$= 0.70, NS; table 5.11).

5.3.15 Effect of acute CDP on sub-chronic PCP treatment on the DI in the NOR test in female rats

One-way ANOVA on the DI data showed a significant effect of drug treatment on the rats ability to discriminate between the familiar and novel objects ($F_{4, 47}$=3.5, P<0.05; figure 5.15). Post-hoc analysis showed a significant reduction in the DI of the sub-chronic PCP treated group (P<0.05) compared to vehicle control. Sub-chronic PCP rats treated with the CDP (2.5 & 5.0 mg/kg) also showed a significant (P<0.05-P<0.001, respectively) reduction in DI compared to vehicle.

5.3.16 Effect of acute CDP on sub-chronic PCP treatment on the number of line crossings in the NOR test in female rats

Figure 5.16 shows the effect of CDP treatment in sub-chronically PCP treated rats on the total number of line crossings during the acquisition and retention trials of the NOR test. One-way ANOVA revealed no significant effect of any treatment on the total number of line crossings ($F_{4, 47}$=1.4, NS).

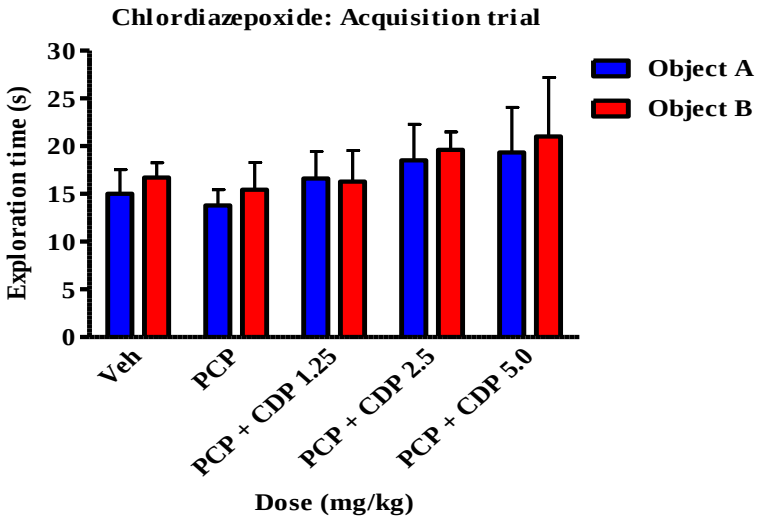


Figure 5.13 The effect of acute CDP (1.25 – 5.0 mg/kg, i.p.) and sub-chronic PCP- (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of identical objects in the 3min acquisition trial of the NOR test. Data are expressed as the mean ± S.E.M. (n=9-10 per group).

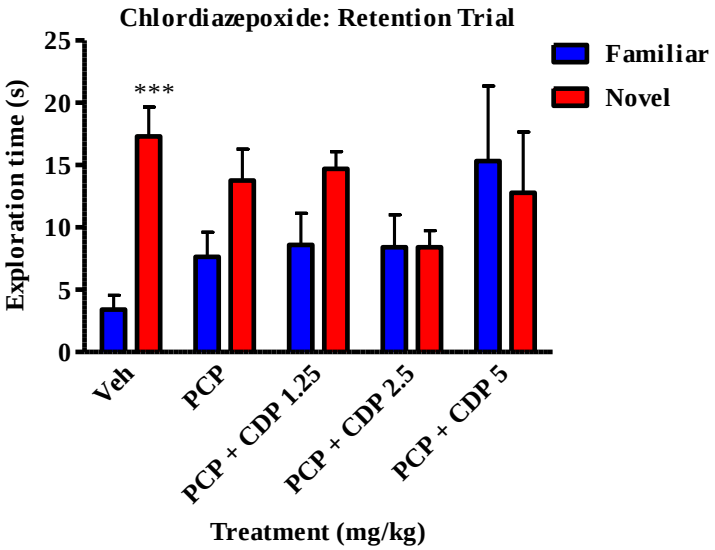


Figure 5.14 The effect of acute CDP (1.25 – 5.0 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of a familiar object and a novel object in the 3min retention trial. Data are expressed as the mean ± S.E.M. (n=9-10 per group). ***P<0.001; significant difference in time spent exploring the novel compared with the familiar object.

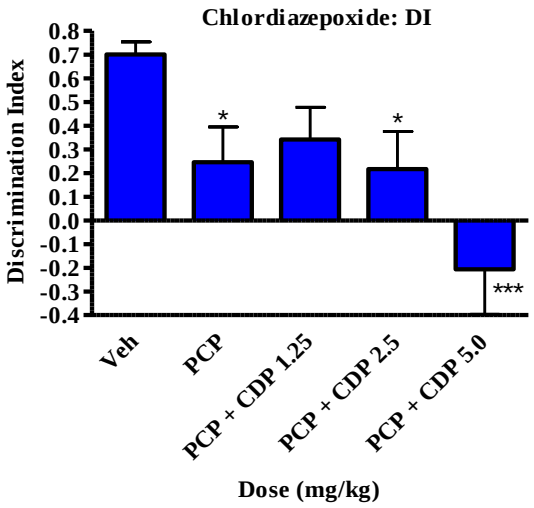


Figure 5.15 The effect of CDP (1.25 – 5.0 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on the DI. Data are expressed as the mean ± S.E.M. (n=9-10 per group). *P<0.05-***P<0.001; significant reduction in DI compared to vehicle.

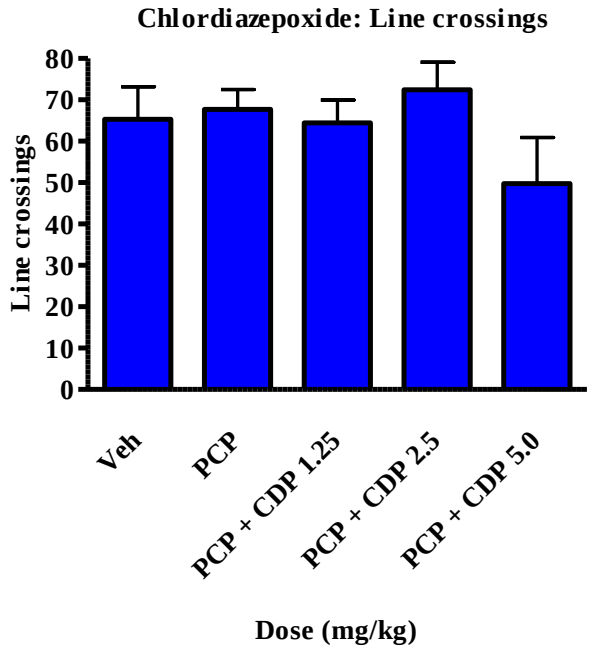


Figure 5.16 The effect of acute CDP (1.25 – 5.0 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on line crossings. Data are expressed as mean ± S.E.M. total number of line crossings in both acquisition and retention trial in the NOR test (n=9-10 per group).

Treatment (mg/kg)	*Total exploration time (s)*	
	Acquisition trial	*Retention trial*
Veh	31.7 ± 3.2	20.7 ± 3.3
PCP	29.2 ± 4.8	19.0 ± 4.5
PCP + CDP	32.9 ± 7.2	23.3 ± 3.1
PCP + CDP	38.1 ± 4.7	16.8 ± 4.0
PCP + CDP	40.1 ± 11.7	28.1 ± 12.6

Table 5.12 The effect of acute treatment with CDP (1.25 - 5.0 mg/kg, i.p.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on the total exploration time in acquisition and retention trial of the NOR test in female rats. Data are expressed as the mean ± S.E.M. (n=9-10 per group).

5.3.17 Effect of acute fluphenazine, fluoxetine, risperidone or modafinil on sub-chronic PCP treatment in the acquisition trial in the NOR test in female rats

An overall two-way ANOVA revealed that administration of fluphenazine (0.2 mg/kg), fluoxetine (5.0 mg/kg), risperidone (0.2 mg/kg) or modafinil (50 mg/kg) to rats treated with sub-chronic PCP did not produce a significant effect on object exploration in the acquisition trial of the NOR test ($F_{5, 46}$=0.4, NS). Rats from both treatment groups spent similar times exploring both the objects (figure 5.17). A one-way ANOVA on the total exploration times in the acquisition trial revealed no significant effect of drug treatment ($F_{5, 51}$= 0.44, NS; table 5.13).

5.3.18 Effect of acute fluphenazine, fluoxetine, risperidone or modafinil on sub-chronic PCP treatment in the retention trial in the NOR test in female rats

A two-way ANOVA revealed that administration of fluphenazine (0.2 mg/kg), fluoxetine (5.0 mg/kg), risperidone (0.2 mg/kg) or modafinil (50 mg/kg) to rats treated with sub-chronic PCP produced a significant effect on object exploration in the retention trial of the NOR test ($F_{5, 46}$=3.9, P<0.01; figure 5.18). Planned post-hoc comparisons showed that rats treated sub-chronically with vehicle spent significantly (P<0.01) more time exploring the novel objects during the retention trial. However this significant preference for the novel compared to the familiar object was not observed in the sub-chronic PCP treated rats and the sub-chronic PCP treated rats which were administered acute fluphenazine and fluoxetine i.e. these rats spent similar amounts of time exploring both objects. Acute treatment with risperidone and modafinil significantly attenuated the sub-chronic PCP-induced impairment such that a significant (P<0.05-P<0.01) increase in time spent exploring the novel compared with the familiar object was again observed. A one-way ANOVA on the total exploration times in the retention trial revealed no significant effect of drug treatment ($F_{5, 52}$=1.1, NS; table 5.13).

5.3.19 Effect of acute fluphenazine, fluoxetine, risperidone or modafinil on sub-chronic PCP treatment on the DI in the NOR test in female rats

A one-way ANOVA on the DI data showed a significant effect of drug treatment on the rats' ability to discriminate between the familiar and novel objects ($F_{5, 51}$=3.1, P<0.05; figure 5.19). Post-hoc analysis showed a significant reduction in the DI of

the sub-chronic PCP group treated with fluoxetine (P<0.01) compared to vehicle control.

5.3.20 Effect of acute fluphenazine, fluoxetine, risperidone or modafinil on sub-chronic PCP treatment on the number of line crossings in the NOR test in female rats

Figure 5.20 shows the effect of fluphenazine, fluoxetine, risperidone or modafinil treatment in sub-chronically PCP treated rats on the total number of line crossings during the acquisition and retention trials of the NOR test. One-way ANOVA revealed a significant effect of drug treatment on the number of line crossings ($F_{5, 51}$=4.9, P<0.01). Post-hoc analysis showed a significant (P<0.05) reduction in the total number of line crossings following treatment with risperidone.

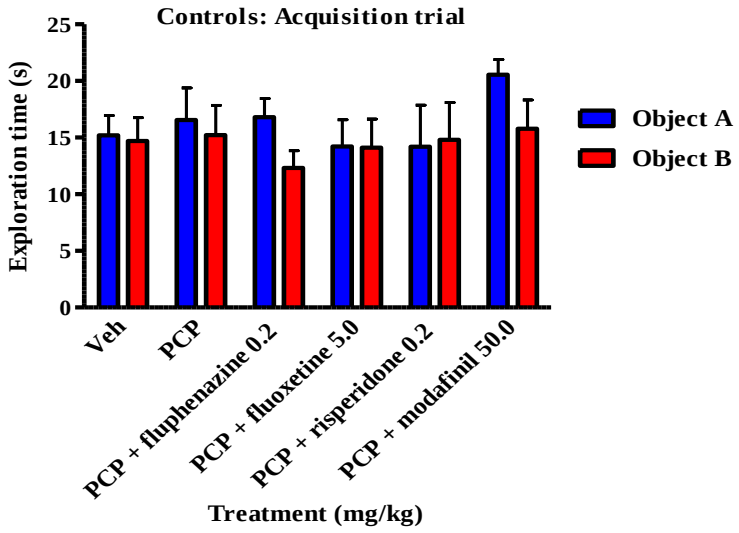


Figure 5.17 The effect of acute fluphenazine (0.2 mg/kg, i.p.), fluoxetine (5.0 mg/kg, i.p.), risperidone (0.2 mg/kg, i.p.) or modafinil (50.0 mg/kg, p.o.) and sub-chronic PCP- (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of identical objects in the 3min acquisition trial of the NOR test. Data are expressed as the mean ± S.E.M. (n=8-10 per group).

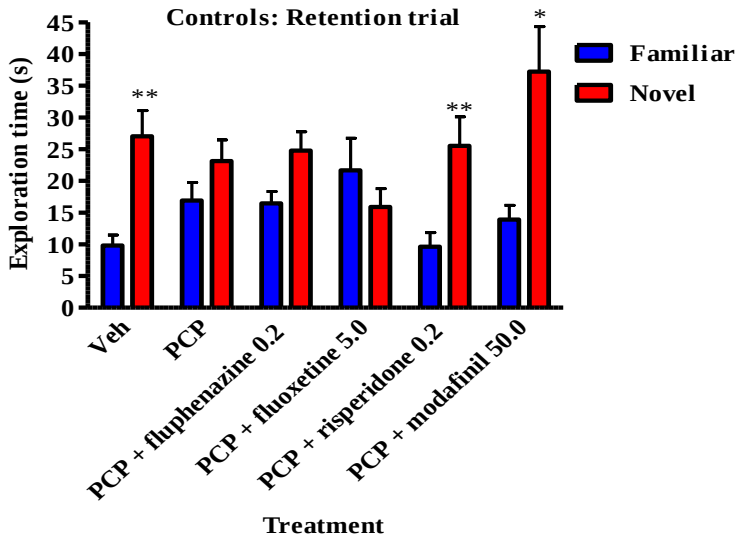


Figure 5.18 The effect of acute fluphenazine (0.2 mg/kg, i.p.), fluoxetine (5.0 mg/kg, i.p.), risperidone (0.2 mg/kg, i.p.) or modafinil (50.0 mg/kg, p.o.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of a familiar object and a novel object in the 3min retention trial. Data are expressed as the mean ± S.E.M. (n=8-10 per group). *P<0.05-**P<0.01; significant difference in time spent exploring the novel compared with the familiar object.

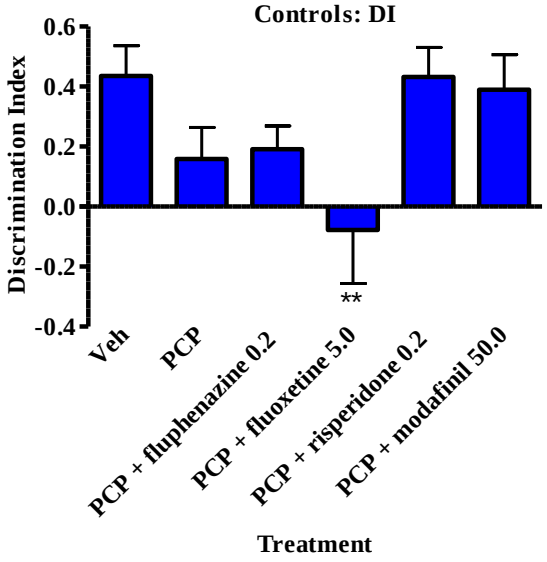


Figure 5.19 The effect of acute fluphenazine (0.2 mg/kg, i.p.), fluoxetine (5.0 mg/kg, i.p.), risperidone (0.2 mg/kg, i.p.) or modafinil (50.0 mg/kg, p.o.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on the DI. Data are expressed as the mean ± S.E.M. (n=8-10 per group). **P<0.01; significant reduction in DI when compared to vehicle.

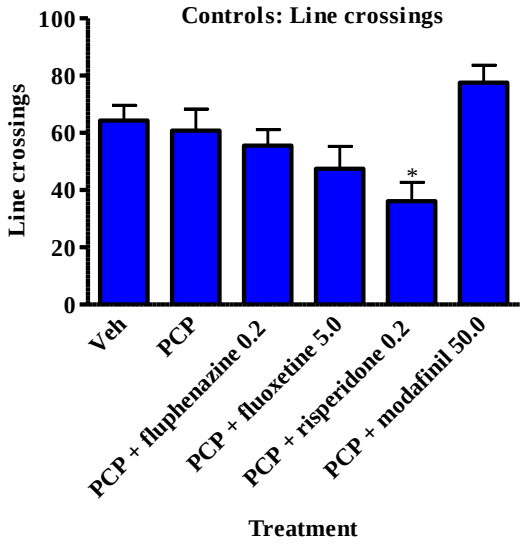


Figure 5.20 The effect of acute fluphenazine (0.2 mg/kg, i.p.), fluoxetine (5.0 mg/kg, i.p.), risperidone (0.2 mg/kg, i.p.) or modafinil (50.0 mg/kg, p.o.) and sub-chronic PCP (2.0 mg/kg, i.p., twice daily for seven days) on line crossings. Data are expressed as ± S.E.M. total number of line crossings in both acquisition and retention trial in the NOR test (n=8-10 per group). *P<0.05; significant reduction in the total number of line crossings compared to vehicle.

Treatment (mg/kg)	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
Veh	29.9 ± 3.2	36.8 ± 3.9
PCP	31.7 ± 4.7	40.0 ± 4.8
PCP + fluphenazine	28.7 ± 2.8	41.2 ± 3.1
PCP + fluoxetine	28.3 ± 4.8	37.6 ± 6.1
PCP + risperidone	29.0 ± 6.8	35.1 ± 6.2
PCP + modafinil	36.3 ± 3.2	51.1 ± 7.0

Table 5.13 The effect of acute fluphenazine (0.2 mg/kg, i.p.), fluoxetine (5.0 mg/kg, i.p.), risperidone (0.2 mg/kg, i.p.) or modafinil (50.0 mg/kg, p.o.) and sub-chronic PCP- (2.0 mg/kg, i.p., twice daily for seven days) on the total exploration time in acquisition and retention trial of the NOR test in female rats. Data are expressed as the mean ± S.E.M. (n=8-10 per group).

5.3.21 Effect of acute PNU-282987 and nicotine on sub-chronic PCP treatment in the acquisition trial in the NOR test in female rats

A two-way ANOVA revealed that administration of PNU-282987 (10 & 20 mg/kg) or nicotine (0.2 mg/kg) to rats treated with sub-chronic PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{4, 59}$=0.2, NS). Rats from all treatment groups spent similar times exploring both the objects (figure 5.21). A one-way ANOVA on the total exploration times in the acquisition trial revealed no significant effect of drug treatment ($F_{4, 63}$=1.3, NS; table 5.14).

5.3.22 Effect of acute PNU-282987 and nicotine on sub-chronic PCP treatment in the retention trial in the NOR test in female rats

A two-way ANOVA revealed that administration of PNU-282987 (10 & 20 mg/kg) or nicotine (0.2 mg/kg) to rats treated with sub-chronic PCP produced a significant effect on object exploration in the retention trial of the NOR test ($F_{4, 59}$=10.1, P<0.001; figure 5.22). Planned post-hoc comparisons showed that rats treated sub-chronically with vehicle spent significantly (P<0.001) more time exploring the novel objects during the retention trial. However, this significant preference for the novel compared to the familiar object was not observed in the sub-chronic PCP treated rats i.e. these rats spent similar amounts of time exploring both objects. Acute treatment with PNU-282987 at both doses (10 & 20 mg/kg) and nicotine (0.2 mg/kg) significantly attenuated the sub-chronic PCP-induced impairment such that a significant (P<0.01) increase in time spent exploring the novel compared with the familiar object was again observed. A one-way ANOVA on the total exploration

times in the retention trial revealed no significant effect of drug treatment ($F_{4, 63}$=1.1, NS; table 5.14).

5.3.23 Effect of acute PNU-282987 and nicotine on sub-chronic PCP treatment on the DI in the NOR test in female rats

One-way ANOVA on the DI data showed a significant effect of drug treatment on the rats ability to discriminate between the familiar and novel objects ($F_{4, 63}$=9.8, P<0.001; figure 5.23). Post-hoc analysis showed a significant reduction in the DI of the sub-chronic PCP treated group (P<0.01) compared to vehicle control. The sub-chronic PCP-induced reduction in DI was significantly (P<0.05 – P<0.001) restored following treatment with PNU-282987 (10 & 20 mg/kg; respectively) and nicotine (P<0.01).

5.3.24 Effect of acute PNU-282987 and nicotine on sub-chronic PCP treatment on the number of line crossings in the NOR test in female rats

Figure 5.24 shows the effect of PNU-282987 (10 & 20 mg/kg) or nicotine (0.2 mg/kg) treatment in sub-chronically PCP treated rats on the total number of line crossings during the acquisition and retention trials of the NOR test. A one-way ANOVA revealed no significant effect of any treatment on the number of line crossings ($F_{4, 63}$=1.5, NS).

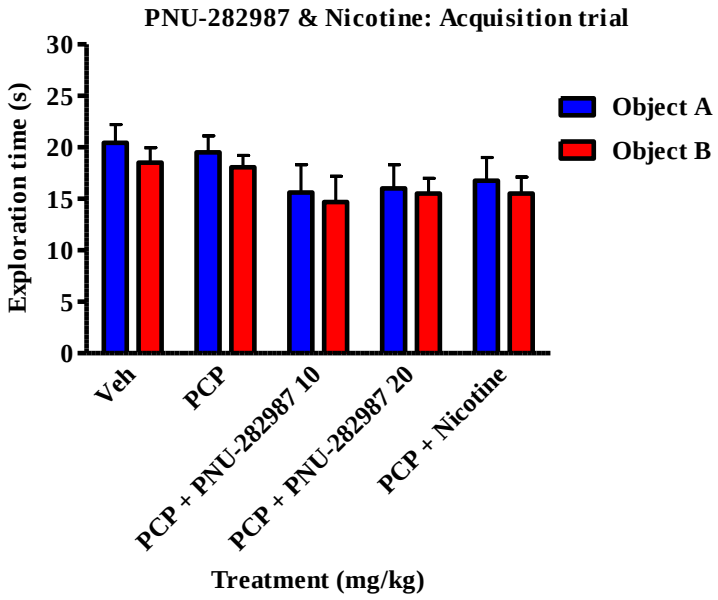


Figure 5.21 The effect of acute PNU-282987 (10 & 20 mg/kg, s.c.) and nicotine (0.2 mg/kg, i.p.) in rats treated sub-chronically with PCP (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of two identical objects in the 3 min acquisition trial of the NOR test. Data are expressed as the mean ± S.E.M. (n=10-18 per group).

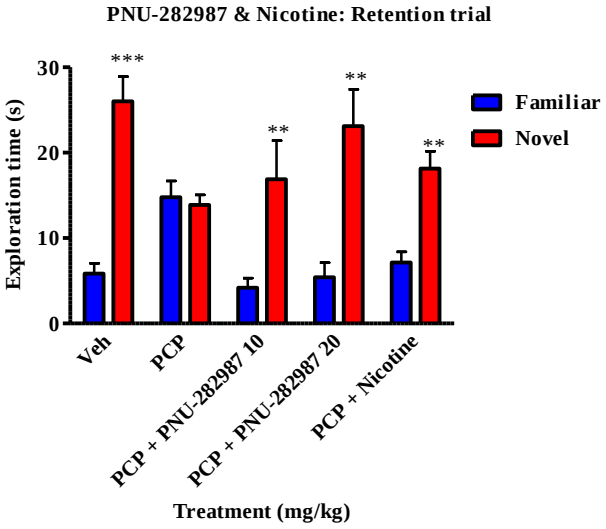


Figure 5.22 The effect of acute PNU (10 & 20 mg/kg, s.c.) or nicotine (0.2 mg/kg, i.p.) in rats treated sub-chronically with PCP (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of a familiar object and a novel object in the 3 min retention trial. Data are expressed as the mean ± S.E.M. (n=10-18 per group). **P<0.05-***P<0.001; significant increase in time spent exploring the novel compared with the familiar object.

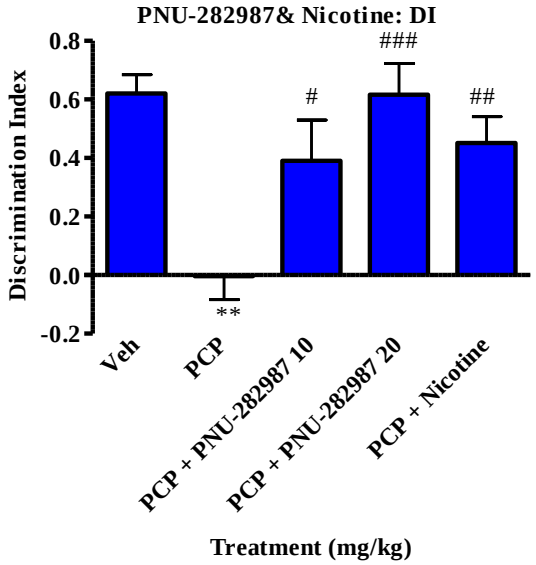


Figure 5.23 The effect of PNU-282987 (10 & 20 mg/kg, s.c.) and nicotine (0.2 mg/kg, i.p.) in rats treated sub-chronically with PCP (2.0 mg/kg, i.p., twice daily for seven days) on the DI. Data are expressed as the mean ± S.E.M. (n=10-18 per group). **P<0.01; significant reduction in DI compared to vehicle. #P<0.05- ###P<0.001; significant increase in DI compared to sub-chronic PCP.

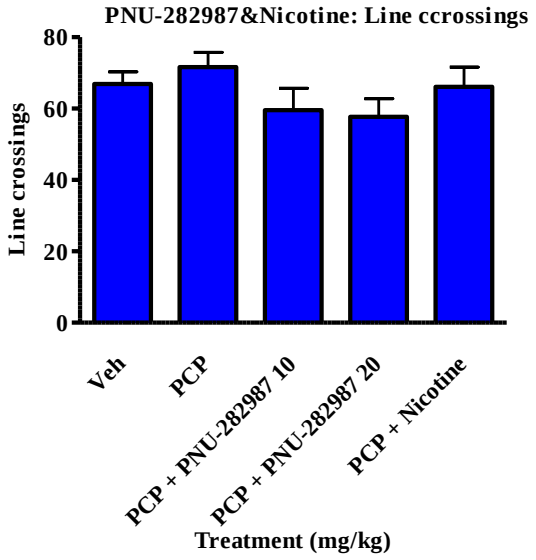


Figure 5.24 The effect of acute PNU-282987 (10 & 20 mg/kg, s.c.) and nicotine (0.2 mg/kg, s.c.) in rats sub-chronically treated with PCP (2.0 mg/kg, i.p., twice daily for seven days) on total number of line crossings in the acquisition and retention trial in the NOR task. Data are expressed as mean ± s.e.m total number of line crossings (n=10-18 per group).

