Chapter 1

Introduction
1. General Introduction

Schizophrenia is one of the most severe psychiatric illnesses causing long-lasting disabilities for its sufferers and significant financial encumbrance to society (Wu et al., 2005). Despite the incidence and devastating effects of schizophrenia, not much is known about the underlying pathology. It is generally considered to be a syndrome, probably a cluster of disorders, which produces varied effects (impairments) in cognition, mood, interpersonal relations, social and work function. Symptoms were initially divided into positive and negative based on the earlier studies of Kraepelin (1913) and Bleuler (1950). However, the disorder has in the ensuing years been modified and categorized into several distinct types including catatonic, paranoid and disorganized (Stahl 2008). Interestingly, the common perception of the disorder relates more to the positive symptoms associated with it, in spite of these being less commonly observed in patients (Harvard Mental Health Letter 2006) and they range from hallucinations, delusions and paranoia (Moller 1995). Although the positive and negative symptoms do form an important aspect, they alone do not represent the degree of the complex dysfunction in schizophrenia. Cognitive impairment which had been previously documented as an important characteristic of schizophrenia is now increasingly being acknowledged as a fundamental and lasting deficit of the disorder (Peuskens et al., 2005). There is a whole body of evidence to suggest that these varying symptom clusters are manifestation of disturbances of several neurotransmitter systems in the brain (reviewed in Lyne et al., 2004).
1.1. Neurotransmitter systems

Much of the research is focused around biochemical theories of schizophrenia as it has been observed that certain chemicals might exacerbate or ameliorate the symptoms by acting on specific neurotransmitter systems (Carlsson et al., 1997). Almost all known neurotransmitters within the brain have been considered, with significant attention being directed towards dopamine (DA) and serotonin systems, substantial support also points towards glutamate systems and gamma-aminobutyric acid (GABA) (Owen and Simpson 1995).

1.1.1. Dopamine hypothesis

This theory was initially proposed when it was first shown that neuroleptics selectively blocked DA receptors. The initial hypothesis was that a hyperdopaminergic state resulting from an excess of DA in the brain was the main cause for the condition (Carlsson and Lindqvist 1963; Seeman 1987). Despite the fact that there is supporting evidence to validate this theory, including an observation that psychotic patients release more DA at the synapse when compared to normal control groups following amphetamine treatment (Thaker & Carpenter 2001), it was also evident that the negative and cognitive symptoms of schizophrenia cannot be accounted for by an excess of DA in the system. This led to the more complex conceptualization of the role of dopaminergic system in the brain, which included four pathways i.e. nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular, formed by dopaminergic neurones and a differential dopaminergic state across these pathways (either hypo or hyper dopaminergic to explain negative and positive symptoms respectively) in primates. However, in rats the distribution of neurone bodies is restricted to two main nuclei (instead of 4 as
seen in man) – the substantia nigra (A9, fig 1) which projects to the striatal regions and the ventral tegmental area (A10, fig 1) which projects to the limbic regions (including cortical and sub-cortical regions) (Dahlstrom & Fuxe 1964). The difference between projections in man and rat is due to the fact that the cortical region in man is comparatively larger and hence has projections divided into mesocortical and mesolimbic innervations. Dopamine exerts its effects by binding to two receptor families, known as the D1 (which include D1 and D5) and D2 receptors (which include D2, D3, and D4) (Jackson and Weslund-Danielsson 1994). Both families exert their biological actions by coupling to and activating different G protein complexes, but while the D1 receptors activate the adenyly cyclase pathway, D2 receptors interact with G proteins to inhibit cAMP production.

Fig 1: Schematic drawing of the major DA-containing pathways in the rat brain (Adapted from University of Plymouth, Department of Psychology website). A9: Substantia nigra; A10: Ventral tegmental area.
The discovery that chlorpromazine (first neuroleptic to be discovered) was a D₂ receptor antagonist had a crucial role in supporting the DA hypothesis (Harrold et al., 1987). In addition to this, indirect DA agonists (e.g. L-DOPA, cocaine and amphetamines) have been shown to induce psychosis in healthy subjects and at very low doses were observed to provoke psychotic symptoms in schizophrenia patients (Carlsson 1997). A close correlation between the therapeutic doses of conventional antipsychotic drugs and their affinities for the D₂ receptor also supported the DA hypothesis (Kapur and Remington 2001). In fact according to previous studies, only D₂ receptor occupancy was implicated and was considered most relevant and also acceptable to explain the effect of both atypical and typical antipsychotic drugs, in some receptor occupancy studies (Farde et al., 1992). This hypothesis also received support from post-mortem and positron emission tomography (PET) studies which clearly demonstrated increased D₂ receptor levels in the brains of schizophrenic patients. Also, these receptor levels (mRNA expression) are known to be upregulated by conventional neuroleptics like haloperidol and molindone and also by the atypical antipsychotic risperidone. Given that these drugs are D₂ antagonists the evidence was not considered to be entirely conclusive. However, it was speculated that this upregulation was a compensatory effect following receptor blockade but with no clear evidence for the same. Another limitation of the theory was the discovery that clozapine (a prototype of atypical antipsychotics) has lower affinity for the D₂ receptor (Meltzer 1999; Kerwin et al., 1994). Recent studies showing a high affinity of clozapine for the D₄ subtype of D₂ receptor class does attempt to resolve this paradox in part (Roth et al., 1995). Also, Kapur and Seeman’s (2002) fast dissociation explanation is useful in clarifying antipsychotic action at the receptor level. According to them, fast dissociation from the D₂
receptor makes an antipsychotic more accommodating of physiological DA transmission, permitting an antipsychotic effect without motor side effects or secondary negative symptoms.

1.1.2. Serotonin (5-HT) hypothesis

With the search for the underlying neurochemistry in schizophrenia underway, there was a renewed interest in the idea of involvement of serotonin (5-HT) to elucidate the symptom clusters. The suggestion of involvement of serotonin in the pathophysiology of the disorder had first been proposed as early as 1954 by Wooley and Shaw based on the psychotomimetic effects of lysergic acid diethylamide (LSD), a compound that is structurally related to 5-HT and is an antagonist at the 5-HT$_{2A}$ receptor. A revised 5-HT hypothesis of schizophrenia however emerged especially as part of the “serotonin-dopamine” hypothesis of schizophrenia. This furthered the idea of increased dopaminergic and serotonergic neurotransmission in subcortical areas in schizophrenia, thereby leading to positive symptoms, and decreased dopaminergic and serotonergic activity, possibly in the prefrontal cortex, leading to the negative symptoms.

The main reason for the renewed interest in the role of 5-HT in schizophrenia was the recognition of several 5-HT receptor subtypes and their widespread impact on multiple neurotransmitters and behaviours. At least 15 5-HT receptor subtypes have been documented to date. These have been divided into seven main groups (5-HT$_1$, 7). The individual units are further divided into subtypes, examples of which are 5-HT$_{2A-C}$, 5HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$. The high affinity of several second generation antipsychotics for the 5-HT$_{2A}$ receptor as a common site of action draws attention to its significance as a potentially critical target in the development of
other therapeutic agents for schizophrenia (Meltzer et al., 2003). Yet another significant outcome of the revised theory was the suggestion that functional modification in the serotonergic system (including both pre and postsynaptic function) influenced numerous neurotransmitter systems (e.g. glutamate, GABA, noradrenaline, acetylcholine and DA) and resulted in the various behavioural disturbances.

1.1.3. Glutamate hypothesis

With the realisation of the significance of negative and cognitive symptoms of schizophrenia, there has also been an increase in studies targeting neurotransmitter systems other than DA and serotonin. The glutamatergic system in particular has come under much scrutiny with regards to the pathophysiology of the disorder. Glutamate is a major excitatory neurotransmitter in the mammalian brain and is implicated in several physiological processes in the body (Ozawa et al., 1998). It exerts its action in the biological system by activating several types of receptors which have been divided into two groups known as “ionotropic” and “metabotropic” receptor subtypes depending on whether they respond to stimuli by forming ion channels (ionotropic) or whether they link indirectly with ion-channels on the plasma membrane of the neurones (metabotropic) through signal transduction mechanisms (Weiler et al., 1997; Ozawa et al., 1998). The ionotropic glutamate receptors are classified based on the glutamate analogue that they selectively bind to, e.g. N-methyl-D-aspartate (NMDA) and alpha amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors which bind NMDA and AMPA respectively. These ionotropic receptors are formed by assemblies of subunits which are accountable for receptor diversity (fig 1.1). This diversity is generated before
and after gene transcription e.g. NMDA receptor subunits are formed by transcription from genes (NR1, NR\textsubscript{2A-D}, NR\textsubscript{3A-B}). The metabotropic receptors are classified based on the types of signal transduction mechanism that modulates glutamatergic transmission. However, this will not be discussed in any greater detail in this thesis as it is less relevant to the work presented here.