Treatment (mg/kg)	*Total exploration time (s)*	
	Acquisition trial	*Retention trial*
Veh	38.9 ± 3.1	31.9 ± 3.6
PCP	37.6 ± 2.5	28.7 ± 2.0
PCP + PNU-282987 10	30.3 ± 5.1	21.1 ± 5.4
PCP + PNU-282987 20	31.5 ± 3.5	28.5 ± 5.5
PCP + nicotine 0.2	32.3 ± 2.5	25.2 ± 1.8

Table 5.14 The effect of acute PNU-282987 (10 & 20 mg/kg, s.c.) and nicotine (0.2 mg/kg, s.c.) in rats sub-chronically treated with PCP (2.0 mg/kg, i.p., twice daily for seven days) on total exploration time (s). Data are expressed as mean ± S.E.M. (n=10-18 per group).

5.3.25 Effect of acute PNU-120596 on sub-chronic PCP treatment in the acquisition trial in the NOR test in female rats

A two-way ANOVA revealed that administration of PNU-120596 (5-20 mg/kg) to rats treated with sub-chronic PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{4, 43}$=1.1, NS). Rats from all treatment groups spent similar times exploring both the objects (figure 5.25). A one-way ANOVA on the total exploration times in the acquisition trial revealed no significant effect of drug treatment ($F_{4, 47}$=0.4, NS; table 5.15).

5.3.26 Effect of acute PNU-120596 on sub-chronic PCP treatment in the retention trial in the NOR test in female rats

A two-way ANOVA revealed that administration of PNU-120596 (5-20 mg/kg) to rats treated with sub-chronic PCP produced a significant effect on object exploration in the retention trial of the NOR test ($F_{4, 43}$=3.4, P<0.05). Planned post-hoc comparisons showed that rats treated sub-chronically with vehicle spent significantly (P<0.05) more time exploring the novel objects during the retention trial. However, this significant preference for the novel compared to the familiar object was not observed in the sub-chronic PCP treated rats i.e. these rats spent similar amounts of time exploring both objects (figure 5.26). Acute treatment with PNU-120596 at 10 mg/kg significantly attenuated the sub-chronic PCP-induced impairment such that a significant (P<0.05) increase in time spent exploring the novel compared with the familiar object was again observed. A one-way ANOVA on the total exploration times in the retention trial revealed no significant effect of drug treatment ($F_{4, 47}$=1.8, NS; table 5.15).

5.3.27 Effect of acute PNU-120596 on sub-chronic PCP treatment on the DI in the NOR test in female rats

One-way ANOVA on the DI data showed a significant effect of drug treatment on the rats ability to discriminate between the familiar and novel objects ($F_{4, 47}$=4.9, P<0.01; figure 5.27). Post-hoc analysis showed a significant reduction in the DI of the sub-chronic PCP treated group (P<0.01) compared to vehicle control. The sub-chronic PCP-induced reduction in DI was significantly (P<0.001 - P<0.05) restored following treatment with PNU-120596 (10 & 20 mg/kg; respectively).

5.3.28 Effect of acute PNU-120596 on sub-chronic PCP treatment on the number of line crossings in the NOR test in female rats

Figure 5.28 shows the effect of PNU-120596 (5-20 mg/kg) treatment in sub-chronically PCP treated rats on the total number of line crossings during the acquisition and retention trials of the NOR test. A one-way ANOVA revealed no significant effect of any treatment on the number of line crossings ($F_{4, 47}$=1.8, NS).

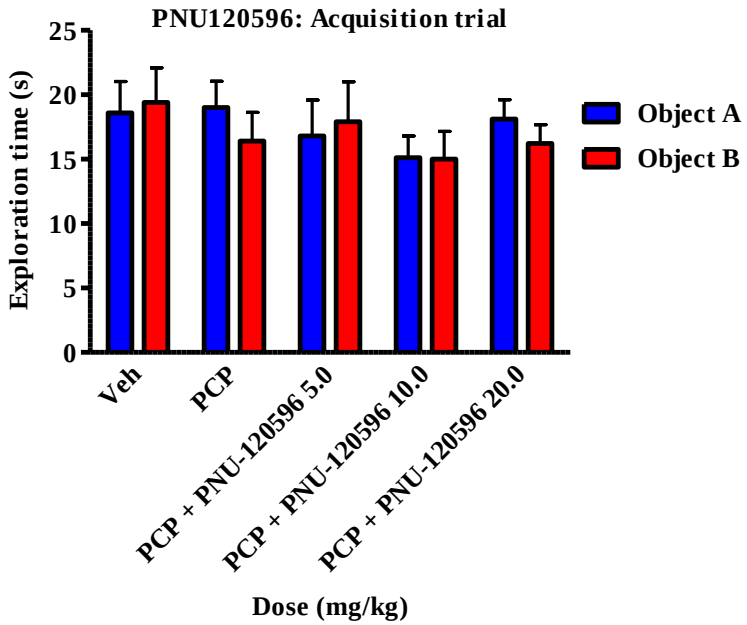


Figure 5.25 The effect of acute PNU-120596 (5.0 - 20.0 mg/kg, p.o.) in rats treated sub-chronically with PCP (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of two identical objects in the 3 min acquisition trial of the NOR test. Data are expressed ± S.E.M. (n=9-10 per group).

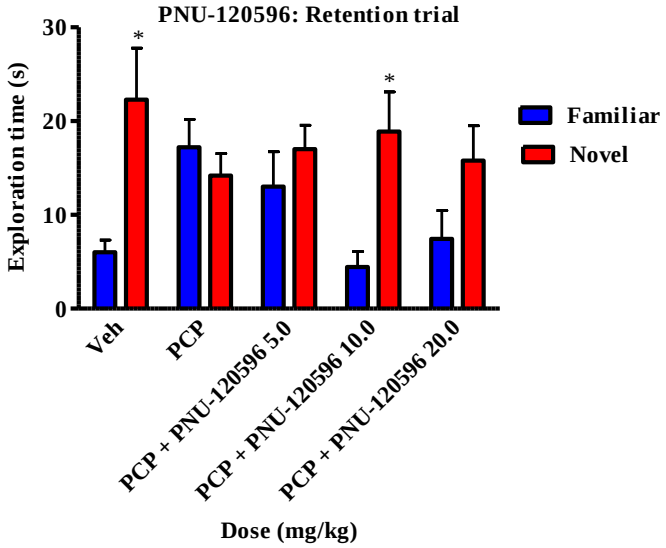


Figure 5.26 The effect of acute PNU-120596 (5.0 - 20.0 mg/kg, p.o.) in rats treated sub-chronically with PCP (2.0 mg/kg, i.p., twice daily for seven days) on exploration time (s) of a familiar object and a novel object in the 3 min retention trial of the NOR test. Data are expressed ± S.E.M. (n=9-10 per group). *P<0.05; significant increase in the time spent exploring the novel compared to the familiar object.

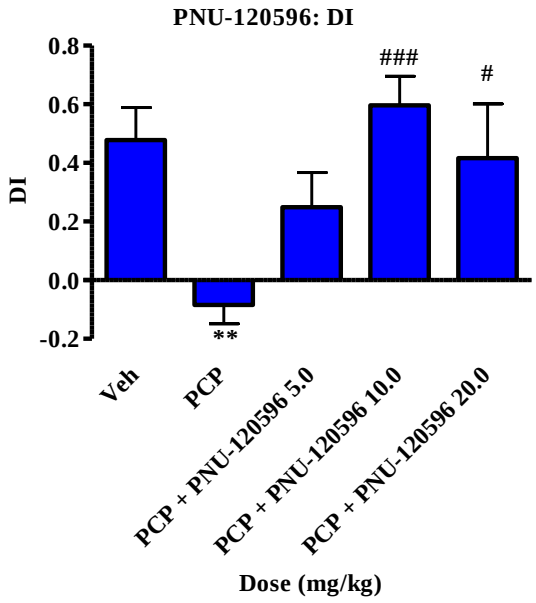


Figure 5.27 The effect of PNU-120596 (5 - 20 mg/kg, p.o.) in rats treated sub-chronically with PCP (2.0 mg/kg, i.p., twice daily for seven days) on the DI. Data are expressed as the mean ± S.E.M. (n=9-10 per group). **P<0.01; significant reduction in DI compared to vehicle. #P<0.05 - ###P<0.001; significant reversal of reduction in DI compared to sub-chronic PCP.

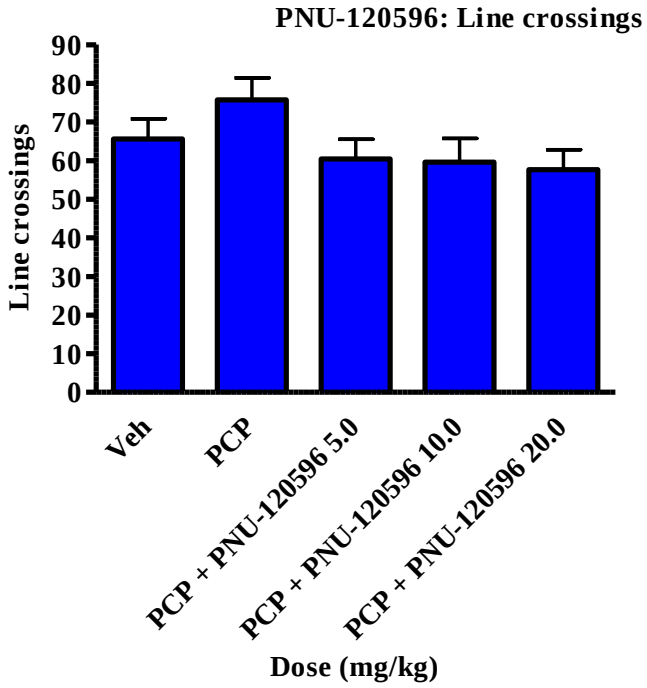


Figure 5.28 The effect of acute PNU-120596 (5 - 20 mg/kg, p.o.) in rats sub-chronically treated with PCP (2.0 mg/kg, i.p., twice daily for seven days) on total number of line crossings in the acquisition and retention trial in the NOR task. Data are expressed as mean ± s.e.m. total number of line crossings (n=9-10 per group).

Treatment (mg/kg)	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
Veh	38.0 ± 5.0	29.3 ± 5.7
PCP	35.4 ± 4.1	32.4 ± 4.9
PCP + PNU-120596 5	34.7 ± 5.4	29.3 ± 5.6
PCP + PNU-120596 10	30.1 ± 3.4	23.3 ± 4.0
PCP + PNU-120596 20	34.3 ± 2.7	23.2 ± 3.9

Table 5.15 The effect of acute PNU-120596 (5-20 mg/kg, p.o.) in rats sub-chronically treated with PCP (2.0 mg/kg, i.p., twice daily for seven days) on the total exploration time (s). Data are expressed as mean ± S.E.M. (n=9-10 per group).

Treatments	*Doses tested (mg/kg)*	*Deficit in the retention trial. Dose (mg/kg)*	*Deficit in the DI. Dose (mg/kg)*	*Effect on line crossings. Dose (mg/kg)*	*Effect on total exploration time. Dose (mg/kg)*
Sub-chronic PCP	**2.0**	**Yes**	**Yes** (Except clozapine study)	**No**	**No**
Haloperidol	**0.05, 0.075**	**Yes** 0.05, 0.075	**Yes** 0.05, 0.075	**No** 0.05, 0.075	**No** 0.05, 0.075
Clozapine	**1, 5**	**No** 1, 5	**No** 1, 5	**No** 1, 5	***Yes** (retention; PCP, 1) **No** 5
Risperidone	**0.05, 0.1, 0.2**	**Yes** 0.05, 0.1 No 0.2	**Yes** 0.05 No 0.1, 0.2	**No** 0.05, 0.1, 0.2	**No** 0.05, 0.1, 0.2
CDP	**1.25, 2.5, 5.0**	**Yes** 1.25, 2.5, 5.0	**No** 1.25, 2.5 **Yes** 5.0	**No** 1.25, 2.5, 5.0	**No** 1.25, 2.5, 5.0
Fluphenazine	**0.2**	**Yes**	**Yes**	**No**	**No**
Fluoxetine	**5.0**	**Yes**	**Yes**	**No**	**No**
Modafinil	**50**	**No**	**No**	**No**	**No**
PNU-282987	**10, 20**	**No**	**No**	**No**	**No**
Nicotine	**0.2**	**No**	**No**	**No**	**No**
PNU-120596	**5, 10, 20**	**Yes** 5, 20 **No** 10	**No**	**No**	**No**

Table 5.16 Summary table showing the effect of sub-chronic treatment with PCP (2.0 mg/kg, i.p., twice daily for seven days) and haloperidol (0.05-0.075 mg/kg), clozapine (1.0-5.0 mg/kg), risperidone (0.05-0.2 mg/kg), CDP (1.25-5.0 mg/kg), fluphenazine (0.2 mg/kg), fluoxetine (5.0 mg/kg), modafinil (50 mg/kg), PNU-282987 (10 & 20 mg/kg), nicotine (0.2 mg/kg) and PNU-120596 (5-20 mg/kg) on female rats on performance in the NOR test. Significant increase (*$P<0.05$-$P<0.01$) compared to the vehicle treated group.

5.4 Discussion

5.4.1 Pharmacology

The sub-chronic PCP-induced object recognition deficits presented in this chapter were robust and consistent, which enabled pharmacological intervention to assist in validation of the sub-chronic PCP impairments in the NOR test and assessment of its use as a test for detecting efficacy of novel targets for this aspect of cognitive dysfunction in schizophrenia.

5.4.2 Haloperidol, clozapine and risperidone

Acute administration of the classical antipsychotic haloperidol was ineffective at reversing the recognition memory deficit induced by sub-chronic PCP in the NOR test. Conversely, acute treatment with the atypical antipsychotic agents, clozapine and risperidone successfully restored the sub-chronic PCP-induced deficit. These results are in agreement with NOR studies performed in mice by Hashimoto et al (2005), whereby NOR deficits were induced by repeated administration of PCP and this deficit was significantly improved by sub-chronic administration of clozapine but not haloperidol. Furthermore, the atypical antipsychotic agents clozapine, olanzapine and ziprasidone were effective at restoring the sub-chronic PCP-induced deficits in the reversal learning task (Abdul-Monim et al, 2003, 2006). The ability of clozapine and risperidone to improve the sub-chronic PCP-induced impairments in recognition memory in the NOR test in the rat are in agreement with some clinical findings stating that clozapine and risperidone can improve certain aspects of cognition in certain schizophrenia patients (Meltzer & McGurk, 1999; Keefe et al, 1999). In marked contrast to these effects, acute administration of the classical

agents haloperidol and chlorpromazine failed to significantly reverse the PCP-induced cognitive impairment in reversal learning in the rat (Abdul-Monim et al, 2006). The pharmacological binding profile of clozapine and risperidone may explain the pro-cognitive effects compared to the lack of effect of the classical agents to reverse the sub-chronic PCP-induced deficits in the NOR test. It has been shown that clozapine and risperidone have a high affinity for 5-HT receptors, particularly the 5-HT_{2A} receptor, whereas haloperidol has low affinity for 5-HT_{2A} receptors and high affinity for D_2 receptors, suggesting involvement of the 5-HT_{2A} receptor in cognition. Atypical antipsychotics have been shown to elevate mPFC dopamine levels via blockade of 5-HT_{2A} and D_2 receptors to promote 5-HT_{1A} stimulation to increase dopamine release (Ichikawa et al, 2001). This increased dopamine release in the mPFC has been hypothesized to contribute to the ability to improve negative symptoms and some domains of cognition in schizophrenia (Moghaddam and Bunney, 1990; Kuroki et al, 1999; Meltzer and McGurk. 1999).

The ability of the atypical antipsychotics, cognitive enhancers and novel agents but not the classical antipsychotics, antidepressants and anxiolytics to reverse the sub-chronic PCP induced recognition deficits in the NOR test provide important predictive validity. However it is of importance to note that atypical antipsychotics such as clozapine, demonstrated by the clinical Antipsychotic Trials of Invention Effectiveness (CATIE) study showed a lack of ability compared to classical agents to improve the cognitive symptoms associated with schizophrenia (Lieberman, 2006; Keefe et al, 2007). This difference between the results obtained in this thesis and such clinical findings is vital. It could be argued that sub-chronic PCP in combination with the NOR test does not accurately represent the clinical situation,

given the lack of efficacy of atypical antipsychotics for improving cognitive deficits in schizophrenia. It is important to bear in mind that we are using animals to mimic an aspect of a complex human disease where patients have genetic and neurodevelopmental predispositions for the disorder, may have co-morbid drug abuse, and are commonly prescribed combinations of different pharmacological agents. In contrast, our rats have no genetic predisposition and no previous drug exposure. Instead they live in optimal conditions (lighting, noise level, humidity etc.) for that species with minimum stress. This makes it even harder to fully mimic a human disorder (particularly a psychiatric disorder) and this caveat is important to recognise (see Moore 2010 for a full review of this issue and Neill et al, 2010).

5.4.3 Fluphenazine

In agreement with the reversal learning studies (Abdul-Monim et al, 2006), the inability of the classical antipsychotic haloperidol to ameliorate sub-chronic PCP-induced deficits in NOR was further established with the use of another classical agent fluphenazine. In the clinical setting, classical antipsychotic agents such as haloperidol, chlorpromazine and fluphenazine are effective at relieving the positive symptoms of schizophrenia and are known to exacerbate cognitive and negative symptomatology (Keefe et al, 1999; Meltzer et al, 1999). Further studies using carefully selected pharmacological agents in the sub-chronic PCP treated rats were conducted in order to provide further validation of the sub-chronic PCP-induced deficit in the NOR test.

5.4.4 Chlordiazepoxide

The benzodiazepine, anxiolytic agent chlordiazepoxide which binds to the $GABA_A$ receptor was shown to be ineffective in this study to reverse the sub-chronic PCP-induced impairments in NOR. The evidence available on the effect of benzodiazepines on cognition in patients with schizophrenia is inconclusive (Baandrup et al, 2011). However, clinical studies (for review see Lister, 1985) have shown that benzodiazepine treatment impairs cognition, particularly long-term episodic memory in healthy controls. A pre-clinical study in rats, looking at the effects of intra-perirhinal cortex injections of the benzodiazepine lorazepam demonstrated impairments of recognition memory (Wan et al, 2004). Results of a NOR study in mice by Bertaina-Anglade (2006), provide further support for the lack of effect of CDP to reverse the sub-chronic PCP-induced deficit in NOR, whereby alprazolam and diazepam prior to the acquisition trial of the NOR test induced impairments in object recognition memory.

5.4.5 Fluoxetine

Following acute treatment with the selective serotonin re-uptake inhibitor (SSRI) antidepressant agent fluoxetine, no improvement of the sub-chronic PCP-induced cognitive deficit was observed; indeed the very poor discriminatory ability of the rats following fluoxetine treatment may have exacerbated the sub-chronic PCP-induced impairments. These results are supported by the clinical data, whereby clinically depressed patients exhibit SSRI-induced deficits in episodic memory (Wadsworth et al, 2005). Furthermore, one study has demonstrated no significant cognitive improvements following adjunctive treatment with the SSRI citalopram added to

atypical medications in patients with schizophrenia (Friedman et al, 2005). Conversely, fluoxetine has been shown to induce cognitive impairments (Mirrow, 1991; Bangs, 1994) and also to improve cognition (Cassano et al, 2002; Doraiswamy et al, 2003) in elderly patients suffering from depression.

5.5.6 Modafinil

In this study, administration of the analeptic agent modafinil which is thought in part to exert its effects via the hypocretin/orexin system was effective in restoring the cognitive deficit induced by sub-chronic PCP. These results support the findings of Dawson et al (2010) and Goetghebeur & Dias (2009), who demonstrated a reversal of sub-chronic PCP-induced impairments in ability to switch attentional set by modafinil. Additionally, acute treatment with modafinil has been shown to ameliorate the PCP-induced deficit in novel object exploration (Redrobe et al, 2010). The pre-clinical data is further substantiated by the promising pro-cognitive effects of modafinil treatment in some specific groups of schizophrenia patients (see review by Morein-Zamir et al, 2007).

5.5.7 Nicotine

Acute administration of the nicotinic receptor agonist, nicotine to the sub-chronic PCP-treated rats was effective at restoring the object recognition deficit. There is extensive literature indicating nicotine's pro-cognitive effects in brain lesion models of cognitive impairment (Decker et al, 1992; Grigoryan et al, 1994), and muscarinic and nicotine antagonist mecamylamine-induced impairments (Zarrindast et al, 1996). Additionally, chronic nicotine treatment has been shown to have pro-cognitive effects in normal rats as measured by the 5CSRTT (Amitai & Markou, 2009).

However, chronic nicotine treatment was ineffective in reversing the sub-chronic PCP-induced cognitive deficits in 5CSRTT (Amitai & Markou, 2008). In another study by Rushforth et al, (2010), nicotine, dose dependently reversed working memory deficits in the odour span test in sub-chronic ketamine treated rats. Research on administration of nicotine to patients with schizophrenia has shown that single administration improves working and declarative memory, whereas second administrations of nicotine are not as effective, due to tachyphylaxis (Tamminga, 2006). Nicotinic receptors appear to modulate neurotransmitter release to improve cognition which suggests that the nicotinic receptor has a modulatory role with respect to cognition. Moreover, it is reported that schizophrenia patients are very heavy cigarette smokers, allegedly self medicating to alleviate side effects of antipsychotics, enhance the therapeutic effects of antipsychotics in alleviating the negative symptoms and to ameliorate the cognitive deficits associated with schizophrenia (Kumari & Postma, 2005). Nicotine may improve cognition by direct effects on attention and by interacting with the presynaptic nAChR to facilitate the release of ACh, dopamine, noradrenaline, glutamate, 5-HT, and GABA, neurotransmitters that have all been implicated in learning and memory (Samuels & Davis 1998; Wonnacott 1997).

5.5.8 PNU-282987 (α7 full agonist)

One of the therapeutic targets identified by the MATRICS is the α7-nicotinic acetylcholine receptor (nAChR). Results presented in thesis show that acute treatment with PNU-282987, a selective full agonist of the human and rat α7 nAChR, improved the sub-chronic PCP-induced impairment in object recognition. There was no effect in the acquisition trial or on the total number of line crossings which

suggests specificity of PNU-282987 for the retention trial without any sedation. These results are supported by work undertaken in our laboratory, whereby, PNU-282987 selectively reversed the sub-chronic PCP-induced impairments in reversal learning (McLean et al, 2011). This compound has previously been shown to reduce amphetamine induced gating deficits in PPI and also improved a scopolamine-induced deficit in a continuous Y-maze task in mice at 10 mg/kg (Redrobe et al., 2009). Results are further substantiated by the work of Buccafusco and Terry (2009) in pigtail monkeys (Macaca nemestrina), whereby the α7 nicotinic receptor agonist GTS-21, produced a dose-dependent attenuation of ketamine-induced decreases in task accuracies in a delayed matching-to-sample test (DMTS). In fact, the highest dose of GTS-21 completely reversed the effects of ketamine. Also, the partial α7 agonist, AZD0328, has been shown to improve cognitive performance in a delayed spatial response task using the Wisconsin General Testing Apparatus in rhesus macaques (*Macaca mulatta*) (Castner et al, 2010).

It has been shown from post-mortem studies that schizophrenia patients have decreased expression of α7 nAChR at the two major sites of expression in the brain, the inhibitory interneurons of the hippocampus and of the nucleus reticularis thalami (Freedman et al, 2008). Activation of the α7 nAChR by PNU-282987 at 1 mg/kg i.v., induced an enhancement in amphetamine-induced theta and gamma oscillations in the CA3 region of the hippocampus and entorhinal cortex; these frequencies of oscillations and these brain regions are believed to be important for cognitive processing (Hajós et al, 2005; Hoffmann et al, 2005). Furthermore, studies in our laboratory have shown that gamma oscillations are reduced following sub-chronic PCP treatment in the CA3 region of the hippocampus (McLean et al, 2009).

5.5.9 PNU-120596 (PAM)

Results presented in this thesis also show that the α7 positive allosteric modulator (PAM) PNU-120596 was effective in reversing the sub-chronic PCP-induced deficit in NOR, which is supported by previous work in our laboratory, showing that PNU-120596 was successful in reversing a sub-chronic PCP-induced cognitive impairment in the attentional set-shifting task (McLean et al, 2011). The pro-cognitive effects of PNU-120596 on the sub-chronic PCP-induced recognition memory deficits in the NOR test are supported by a study showing that PNU-120596

increased the potency of ACh by ~10-fold and reversed an amphetamine-induced deficit in auditory gating in rats (Hurst et al, 2005). PNU-120596 has also been reported to enhance the increase in dopamine release in the mPFC induced by choline and an α7 nAChR agonist (compound A; (R)-N-(1-azabicyclo[2.2.2]oct-3-yl)(5-(2-pyridyl)thiophene-2-carboxamide); it was also shown that PNU-120596 when administered systemically (but not locally) produced an increase in dopamine overflow in the mPFC in the absence of an agonist (Livingstone et al, 2009). This is important because the PCP-induced deficit in NOR memory is accompanied by impaired dopamine neurotransmission in the PFC during the retention trial of the task (Snigdha et al, 2008).

CHAPTER 6 - Investigation of NOR deficits produced by another animal model of schizophrenia symptomatology: Rats reared in social isolation

6.1 Introduction

An important finding of neurodevelopment research is that the basic architecture of the brain is set in-utero, but the wiring of the brain is only partially complete by birth. Neurodevelopment continues after birth and is constantly modified by environmental influences, possibly the most significant factor in development is the introduction of stress in early life. There is a large body of evidence indicating that early environmental stress plays a pivotal role in the pathogenesis of psychiatric disorders such as schizophrenia and depression (Agid et al, 2000; Yuii et al, 2007; van OS et al, 2010). Isolation rearing of rats at weaning, results in a lack of the essential sensory information coming from social contact with cage mates. The olfactory information and environmental stimuli available to isolation reared rats may not be sufficient to drive the process of normal brain development and neurotransmitter function (Muchimapura & Marsden, 2004). Isolation rearing of rats is considered to be an environmental stressor and has been shown to consistently result in hyperactivity at adulthood when placed into a novel environment (Smith et al, 1997; Lapiz et al, 2003; Elliot & Grunberg, 2005; Bianchi et al, 2006, Marsden et al, 2011). This behavioural effect is undoubtedly the most robust effect of isolation rearing and has been shown to appear as early as 2 weeks of isolation in Hooded-Lister rats (Bakshi & Geyer, 1999). Isolation rearing provides a non-pharmacological method of inducing long-term alterations of relevance to symptomology of schizophrenia (Geyer et al, 1993). Prepulse inhibition of the startle reflex (PPI) is an operational measure of sensorimotor gating which has been shown to be disrupted in rats reared in isolation (Cilia et al, 2001; Varty et al, 1995). Results from earlier studies in our laboratory demonstrate impairments in the EDS phase of the attentional set-shifting

task in isolation reared rats, they required significantly more trials to reach criterion compared to socially housed animals; indicative of a selective deficit in set-shifting ability (McLean et al, 2010). Additionally, other laboratories have shown isolation rearing induced impairments in spatial memory and reversal learning in the Morris water maze (Hellemans et al, 2004; Quan et al, 2010). However, there have been studies to contradict these findings, for example; Wongwitdecha & Marsden (1996) showed that place learning and reversal learning in the Morris water maze was enhanced in isolation reared rats compared to socially housed controls. Bianchi and colleagues (2006), showed deficits in object recognition following a 1 h ITI, but not at a 1 min ITI in male rats reared in isolation compared to social controls. Isolation reared rats have been shown to exhibit an enhanced behavioural reactivity to dopamine agonists (Bowling and Bardo, 1994; Jones et al, 1992) which is also observed in schizophrenia patients (Lieberman et al, 1987).