![Glutamate Receptors Diagram](image)

**Fig. 1.1** Classification of different types of glutamate receptors and subunit composition of ionotropic receptors.

### 1.1.4 Glutamate in schizophrenia

The origin of the idea that glutamatergic neurotransmission is disrupted in schizophrenia dates back to the 1950s when it was first reported that the anaesthetic phenylclidine (PCP) could produce psychotic effects in people (Luby et al., 1959). Studies that proposed the use of PCP as a pharmacological tool to mimic schizophrenia and the underlying cause of the PCP-induced symptoms including the role of glutamate receptors were discovered much later (Kim et al., 1980; Javitt et al. 2007).
Although DA agonists (e.g. amphetamine) and glutamate receptor antagonists (e.g. PCP) both replicated psychosis most effectively, the latter was able to better produce the negative and cognitive deficits associated with schizophrenia. Subsequently, a great deal of research emphasis is now being placed on the role of glutamate in schizophrenia. Olney & Farber (1995) proposed a mechanism, which could clarify in part, the symptoms and natural course of schizophrenia centred on NMDA receptor hypofunction (NRHypo), according to which a dysfunction of the NMDA receptor could be reproduced by blocking NMDA receptors pharmacologically with PCP, ketamine or MK-801. Also, the organisation and functions of the forebrain glutamatergic systems implicate them in the pathophysiology of schizophrenia. In addition, there are several post-mortem studies and genetic findings which support the hypothesis that disruption in the normal functioning of subtypes of NMDA receptors contribute to the “systemic manifestations of schizophrenia”. With growing evidence in support of the glutamatergic theory of schizophrenia, it is now a pharmacological target for development of drugs which modulate/enhance activity at the NMDA receptor. Furthermore, studies on substances that indirectly enhance NMDA receptor function via the glycine modulatory site have been known to reduce negative symptoms and even improve cognitive functioning in schizophrenic patients treated with antipsychotic agents (Goff & Coyle 2001). Other novel sites for pharmacological manipulation of these and other glutamate receptors are also being studied as potential therapeutic targets of schizophrenia.
1.1.5. GABAergic system

The glutamate hypothesis of schizophrenia emphasizes primarily, a considerable decrease in the activity of the glutamate neurotransmitter system, particularly the NMDA receptors. This deficit also influences other neurotransmitter systems in the brain, such as the GABAergic system. The latter is controlled by the glutamate system and hence may be reduced in its activity as a consequence of a decline in glutamate neurotransmission (Olney et al., 1999). The GABAergic cells which are exclusively interneurons in the prefrontal cortex and in the hippocampal formation play a crucial role in regulating the activity of the projecting glutamatergic pyramidal cell. A very significant question in trying to understand the pathophysiology of schizophrenia is which of the two modifications are etiologic and which is a consequence.

GABAergic interneurons are made up of subsets of neurones that differ in their morphology, distributions, connections and electrical properties. They are known to co-express calcium-binding proteins including parvalbumin, calretinin and calbindin. The large basket and chandelier cells are observed to express parvalbumin most commonly (Benes and Berretta, 2001). Also, double bouquet cells show the presence of calbindin (Defelipe et al., 1999) while calretinin-immunoreactive neurones are normally double bouquet, and Cajal-Retzius cells (Jacobowitz & Winsky 1991).

It has been observed that the expression of parvalbumin is reduced in the prefrontal cortex in schizophrenia (Hashimoto et al., 2003) with a similar decrease in parvalbumin being observed in the hippocampal region of the temporal lobe and in calbindin cells (Zhang et al., 2002). However, no loss of calretinin-immunoreactive
neurones has been observed in schizophrenic subjects. This has led to suggestions that GABAergic deficits observed in schizophrenia could be attributed to specific reductions in parvalbumin-immunoreactive and perhaps calbindin cells. Therefore, there is a convincing substantiation of hypofunction of some GABAergic interneurons in the frontal cortex and temporal lobe, and hypofunction of NMDA receptors in these regions in schizophrenia. There is however no definitive evidence to associate the two mechanisms with each other, with the exception of speculations which advocate certain susceptibility of GABAergic interneurons to the effects of NMDA receptor antagonists (Moghaddam 2003) and these should be investigated in future.

1.1.6. Other biochemical hypotheses

There are several lines of evidence that correlate a number of neurochemicals (other than those mentioned previously) implicated in the pathophysiology of schizophrenia. Mechanisms involving acetylcholine, glycine and even endorphins and their corresponding receptors may be related to events associated with the disorder. Although the inhibitory influence of GABAergic neurones on dopaminergic neurones has led to the hypothesis that decreased GABAergic activity may be involved in producing schizophrenic symptoms, there are also suggestions of elevated concentrations of noradrenaline in specific areas of the brain and in the spinal fluid which have led to the hypothesis of possible implication of noradrenaline in the pathology of schizophrenia. There are also speculations about the possible role of both an excess and a deficiency of endorphin activity in schizophrenia, and rather uncertain evidence has been used to support both hypotheses. It is unclear whether these neurochemical findings represent primary (etiological) or secondary
(pathological changes as a result of environmental influences and compensatory mechanisms) outcomes of the condition. This uncertainty involves risk factors other than the above-mentioned biochemical agents.

1.2. Neuropathological factors in schizophrenia

Neuropathological analyses of schizophrenia (Arnold et al., 1998) have not found any evidence of the usual features of neurodegenerative diseases, such as dystrophic neuritis or reactive gliosis. The neuropathological factors provides us with conflicting evidence of subtle cytoarchitectural anomalies in gray matter (Arnold et al., 1997) and in corticolimbic regions, and an abnormally high frequency of aberrant neurones in the white matter underlying prefrontal cortex (Akbarian et al., 1996), temporal and parahippocampal regions (Arnold et al., 2005) and also present evidence for subtle abnormalities in neurodevelopment in schizophrenia, such as disordered cortical neuronal migration, consistent with behavioural, and neurological anomalies. Previous studies have identified the prefrontal cortex as the crucial node in these neuronal networks (Goldman-Rakic 1999). Magnetic resonance imaging (MRI) studies have revealed morphological and volumetric peculiarities in the brains of schizophrenic patients. A recent study also focussed on the fact that the pathology of the hippocampal complexin proteins might play an important role in schizophrenia, especially the cognitive disturbances (Sawada et al., 2005).

The implication of several brain regions is consistent with the multiple forms and extensive symptoms of schizophrenia. Despite the suggested involvement of numerous circuits, almost all of these directly impact connections and functions of the prefrontal cortex, more specifically, the cortico-cortical and corticosubcortical
pyramidal neurone. The neuronal systems innervating these regions are local GABAergic interneuronal inputs, dopaminergic inputs and glutamatergic extensions. Other cortical areas of the temporal lobe also impact (albeit to a lesser degree) the overall neuronal dysfunction in schizophrenia. It has been believed for several years that the cortical regions have been implicated in schizophrenia. However, some early studies failed to demonstrate hippocampal innervations by dopaminergic fibres (Lindvall et al., 1978; Loy et al., 1980) and the role of the hippocampal dysfunction in schizophrenia was only discovered later. It therefore seems clear that the prefrontal cortex and the hippocampal formation are two of the main areas implicated in schizophrenia. However, the neuropathology of the complex dysfunction is not restricted to the neurotransmitter system but also is influenced by the neuronal innervations in different brain regions. While a pattern of widespread structural abnormalities specific for positive symptoms was observed in areas of a frontotemporal network, computed tomography (CT scan) studies showed that the brains of patients with schizophrenia have lateral and third ventricular enlargement and some reduction in cortical volume. Although, morphological differences have not been conclusively linked with one or the other symptom clusters in schizophrenia CT scan abnormalities (mentioned above) have been correlated with the presence of negative symptoms. Negative symptoms also appear to be correlated with left temporal lobe atrophy (Turetsky et al., 1995). Similarly, the hippocampal formation and surrounding structures of the temporal lobe are known to be involved in a variety of learning and memory tasks (Eichenbaum 2004; Forwood et al., 2005; Shenton et al., 2001) and impairments in these regions are thought to be implicated in the cognitive symptoms of schizophrenia. The pattern of abnormalities and symptoms associated with schizophrenia is best explained by
disruption of connectivity within and between the prefrontal, temporal and subcortical regions of the brain (Lawrie et al., 2002; Boksman et al., 2005; Honeet al., 2005). The simultaneous study of these regions seems relevant since the prefrontal and temporal lobes are closely connected with each other and with the basal ganglia and thalamus. It is likely that this underlying neuropathology emerges due to interactions between adverse environmental conditions and associated genetic factors.