Isolation rearing has been shown to disrupt behaviour which is accompanied by alterations in neurochemistry and neuropathology associated with schizophrenia (Harte et al, 2006, 2007; Fone & Porkess, 2008). Rats reared in isolation have been shown to have increased steady-state levels of dopamine and decreased basal dopamine turnover which indicates reduced metabolic activity in the medial prefrontal cortex (Heidbreder et al, 2000). A reduction in metabolic activity in the prefrontal cortex is also observed in schizophrenia patients and may be indicative of hypofrontality (Andreasen et al 1997). Studies have demonstrated that isolated rats exhibit PPI deficits and reductions in parvalbumin and calbindin-immunoreactive cells in the hippocampus, with no significant change in calretinin. These findings reveal selective abnormalities of sub-populations of GABAergic interneurons in the

hippocampus of isolation reared rats, which resemble the neuronal deficits seen in this region in schizophrenia (Harte et al, 2007).

The aim of these studies was to assess the effects of isolation rearing compared to socially housed controls on the cognitive performance in the NOR test following increasing ITI periods. Furthermore, in view of the fact that previous studies have demonstrated that isolation reared rats demonstrate an elevated response to psychotomimetic agents, the second aim was to investigate the effects of isolation rearing in response to acute treatment with d-amphetamine and PCP on cognitive performance in the NOR test.

6.2 Materials and Methods

6.2.1 Experimental Animals and Design

All experiments (using cohorts 12 and 13; see table 6.1) were conducted using female hooded-Lister rats (Charles River, UK), obtained at weaning (21-25 days) and weighing between 75-100 g. For details regarding the isolation rearing protocol, housing conditions and experimental timeline see section 2.1.6, 2.1.1 and figure 6.0 respectively.

Experiment number	*Cohort number*	*Total number of rats used*	*Weight range during testing (g)*	*Experiment, ITI duration or compound tested (mg/kg)*
1	12	n=20 isolates; n=30 socials	220-260	Locomotor activity (60 min test)
2	12	n=10 isolates; n=10 socials	220-260	1 min ITI
3	12	n=10 isolates; n=10 socials	220-260	1 h ITI
4	12	n=10 isolates; n=10 socials	220-260	3.5 h ITI
5	12	n=10 isolates; n=10 socials	220-260	4 h ITI
6	12	n=10 isolates; n=10 socials	220-260	5 h ITI
7	12	n=10 isolates; n=10 socials	220-260	6 h ITI
8	12	n=20 isolates; n=20 socials	240-280	PCP (lower doses; 0.5-1.5)
9	12	n=16 isolates; n=16 socials	240-280	PCP (higher dose; 2.0)
10	12	n=16 socials	190-225	PCP (repeat; 0.5)
11	13	n=10 isolates; n=10 socials	240-280	d-amph (0.25-0.75)

Table 6.1 Details of the rats used in these studies.

Timeline of the isolation rearing studies

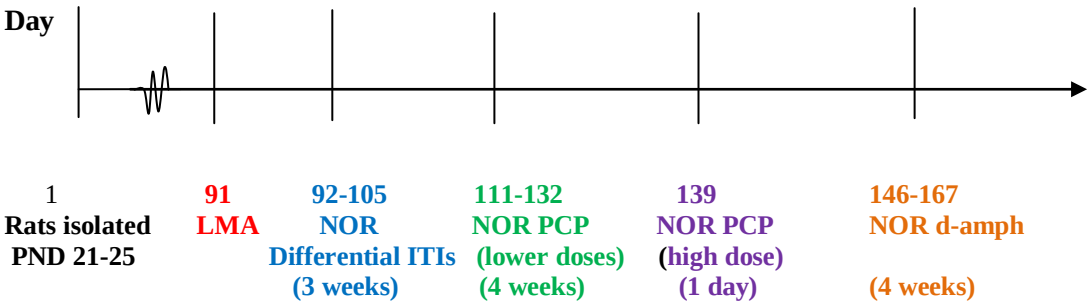


Figure 6.0 Timeline showing days after isolation on which behavioural tests were conducted. LMA: Locomotor Activity in a novel environment, NOR: Novel Object Recognition. The repeat study for PCP (0.5 mg/kg) is not included in timeline as the experiment was performed in a separate cohort of rats (i.e. cohort 13).

6.2.2 NOR testing (social v isolates; differential ITI, experiment 2-6)

Both groups of rats were habituated and tested in the NOR apparatus as previously described (see section 2.1.4) and a range of ITIs were used (1 min, 1, 3.5, 4, 5 and 6 h; see table 6.2). These ITIs were selected and based upon previous data from our laboratory, which showed that group housed rats were able to discriminate between the novel and familiar up to an ITI of 3 h (Sutcliffe, et al, 2007). The isolation reared group rats were tested up to 4 h ITI and the group housed rats were tested up to an ITI of 6 h. The duration of ITI experiments were separated by at least 7 days and due to the small total number of animals available for the isolation rearing ITI study, rats were tested twice and the study took 2 weeks to complete. For the ITI study, a within subjects design would have been ideal, however the time available using the NOR apparatus was limited.

		Social		***Isolates***	
Experiment & order	***ITI***	***Initial number of rats***	***Number of rats excluded***	***Initial number of rats***	***Number of rats excluded***
2	**1 min**	10	0	10	0
3	**1 h**	10	0	10	0
4	**3.5 h**	10	0	10	0
5	**4 h**	10	0	10	0
6	**5 h**	10	0		
7	**6 h**	10	0		

Table 6.2 The number of rats excluded from from each housing group (social and isolates) during the differential ITI's (1 min- 6h) selected for use in this NOR study.

6.2.3 NOR testing (social v isolates; acute PCP, experiment 8)

In this experiment a within subjects experimental design was used. Both groups of rats were administered vehicle (0.9% saline, i.p.) or PCP (0.5-1.5 mg/kg, i.p.), 30 min prior to NOR testing in a volume of 1 ml/kg, calculated as the base equivalent weight (see table 6.1 for doses). Due to lack of efficacy of the first dose range of PCP to produce a NOR deficit in the socially reared rats, a second separate experiment was carried out in the social rats using a higher dose of PCP (2 mg/kg; see table 6.4).

	Experiment 8			
Dose of PCP (mg/kg)	***Social***		***Isolates***	
	Initial number of rats	***Number of rats excluded***	***Initial number of rats***	***Number of rats excluded***
Veh	20	0	20	0
0.5	20	0	20	0
1.0	20	0	20	0
1.5	20	0	20	0

Table 6.3 The number of rats excluded from each housing group (social and isolates) in the NOR study following acute administration of PCP (0.5-1.5 mg/kg, i.p.), or vehicle (0.9% saline, i.p.).

6.2.4 NOR testing (social v isolates; acute PCP, experiment 9)

Both groups of rats were administered vehicle (0.9% saline, i.p.) or PCP (2.0 mg/kg, i.p.), 30 min prior to NOR testing in a volume of 1 ml/kg, calculated as the base equivalent weight (see table 6.4 for doses).

	Experiment 9			
Dose of PCP (mg/kg)	***Social***		***Isolates***	
	Initial number of rats	***Number of rats excluded***	***Initial number of rats***	***Number of rats excluded***
Veh	8	0	8	0
2.0	8	0	8	0

Table 6.4 The number of rats excluded from each housing group (social and isolates) in the NOR study following acute administration of PCP (2.0 mg/kg, i.p.), or vehicle (0.9% saline, i.p.).

6.2.5 NOR testing (social v isolates; acute PCP, experiment 10).

It was demonstrated in chapter 4 (summary table 4.15) that acute treatment with PCP (0.5-1.5 mg/kg) induced an NOR deficit in socially reared female rats. However, studies in this chapter showed there was a lack of efficacy of acute PCP (0.5-1.5 mg/kg) to induce a deficit in the NOR test in the socially reared rats. It is unclear as to why there was a lack of efficacy of acute PCP to induce a cognitive deficit at the lower doses (i.e. 0.5-1.5 mg/kg). Therefore, the lowest dose of acute PCP was selected to be re-tested since this had previously been shown to induce a cognitive deficit in socially reared female rats (see chapter 4). Both groups of rats were administered vehicle (0.9% saline, i.p.) or PCP (0.5 mg/kg, i.p.), 30 min prior to NOR testing in a volume of 1 ml/kg, calculated as the base equivalent weight (see table 6.5 for doses).

	Experiment 10			
Dose of PCP (mg/kg)	Social		Isolates	
	Initial number of rats	Number of rats excluded	Initial number of rats	Number of rats excluded
0	8	0	8	0
0.5	8	0	8	0

Table 6.5 The number of rats excluded from each housing group (social and isolates) in the NOR study following acute administration of PCP (0.5 mg/kg, i.p.), or vehicle (0.9% saline, i.p.).

6.2.6 NOR testing (social v isolates; acute d-amph, experiment 11).

In this experiment a within subject experimental design was used, both groups of rats were administered vehicle (0.9% saline, i.p.) or d-amph (0.25-0.75 mg/kg, i.p.), 30 min prior to NOR testing in a volume of 1 ml/kg, calculated as the base equivalent weight (see table 6.6 for doses).

	Experiment 11			
Dose of d-amph (mg/kg)	Socials		Isolates	
	Initial number of rats	Number of rats excluded	Initial number of rats	Number of rats excluded
0	10	0	10	2
0.25	10	0	10	2
0.5	10	0	10	2
0.75	10	0	10	2

Table 6.6 The number of rats excluded from each housing group (social and isolates) in the NOR study following acute administration of d-amphetamine (0.25-0.75 mg/kg, i.p.), or vehicle (0.9% saline, i.p.).

6.2.7 NOR apparatus

For details regarding NOR apparatus see section 2.1.4.

6.2.8 NOR testing

For details regarding NOR testing see section 2.1.5.

6.2.9 Statistical analysis

All data are expressed as the mean ± S.E.M. For the NOR experiments, exploration data were analysed by a two-way ANOVA or a repeated measures two-way ANOVA. This detected the main effect of variable ITI, main effect of the task (exploration of both objects) and the interaction between variable ITI and the two trials (acquisition and retention). Planned comparisons using a post-hoc Student's t-test was carried out to compare the time spent exploring the novel and familiar objects.

Discrimination index, total exploration time and line crossing data was analysed by a one-way ANOVA, followed by a post-hoc Dunnett's t-test.

6.3 Results

6.3.1 Effect of rearing conditions in response to a novel environment

Figure 6.1 shows the effect of housing conditions in response to a novel environment. An independent samples Student's t-test revealed a significant ($P<0.05$) increase in LMA response to a novel environment in isolation-reared rats compared to social controls.

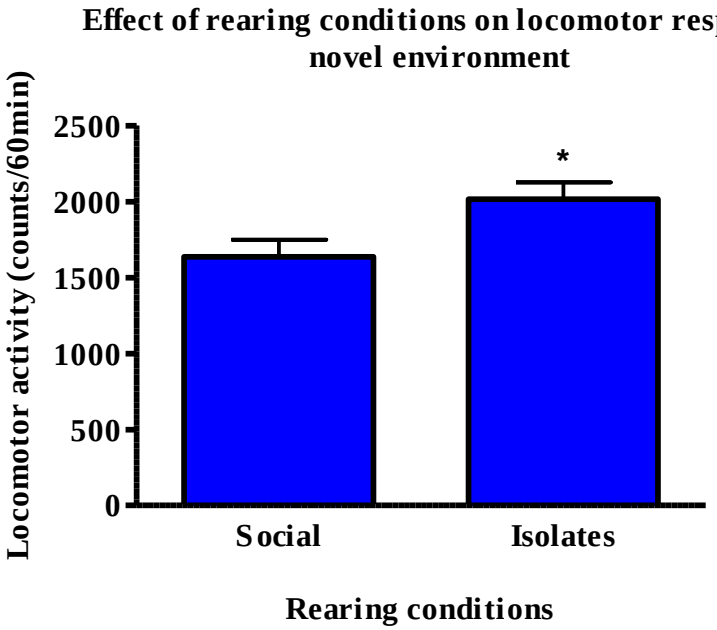


Figure 6.1 The response to a novel environment in rats reared socially and in isolation. Data are expressed as the mean ± S.E.M. (n=20 per group). *P<0.05; significant increase in LMA in the isolates compared to social controls.

6.3.2 Effect of increasing the ITI on social controls in the acquisition trial of the NOR test in female rats

A two-way ANOVA revealed a significant interaction between object exploration and ITI in the acquisition trial of the NOR test ($F_{5, 51}$ =2.54, P<0.05). However, rats from all ITI groups spent similar times exploring both the objects (figure 6.2). A one-way ANOVA on the total exploration time revealed a significant effect ($F_{5, 56}$=2.74, P<0.05). Post-hoc analysis showed a significant (P<0.01) reduction in the time spent exploring the identical objects at 6 h ITI when compared to the 1 min ITI (table 6.7).

6.3.3 Effect of ITI on social controls in the retention trial of the NOR test in female rats

An overall two-way ANOVA revealed a significant interaction between object exploration and ITI in the retention trial of the NOR test ($F_{5, 51}$=4.7; P<0.01). Planned post-hoc comparisons revealed that rats spent significantly more time exploring the novel objects during the retention trial at ITIs of 1 min (P<0.001), 1 h (P<0.001), 3.5 h; (P<0.01) and 4 h (P<0.01) but not at 5 h and 6 h (figure 6.3). This significant preference for the novel object compared to the familiar object was not observed in the rats from the 5 h and 6 h ITI groups i.e. these rats spent similar amount of time exploring both objects. Analysis of the total exploration of the objects in the retention trial by a one-way ANOVA revealed a significant effect of ITI ($F_{5, 56}$=2.54, P<0.05; table 6.7). However, planned post-hoc analysis failed to reveal any individual significant differences in total exploration time in the retention trial when compared to the 1 min ITI.

6.3.4 Effect of ITI duration on the DI in social controls in the NOR test in female rats

One-way ANOVA on the DI data showed no significant effect of increasing the ITI duration on the rats' ability to discriminate between the familiar and novel objects ($F_{5, 56}$=1.63, NS; figure 6.4). There was a marked trend in reduction of DI; however this failed to reach statistical significance. Planned post-hoc analysis failed to reveal any individual significant differences in DI.

6.3.5 Effect of ITI duration on the line crossings in social controls in the NOR test in female rats

Figure 6.5 shows the effect of increasing the ITI duration on the total number of line crossings of the rats during the acquisition and retention trials of the NOR test. One-way ANOVA revealed no significant differences when compared to the 1 min ITI ($F_{5, 56}$=0.42, NS).

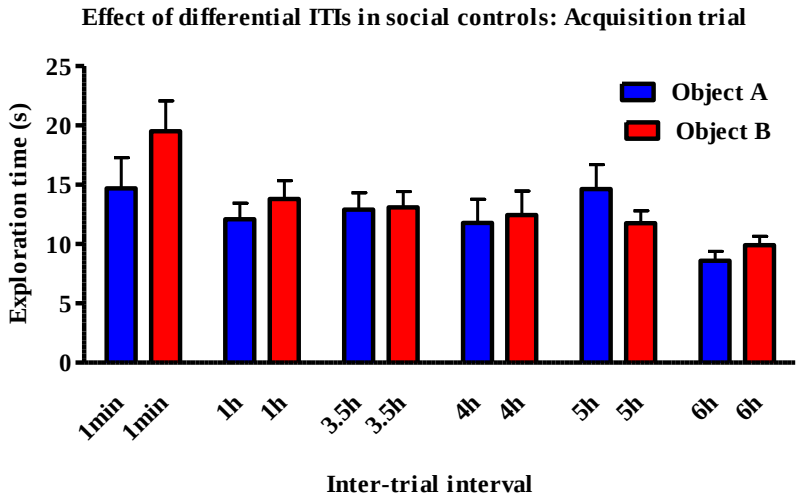


Figure 6.2 The effect of **social** rearing conditions on exploration of two identical objects in the 3 min acquisition trial prior to ITIs of 1 min, 1 h, 3.5 h, 4 h, 5 h and 6 h in the NOR test in female rats. Data are shown as mean ± S.E.M. (n=8-10 per group).

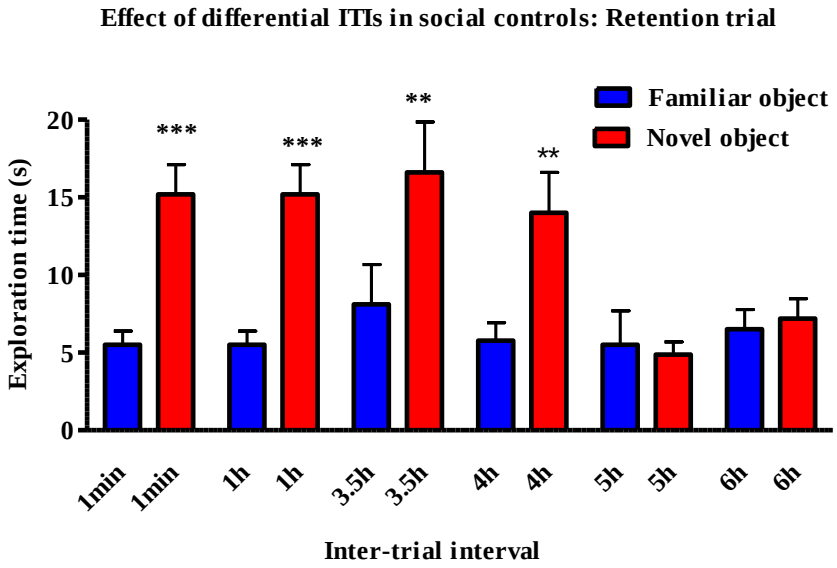


Figure 6.3 The effect of **social** rearing conditions on exploration of a novel and a familiar object following ITIs of 1 min, 1 h, 3.5 h, 4 h, 5 h and 6 h during the 3 min retention trial in the NOR test in female rats. Data are shown as mean ± S.E.M (n=8-10 per group). **P<0.01-***P<0.001; significant increase in time spent exploring the novel compared with the familiar object.

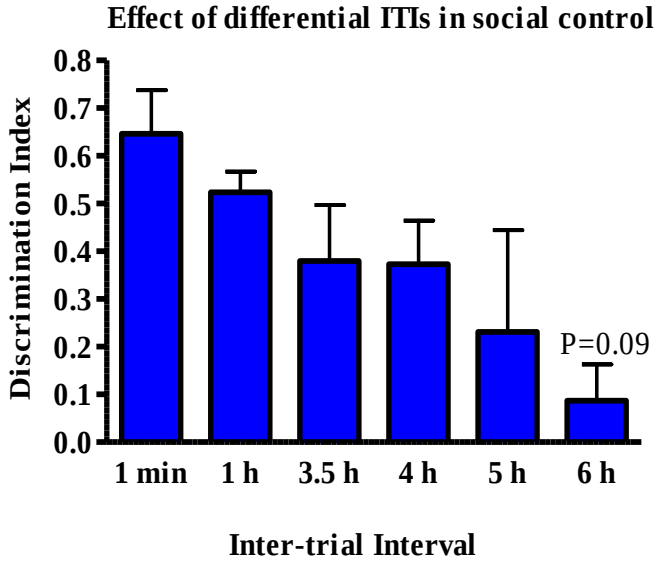


Figure 6.4 The effect of increasing the ITI duration on the DI in the NOR test in female rats reared in social groups. Data are expressed as the mean ± S.E.M. (n=10 per group).

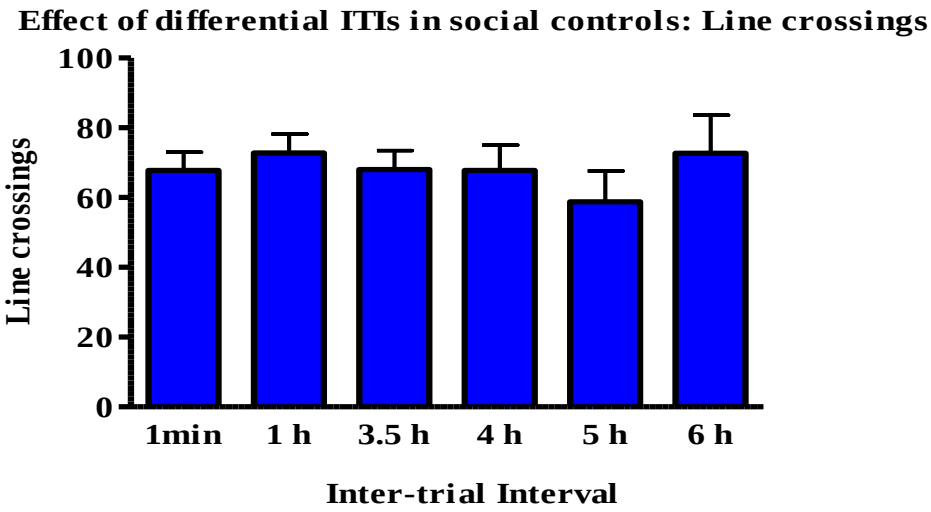


Figure 6.5 The effect of increasing the ITI duration on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats reared in social groups. Data are expressed as the mean ± S.E.M. (n=10 per group).

ITI	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
1 min	34.2 ± 4.7	20.8 ± 2.1
1 h	25.8 ± 2.6	20.7 ± 2.2
3.5 h	26.0 ± 2.5	24.7 ± 5.4
4 h	24.1 ± 3.7	19.8 ± 3.2
5 h	26.4 ± 2.5	10.3 ± 2.6
6 h	18.5 ± 1.2**	14.0 ± 2.2

Table 6.7 The effect of increasing the ITI duration on the total exploration time in **social** controls in the acquisition and retention trials of the NOR test in female rats. Data are expressed as the mean ± S.E.M. (n=10 per group). **P<0.01; significant reduction in total object exploration time compared to the 1 min ITI.

6.3.6 Effect of ITI duration on the acquisition trial of the NOR test in female rats reared in isolation

A two-way ANOVA revealed no significant interaction between object exploration and ITI in the acquisition trial of the NOR test ($F_{3, 36}$=0.40, NS). Rats from all ITI groups spent similar times exploring both the objects (figure 6.6). A one-way ANOVA on the total exploration time revealed a significant effect ($F_{3, 39}$=5.05, P<0.01). Planned post-hoc analysis showed significant reductions in the time spent exploring the identical objects at 1 h ITI (P<0.05), 3.5 h ITI (P<0.01) and 4 h ITI (P<0.01) when compared to the 1 min ITI (table 6.8).

6.3.7 Effect of ITI duration on the retention trial of the NOR test in female rats reared in isolation

An overall two-way ANOVA revealed a significant interaction between object exploration and ITI in the retention trial of the NOR test ($F_{3, 36}$=11.12, P<0.001). Planned post-hoc comparisons revealed that rats spent significantly more time exploring the novel object during the retention trial at ITIs of 1 min (P<0.001) and 1 h (P<0.001) but not following ITIs of 3.5 h and 4 h (figure 6.7). A one-way ANOVA on the total time spent exploring both the novel and familiar objects in the retention trial revealed a significant effect ($F_{3, 39}$=5.01, P<0.01). However, planned post-hoc analysis showed no significant effect in the time spent exploring the novel and familiar objects following all ITIs (1 h, 3.5 h and 4 h) when compared to the 1 min ITI (table 6.8).

6.3.8 Effect of ITI duration on the DI in the NOR test in female rats reared in isolation

One-way ANOVA on the DI data showed no significant effect of increasing the ITI duration on the rats' ability to discriminate between the familiar and novel objects ($F_{3, 39}$=0.67, NS).

6.3.9 Effect of ITI duration on the total number of line crossings in the NOR test in female rats reared in isolation

Figure 6.9 shows the effect of increasing the ITI duration on total number of line crossings of the rats during the acquisition and retention trials in the NOR test. One-way ANOVA revealed no significant differences when compared to the 1 min ITI ($F_{3, 39}$=0.93, NS).

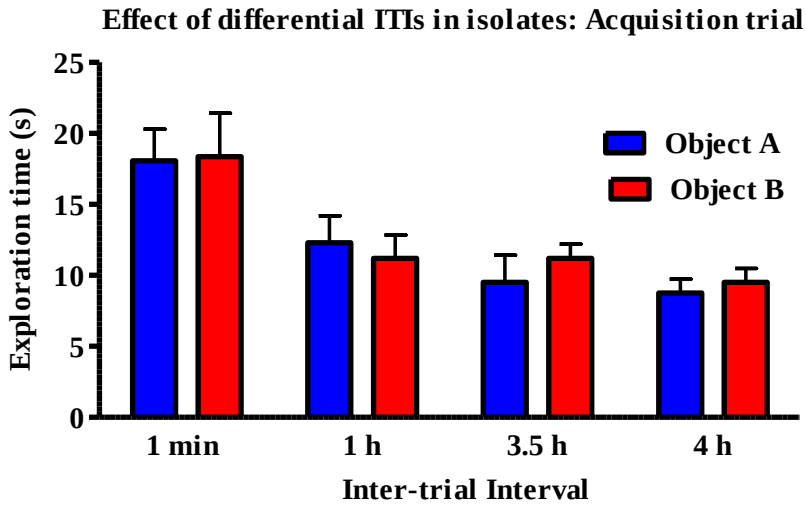


Figure 6.6 The effect of **isolation** rearing conditions on exploration of two identical objects in the 3 min acquisition trial prior to the ITIs of 1 min, 1 h, 3.5 h and 4 h in the NOR test in female rats. Data are shown as mean ± S.E.M. (n=10 per group).