1.3. Genetics and Environmental risk factors

The etiology of schizophrenia remains unknown. However, studies using genetic approach propose that there are heritable components in the pathophysiology of schizophrenia which appear to follow the polygenic model of inheritance (Procopio 2005). Other studies have shown interaction between environmental factors such as season of birth or obstetric complications and specific gene polymorphisms (Cannon et al., 2006; Chotai et al., 2003). There are several arguments against the relevance of environmental factors for schizophrenia (Crow et al., 2007); the most well known study which observed that the disease process was independent of environmental factors was conducted by the World Health Organisation which concluded that “schizophrenic illnesses are ubiquitous, appear with similar incidence in different cultures and have clinical features that are more remarkable by their similarity across cultures than by their difference” (Jablensky et al., 1992). However, data based on genetic, biochemical and animal studies have consistently shown that genetic factors contribute to schizophrenia. There is a higher risk of schizophrenia in biological relatives of patients (Riley and Kendler 2006) and linkage studies have shown different chromosomal regions as possible candidate locus for susceptibility.
genes in schizophrenia (Schosser & Aschauer 2004). Genes such as dysbindin, neugulin -1 (NRG-1), disrupted in schizophrenia-1 (DISC1), catechol-O-methyl transferase (COMT) have been consistently reported to have functional polymorphisms relevant to schizophrenia (Harrison & Owen 2003; Hashimoto 2007). Studies have also associated brain-derived neurotrophic factor (BDNF) polymorphism with schizophrenia. The BDNF gene is involved in regulation of both DA (Bustos et al., 2004) and glutamate neurones (Jiang et al., 2003). The autosomal recessive mouse mutant reeler has led to the identification of the protein Reelin (RELN), which is considered to play a potential role in schizophrenia (Lewis et al., 2000). It has been demonstrated that there is an significant (~ 50%) deficit in the RELN content in specific areas of brains in schizophrenic individuals (Guidotti et al., 2000). Due to the fact that this protein is in general operational during embryonic corticogenesis and throughout the patient’s lifespan, the morbidity risk of schizophrenia, in agreement with the implication that embryonic factors contribute to the aetiology of schizophrenia (Lewis et al., 2000), may arise as a consequence of compromised RELN expression. A review of all candidate genes identified so far is beyond the scope of this thesis. There are several susceptibility loci and the mode of interaction of these loci is yet unknown. The extent of epigenetic consequences in the mode of transmission is also poorly understood. However, a summary showing the chromosomal loci of some most promising candidate genes is shown below (table 1)
<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosomal location</th>
<th>Evidence in schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysbindin</td>
<td>6p22</td>
<td>++++</td>
</tr>
<tr>
<td>Neuregulin1</td>
<td>8p12</td>
<td>++++</td>
</tr>
<tr>
<td>DISC 1</td>
<td>1q42</td>
<td>+++</td>
</tr>
<tr>
<td>RGS4</td>
<td>1q23</td>
<td>++</td>
</tr>
<tr>
<td>COMT</td>
<td>22q11</td>
<td>++</td>
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</tbody>
</table>

Table 1: From Craddock et al., 2006. + indicates the number of experiments, i.e. more + symbols, greater the evidence. (DISC 1: Disrupted in schizophrenia 1; RGS4: Regulator of G protein signalling; COMT: Catechol-O-methyl transferase)

As seen in table 1, genes expressing NMDA receptor subunits are not listed here. This is due to the lack of strong evidence of association between NMDA receptor genes and schizophrenia has been found yet. However, there are some positive reports for mGluR receptors and AMPA subunit changes (Schiffer 2002, Harrison and Owen 2003), however, neither the sample size of these investigations nor reproducibility of the data aids in identifying NMDA receptor subunit genes as strong candidate genes for schizophrenia. Consequently, the question that needs to be addressed is why then is the NMDA receptor hypofunction model being used to mimic aspects of the disease in animal models.

Genetic variation results in susceptibility of the disease in one of two ways—either the structure of the encoded proteins are changed or expression of the gene is altered (either via transcription or translation). It is apparent that both processes exert their effects by varying function of the protein. Since the end result causing disease susceptibility is a result of changes in protein levels, genetic variants are not the only basis behind diseased state. Molecules encoded by the genes are a single point in a convergent pathway that can be adversely affected by numerous other entry
points, including variations in other proteins that eventually feed into these pathways, and lead to compensatory or secondary changes. It is likely that the changes caused by NMDA receptor antagonism by PCP result in several long lasting changes that feed into the genetic variation pathway at some point resulting in similar phenotypic changes. Understanding the basis of such effects is thus necessary to improve our understanding of various symptoms of schizophrenia and develop pharmacotherapies which may help provide improved drug treatment for patients.

1.4 Cognitive and negative symptoms in schizophrenia

Cognition refers to the mental processes involving perception, storage and retrieval of information around us. It encompasses several neurological domains such as attention, working memory, reference memory, language skills and executive functioning. Social skills (interaction), which are vital for integration of a person into society, are dependent on normal cognitive function. Deficits in cognitive processing are one of the central features of schizophrenia (Goldberg et al., 1995). This feature is becoming increasingly vital due to the fact that impaired cognition has been implicated in poor long-term outcome (Marder & Fenton, 2004). Virtually every act of daily living requires the ability to remember past events and put them into context, e.g., episodic memory. Therefore, it is evident that a clear picture of cognitive function is essential not just to understand, but also to restore normal brain functioning. Hence, there is now increased focus on targeting cognitive dysfunction in schizophrenia for therapeutic purposes. The collaboration between the National Institute of Mental Health, the University of California, Los Angeles, and the United States Food and Drug termed – Measurement and Treatment Research to Improve
Cognition in Schizophrenia (MATRICS), an initiative dedicated to encouraging the development of cognition-enhancing drugs for schizophrenia is one such example (Marder & Fenton 2004). This initiative has identified seven cognitive domains as being most severely affected in schizophrenia (MATRICS initiative, Marder & Fenton 2004). These domains (attention vigilance, verbal learning and memory, reasoning and problem solving, working memory, speed of processing, visual learning and social cognition) are now being considered as ‘reference points’ to guide research and investigations on cognitive dysfunction in schizophrenia. Overall, the core purpose of creation of MATRICS was to identify new cognitive neuroscience-based approaches to address issues regarding the animal and human models and biomarkers required for understanding and measuring cognitive processes.

The next major step of the MATRICS initiative aimed at addressing the problem of negative symptoms of schizophrenia as an unmet treatment need. The January 2006 issue of Schizophrenia Bulletin (Kirkpatrick et al., 2006) reported a consensus view on the components of negative symptom construct that was developed during 2005. The domains of negative symptoms as listed by the MATRICS included anhedonia, blunted affect, alogia, and avolition. The relationship among these domains would be an important issue for treatment and for the development of animal models (more on negative symptomatology will be discussed in chapter 3).

Methods employed by the MATRICS initiative for the assessment of cognitive and functional outcomes in schizophrenia were used in a series of projects jointly termed Treatment Units for Research in Neurocognition in Schizophrenia (TURNS). This
was used to evaluate the usefulness of the MATRICS methods and testing the
efficacy of the compounds selected (Green et al., 2008).

Notably, negative and cognitive deficit symptoms in patients with schizophrenia are
recognised as a core part of the disorder, and are believed to have significant bearing
on the patient’s recovery and re-integration into society. They are also considered
the best indicators of relapse and employability of patients (McGurk & Meltzer
2000). Experimental manipulations that represent the biochemical and molecular
biological changes associated with these symptoms can now be reasonably
reproduced in animals, with rodents and primates (other than man) being used as
subjects for the purpose of such studies.