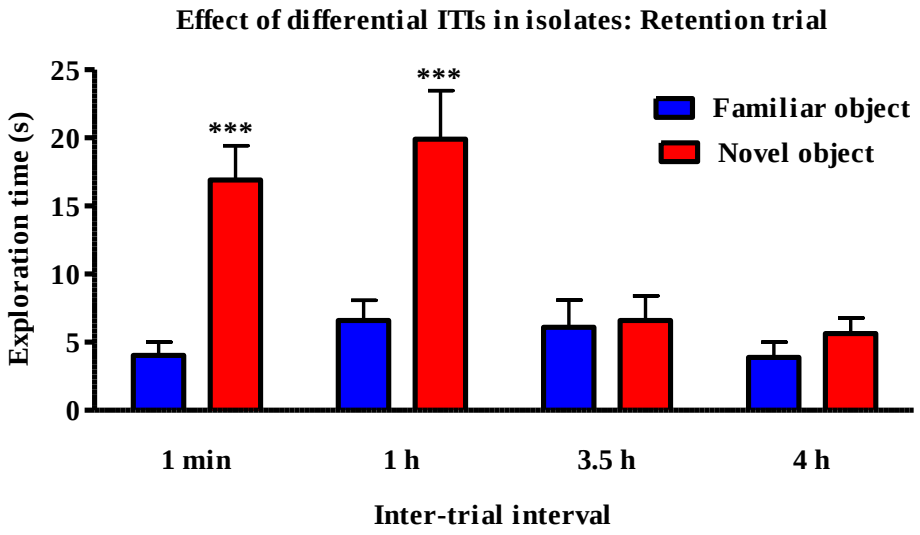


Figure 6.7 The effect of **isolation** rearing conditions on exploration of a novel and a familiar object following ITIs of 1 min, 1 h, 3.5 h and 4 h during the 3 min retention trial in the NOR test in female rats. Data are shown as mean ± S.E.M. (n=10 per group). ***P<0.001; significant increase in time spent exploring the novel compared with the familiar object.

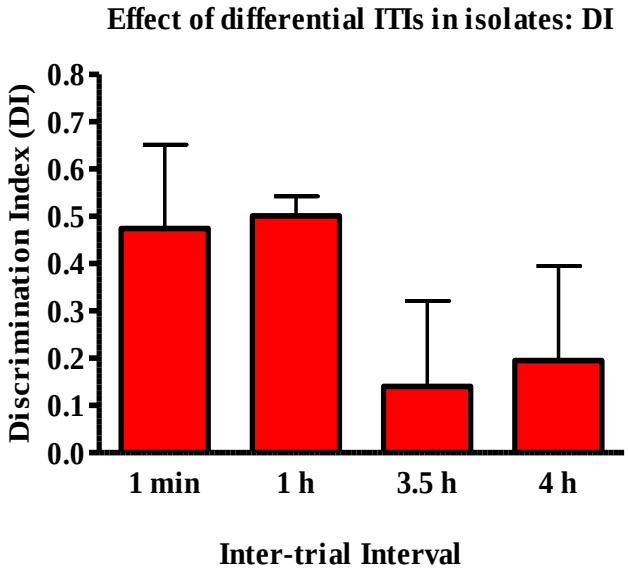


Figure 6.8 The effect of increasing the ITI duration on the DI in the NOR test in female rats reared in isolation. Data are expressed as the mean ± S.E.M. (n=10 per group).

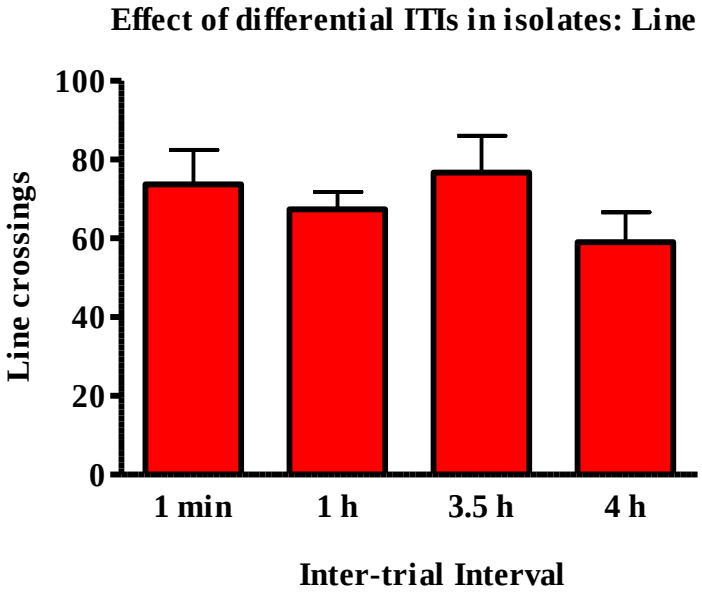


Figure 6.9 The effect of increasing the ITI duration on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats reared in isolation. Data are expressed as the mean ± S.E.M. (n=10 per group).

ITI	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
1 min	35.9 ± 4.9	20.2 ± 3.0
1 h	23.6 ± 3.5*	26.5 ± 4.9
3.5 h	20.7 ± 2.4**	12.7 ± 3.1
4 h	18.3 ± 1.4**	9.5 ± 1.7 (P=0.1)

Table 6.8 The effect of increasing the ITI duration on the total exploration time in the acquisition and retention trials of the NOR test in female rats reared in **isolation**. Data are expressed as the mean ± S.E.M. (n=10 per group). *P<0.05-**P<0.01; significant reduction in the total object exploration time compared to the 1 min ITI.

6.3.10 Effect of acute PCP treatment (0.5-1.5 mg/kg) in the acquisition trial in female rats housed in social groups

A two-way ANOVA revealed that acute treatment with PCP did not produce a significant effect on object exploration in the acquisition trial of the NOR test ($F_{3, 57}$=1.29, NS). Rats from all treatment groups spent similar times exploring both the objects (figure 6.10). A one-way ANOVA on the total time spent exploring both identical objects in the acquisition trial revealed no significant effect of PCP treatment ($F_{3, 57}$=0.83, NS; table 6.9).

6.3.11 Effect of acute PCP treatment (0.5-1.5 mg/kg) in the retention trial in female rats housed in social groups

An overall two-way ANOVA revealed that treatment with acute PCP did not produce a significant effect on object exploration in the retention trial of the NOR test ($F_{3, 57}$=0.89, NS; figure 6.11). Planned post-hoc analysis revealed that vehicle control rats had a significant preference for the novel compared with the familiar object, ($P<0.01$). This preference in the vehicle treated rats for the novel object was also observed in all the PCP treated rats at all of the doses tested (0.5–1.5 mg/kg), i.e. these rats spent significantly ($P<0.01$-$P<0.05$) more time exploring the novel object compared to the familiar object. A one-way ANOVA on the total time spent exploring both the novel and familiar objects in the retention trial revealed no significant effect of PCP treatment ($F_{3, 57}$=1.08, NS; table 6.9).

6.3.12 Effect of acute PCP treatment (0.5-1.5 mg/kg) on the DI in female rats housed in social groups

A one-way ANOVA revealed no significant effect of PCP treatment on the DI ($F_{3, 57}$=2.2, NS). Subsequent planned post-hoc analysis revealed a near significant (P=0.09) reduction in DI following treatment with PCP at 1.5 mg/kg when compared to vehicle control (figure 6.12).

6.3.13 Effect of acute PCP treatment (0.5-1.5mg/kg) on the total number of line crossings in female rats housed in social groups

A one-way ANOVA revealed no significant effect of PCP treatment on the total number of line crossings ($F_{3, 57}$=0.48, NS; figure 6.13).

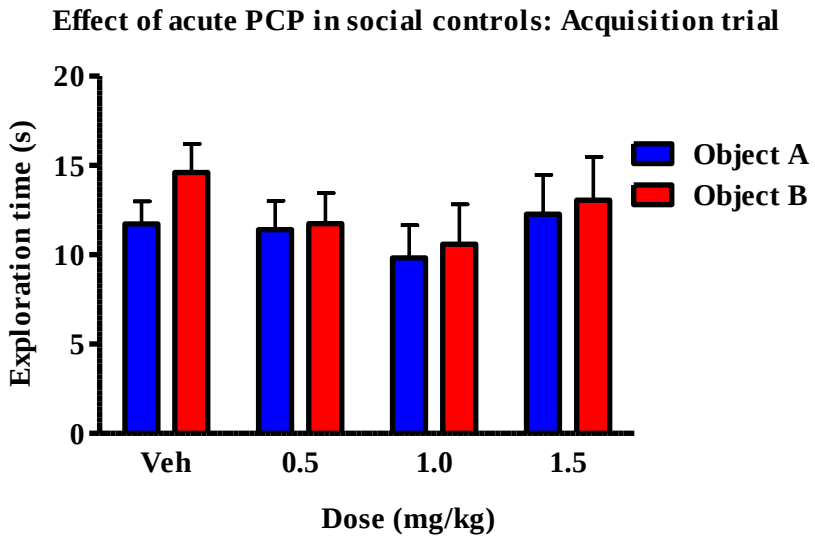


Figure 6.10 The effect of acute PCP (0.5-1.5 mg/kg, i.p) and 0.9% saline (veh, i.p) on the exploration of two identical objects in the 3 min acquisition trial in the NOR test in female rats housed in **social** groups. Data are shown as mean ± S.E.M. (n=20 per group).

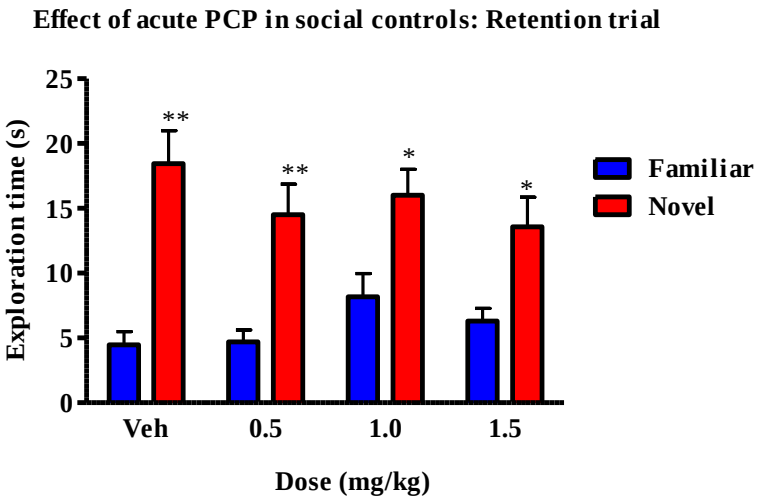


Figure 6.11 The effect of acute PCP (0.5-1.5 mg/kg, i.p) and 0.9% saline (veh, i.p) on the time (s) spent exploring a familiar and novel object in the 3 min retention trial in female rats housed in **social** groups. Data are shown as mean ± S.E.M. (n=20 per group). *P<0.05-**P<0.01; significant increase in the time (s) spent exploring the novel compared with the familiar object.

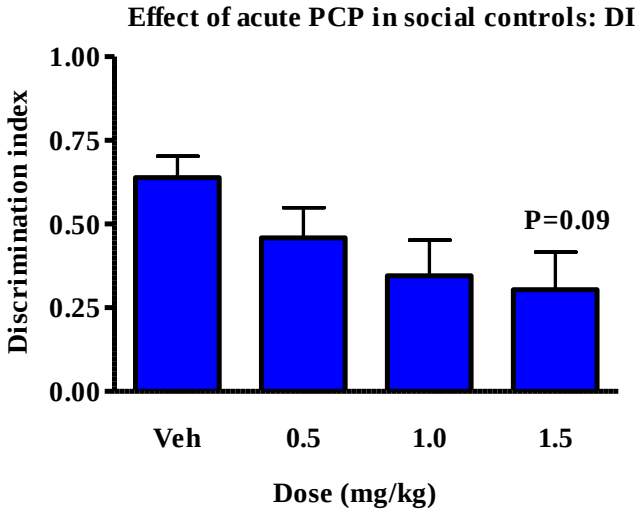


Figure 6.12 The effect of acute treatment with PCP (0.5-1.5 mg/kg, i.p) and 0.9% 0.9% saline (veh, i.p) on the DI in the NOR test in female rats housed in **social** groups. Data are expressed as the mean ± S.E.M. (n=20 per group). P=0.09; approaching significant reduction in DI compared to the vehicle control.

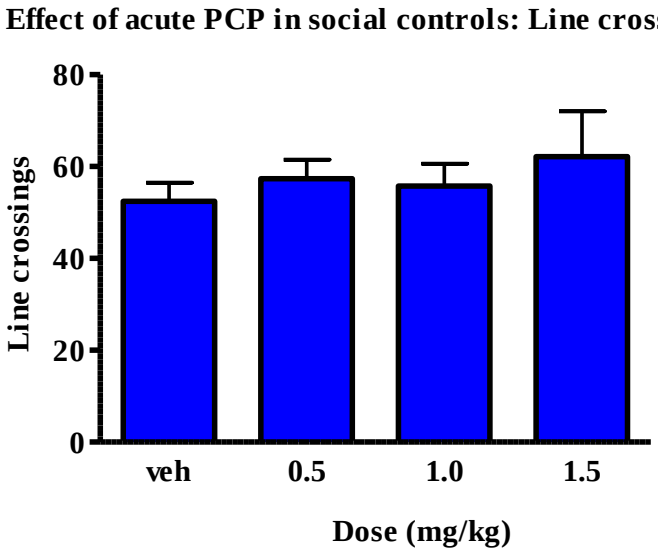


Figure 6.13 The effect of acute treatment with PCP (0.5-1.5 mg/kg, i.p) and 0.9% saline (veh, i.p) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats housed in **social** groups. Data are expressed as the mean ± S.E.M. (n=20 per group).

Treatment	***Total exploration time (s)***	
PCP (mg/kg)	***Acquisition trial***	***Retention trial***
Veh	26.4 ± 2.6	22.9 ± 3.2
0.5	23.1 ± 3.2	19.2 ± 2.4
1.0	20.4 ± 3.9	24.1 ± 2.6
1.5	25.3 ± 4.1	19.8 ± 2.7

Table 6.9 The effect of acute treatment with PCP (0.5-1.5 mg/kg, i.p) and 0.9% 0.9% saline (veh, i.p) on the total exploration time in the acquisition and retention trials of the NOR test in female rats reared in **social** groups. Data are expressed as the mean ± S.E.M (n= 20 per group).

6.3.14 Effect of acute PCP treatment (2.0 mg/kg) in the acquisition trial in female rats housed in social groups

Since there was no effect of PCP (0.5–1.5 mg/kg) to induce a deficit in object recognition memory a higher dose of PCP (2.0 mg/kg) was tested. A two-way ANOVA revealed that acute treatment with PCP (2.0 mg/kg) did not produce a significant effect on object exploration in the acquisition trial of the NOR test ($F_{1, 14}$=3.22, NS). Rats from both treatment groups spent similar times exploring both the objects (figure 6.14). An independent Student's t-test on the total time spent exploring identical objects in the acquisition trial showed no significant effect of PCP treatment (table 6.10).

6.3.15 Effect of acute PCP treatment (2.0 mg/kg) in the retention trial in female rats housed in social groups

An overall two-way ANOVA revealed that treatment with acute PCP did not produce any significant effect on object exploration in the retention trial of the NOR test ($F_{1, 14}$=1.27, NS; figure 6.15). However, planned post-hoc analysis revealed that vehicle control rats had a significant preference for the novel compared with the familiar object ($P<0.05$), this preference was not observed following treatment with PCP at 2.0 mg/kg. An independent Student's t-test on the total time spent exploring the objects in the retention trial showed no significant effect of PCP treatment (table 6.10).

6.3.16 Effect of acute PCP treatment (2.0 mg/kg) on the DI in female rats housed in social groups

An independent samples Student's t-test revealed a significant ($P<0.05$) decrease in the DI compared to vehicle control rats following acute treatment with PCP (2.0 mg/kg; figure 6.16).

6.3.17 Effect of acute PCP treatment (2.0 mg/kg) on the total number of line crossings in female rats housed in social groups

An independent samples Student's t-test revealed no significant effect of acute treatment with PCP (2.0 mg/kg) on the total number of line crossings in the acquisition and retention trials of the NOR test (figure 6.17).

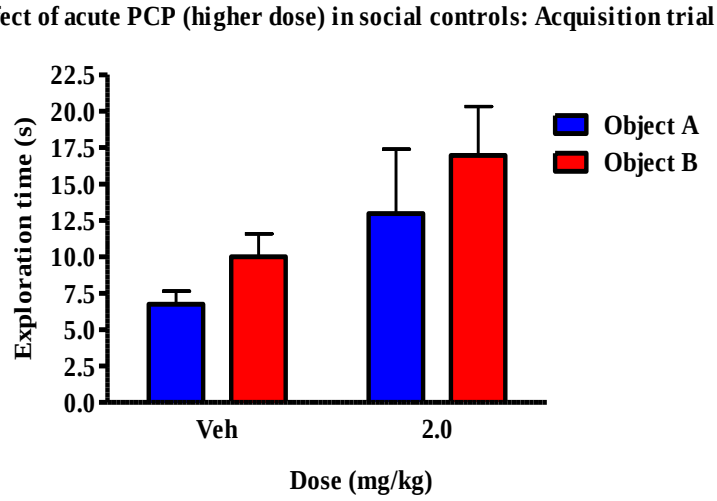


Figure 6.14 The effect of acute PCP (2.0 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on exploration of two identical objects in the 3 min acquisition trial in a NOR test in female rats housed in **social** groups. Data are shown as mean ± S.E.M. (n=8 per group).

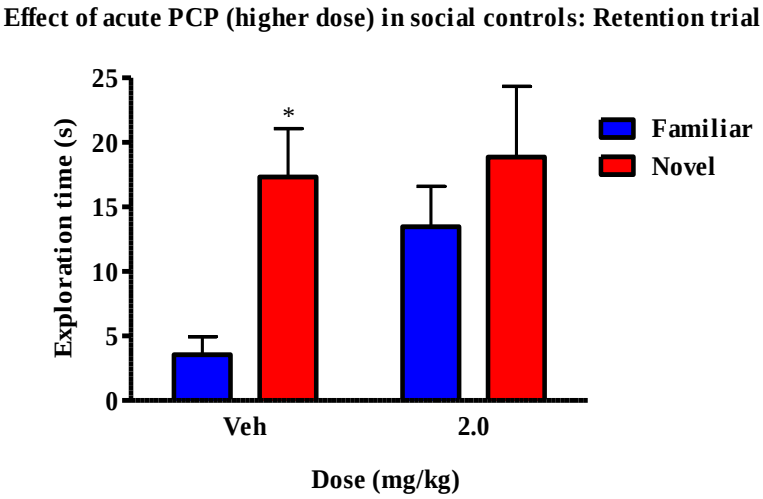


Figure 6.15 The effect of acute PCP administration (2.0 mg/kg, i.p) and 0.9% saline (veh, i.p.) on time (s) spent exploring a familiar and novel object in the 3 min retention trial of the NOR test in female rats housed in **social** groups. Data are shown as mean ± S.E.M. (n=8 per group). *P<0.05; significant difference between the time spent exploring the familiar compared with the novel object.

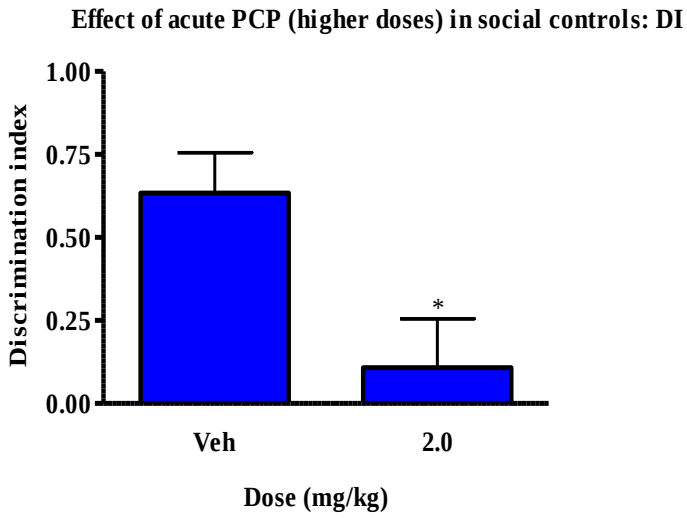


Figure 6.16 The effect of acute treatment with PCP (2.0 mg/kg, i.p) and 0.9% saline (veh, i.p.) on the DI in the NOR test in female rats housed in **social** groups. Data are expressed as the mean ± S.E.M. (n=8 per group). *P<0.05; significant reduction in DI compared with vehicle.

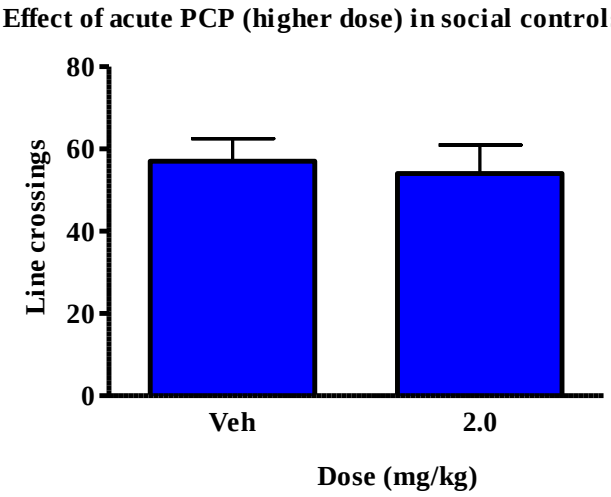


Figure 6.17 The effect of acute treatment with PCP (2.0 mg/kg, i.p) and 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats housed in **social** groups. Data are expressed as the mean ± S.E.M. (n=8 per group).

Socials	***Total exploration time (s)***	
PCP (mg/kg)	***Acquisition trial***	***Retention trial***
Veh	16.7 ± 2.1	20.9 ± 3.5
2.0	29.9 ± 7.0 (P=0.09)	32.3 ± 6.7 (P=0.1)

Table 6.10 The effect of acute treatment with PCP (2.0 mg/kg, i.p) and 0.9% saline (veh, i.p) on the total exploration time (s) in the acquisition and retention trials of the NOR test in female rats housed in **social** groups. Data are expressed as the mean ± S.E.M. (n=8 per group).

6.3.18 Effect of acute PCP treatment (0.5 mg/kg) in the acquisition trial in female rats housed in social groups

Previously in chapter 4, socially reared female rats were sensitive to the object recognition deficit induced by acute PCP at all the doses tested (0.5-2.0 mg/kg). However, due to the lack of effect of PCP (0.5–1.5 mg/kg) to induce a deficit in

object recognition memory in this study (cohort 12), a repeat experiment in a separate group of rats (cohort 13) was performed; to determine the effects of acute PCP in socially reared rats at 0.5 mg/kg.

A two-way ANOVA revealed that acute treatment with PCP (0.5 mg/kg) did not produce a significant effect on object exploration in the acquisition trial of the NOR test ($F_{1, 14}$=0.24, NS). Rats from both treatment groups spent similar times exploring both the objects (figure 6.18). An independent Student's t-test on the total time spent exploring identical objects in the acquisition trial showed no significant effect of PCP treatment (table 6.11).

6.3.19 Effect of acute PCP treatment (0.5 mg/kg) in the retention trial in female rats housed in social groups

An overall two-way ANOVA revealed that acute treatment with PCP (0.5 mg/kg) produced a significant effect on object exploration in the retention trial of the NOR test ($F_{1, 14}$=16.4, P<0.01; figure 6.19). Planned post-hoc analysis revealed that vehicle control rats had a significant preference for the novel compared with the familiar object (P<0.01), this preference was not observed following treatment with PCP at 0.5 mg/kg. An independent Student's t-test showed a significant (P<0.05) decrease in total exploration time following PCP treatment in the retention trial (table 6.11).

6.3.20 Effect of acute PCP treatment (0.5 mg/kg) on the DI in female rats housed in social groups

An independent samples Student's t-test revealed a significant ($P<0.01$) decrease in the DI compared to vehicle control rats following acute treatment with PCP (0.5 mg/kg; figure 6.20).

6.3.21 Effect of acute PCP treatment (0.5 mg/kg) on the total number of line crossings in female rats housed in social groups

An independent samples Student's t-test revealed no significant effect of acute treatment with PCP (0.5 mg/kg) on the total number of line crossings in the acquisition and retention trials of the NOR test (figure 6.21).

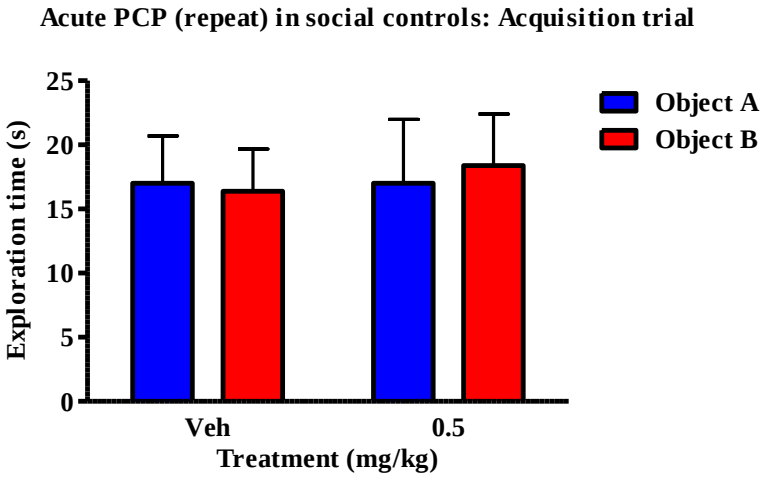


Figure 6.18 The effect of acute PCP (0.5 mg/kg, i.p) and 0.9% saline (veh, i.p) administration on the exploration of two identical objects in the 3 min acquisition trial of the NOR test in female rats housed in **social** groups. Data are shown as mean ± S.E.M. (n=8 per group).

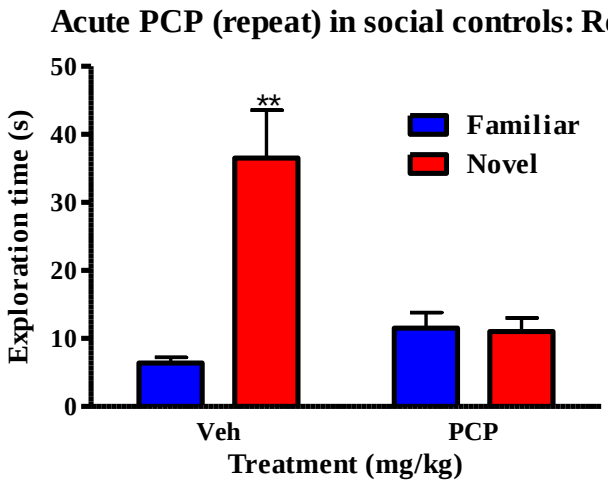


Figure 6.19 The effect of acute PCP administration (0.5 mg/kg, i.p) and 0.9% saline (veh, i.p) on time (s) spent exploring a familiar and a novel object in the 3 min retention trial of the NOR test in female rats housed in **social** groups. Data are shown as mean ± S.E.M. (n=8 per group). **P<0.01; significant increase in time (s) spent exploring the novel compared with the familiar object.

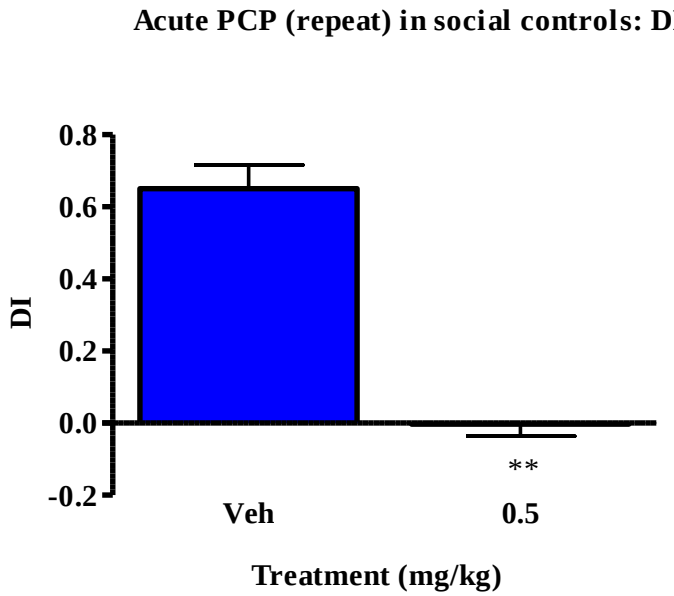


Figure 6.20 The effect of acute treatment with PCP (0.5 mg/kg, i.p.) and 0.9% saline (veh, i.p) on the DI in the NOR test in female rats housed in **social** groups. Data are expressed as the mean ± S.E.M. (n=8 per group). *P<0.01; significant reduction in DI compared with vehicle.

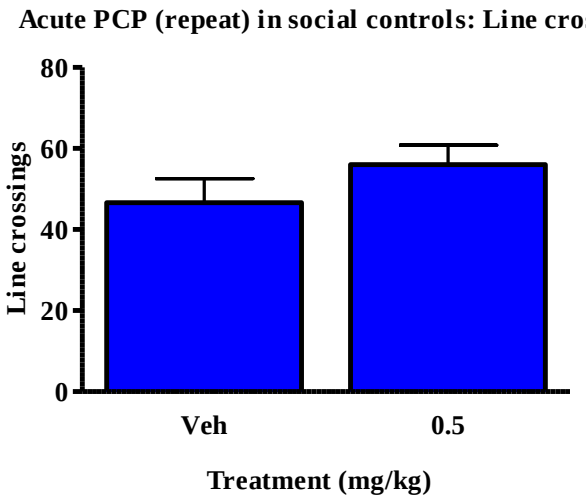


Figure 6.21 The effect of acute treatment with PCP (0.5 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials in the NOR test in female rats housed in **social** groups. Data are expressed as the mean ± S.E.M. (n=8 per group).

Socials	***Total exploration time (s)***	
PCP (mg/kg)	***Acquisition trial***	***Retention trial***
Veh	33.4 ± 5.9	42.9 ± 6.7
0.5	35.4 ± 8.9	22.5 ± 4.2 *

Table 6.11 The effect of acute treatment with PCP (0.5 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the total exploration time (s) in the acquisition and retention trials of the NOR test in female rats housed in **social** groups. Data are expressed as the mean ± S.E.M. (n=8 per group). *P<0.05; significant reduction in total exploration time compared to vehicle.

6.3.22 Effect of acute PCP treatment (0.5-1.5 mg/kg) in the acquisition trial in female rats reared in isolation

A two-way ANOVA revealed that acute treatment with PCP did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{3, 57}$=1.10, NS). Rats from all treatment groups showed similar times exploring both the

objects (figure 6.22). A one-way ANOVA on the total time spent exploring both identical objects in the acquisition trial revealed no significant effect ($F_{3, 57}$=2.08, NS; figure 6.12).

6.3.23 Effect of acute PCP treatment (0.5-1.5mg/kg) in the retention trial in female rats reared in isolation

An overall two-way ANOVA revealed that treatment with PCP produced a near significant effect on object exploration in the retention trial of the NOR test ($F_{3, 57}$=152.77, P=0.05; figure 6.23). Planned post-hoc analysis revealed that vehicle control rats had a significant preference for the novel compared with the familiar object, (P<0.01). This preference in the vehicle treated rats for the novel object was also observed in all the PCP treated rats at all of the doses tested (0.5–1.5 mg/kg), i.e. these rats spent significantly (P<0.01-P<0.05) more time exploring the novel object compared to the familiar object. A one-way ANOVA on the total time spent exploring both the novel and familiar objects in the retention trial revealed no significant effect of PCP treatment ($F_{3, 57}$=0.79, NS; table 6.12).

6.3.24 Effect of acute PCP treatment (0.5-1.5 mg/kg) on the DI in female rats reared in isolation

A one-way ANOVA revealed no significant effect of PCP treatment on the DI ($F_{3, 57}$=2.2, NS; figure 6.24).

6.3.25 Effect of acute PCP treatment (0.5-1.5 mg/kg) on the total number of line crossings in female rats reared in isolation

A one-way ANOVA revealed a significant effect of PCP treatment on the total number of line crossings in the acquisition and retention trials of the NOR test ($F_{3, 57}$=3.78, P<0.05; figure 6.25). Planned post-hoc analysis revealed a significant (P<0.05) increase in the total number of line crossings following treatment with the lowest dose of PCP (0.5 mg/kg) when compared with vehicle control.

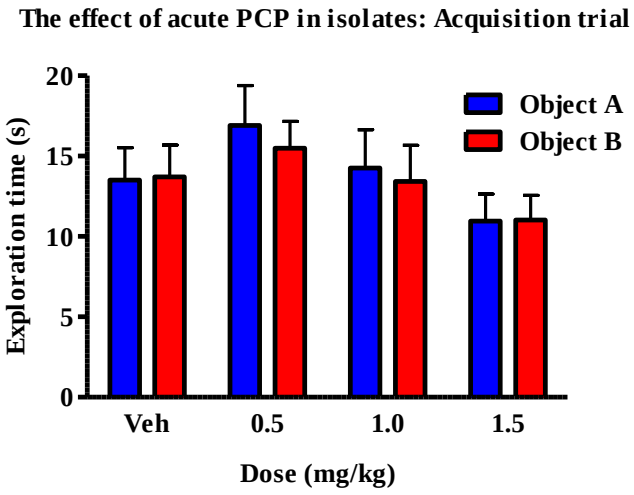


Figure 6.22 The effect acute treatment with PCP (0.5-1.5 mg/kg, i.p.) and 0.9% saline (veh, i.p) on exploration of two identical objects in the 3 min acquisition trial of the NOR test in female rats housed in **isolation**. Data are shown as mean ± S.E.M. (n=20 per group).

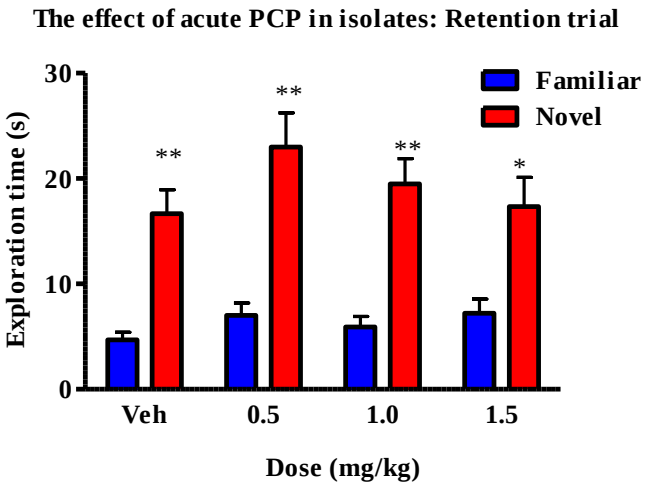


Figure 6.23 The effect of acute treatment with PCP (0.5-1.5 mg/kg, i.p) and 0.9% saline (veh, i.p.) on the time (s) spent exploring a familiar and a novel object in the 3 min retention trial in female rats housed in **isolation**. Data are shown as mean ± S.E.M. (n=20 per group). *P<0.05-P<0.01; significant increase in the time spent exploring the novel compared with the familiar object.

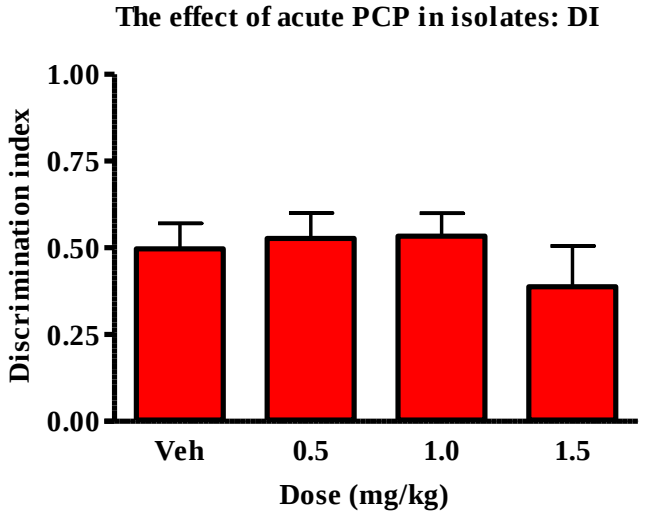


Figure 6.24 The effect of acute treatment with PCP (0.5-1.5 mg/kg, i.p) and 0.9% saline (veh, i.p.) on the DI in the NOR test in female rats housed in **isolation**. Data are expressed as the mean ± S.E.M. (n=20 per group).

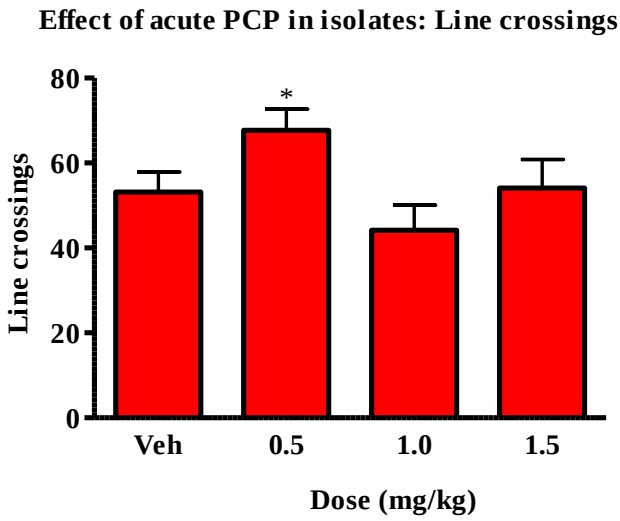


Figure 6.25 The effect of acute treatment with PCP (0.5-1.5 mg/kg, i.p) and 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats reared in **isolation**. Data are expressed as the mean ± S.E.M. (n=20 per group). P<0.05; significant increase in line crossings when compared to the vehicle control group.

PCP (mg/kg)	***Total exploration time (s)***	
	Acquisition trial	***Retention trial***
Veh	27.2 ± 3.3	21.2 ± 2.6
0.5	32.3 ± 3.8	23.7 ± 2.9
1.0	27.7 ± 4.3	27.2 ± 3.3
1.5	21.9 ± 2.9	32.3 ± 3.8

Table 6.12 The effect of acute treatment with PCP (0.5-1.5 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the total exploration time (s) in the acquisition and retention trials in the NOR test in female rats reared in **isolation**. Data are expressed as the mean ± S.E.M. (n= 20 per group).

6.3.26 Effect of acute PCP treatment (2.0 mg/kg) in the acquisition trial in female rats housed in isolation

A two-way ANOVA revealed that acute treatment with PCP (2.0 mg/kg) did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{1, 14}$=1.23, NS). Rats from both treatment groups spent similar times exploring both the objects (figure 6.26). An independent Student's t-test on the total time spent exploring identical objects in the acquisition trial showed no significant effect of PCP treatment (table 6.13).

6.3.27 Effect of acute PCP treatment (2.0 mg/kg) in the retention trial in female rats housed in isolation

An overall two-way ANOVA revealed that acute treatment with PCP produced a significant effect on object exploration in the retention trial of the NOR test ($F_{1, 14}$=100.0, P<0.001; figure 6.27). Post-hoc analysis revealed that vehicle control rats had a significant preference for the novel compared with the familiar object (P<0.05) this preference was not observed following treatment with PCP at 2.0 mg/kg. An independent Student's t-test on the total time spent exploring the objects in the retention trial showed no significant effect of PCP treatment (table 6.13).

6.3.28 Effect of acute PCP treatment (2.0 mg/kg) on the DI in female rats housed in isolation

An independent samples Student's t-test revealed a significant (P<0.05) decrease in the DI compared to vehicle control rats following acute treatment with PCP (2.0 mg/kg; figure 6.28)

6.3.29 Effect of acute PCP treatment (0.5 mg/kg) on the total number of line crossings in female rats housed in isolation

An independent samples Student's t-test showed no significant effect of acute PCP treatment on the total number of line crossings compared to vehicle control rats (figure 6.29).

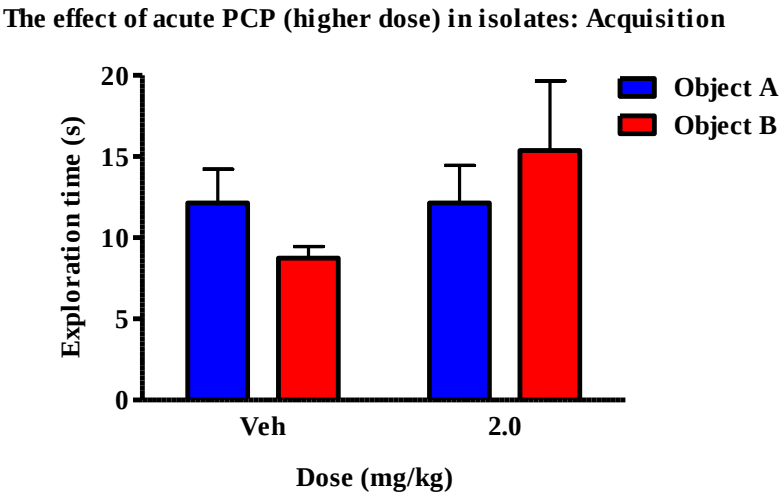


Figure 6.26 The effect acute treatment with PCP (2.0 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on exploration of two identical objects in the 3 min acquisition trial in the NOR test in female rats housed in **isolation**. Data are shown as mean ± S.E.M. (n=8 per group).

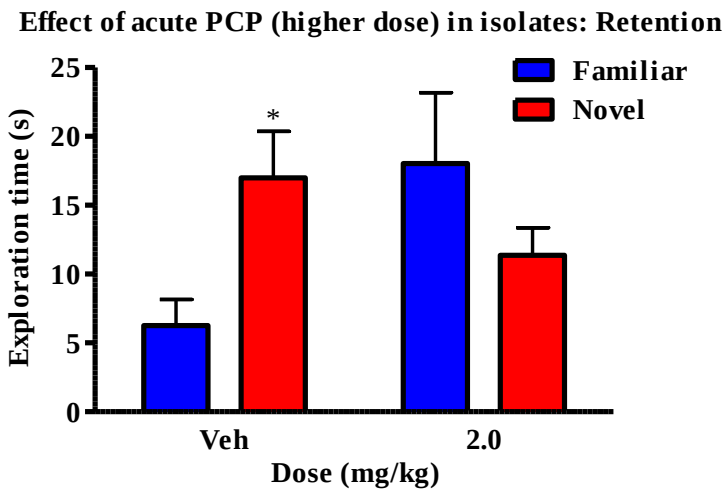


Figure 6.27 The effect of acute PCP administration (2.0 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the time (s) spent exploring a familiar and novel object in the 3 min retention trial in the NOR test in female rats housed in **isolation**. Data are shown as mean ± S.E.M. (n=8 per group). *P<0.05; significant increase in time spent exploring the novel compared with familiar object.

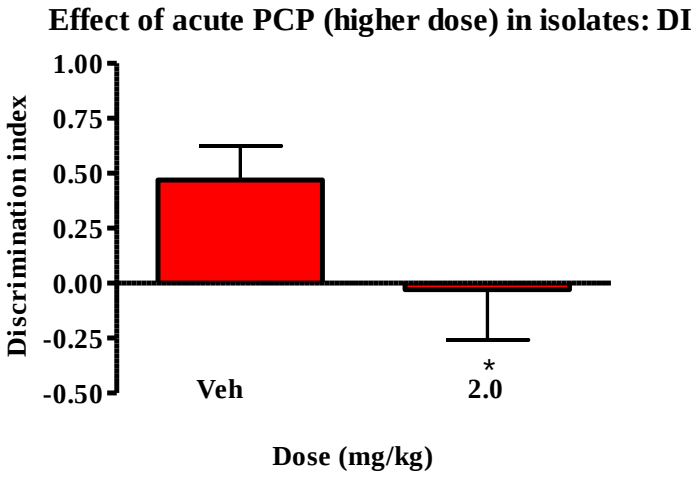


Figure 6.28 The effect of acute treatment with PCP (2.0 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the DI in the NOR test in female rats housed in **isolation**. Data are expressed as the mean ± S.E.M. (n=8 per group). *P<0.05; significant reduction in DI compared with vehicle.

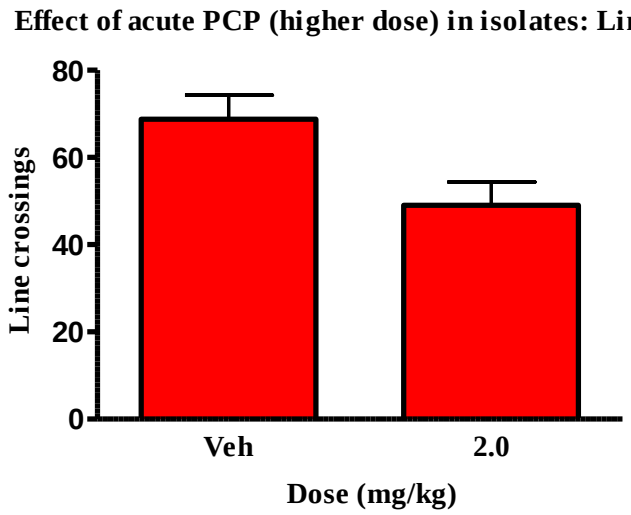


Figure 6.29 The effect of acute treatment with PCP (2.0 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats housed in isolation. Data are expressed as the mean ± S.E.M. (n=8 per group).

Isolates	***Total exploration time (s)***	
PCP (mg/kg)	***Acquisition trial***	***Retention trial***
Veh	20.9 ± 2.1	23.3 ± 3.5
2.0	27.5 ± 5.6	29.4 ± 3.9

Table 6.13 The effect of acute treatment with PCP (2.0 mg/kg, i.p) and 0.9% saline (veh, i.p) on the total exploration time in the acquisition and retention trials of the NOR test in female rats housed in isolation. Data are expressed as the mean ± S.E.M. (n=8 per group).

6.3.30 Effect of acute d-amph treatment (0.25-0.75 mg/kg) in the acquisition trial in female rats housed in social groups

A two-way ANOVA revealed that acute treatment with d-amph did not produce a significant effect on object exploration in the acquisition trial of the NOR test ($F_{3, 21}$=0.39, NS). Rats from all treatment groups spent similar times exploring both the

objects (figure 6.30). Analysis of the total object exploration time in the acquisition trial by a one-way ANOVA revealed a significant effect of drug treatment ($F_{3, 21}$=7.93, P<0.01). Post-hoc analysis showed that all doses of d-amph (0.5 - 0.75 mg/kg) significantly (P<0.05-P<0.001) reduced the total exploration time of the identical objects when compared to vehicle control (table 6.14).

6.3.31 Effect of acute d-amph treatment (0.25-0.75 mg/kg) in the retention trial female rats housed in social groups

A two-way ANOVA revealed that treatment with d-amph produced a significant effect on object exploration (figure 6.31) in the retention trial of the NOR test ($F_{3, 21}$=3.6, P<0.05). Further planned post-hoc analysis showed that rats treated with vehicle control and the lowest dose of d-amph (0.25 mg/kg) had a significant (P<0.05 and P<0.01, respectively) preference for the novel compared with the familiar object. This significant preference in the vehicle treated rats for the novel object was not observed in the rats treated with higher doses of d-amph (0.5 - 0.75 mg/kg), i.e. these rats spent similar amounts of time exploring both objects. Analysis of the total exploration time of the objects in the retention trial by a one-way ANOVA revealed no significant effect ($F_{3, 21}$=1.33, NS). Post-hoc analysis on the observed reduction in total exploration time following treatment with the highest dose of d-amph (0.75 mg/kg) failed to reach significance (P=0.15) when compared to vehicle (table 6.14).

6.3.32 Effect of acute d-amph treatment (0.25-0.75 mg/kg) on the DI in female rats housed in social groups

One-way ANOVA on the DI data showed a significant effect of d-amph treatment ($F_{3, 21}$=4.02, P<0.05; figure 6.32). Further post-hoc analysis of the DI showed that the highest dose of d-amph (0.75 mg/kg) induced a significant (P<0.05) reduction in DI when compared to vehicle control.

6.3.33 Effect of acute d-amph treatment (0.25-0.75 mg/kg) on the total number of line crossings in female rats housed in social groups

Figure 6.33 shows the effect of acute d-amph treatment on total number of line crossings of the rats during the acquisition and retention trials of the NOR test. One-way ANOVA revealed a significant effect of d-amph on total number of line crossings ($F_{3, 21}$=10.4, P<0.001). Further post-hoc analysis of the total number of line crossings revealed that the two highest doses of d-amph (0.5 and 0.75 mg/kg) induced a significant (P<0.01 and P<0.001 respectively) increase in the number of line crossings.

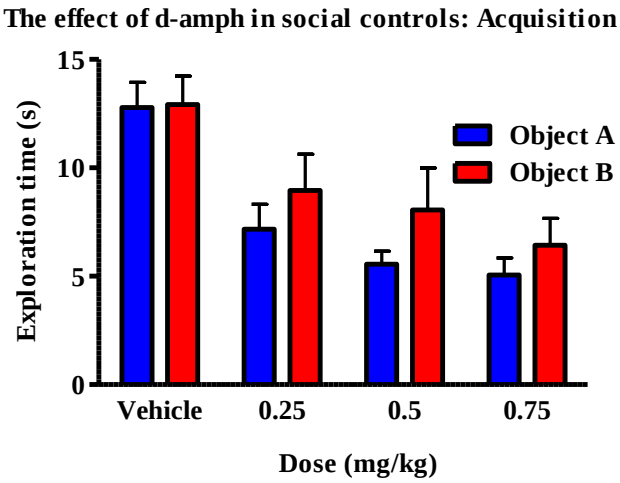


Figure 6.30 The effect of acute treatment with d-amph (0.25-0.75 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on exploration of two identical objects in the 3 min acquisition trial of the NOR test in female rats housed in **social** groups. Data are shown as mean ± S.E.M. (n=8 per group).

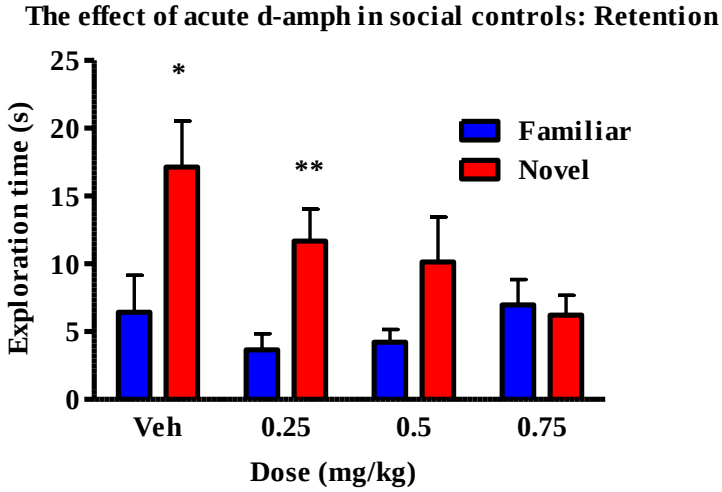


Figure 6.31 The effect of acute treatment with d-amph (0.25-0.75 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on time (s) spent exploring a familiar and a novel object in the 3 min retention trial in female rats housed in **social** groups. Data are shown as mean ± S.E.M. (n=8 per group). *P<0.05-**P<0.01; significant increase in the time (s) spent exploring the novel compared with the familiar object.

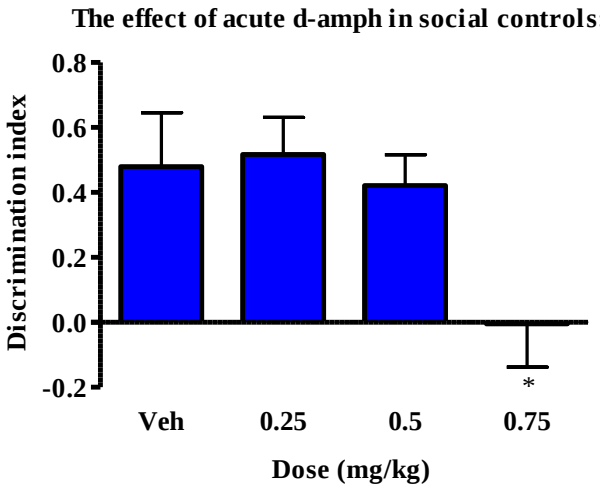


Figure 6.32 The effect of acute treatment with d-amph (0.25-0.75 mg/kg, i.p.) and 0.9% saline (veh, i.p) on the DI in the NOR test in female rats housed in **social** groups. Data are expressed as the mean ± S.E.M. (n=8 per group). *P<0.05; significant reduction in DI compared with vehicle.

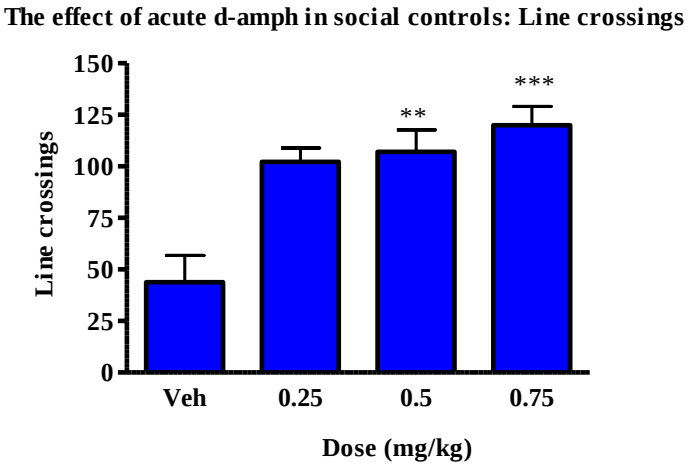


Figure 6.33 The effect of acute treatment with d-amph (0.25-0.75 mg/kg, i.p) and 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats housed in **social** groups. Data are expressed as the mean ± S.E.M. (n=8 per group). **P<0.01-**P<0.001; significant increase compared to vehicle control.