1.5. Modelling the symptoms of schizophrenia

Animal models of negative symptoms and cognitive processes need to mimic the
highest of behavioural functions in humans so as to be of therapeu tic relevance.
Primitive functions like habituation and sensitization are comparatively easy to
mimic in almost all species (Le Merrer & Nogues 2000). However, to model a
complex psychiatric condition like schizophrenia, the higher cognitive functions
which require an ability to use information in a very specific and flexible manner
need to be replicated. Similarly, modelling negative symptoms successfully requires
measures of socially derived emotional animal behaviour. Although there are severe
limitations in reproducing cognitive and negative dysfunction in animals accurately,
these models have proved to be indispensible tools for the testing of the various
hypotheses advanced in schizophrenia research. However, there is this critical
question of validity of these models with respect to schizophrenia. The three major
aspects which define and determine the significance of animal models are construct,
face and predictive validity. The preliminary development of animal models of psychiatric disorders is usually centred on the “face validity”, which is a reflection of the parallel behavioural patterns observed in humans and animals. “Construct validity”, which is derived from the correlation in behaviour and neural events or biochemical mechanisms between the animal model and patients of the disorder, forms the basis of proposed models. The practical value of such models is measured by the “predictive validity” which is an indicator of the therapeutic efficacy of new treatments for psychiatric disorders in man based on the animal models (Yamamoto 2002). While animal models are fairly accurate in terms of face validity, construct validity has been relatively harder to achieve. This is primarily due to the limited knowledge relating to the causes underlying the symptoms of schizophrenia. Predictive validity refers to the selectivity whereby an animal model responds to specific classes of compounds (Willner 1984). For instance, several animal models of schizophrenia respond to new generation but not the old generation of antipsychotics showing some level of the predictive validity, although predictive validity of several other tests based on similar constructs have been known to be flawed on occasions (Sarter et al., 1991, 1992).

In the last decade however more promising models have been introduced and existing models have been refined. Models based on newer findings implicating alterations in DA, glutamate and serotonin function in the pathophysiology and pharmacotherapy of schizophrenia are now better supported and potentially more productive with respect to mimicking the psychopharmacology of schizophrenia.
Present day animal models of schizophrenia can be divided into three categories depending on the method used for their construct. These are pharmacological models (using psychotomimetic drugs), non-pharmacological models (using lesions or disturbances in social communication) and genetic mouse models.

1.5.1. Pharmacological models

The use of dopaminergic drugs to elicit a behavioural response in animals has been the basis in developing animal models of schizophrenia. These models have been an effective tool for drug development and indeed many current antipsychotic drugs were developed on the basis of these models. The glutamate/NMDA antagonist PCP has now emerged as a more reliable model of schizophrenia in that it can induce both negative as well as positive symptoms following treatment with amphetamine (Jentsch & Roth 1999). Both in terms of predictive and pathological validity, the PCP model is considered to be effective. While abnormalities of glutamatergic system including deficits in cortico-striatal innervation can be replicated by PCP, it has also been evidenced that some of the PCP-induced behaviours can be effectively reversed by certain atypical, but not typical antipsychotics (Abdul-Monim et al., 2007; Grayson et al., 2007; Hashimoto et al., 2005). This is in line with the effect of newer antipsychotics on patients where it is observed that negative and cognitive symptoms respond less well to the typical drugs.

1.5.2. Non-pharmacological models
Abnormal development of or changes in the structural components of the brain during gestation or soon after contribute to the manifestation of schizophrenia throughout the entire lifespan (Chaturvedi & Thakur 2003). While several animal models have been useful in explaining the core mechanisms of these changes in the developing brain, the models which determine the effects of specific non-pharmacological insults have proved to be an indispensable approach. Lesioning of hippocampal pathways and the frontal cortex are examples of such models. Although the lesions can be in the form of electrolytic or aspiration lesions, most typically excitotoxic agents, which destroy neuronal tissue through stimulation of excitatory glutamate release or by acting as direct glutamate receptor agonists are used. Hence, depending on the method used this may be a pharmacological or non-pharmacological model.