Socials	***Total exploration time (s)***	
d-amph (mg/kg)	***Acquisition trial***	***Retention trial***
Veh	25.7 ± 1.9	21.9 ± 3.2
0.25	16.1 ± 2.5 *	15.3 ± 2.6
0.5	13.6 ± 2.1 **	14.4 ± 4.0
0.75	11.5 ± 1.9 ***	13.2 ±2.8 (P=0.15)

Table 6.14 The effect of acute treatment with d-amph (0.5-0.75 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats housed in social groups. Data are expressed as the mean ± S.E.M. (n=8 per group). *P<0.05-***P<0.001; significant reduction in the total exploration time when compared to vehicle control.

6.3.34 Effect of acute d-amph treatment (0.25-0.75 mg/kg) in the acquisition trial in female rats housed in isolation

A two-way ANOVA revealed that treatment with d-amph did not produce any significant effect on object exploration in the acquisition trial of the NOR test ($F_{3, 21}$=1.6, NS). Rats from all treatment groups spent similar times exploring both objects (figure 6.34). Analysis of the total exploration time in the acquisition trial by a one-way ANOVA revealed no significant effect ($F_{3, 21}$=1.47, NS). However, planned post-hoc analysis showed that the highest dose of d-amph (0.75 mg/kg) significantly (P<0.05) reduced the total exploration time of the identical objects when compared to vehicle control (table 6.15).

6.3.35 Effect of acute d-amph treatment (0.25-0.75 mg/kg) in the retention trial female rats housed in isolation

A two-way ANOVA revealed that treatment with d-amph produced a significant effect on object exploration in the retention trial of the NOR test ($F_{3, 21}$=3.06, P<0.05). Planned post-hoc comparisons revealed that rats spent significantly (P<0.05) more time exploring the novel object during the retention trial in the vehicle control group only (figure 6.35). This significant preference in the vehicle treated rats for the novel object was not observed in the rats treated with all doses of d-amph (0.25-0.75 mg/kg), i.e. these rats spent similar amount of time exploring both objects. Analysis of the total exploration time in the retention trial by a one-way ANOVA revealed a significant effect of drug treatment ($F_{3, 21}$=4.64, P<0.05). Post-hoc analysis showed that the highest doses of d-amph (0.5 and 0.75 mg/kg) significantly (P<0.05 and P<0.01, respectively) reduced the total exploration time of the objects in the retention phase when compared to vehicle control (table 6.15).

6.3.36 Effect of acute d-amph treatment (0.25-0.75 mg/kg) on the DI in female rats housed in isolation

One-way ANOVA on the DI data showed no significant effect of d-amph treatment ($F_{3, 21}$=1.33, NS; figure 6.36).

6.3.37 Effect of acute d-amph treatment (0.25-0.75 mg/kg) on the total number of line crossings in female rats housed in isolation

Figure 6.37 shows the effect of d-amph treatment on total number of line crossings of the rats during the acquisition and retention trials in the NOR test. One-way

ANOVA revealed a significant effect of d-amph on line crossings ($F_{3, 21}$=8.14, P<0.01). Further post-hoc analysis of the total line crossings revealed that all the doses of d-amph (0.25 - 0.75 mg/kg) induced a significant (P<0.01 - P<0.001) increase in number of line crossings compared to vehicle.

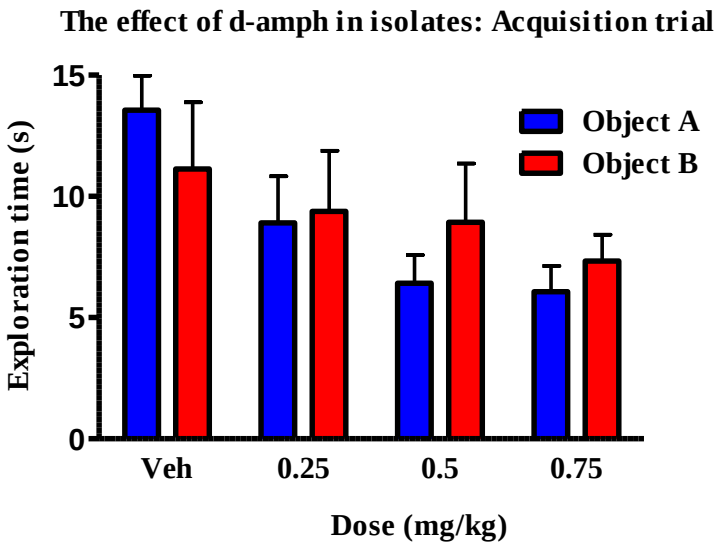


Figure 6.34 The effect of acute treatment with d-amph (0.25-0.75 mg/kg, i.p.) and 0.9% saline (veh, i.p) on the exploration of two identical objects in the 3 min acquisition trial in the NOR test in female rats housed in **isolation**. Data are shown as mean ± S.E.M. (n=8 per group).

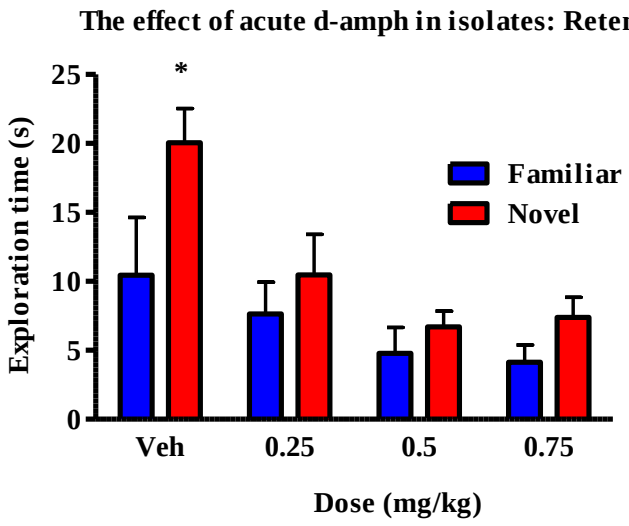


Figure 6.35 The effect of acute treatment with d-amph (0.25-0.75 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the time (s) spent exploring a familiar and a novel object in the 3 min retention trial in female rats housed in **isolation**. Data are shown as mean ± S.E.M. (n=8 per group). *P<0.05; significant increase in the time spent exploring the novel compared with the familiar object.

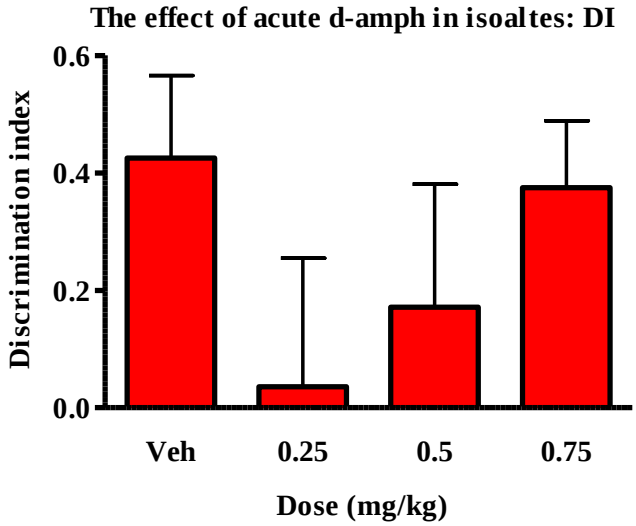


Figure 6.36 The effect of acute treatment with d-amph (0.25-0.75 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the DI in the NOR test in female rats housed in **isolation**. Data are expressed as the mean ± S.E.M. (n=8 per group).

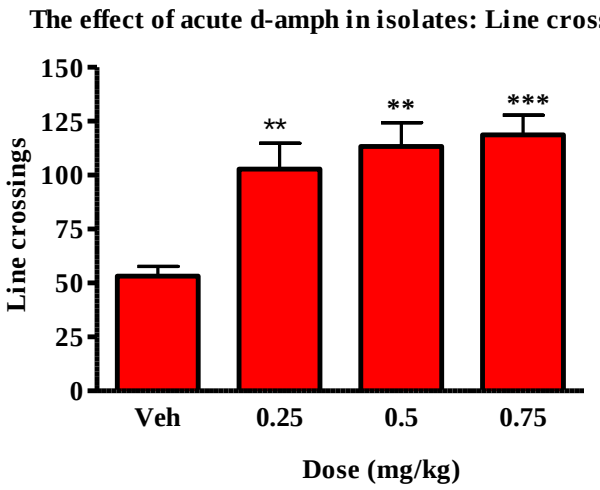


Figure 6.37 The effect of acute treatment with d-amph (0.25-0.75 mg/kg, i.p.) and 0.9% saline (veh, i.p.) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats housed in **isolation**. Data are expressed as the mean ± S.E.M. (n=8 per group). **P<0.01-***P<0.001; significant increase the line crossings when compared to vehicle control.

Isolates	***Total exploration time (s)***	
d-amph (mg/kg)	***Acquisition trial***	***Retention trial***
Veh	24.6 ± 3.3	30.5 ± 6.2
0.25	18.3 ± 4.3	18.1 ± 4.9
0.5	15.4 ± 2.9	11.5 ± 2.5 *
0.75	13.4 ± 2.0 *	11.5 ± 2.4 **

Table 6.15 The effect of acute treatment with d-amph (0.5-0.75 mg/kg, i.p.) and 0.9% saline (veh, i.p) on the total number of line crossings in the acquisition and retention trials of the NOR test in female rats housed in **isolation**. Data are expressed as the mean ± S.E.M. (n=8 per group). *P<0.05-**P<0.01; significant reduction in total exploration time when compared to vehicle control.

Housing conditions	*LMA response to novel environment*	*Onset of deficit in the retention trial*	*Deficit in the DI*	*Effect of ITI on line crossings*	*Effect of ITI on total exploration time*
Isolates	***Yes**	3.5 h	No (trend; NS)	**No**	# (acquisition; 1h, 3.5h & 4h *cf.* 1 min)
Socials	**No**	5 h	No (trend; NS)	**No**	# (acquisition; 6h *cf.* 1 min)

Table 6.16 Summary table showing the effect of differential ITIs on the performance in the NOR test in female rats raised in **isolation** or in **social** groups. Significant (*P<0.05) increase in LMA in the isolates when compared to the social control group. Significant decrease (#P<0.05-P<0.01) compared to 1 min ITI.

Treatment	*Doses tested (mg/kg)*	*Effective in the retention trial. Dose (mg/kg)*	*Deficit in the DI. Dose (mg/kg)*	*Effect on line crossings. Dose (mg/kg)*	*Effect on total exploration. Dose (mg/kg)*
Acute PCP	**0.5, 1.0, 1.5**	**No**	1.5; P=0.09, NS	**No**	**No**
Acute PCP (higher dose)	**2.0**	**Yes**	**Yes** 2.0	**No**	* (Acquisition; P=0.09. Retention; P=0.1)
Acute PCP (repeat)	**0.5**	**Yes**	**Yes** 0.5	**No**	**No**
Acute d-amph	**0.25, 0.5, 0.75**	**Yes** 0.5, 0.75	**Yes** 0.75	*0.5, 075	# (Acquisition; 0.25, 0.5 0.75, retention; 0.75 P=0.15)

Table 6.17 Summary table showing the effect of acute treatment with PCP (0.5-2.0 mg/kg or d-amph (0.25-0.75 mg/kg) on female rats raised in **social** groups on performance in the NOR test. Significant decrease# or increase* (P<0.05-P<0.001) compared to the vehicle treated group.

Treatment	*Doses tested (mg/kg)*	*Deficit in the retention trial. Dose (mg/kg)*	*Deficit in the DI. Dose (mg/kg)*	*Effect on line crossings. Dose (mg/kg)*	*Effect on total exploration. Dose (mg/kg)*
Acute PCP	0.5, 1.0, 1.5	**No**	**No**	*0.5	**No**
Acute PCP (higher dose)	2.0	**Yes**	**Yes**	**No**	**No**
Acute d-amph	0.25, 0.5, 0.75	**Yes** 0.25, 0.5, 0.75	**No**	*0.25, 0.5, 0.75	#(acquisition; 0.75, retention; 0.5, 0.75)

Table 6.18 Summary table showing the effect of acute treatment with PCP (0.5-2.0 mg/kg or d-amph (0.25-0.75 mg/kg) on female rats raised in **isolation** on performance in the NOR test. Significant decrease# or increase* (P<0.05-P<0.001) compared to the vehicle treated group.

6.6 Discussion

6.6.1 LMA in a novel environment

Results demonstrate that isolation rearing induced an enhanced locomotor reactivity to a novel environment (LMA box) compared to socially housed controls. The isolated rats were more active compared to the social controls when comparing the mean locomotor counts over a 60 min period. These results are supported by many studies into the effects of isolation rearing (Phillips et al, 1994; Hall et al, 1998; Lapiz et al, 2000; Fabricius et al 2011; review by Fone and Porkess, 2008) and indicate that isolated rats show a differential habituation response to a novel environment. The rationale behind measuring the rat's locomotor activity response to a novel environment, prior to behavioural experimentation and subsequent drug administration was to ensure the efficacy of the isolation procedure. Hyperactivity

response to a novel environment induced by isolation rearing has been shown to be dependent on dopaminergic function (Burns et al, 1994), and indicates that isolation rearing of rats induces an alteration of the mesolimbic dopamine system.

6.6.2 Socials and isolates; Differential ITI

In this study, there was no effect of rearing conditions on the acquisition trial or on line crossings following any ITI which suggests specificity of effects in the NOR test. However there were reductions in the total exploration time in the acquisition trial in the socially reared and to a greater extent in the isolation reared rats which could be explained as an exploratory habituation effect of repeated (twice) testing of the rats. Work carried out in our laboratory has previously shown that female rats reared in social groups can discriminate between novel and familiar objects when the ITI is 3 h, but were unable to discriminate at 4 h (Sutcliffe et al, 2007). However, in the present study, female rats reared in social groups were successful at discriminating between the novel and familiar object at a greater ITI of 4 h and incapable of discriminating at 5 h. The rats were supplied for the studies by two different companies (Harlan & Charles River) which may explain the difference in the discriminatory abilities of the rats in these two studies. In contrast, the rats reared in isolation were only successful at discriminating between novel and familiar objects up to an ITI of 1 h, but were not capable of discriminating between the novel and familiar objects at 3.5 h, indicating an impairment in object recognition memory in rats reared in isolation. A previous study into the effects of isolation rearing in NOR using male Hooded-Lister rats showed deficits in their ability to discriminate between the novel and familiar objects following at 1 h ITI (Bianchi et al, 2006). Studies in our laboratory have previously shown that male rats can discriminate

between the novel and familiar objects up to an ITI of 30 min and are unable to discriminate between the novel and familiar objects following an ITI of 1h. This difference in discriminatory ability of male and female isolation reared rats may be explained by evidence showing that in general female rats are superior at discriminating between the novel and familiar objects at longer ITIs (Sutcliffe et al, 2007).

6.6.3 Isolation rearing; acute PCP

The first dose range (0.5-1.5 mg/kg) of acute PCP when administered to socially reared rats failed to produce an object recognition memory deficit, albeit a reduction in the DI was observed, when compared to vehicle controls, but this failed to reach statistical significance (P=0.09). The lack of effect of acute PCP to produce an object recognition impairment was unusual, unexpected and contradicted the results from chapter 4, which clearly showed that acute PCP treatment induced an object recognition memory deficit at all the doses tested (0.5-1.5 mg/kg). The female rats used in chapter 4 had a lighter bodyweight (g), and were younger than the social controls used in this study. It was therefore decided to test a higher dose of PCP (2 mg/kg) in the same cohort of rats (cohort 12), and to re-test the lowest dose of PCP (0.5 mg/kg) in a new cohort of younger and lighter (g) social controls (cohort 13). This time, both treatments resulted in clear object recognition memory deficits. The differences in bodyweight and age may provide some explanation for the lack of consistency in the results obtained following treatment with acute PCP in the separate cohorts of rats. In the isolation reared rats, acute PCP treatment did not affect exploration levels in the acquisition trial and failed to induce an object recognition deficit as measured in the retention trial at all of the doses tested.

However, there was a small but significant increase in the total number of line crossings following administration of the lowest dose of PCP (0.5 mg/kg), which may suggest that acute treatment with PCP at this dose is inducing hyperactivity. As with the social control rats, a higher dose of PCP (2 mg/kg) was tested in the isolation reared rats and a clear object recognition deficit was observed in the absence of any non specific drug effects in the acquisition trial, line crossings and total exploration time. The apparent lack of sensitivity of the isolation reared rats to the object recognition deficits induced by PCP in the social controls is not supported by previous studies in our laboratory, whereby the identical doses of acute PCP impaired reversal task performance of the reversal learning task in social and isolation reared rats (Abdul-Monim, et al, 2003). It is not clear why the isolation reared rats were less sensitive to the acute PCP-induced recognition memory impairments than socially reared controls in the NOR test. However, acute PCP treatment enhances dopamine in the mPFC (Jentsch et al, 1997; Balla et al, 2003; Kalinichev et al, 2008) and isolation rearing induces a reduction of dopamine turnover and function in the mPFC (Heidbreder et al, 2000; Muira et al, 2002; Fone & Porkess, 2008). Acute treatment with PCP could enhance the dopamine function in the mPFC back to control levels, but not induce a hyperdopaminergic state, thus sparing the isolation reared rats from the cognitive impairments observed in the social controls. The mPFC is a region known to be important for cognition (Goldman-Rakic et al, 2000) and is connected to the perirhinal cortex via excitatory projections (Aspergis-schoute et al, 2006). Conversely, the recognition deficits induced by acute PCP treatment in social control rats may be explained by acute PCP inducing a hyperdopaminergic state with levels of dopamine above the optimum levels for cognition. The optimum levels of dopamine required for performance in

the NOR test follows an inverted U-shape and reflects the Yerkes-Dodson law (Kimberg et al, 2001).

6.6.4 Isolation rearing; acute d-amphetamine

Object recognition impairments were observed following the administration of d-amphetamine to both social and isolation reared rats. However, isolation reared rats exhibited object recognition deficits at a lower dose of d-amphetamine compared to social controls, indicating an enhanced sensitivity to the cognitive impairment effects of the isolation reared rats to d-amphetamine. Additionally, both groups of rats demonstrated enhanced total number of line crossings following treatment with d-amphetamine, signifying a lack of specificity of the cognitive impairment induced by d-amphetamine. Conversely, only the isolation reared rats showed increased line crossings at a lower dose of d-amphetamine, indicating an enhanced sensitivity to the locomotor stimulant effects of d-amphetamine. Furthermore, the effect of d-amphetamine to induce recognition deficits in the NOR test is confounded by the reduced total exploration time of the objects. These results are in agreement with previous work carried out in our laboratory (Smith et al, 1997) and other laboratories (Jones et al, 1990; 1992), demonstrating that isolation reared rats have an enhanced response to the d-amphetamine in terms of increased locomotor activity, although there are some conflicting reports (Bowling & Bardo 1994). Both groups of rats demonstrated decreased total exploration time of the objects in the acquisition and retention trials following treatment with d-amphetamine, further substantiating the lack of specificity of the cognitive impairment induced by acute d-amphetamine in the NOR test. Reversal learning studies in our laboratory have shown that administration of d-amphetamine to socially reared rats selectively impairs the

performance of an operant reversal-learning task an effect which was subsequently reversed by the administration of the D_2 receptor antagonist haloperidol. (Idris et al, 2005). Microdialysis studies have shown that acute administration of d-amphetamine elevates dopamine levels in the mPFC (Pehek, 1999), thus inducing a hyerdopaminergic state which may account for the recognition memory deficits observed in the NOR test.

CHAPTER 7 - Investigation of psychotomimetic-induced deficits in another test of cognitive function impaired in psychiatric disorders: Working memory using the 16-holeboard maze

7.1 Introduction

Working memory deficits are one of the most robust and measurable cognitive deficits observed in schizophrenia patients and their relatives (Goldman-Rakic, 1994; Barch, 2005, Lee & Park, 2005). Evidence for the role of the prefrontal cortex in working memory comes from pre-clinical and clinical studies such as human neuropsychological, brain lesion, functional brain imaging studies and lesion studies in the primate (Perlstein et al, 2001). The role of the prefrontal cortex in schizophrenia comes from studies that demonstrate poor performance of patients on tasks that are sensitive to frontal lobe damage such as the WCST (van der Does & van den Bosch, 1992) and delayed response tasks (Park & Holzman, 1992; Carter et al, 1996). Furthermore, schizophrenia patients showed a physiological deficit in activation of the right dorsolateral prefrontal cortex under task performance of the "n-back" sequential letter working memory task when compared to healthy controls (Perlstein et al, 2001).

Primarily the holeboard has previously been used to assess altered exploratory activity and anxiety and not spatial learning and memory (Rogers et al, 1999; Casarrubea et al, 2010). However, there have been a number of studies reported using the holeboard maze for measuring experimental manipulations on learning and memory in mice and rats (Oades and Isaacson, 1978; Kuc et al, 2006). A large variety of measures can be assessed using the holeboard maze (see review by van der Staay et al, 2012). The working memory measure has been calculated in a variety of ways, for results shown in this thesis, working memory score is calculated as: rewarded hole visits/ total hole visits (see chapter 2, section 2.1.8.4.2).

Selected pharmacological agents and specific brain lesions have been shown to disrupt learning and memory on the holeboard maze. Studies by Kuc and colleagues (2006) established that the muscarinic antagonist scopolamine at 0.1 and 1.0 mg/kg significantly increased the working memory score i.e. the mice performed worse compared to controls.

The cone field test is a variant of the holeboard maze which instead of holes, has 16 cones upon which small cups hold the food rewards (van der Staay et al, 1990). A study by Blokland et al (1998) demonstrated impairments in working memory following treatment with d-amphetamine in the cone field test. Furthermore, deficits in working memory were observed in the cone field test following treatment with scopolamine, which were reversed by the acetylcholinesterase inhibitor metrifonate (van der Staay & Bouger, 2005). In another study, a significant increase in the number of working memory errors was observed following a repeated (once daily for 14-days) dosing regimen with the NMDA receptor antagonist MK801 in a modified version of the holeboard maze (Rujescu et al, 2006).

It has been shown that deactivation of the medial prefrontal cortex with muscimol selectively impairs working memory in a delayed alternation task in the rat (Yoon et al, 2008). Additionally, lesions of the prelimbic and infralimbic but not the medial prefrontal cortex region of the frontal cortex has been shown to induce profound impairments in working memory in a delayed non-matching-to-sample test in the rat (Kesner et al, 1996). Delayed discrimination tasks have also revealed to important role for the prefrontal cortex. For example, lesions of the prefrontal cortex have been shown to impair performance in a delayed alternation task (Joel et al, 1997; Sanchez-Santed et al, 1997). The effect of prefrontal cortex lesions has not been assessed

using the holeboard maze. However, lesions of the hippocampus, a brain region also implicated in working memory and schizophrenia, resulted in increased working memory errors in a 16-holeboard maze in the rat (Oades, 1981). Working memory on the holeboard maze is not only impaired after direct lesions of the hippocampus, but also lesions of the hippocampal afferents (van der Staay et al, 1989).

The aim of this study was to determine the effects of acute treatment with psychotomimetic agents d-amph, PCP and the muscarinic antagonist scopolamine on working memory score in the 16-holeboard maze. Furthermore, the cognitive effects of the sub-chronic PCP dosing regimen were evaluated in this paradigm to detect impairments in working memory score.

7.2 Materials and methods

7.2.1 Experimental Animals and Design

Studies used two cohorts (cohort 16 & 17) of adult (n=28; 200-245g) female hooded-Lister rats (Harlan, UK), details are shown in tables 7.1 & 7.2. For housing conditions see section 2.1.1 and for information regarding the drugs used in this study see table 7.3, 7.4 and 2.2. For the sub-chronic PCP dosing regimen see section 2.1.3.

Experiment number	**Cohort number**	**Total number of rats used**	**Weight range (g)**	**Compound tested and dose (mg/kg)**
1	1	16	200-245	PCP 2.0
2	1	16	200-245	d-amph 0.75
3	1	16	200-245	Scopolamine 0.25

Table 7.1 Details of the rats used in the acute studies.

Experiment number	*Cohort number*	*Total number of rats used*	*Weight range (g)*	*Compound tested and dose (mg/kg)*
4	2	12	210-245	Sub-chronic PCP 2.0

Table 7.2 Details of the rats used in the sub-chronic PCP studies.

7.2.2 Drugs

The individual experiments (1-3) were separated by 1 week, rats were tested three times and the study took 3 weeks to complete.

In experiment 1, rats were randomly assigned to receive either an acute injection of PCP (2.0 mg/kg, i.p.) or vehicle (0.9% saline, i.p.). In experiment 2, rats were randomly assigned to receive either an acute injection of d-amph (0.75 mg/kg, i.p.) or vehicle (0.9 % saline, i.p.) and in experiment 3, rats were again randomly assigned to receive scopolamine (0.25 mg/kg, i.p.) or vehicle (0.9 % saline, i.p.). All compounds were administered 30 min prior to testing on the 16-holeboard maze (see table 2.2). The doses of PCP and d-amph used in this study were chosen based upon previous studies whereby similar doses of PCP (1.5 mg/kg) and d-amph (0.75 mg/kg) induced a psychotomimetic reversal learning deficit in rats (McLean et al, 2010). The dose of scopolamine was chosen based on a study whereby scopolamine (0.2 mg/kg) impaired NOR in young (3-6 mths old), male Wistar rats (Vannucchi et al, 1997).

Experiment number	*Acute Treatment*	*Dose (mg/kg)*	*Dose volume (ml/kg)*	*Initial number of rats*	*Number of rats excluded*
1	***Vehicle***	0	1	8	0
1	***PCP***	2.0	1	8	0
2	***Vehicle***	0	1	8	0
2	***d-amph***	0.75	1	8	0
3	***Vehicle***	0	1	8	0
3	***Scopolamine***	0.25	1	8	0

Table 7.3 The acute treatments and number of rats in each treatment group.

In experiment 4, a total of 12 rats were randomly assigned to receive either vehicle (n=6; 0.9% saline twice a day, i.p.) or PCP (n=6; 2mg/kg twice a day, i.p.) in a volume of 1 ml/kg for 7 days. Subsequently, animals were given a 7-day drug free period prior to 16-holeboard maze testing. Training data is not shown for cohort 17 as it was almost identical to the training data generated from cohort 16.

Experiment number	*Treatment*	*Dose (mg/kg)*	*Initial number of rats*	*Number of rats excluded*
4	***Veh***	0	6	0
4	***PCP***	2.0	6	0

Table 7.4 The dose of sub-chronic PCP and number of rats in each treatment group.

7.2.3 16-Holeboard Maze apparatus

For details regarding the apparatus, see section 2.1.8.1.

7.2.4 16-Holeboard Maze protocol

For details regarding the protocol, see section 2.1.8.3.

7.2.5 Statistical analysis

For details regarding the statistical analysis, see section 2.1.8.5.

7.3 Results

7.3.1 Effect of daily training sessions on WMS in the 16-holeboard maze in female rats

A one-way ANOVA revealed that daily training sessions had a significant ($F_{8, 143}$=10.2, P<0.001) effect on the WMS in the 16-hole board maze. Planned post-hoc comparisons showed a significant increase in WMS on days 3 (P<0.05), 4 (P<0.01) and 5-9 (P<0.001) when compared to day 1 (figure 7.1).

7.3.2 Effect of daily training sessions on latency to complete the task in the 16-holeboard maze in female rats

A one-way ANOVA revealed that daily training sessions had a significant ($F_{8, 143}$=37.7, P<0.001) effect on latency to complete the task in the 16-holeboard maze. Planned post-hoc comparisons showed a significant decrease in latency on days 4-9 (P<0.001) compared to day 1 (figure 7.2).

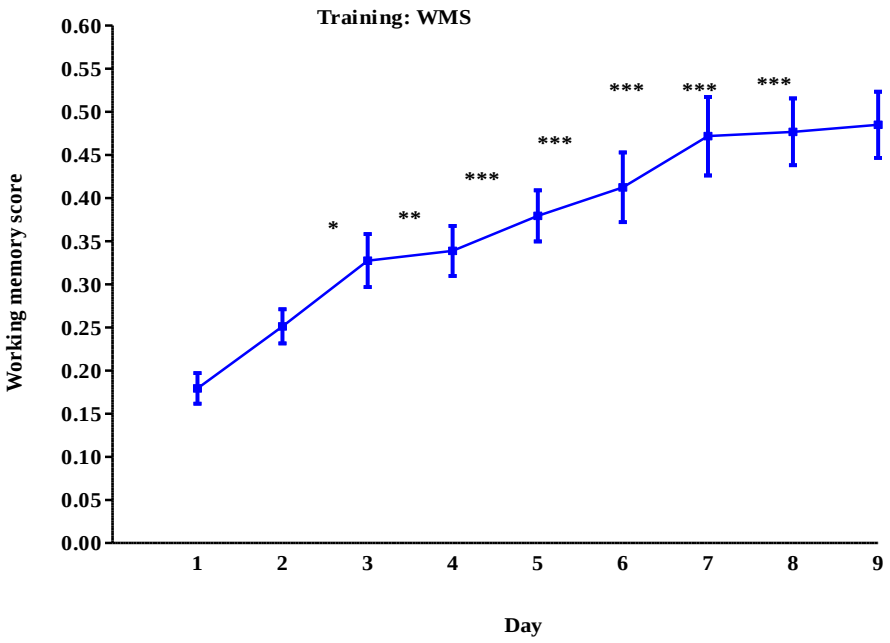


Figure 7.1 The effect of daily training sessions on WMS in the 16-holeboard maze. Data are expressed as the mean of 10 trials per day over 9-days ± S.E.M.. (n=16). *P<0.05-***P<0.001; significant increase in WMS compared to day 1.

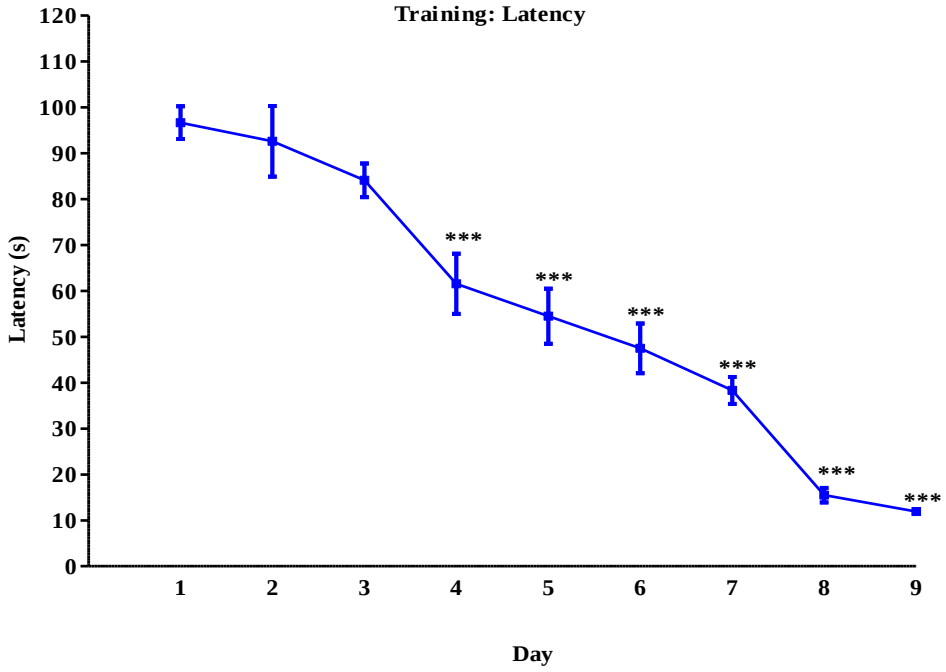


Figure 7.2 The effect of daily training sessions on latency to complete the task (i.e. collect all 4 food pellets) in the 16-holeboard maze. Data are expressed as the mean of 10 trials per day over 9-days ± S.E.M. (n=16). ***P<0.001; significant decrease in latency to complete the task compared to day 1.

7.3.3 Effect of acute PCP on WMS in the 16-holeboard maze in female rats

A two-way ANOVA revealed that acute administration of PCP (2.0 mg/kg) to rats produced an overall significant effect on WMS in the 16-holeboard maze ($F_{1, 14}$=57.7, P<0.001; figure 7.3). Further planned post-hoc Student's t-test analysis revealed a significant reduction in WMS in the PCP treated rats compared to vehicle controls in trials 1 & 3 (P<0.001), 4 (P<0.05), 5 (P<0.01) and 6-9 (P<0.05).

7.3.4 Effect of acute PCP on latency to complete the task in the 16-hole board maze in female rats

A two-way ANOVA revealed that acute administration of PCP (2.0 mg/kg) to rats produced an overall significant effect on latency to complete the task in the 16-holeboard maze ($F_{1.14}$=9.7, P<0.01; figure 7.4). Further planned post-hoc Student's t-test analysis revealed a significant increase in latency to complete the task in the PCP treated rats compared to vehicle controls in trials 1 (P<0.001), 4 (P<0.05) and 9 (P<0.05).

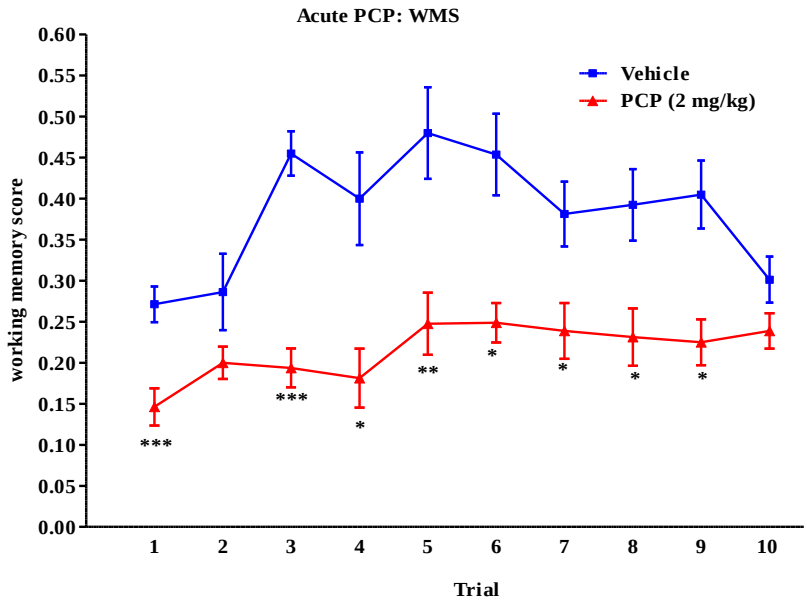


Figure 7.3 The effect of acute treatment with PCP (2 mg/kg, i.p.) on WMS over 10 trials in the 16-holeboard maze. Data are expressed as mean ± S.E.M. (n=8). *P<0.05-P<0.001; significant reduction in WMS compared to vehicle control.

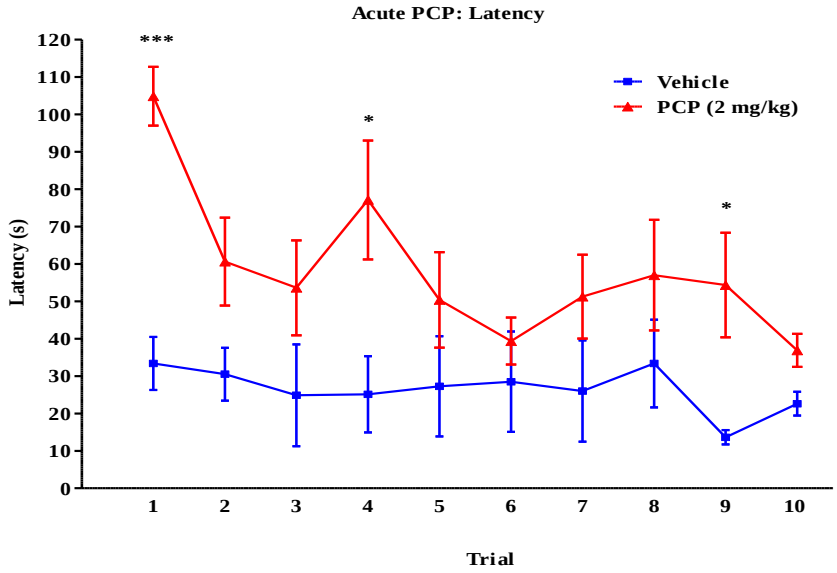


Figure 7.4 The effect of acute treatment with PCP (2 mg/kg, i.p.) on latency to complete the task (i.e. collect all 4 food pellets) over the 10 trials on the 16-holeboard maze. Data are expressed as mean ± S.E.M. (n=8). *P<0.05-***P<0.001; significant increase in latency to complete the task compared to vehicle control.

7.3.5 Effect of acute d-amph on WMS in the 16-holeboard maze in female rats

A two-way ANOVA revealed that acute administration of d-amph (0.75 mg/kg) to rats produced an overall significant effect on WMS in the 16-holeboard maze ($F_{1, 14}$=20.1, P<0.01; figure 7.5). Further planned post-hoc Student's t-test analysis revealed a significant reduction in WMS in the d-amph treated rats compared to vehicle control in trials 6-8 (P<0.05) and 10 (P<0.001).

7.3.6 Effect of acute d-amph on latency to complete the task in the 16-holeboard maze in female rats

A two-way ANOVA revealed that acute administration of d-amph (0.75 mg/kg) to rats produced an overall significant effect on latency to complete the task in the 16-holeboard maze ($_{F1.14}$=43.9, P<0.001; figure 7.6). Further planned post-hoc Student's t-test analysis revealed a significant increase in latency to complete the task in the d-amph treated rats compared to vehicle controls in trials 1-3 and 5 (P<0.05), 6 (P<0.01), 8 (P<0.05), 9-10 (P<0.01).

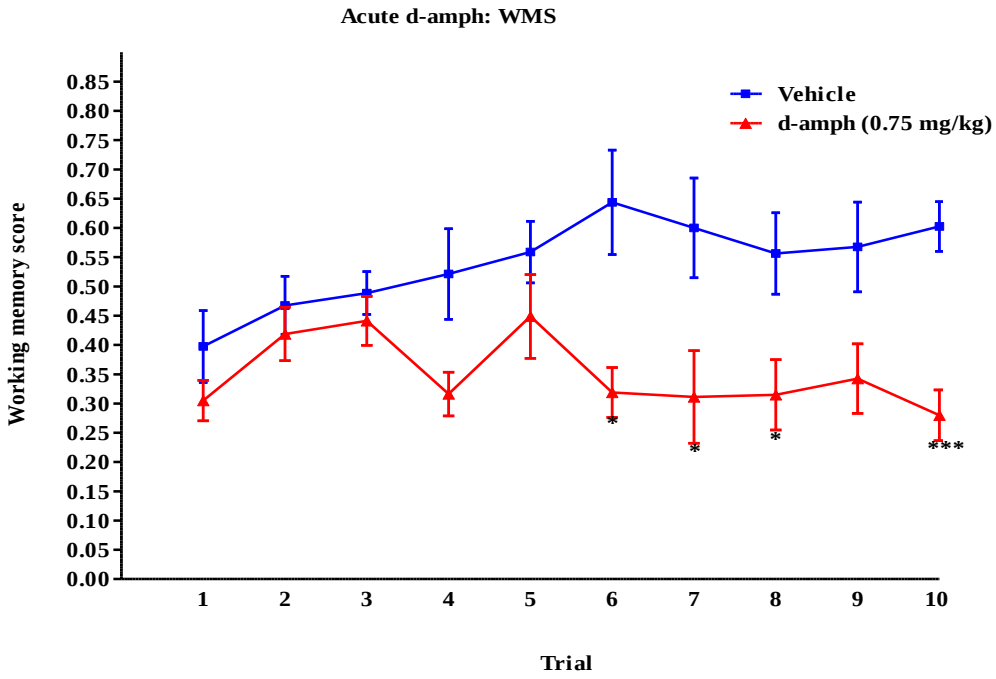


Figure 7.5 The effect of acute treatment with d-amph (0.75 mg/kg, i.p.) on WMS over 10 trials in the 16-holeboard maze. Data are expressed as mean ± S.E.M. (n=8). *P<0.05-P<0.001; significant reduction in WMS compared to vehicle control.

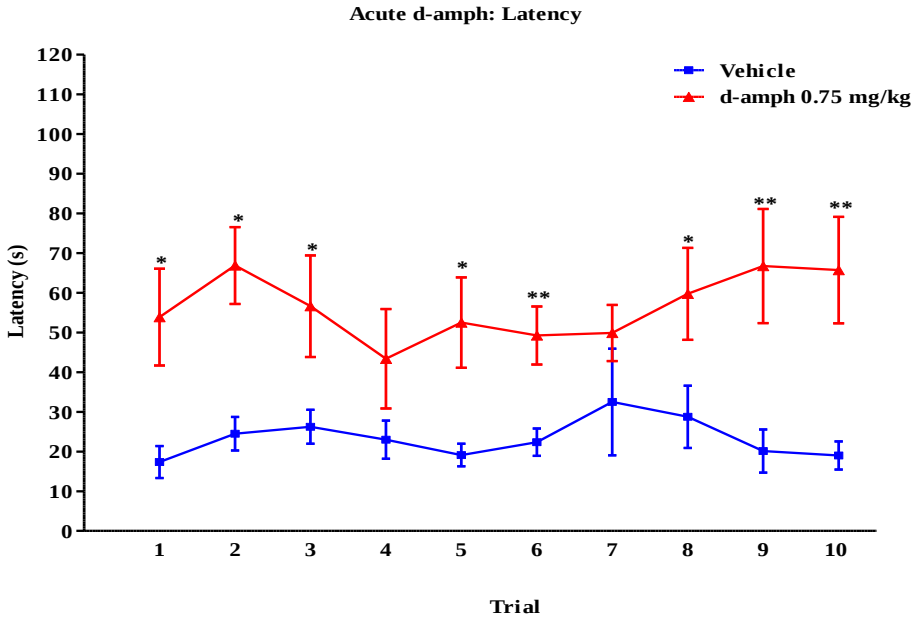


Figure 7.6 The effect of acute treatment with d-amph (0.75 mg/kg, i.p.) on latency to complete the task (i.e. collect all 4 food pellets) over the 10 trials on the 16-holeboard maze. Data are expressed as mean ± S.E.M. (n=8). *P<0.05-**P<0.01; significant increase in latency to complete the task compared to vehicle control.

7.3.7 Effect of acute scopolamine on WMS in the 16-holeboard maze in female rats

A two-way ANOVA revealed that acute administration of scopolamine (0.25 mg/kg) to rats produced an overall significant effect on WMS in the 16-holeboard maze ($F_{1, 14}$=20.3, P<0.001; figure 7.7). Further planned post-hoc Student's t-test analysis revealed a significant reduction in WMS in the scopolamine treated rats compared to vehicle control in trials 1 (P<0.05), 2-4 (P<0.01), 5 (P<0.001), 7 (P<0.01), 9 and 10 (P<0.05).

7.3.8 Effect of acute scopolamine on latency to complete the task in the 16-hole board maze in female rats

A two-way ANOVA revealed that acute administration of scopolamine (0.25 mg/kg) to rats produced an overall significant effect on latency to complete the task in the 16-holeboard maze ($_{F1.14}$=47.1, P<0.001; figure 7.8). Further planned post-hoc Student's t-test analysis revealed a significant increase in latency to complete the task in the scopolamine treated rats compared to vehicle controls in trials 2 (P<0.01), 3 (P<0.05), 4 (P<0.01) 5 and 6 (P<0.05), 7 (P<0.01), and 9 (P<0.05).

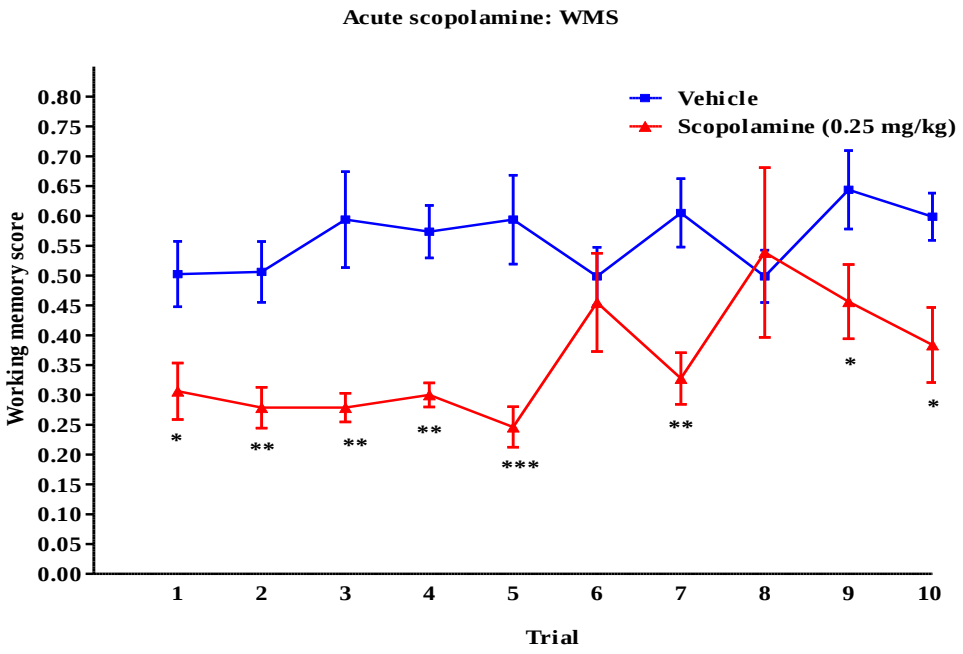


Figure 7.7 The effect of acute treatment with scopolamine (0.25 mg/kg, i.p.) on WMS over 10 trials in the 16-holeboard maze. Data are expressed as mean ± S.E.M. (n=8). *P<0.05-***P<0.001; significant reduction in WMS compared to vehicle control.

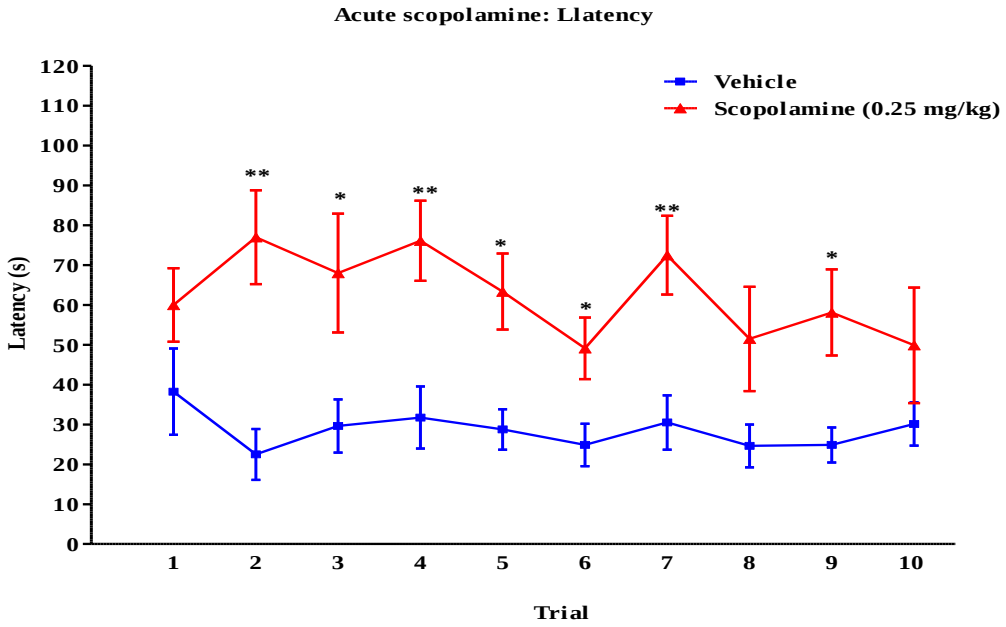


Figure 7.8 The effect of acute treatment with scopolamine (0.25 mg/kg, i.p.) on latency to complete the task (i.e. collect all 4 food pellets) over the 10 trials on the 16-holeboard maze. Data are expressed as mean ± S.E.M. (n=8). *P<0.05-**P<0.01; significant increase in latency compared to vehicle control.

7.3.9 Effect of sub-chronic PCP-treatment on WMS in the 16-holeboard maze in female rats

A two-way ANOVA revealed that sub-chronic PCP treatment produced an overall significant effect on WMS in the 16-holeboard maze ($F_{1, 10}$=9.3, P<0.05; figure 7.9). Further planned post-hoc Student's t-test analysis revealed a significant reduction in WMS in the sub-chronic PCP treated rats compared to vehicle controls in trials 1 (P<0.01), 6, 8 and 9 (P<0.05).

7.3.10 Effect of acute sub-chronic PCP-treatment on latency to complete the task in the 16-hole board maze in female rats

A two-way ANOVA revealed that sub-chronic PCP treatment produced an overall significant effect on latency to complete the task in the 16-holeboard maze ($_{F1.10}$=8.8, P<0.05; figure 7.10). Further planned post-hoc Student's t-test analysis revealed a significant increase in latency to complete the task in the sub-chronic PCP treated rats compared to vehicle controls in trials 1 (P<0.01), 4 (P<0.05) and 5 (P<0.01).

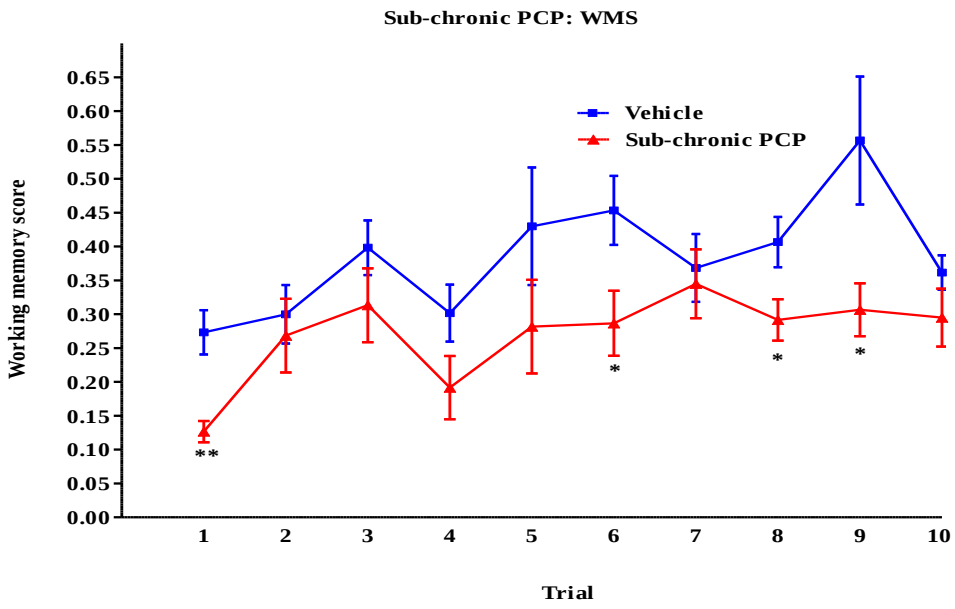


Figure 7.9 The effect sub-chronic PCP treatment (2mg/kg i.p. twice daily for 7-days) on WMS over 10 trials in the 16-holeboard maze. Data are expressed as mean ± S.E.M. (n=6). *P<0.05-**P<0.01; significant reduction in WMS compared to vehicle control.

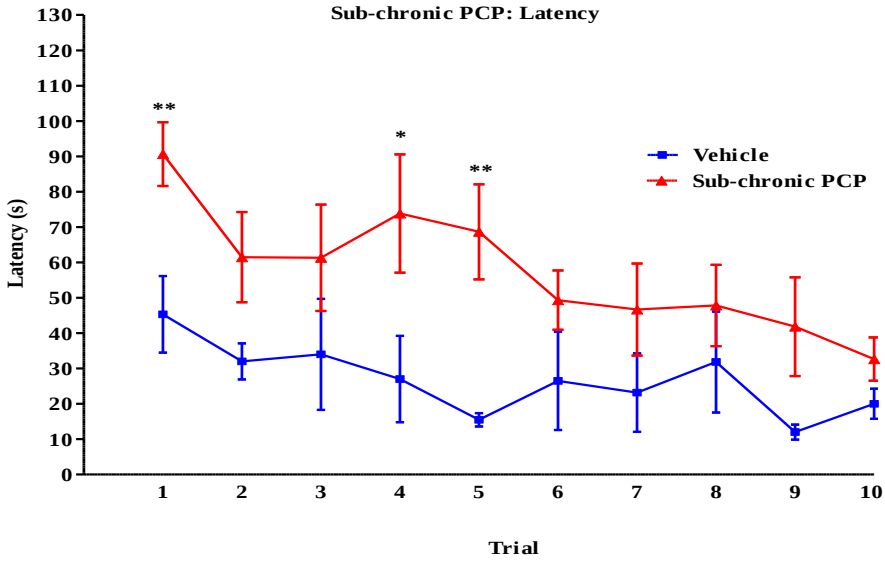


Figure 7.10 The effect sub-chronic PCP treatment (2mg/kg i.p. twice a day for 7-days followed by a 7-day drug free washout period) on latency to complete the task (i.e. collect all 4 food pellets) over the 10 trials in the 16-holeboard maze. Data are expressed as mean ± S.E.M. (n=6). *P<0.05-**P<0.01; significant increase in latency compared to vehicle control.

Treatments	*Number of trials showing a deficit in the WM S*	*Increase in latency to complete the task (time s)*
Acute PCP	8/10 trials (*-***)	3/10 trials(*-***)
Acute d-amph	4/10 trials (*-***)	8/10 trials (*-***)
Acute scopolamine	8/10 trials (*-***)	7/10 trials (*-**)
Sub-chronic PCP	4/10 trials (*-**)	4/10 trials (*-**)

Table 7.5 Summary table showing the effect of acute treatment with PCP, d-amph, scopolamine and sub-chronic PCP in female rats performance in the NOR test. *P<0.05-***P<0.001; significant deficit in WMS compared to vehicle control. *P<0.05-***P<0.001; significant increase in the latencey to complete the task**.**

7.4 Discussion

7.4.1 16-Holeboard maze

Studies demonstrate that daily training sessions to find the food rewards on the 16-holeboard maze increased the working memory score (WMS) compared to training day 1, which demonstrates the ability of the rats to learn the location of the 4 food rewards in the 16-holeboard maze and their ability to utilise their spatial working memory to remember which food rewards had already been eaten and not to re-visit these previously visited food rewarded holes. In addition, repeated daily training sessions on the 16-holeboard maze was seen to reduce the latency to find and eat the 4 food pellets during the training sessions compared to training day 1, which again provides further evidence of the ability of the rats to learn the test.

7.4.2 Acute PCP, d-amphetamine, scopolamine

Acute treatment with PCP was shown to impair the WMS throughout the test (8/10 trials), demonstrating a powerful effect of acute PCP to produce a cognitive impairment in the 16-holeboard maze. Latency to complete the task was generally increased following acute treatment with PCP and reached significance at 3/10 trials. This increase in latency is probably due to the rats making more errors and is a secondary consequence of the increased WMS observed in the acute PCP treated rats. It cannot be ignored that the reduction in latency to complete the task could be explained by a direct decrease in locomotor activity. Nevertheless, this is unlikely since acute PCP has been shown to increase locomotor activity in rats, usually at higher doses than used in this study (Javitt et al, 1997). However, the dose of PCP used in this study was selected based on the results in chapter 4 whereby, PCP at a dose of 2 mg/kg induced object recognition deficits in the absence of an increase in locomotor activity. The impairments in WMS following acute treatment with d-amphetamine were observed in 4/10 trials. The pattern of WMS impairment in the d-amphetamine treated rats differed from the overall general impairment observed in the acute PCP treated rats, such that WMS impairments following acute administration of d-amphetamine were only seen in the later trials (trials 6-8, 10). However, the increase in latency to complete the task induced by acute d-amphetamine treatment was observed in 8/10 trials, an effect that was evenly distributed throughout the 10 trials. In contrast to the acute PCP treated rats, the increase in latency to complete the task following acute d-amphetamine treatment is likely to be affected by the locomotor stimulant effect; since the dose of 0.5 mg/kg increased line crossings in the NOR test in chapter 4. Furthermore, it is widely

accepted that d-amphetamine is a powerful enhancer of locomotor acivity (Antoniou et al, 1998; Frey et al, 2006).

Administration of scopolamine induced deficits in WMS in 8/10 trials in the 16-holeboard maze. The pattern of WMS impairment over the 10 trials was different to those observed following acute treatment with PCP and d-amphetamine. The increased latency to complete the task was observed somewhat uniformly throughout the 10 trials of the 16-holeboard maze test and most likely is a result of the increased number of errors producing a reduction in the WMS, which has a secondary effect on latency to complete the task. Previous studies provide further support, showing that scopolamine-induced increases in locomotor activity in mice on the COGITAT hole-board system which is a modified automated version of the hole-board maze, decreased attention and resulted in a higher number of non-baited hole inspections (Post et al, 2011). Scopolamine induced memory deficits are related to reduced cholinergic activity through blockade of muscarinic receptors and studies in the clinic have shown that scopolamine induces working memory impairments in man (Thomas et al, 2008) and rodent (see review by Klinkenburg & Blokland, 2010). Scopolamine has been shown to increase locomotor activity at 0.56 mg/kg (Sipos et al, 1999), somewhat higher than the dose tested in this study. Nevertheless, we cannot rule out the possibility of non-specific locomotor effects of acute scopolamine in the 16-holeboard maze. The reduction in the scopolamine induced WMS observed in these studies is supported by numerous studies demonstrating increases in working memory errors in the 8-arm radial maze following acute treatment with scopolamine (Watts et al, 1981; Wang & Tang, 1998; Olsen & Cero, 2010).

7.4.2 Sub-chronic PCP

Following treatment with sub-chronic PCP, WMS deficits were observed in 4/10 trials, latency to complete the task increased in 3/10 trials. Sub-chronic PCP treatment did not affect the total number of line crossings in the NOR test. Therefore, it is unlikely that the increase in latency to complete the trials following treatment with sub-chronic PCP, in the hole board maze, is a direct locomotor effect of PCP and more likely to be secondary effect of the decrease in WMS. The reduction in the WMS induced by sub-chronic PCP is supported by Jentsch and Taylor (2001) who have demonstrated that rats display impairments in spatial working memory in the T-maze following sub-chronic PCP treatment. Furthermore, studies using a non-delayed 4-arm baited radial maze have reported working memory impairments in male Sprague-Dawley rats after 14 day treatment regimen of PCP (Noda et al, 2000).

CHAPTER 8 - General Discussion

8.1 General discussion

The work presented in this thesis investigates the NOR test as a potential paradigm for studying recognition memory in the rat. Initial pilot studies were carried out to provide validation initially this involved determination of innate preference for various different objects and then the effect of altering the inter-trial-interval was studied. Behavioural manipulations and psychotomimetic dosing regimens were performed in an attempt to induce cognitive impairments of relevance to psychiatric disorders, especially schizophrenia. Further studies aimed to attenuate the sub-chronic PCP-induced recognition memory deficits with a series of carefully selected pharmacological agents in an attempt to provide further understanding of the pharmacological mechanisms involved in the sub-chronic PCP induced cognitive deficit in the NOR test. Additional studies were carried out into the 16-holeboard maze as a potential paradigm for studying working memory which is impaired in many neurological and psychiatric disorders e.g. schizophrenia and Alzheimer's disease. A summary of all the results presented in this thesis from the individual chapters are shown in tables 3.3, 3.9, 4.13, 4.14, 4.15, 4.16, 5.16, 6.16, 6.17, 6.18 and 7.5.

Hooded-Lister rats were selected for use in all the studies, since these animals have been found to be superior when compared to other rat strains in a simple a simple T-maze task (Deacon & Rawlings, 2006). Additionally, in our laboratory female hooded-Lister rats have consistently been shown to learn quickly and perform reliably in various cognitive tasks (see review by Neill et al, 2010). The preliminary work in this thesis concentrated on the development of a suitable testing protocol for the NOR paradigm.

The importance of pilot studies prior to behavioural research work is paramount, undertaking careful initial studies allow the parameters of a paradigm to be adjusted and the observed animal behaviour fully understood. One of the main advantages of conducting pilot studies is that they give advance warning about where the main research project could fail, or whether proposed methods and measurement tools or instruments are inappropriate or too complicated (Festing & Altman, 2004).

The main findings of this thesis are summarised below:

(1) In summary, the results of the object preference studies in chapter 3 highlight the importance of undertaking preliminary pilot studies. It was vital to determine the object preference of both sub-chronic PCP treated rats and not just controls since one of the core feature of the thesis was to investigate the effect of sub-chronic PCP treatment in the NOR test. The results demonstrated a preference for the wood cone in both sub-chronic PCP and vehicle control treated rats, furthermore a significant increase in total exploration time of the wood cone was observed in the sub-chronic PCP treated rats when all the total exploration times were pooled from the series of object preference experiments. If this preliminary study had not been carried out, the entire data set where we used the wooden cone would have been fundamentally flawed.

(2) It could be hypothesised that sub-chronic PCP-induced recognition memory deficits observed after a short ITI such as 1 min in the NOR test may be attributed to impaired attention processes. Impaired attention in the NOR test would manifest as a decreased ability of the rats to encode information in the acquisition trial. However, it is unlikely that sub-chronic PCP-induced deficits in NOR are solely attributed to a lack of attention since the total exploration time in the acquisition and retention trials

in the sub-chronic PCP-treated groups of rats are not lower compared to control groups.

(3) A series of experiments was performed to determine if the recognition memory deficits observed in the sub-chronic PCP treated rats following the short 1 min ITI were as a result of disruption in the ability of the rats to encode the information (identical objects) in the acquisition trial. Therefore, if rats were unable to encode the information in the acquisition trial, any recognition deficits observed following sub-chronic PCP treatment could not be attributed to memory impairment. Results suggest that following a 0 or 1 min ITI, i.e. if rats remain in the NOR box whilst the acquisition objects (two identical objects) were carefully exchanged for the retention trial objects (familiar and novel objects), sub-chronic PCP treated rats were able to discriminate between the novel and familiar object. These animals are therefore successful in encoding the information presented in the acquisition trial. Furthermore, it was discovered that the sub-chronic PCP-induced deficit in recognition memory in the NOR test during the short ITIs (0 – 1 min) may be in part due to distraction. For example, the sub-chronic PCP treated rats only demonstrated object recognition deficits when they were either removed from the NOR test box by an experimenter or remained in the NOR test box with a distracter object for the duration of the ITI.

These careful pilot studies are vital components to the overall infrastructure in developing and validating a behavioural paradigm.

(4) Based on the pilot study results in chapter 3, acute treatment with PCP (0.5-5.0 mg/kg) and d-amph (0.1-2.5 mg/kg) were examined for their ability to induce cognitive deficits in both male and female rats in the NOR test (chapter 4). Results showed that that acute treatment with d-amph and PCP induces recognition deficits

in the NOR test with lower sensitivity in male compared to female rats, clearly signifying a gender difference in the PCP and d-amph–induced impairments in cognition. Sub-chronic PCP treatment induces recognition deficits in both sexes of rats, but more pronounced sub-chronic PCP-induced NOR deficits were observed in female rats.

(5) The effect of isolation rearing on performance in the NOR test was examined in chapter 6. The principal findings show that rats reared in social groups exhibit an enhanced capacity to discriminate between novel and familiar objects at a longer ITI in the NOR test than rats reared in isolation. Furthermore, rats reared in isolation were less sensitive to the acute PCP-induced recognition memory deficits and more sensitive to the acute d-amph-induced recognition memory deficits when compared to social controls in the NOR test.

(6) The results from chapter 5 show that sub-chronic PCP-induced cognitive deficits observed in the NOR test were successfully reversed by the atypical antipsychotic agents clozapine and risperidone, the cognitive enhancing agents modafinil and nicotine, the novel agents α7 nAChR agonist PNU-282987 and the α7 PAM PNU-120986. Furthermore, the sub-chronic PCP-induced cognitive deficits in NOR were not reversed by the classical antipsychotic agents haloperidol and fluphenazine, the benzodiazepine anxiolytic agent CDP or the SSRI antidepressant agent fluoxetine.

(7) In chapter 7, the 16-holeboard maze was selected to examine the acute and sub-chronic effects of PCP, acute treatment with d-amph or the muscarinic receptor antagonist scopolamine. Results demonstrate that acute treatment with d-amph, PCP and scopolamine induce robust reductions in working memory score in the holeboard

maze. In addition, sub-chronic PCP treatment also produced reductions in working memory score in this paradigm.

There has been a surge of interest in the NOR test by psychologists and neuroscientists in the last decade. The NOR test is a very simple, non-rewarded 1-trial test, requires little habituation, involves no training and experiments using this paradigm can be completed rapidly (Silvers et al, 2007). The NOR test utilises the rats' innate preference for novelty and measures the rats' ability to recognise a novel object within its environment. The unconditioned preference of rats and mice for the novel object is an indication that an account of the familiar object exists in the memory. The NOR test involves memory for a familiar object and detection and processing of information about a novel object. The ITI-delay/pharmacological dependent decrease in recognition memory results from a decay in memory of the familiar object. The novel object needs to be detected and encoded whereas the familiar object will need to be updated and reconsolidated after delay intervals or other manipulations (Ennaceur, 2010).

There has been much discussion over the type of memory that is measured using the NOR test (Ennaceur, 2010; Antunes & Biala, 2011) and it has been described as measuring working memory (Ennaceur & Delacour, 1987), episodic memory (Bertaina-Angalade et al, 2011), and novelty preference (Gaskin et al, 2003). Episodic memory is described as the ability remember an event in time and place i.e. what-where-when, however the 1-trial NOR test only measures what- (Le Cozannet et al, 2010). Assessing the temporal aspect of an episode is very challenging. A novelty preference test infers the rats may have a generic reaction of familiarity in the absence of a specific recollection of the memory of the familiar object. It is now

agreed that the NOR test is not a working memory test because the objects are apparently not encoded for the explicit purpose to achieve an ongoing task (Ennaceur, 2010).

In all experiments (chapter 1-6) using sub-chronic PCP treated rats, a robust recognition memory deficit in the retention trial was observed in the NOR test. Non-specific effects in the acquisition trial, line-crossings and total object exploration time were not observed following treatment with sub-chronic PCP in the NOR test. On the contrary, non-specific effects were observed following acute treatment with PCP and d-amph in the NOR test. Furthermore, results in chapter 4 demonstrate that male rats were insensitive to the acute PCP induced NOR deficits observed in female rats. However, male rats demonstrated sensitivity to the sub-chronic PCP induced NOR deficits. Both male and female rats demonstrated NOR deficits following acute treatment with d-amph, however, lower doses of d-amph induced NOR deficits in the female rats. These results provide evidence that sub-chronic PCP dosing regimen is superior to acute PCP treatment in producing NOR deficits in both sexes of rats. The possible reasons for the sex differences in the effects of PCP and d-amph are discussed in chapter 4. Since schizophrenia is generally shown to be more prevalent in males compared to female patients (Nicole et al, 1992), the sub-chronic PCP treatment regimen demonstrates better face validity compared to acute PCP treatment. Overall this suggests that the NOR test is a sensitive paradigm with ability to assess the sub-chronic PCP induced recognition memory deficits. The sub-chronic PCP-induced recognition deficits measured by the NOR test provide good face validity for the cognitive deficits associated with schizophrenia on the basis that

schizophrenia patients demonstrate impairments in a 2D-visual object recognition task (Tek et al, 2002).

In an attempt to provide further validation of and to explore the reliability of the NOR test to detect cognitive deficits associated with schizophrenia, it was important to assess object recognition memory in isolation reared rats; another model with translational relevance to some of the core symptoms of schizophrenia. Results are consistent with previous studies (Bianchi et al, 2006) demonstrating that isolation rearing of rats from weaning produced ITI dependent deficits in NOR when compared to social controls i.e. isolation reared rats could discriminate between novel and familiar objects up to ITIs of 3.5 h compared to 5 h in the social controls. The NOR deficits induced by isolation rearing were not comparable to the sub-chronic PCP-induced recognition deficits following a much shorter ITI of 1 min. A study by Jones et al (1990) may help to explain these differences; isolation reared rats were less susceptible to disruptions such as reduction in stimulus lights or the introduction of distracting stimulus during the acquisition of a conditional visual discrimination task. Therefore, the lack of NOR deficit observed in the isolation reared rats following a 1 min ITI could be explained by the resistance to distraction during the ITI; an opposite effect is observed following sub-chronic PCP treatment. Additionally, it may be suggested that sub-chronic PCP may induce greater neurophysiological disruptions compared to isolation rearing in rats and therefore demonstrate more powerful object recognition deficits in the NOR test.

An essential feature of an animal model of a human disease is the predictive validity. The predictive validity of any cognitive test will be limited by the quality of the rodent model and the degree to which they reproduce the biological basis of the

cognitive impairments associated with the disease state being modelled. Therefore, if the NOR test is to be classed as an important screening paradigm then it is imperative that the NOR test can detect compounds that demonstrate efficacy in the clinic and not generate false positives by detecting compounds that would not show efficacy in the clinic. Many genetic, pharmacological and environmental manipulations, thought to model aspects of cognitive dysfunction associated with schizophrenia have demonstrated impairments in the NOR test. Importantly, a number of pharmacological agents of relevance to therapy of schizophrenia have been shown the restore the cognitive deficits (see review by Lyon et al, 2011).

In chapter 5, a selection of agents with diverse pharmacology and mechanisms of action were selected to elucidate the potential of the sub-chronic PCP-induced cognitive deficit measured in the NOR test as a useful preclinical model for detection of novel pro-cognitive agents for the treatment of cognitive impairments in schizophrenia. Results in chapter 5 reveal that atypical agent's clozapine and risperidone but not the classical antipsychotic agent's haloperidol and fluphenazine were effective in reversing the sub-chronic PCP-induced impairment. These data are consistent with some studies in the clinic (Keefe et al, 2007). Unfortunately, the CATIE study which compared four atypical antipsychotics to the classical agent perphenazine in over 1400 schizophrenia patients revealed that, although all drugs improved cognition minimally, no difference between atypicals and classical antipsychotics with respect to cognition was observed. The wake promoting agent modafinil licensed to treat the symptoms of narcolepsy has demonstrated some positive results in enhancing performance in several domains of cognition but can also exacerbate the positive symptoms in some schizophrenia patients (see review by

Morein-Zamir et al, 2007). Results presented in this thesis demonstrate that modafinil was effective at restoring the sub-chronic PCP-induced cognitive deficit in the NOR test which provides support for the clinical data and the predictive validity for the combination of the NOR test and sub-chronic PCP treatment regimen. Furthermore, additional predictive validity was demonstrated when the SSRI fluoxetine, licensed to treat depression and the anxiolytic, benzodiazepine chlordiazepoxide were ineffective in ameliorating the NOR deficits induced by sub-chronic PCP. Negative controls were used to confirm that the sub-chronic PCP treatment regimen in combination with the NOR test is not detecting any unrelated effects and therefore minimises the risk of detecting false positives in the future. The results in this thesis outline the importance of assessing the effects of negative controls when validating animal models of human disease.

The α7-nAChR receptor has been selected as a most important target for drug development by the MATRICS initiative (Decker et al, 2008). In clinical trials, nicotine and the selective α7-nicotinic receptor agonists have produced the most convincing improvements cognition in schizophrenia patients (see review by Lyons et al, 2011). Furthermore, adjunct treatment with galantamine a positive allosteric modulator of the α7-nicotinic receptor and AChE inhibitor have been shown to improve the negative and cognitive symptoms of schizophrenia (Schilstrom et al, 2007), although few studies measured visual memory. Unfortunately, despite these initial positive findings, subsequent results with galantamine have been mixed (Gray & Roth, 2007). PNU-120596, a selective positive allosteric modulator of the α7-nicotinic receptor has demonstrated efficacy to improve sub-chronic PCP-induced cognitive deficits in attentional set-shifting; an animal model selected by the TURNS

initiative as a test that can be used to determine the problem solving deficits described in the MATRICS cognitive battery (McLean et al, 2011). Results from data presented in this thesis show that both activation and modulation of the α7-nicotinic receptor by PNU-282987 and PNU-120596 improve the sub-chronic PCP-induced deficit in NOR. These data are supported by the recent phase 2b clinical trials, whereby the α7-nicotinic receptor agonist; EVP-6124 demonstrated efficacy to improve cognition in schizophrenia patients (Watertown, 2011). This demonstrates that alleviation of the sub-chronic PCP-induced NOR deficit in rats may have some useful predictive validity for detecting novel targets with efficacy for cognition in schizophrenia.

Overall, it can be seen that some improvements in cognition have been reported in clinical trials using compounds that can also improve the recognition memory deficits in the NOR test observed following sub-chronic PCP treatment. The clinical improvements in cognition observed in schizophrenia patients following treatment with the same compounds that were efficacious to improve the sub-chronic PCP-induced recognition deficits presented in this thesis are generally smaller. Unfortunately, the full reversal of the sub-chronic PCP-induced recognition memory deficits observed in the NOR test by acute treatment with some pharmacological agents, is not mirrored in the clinic. It is currently believed that exposure to a combination of risk genes and environmental factors early in life can, in the future, lead to the development of schizophrenia. It is therefore unlikely that pharmacological animal models, especially in the rat which are neurobiologically different from humans, can be expected to fully recapitulate all of the salient features

of a human mental illness and have perfect correspondence with respect to individual behavioral symptoms (Nestler & Hyman, 2010).

Working memory dysfunction has emerged as the core cognitive domain of schizophrenia and other disorders. The preliminary results from this thesis suggest that the 16-holeboard maze may be a useful tool in measuring working memory deficits associated with schizophrenia. The working memory aspect of this food rewarded paradigm holds information that is only relevant within a specific trial, such as a list of locations that have already been visited. To obtain a high working memory score in the 16-holeboard maze the rats must process the temporal context associated with an event i.e. "what happened and when did it happen" and remember which holes have already been visited in attempt to negotiate the spatial working memory aspect of this test. Acute administration of scopolamine, PCP, d-amph and sub-chronic PCP treatment resulted in the rats forgetting which food rewarded holes they had already visited, therefore, rats returned to empty, previously visited food-baited holes. These impairments were expressed as reductions in working memory scores compared to controls. These initial studies may imply face validity of the 16-holeboard maze to detect the working memory impairments observed in schizophrenia.

8.2 Limitation of the NOR test

The moderately low exploration times sometimes observed can reduce the value of the data obtained in the NOR test which may impact on drug effects in the retention trial. It has been suggested that using a low level of food deprivation may increase the amount of exploration during the test (see review by Lyon et al, 2011).

However, making these changes to the testing protocol will invariably introduce new problems. Food deprivation is a mild stressor and has been indicated to exert various effects on brain neurotransmitters and increase the levels of the dopamine metabolite 3, 4-dihydroxyphenylacetic acid in the mPFC (Carlson et al, 1987). Furthermore, behaviours and exploration levels of rats in the NOR test can be variable; this is because the test relies on the spontaneous behaviour of the rats to explore the novel and familiar objects.

In the pharmacology section (chapter 5), careful single dose selections were made based on previous studies in our laboratory and the available literature. However, single dose pharmacology is not ideal and is a limitation of the test. If animal numbers had allowed, it would have been preferable to test full dose ranges of all compounds.

8.3 Future studies using the NOR test

Detailed *in-vivo* microdialysis studies would allow a better understanding of the neurotransmitter alterations following sub-chronic PCP treatment during the NOR test. Furthermore, the examination of neurotransmitter levels in rats during the restoration of sub-chronic PCP induced recognition memory deficits by various pharmacological agents would provide valuable information on the mechanism of action of the potential pro-cognitive agents.

Since the effects of pro-cognitive compounds provide complete restoration of sub-chronic PCP-induced recognition memory deficits, it would be very useful to develop a recognition memory deficit in the NOR test that was more difficult to reverse. This could be achieved by combining two separate procedures that are

known to induce cognitive deficits of relevance to schizophrenia; sub-chronic PCP treatment and prefrontal cortex lesions (Schwabe et al, 2006).

8.4 Limitations of the 16-holeboard maze

The somewhat small differences in working memory scores between control and drug–induced reductions in working memory scores means the available window of improvement is narrow. This is a limitation of the test and would need addressing if pharmacological reversals of impairments were to be detected and measured i.e. if the test was to be used routinely to detect novel cognitive enhancers. The number and pattern of baited holes could be increased in attempt to make the test more difficult and thus induce more errors. However, this would probably increase the training time to attain a high WMS baseline.

8.5 Future studies using the 16-holeboard maze.

Further pilot and validation studies are necessary to provide evidence for the predictive validity of the 16-holeboard maze. As in the NOR experiments presented in this thesis, pharmacological agents that have demonstrated positive effects on working memory in schizophrenia patients would need to be assessed to provide the necessary evidence to determine the predictive validity of the test.

Learning of the 16-holeboard test has been shown to be dependent on the hippocampus, hippocampal afferents and VTA (Oades et al, 1981, 1982), however, specific brain lesion studies would be important to determine which region is involved in the sub-chronic PCP-induced working memory deficits following the training period of the test.

8.6 Conclusions

Results from the data presented in this thesis demonstrate the importance of carrying out preliminary validation studies and shows the processes that contribute towards increasing the validity of animal tests of cognition. The initial analysis of behavioural parameters revealed that there was an object preference with the wooden block being preferred, explored more than any other object. Clearly if we had not carried out this preliminary experiment, and used this object, some of our data would have been biased. Initial studies also showed that sub-chronic PCP treated rats successfully encoded object information in the acquisition trial and that males were less sensitive to the impairments in recognition memory induced by acute d-amphetamine, acute PCP and most importantly for these studies, by the sub-chronic PCP treatment regimen compared with females. The data suggest that the sub-chronic PCP dosing regime in female rats is superior in mimicking the cognitive deficits observed in schizophrenia, compared to acute treatments with d-amphetamine and PCP. Further validation demonstrated the ability of the NOR test to detect isolation rearing induced-recognition memory deficits, which is another model of relevance to schizophrenia symptomatology.

Pharmacological studies using both positive and negative controls are vitally important when validating an animal model. Results demonstrated that sub-chronic PCP in combination with the NOR test induces recognition memory deficits that are highly robust, reproducible with ability to detect compounds that show some clinical efficacy to improve cognition in schizophrenia patients and importantly does not detect agents that do not have efficacy to improve cognition. These results suggest

that the sub-chronic PCP deficits in the NOR test has the potential to predict the clinical response to potential novel pro-cognitive enhancers.

It is essential when detecting the efficacy of potential cognitive enhancers that differential domains of cognitive impairments related to schizophrenia can be measured, NOR only measures visual recognition memory. With further validation, the 16-holeboard maze may provide a useful tool to assess the working memory deficits associated with schizophrenia.

In summary, the sub-chronic PCP dosing regimen in female Hooded-Lister rats can mimic certain cognitive deficits of schizophrenia. When used in combination with the NOR test and the 16-holeboard maze, this provides a relatively valid animal model of recognition and working memory. Further studies are clearly required to refine the PCP model and to explore further behavioural tests of cognition in order to encompass all the domains affected in this and other disorders.

CHAPTER 9 - References

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