The neonatal ventral hippocampal lesion model (NVHL) uses an infusion of the excitotoxic compound, ibotenic acid directly into the rat ventral hippocampus (bilaterally) on postnatal day (PND) 7. This led to the post-pubertal development of a number of behaviours akin to those seen in schizophrenia (Lipska, 2003), such as increased sensitivity to MK-801 and apomorphine down-regulation of striatal D2 receptors (Schroeder et al., 1999), disrupted DA/glutamate interactions in the PFC (Tseng & O’Donnell, 2007), decreased dendritic branching (Flores et al., 2005) deficits in social behaviour and cognitive impairment (Le Pen and Moreau, 2002). Yet another typical example of a non-pharmacological animal model is isolation rearing. It assesses the environmental or developmental contributions to the symptom cluster in schizophrenia and is particularly relevant in the context of the neurodevelopmental hypothesis of the disorder (Weinberger 1987). It has been
observed that isolation rearing of rats, i.e. rearing of rats alone with one rat in a cage, from weaning through adulthood results in social withdrawal tendencies (Fone et al., 2008), deficits in prepulse inhibition (PPI) (Geyer et al., 1993), alterations in the expression of behavioural sensitization to repeated amphetamine (Weiss et al., 2000) and hyperlocomotion in novel environments (Del Arco et al., 2004) along with changes at the morphological and neurochemical levels (Fulford & Marsden 1998; Silva- Gomez et al., 2003). The deficits are further likened to schizophrenia since they appear only at or after puberty, as is the case with most patients. Interestingly, this model does not show differences in the ability of conventional and atypical antipsychotics to reverse the deficits. It has however been suggested that isolation rearing may prove to be a sensitive index of novel antipsychotics that do not act at any known receptor system as it does not rely on drug treatment to produce the behavioural deficits. A large number of studies have shown that exposure of animals to early-life adversity (insults), such as maternal separation or social isolation from conspecifics, adversely affects brain development and adult behaviour (Rapoport et al., 2005). The term neurodevelopment is associated with risk for schizophrenia has become widely accepted among researchers (more details on neurodevelopmental models will be detailed in the next section).

Neurodevelopmental hypotheses can be divided into two; one that argues for disruptions in brain maturation arising in late adolescence and the other that favours an early (pre-or perinatal) brain lesion model (Rapoport et al., 2005). In experimental animals, a number of methods have been used to model the developmental origin of schizophrenia symptomatology. These models attempt to replicate, in experimental scenarios some of the factors that are suspected to cause schizophrenia, such as early stressful experience (Lehmann & Feldon 2000). As
mentioned above, models have been developed that focus on neonatal damage of brain regions especially the hippocampus (Lipska et al., 2001) and other structures such as the prefrontal cortex (Lipska et al., 1998), amygdala (Diergaarde et al., 2005) which are implicated as a site of the neuropathology in schizophrenia. Yet another neurodevelopmental model of interest is prenatal methylazoxymethyl (MAM) model. Treatment with MAM at specific time points during the gestational period was shown to induce region specific disruption of brain development. MAM treatment on gestation day (G)15 led to the development of increased striatal DA and deficits in sensorimotor gating and cognitive processes (Moore et al., 2006). Apart from the behavioural deficits seen in these rats, they also showed region specific volume reductions (including in the hippocampus and prefrontal cortex), a decrease in hippocampal parvalbumin-expressing neurones and altered response to amphetamine (Flagstad et al., 2004). Thus the MAM can induce behavioural and neurochemical changes with relevance to schizophrenia.

1.5.3. Genetic mouse models

Genetic contributions to schizophrenia have also been the focus of considerable research, although the application of linkage analyses to schizophrenia have not proven successful yet, much insight into the mechanisms of neural signalling has been gained from transgenic animals (Mohn et al., 1999). Genetic linkage and association studies in man have shown that multiple chromosomal regions contain candidate susceptibility genes for schizophrenia. These genes encode for proteins regulating synaptic plasticity, neurotransmission (hypoglutamatergic and hyerdopaminergic) and neurodevelopment, which are all known to be disrupted in
schizophrenia. Although there are several genes implicated in the process, notable among these are those encoding caeochol-O-methyltransferase (COMT), dysbindin-1, D-amino acid oxidase (DAO), regulator of G-protein signalling 4 (RGS4), calcineurin, neuregulin 1 (NRG1) and Disrupted in Schizophrenia 1 and 2 (DISC-1 & DISC-2) (Robertson et al., 2006). Generation of mice lacking these proteins using gene knockout or gene inactivation methods have also created a novel set of animal model of schizophrenia. However, this will not be discussed in detail in this thesis as it is less relevant to the project presented here.

1.6. Behavioural assays of animal models (tests employed)

The behavioural assessments are the most common approach for developing animal models. Indeed a major problem in the behavioural analysis of models of negative and cognitive deficits is that they involve several domains. As a result of this any one test cannot accurately describe or assess these multiple dimensions of cognition. It is therefore preferable to use several behavioural tests so that it is possible to characterize multiple functional domains such as changes in information processing, attention or cognition and social behaviours, which are all disrupted in schizophrenia. Some of these tests which assess regulation of significant information for maintenance and retrieval (which is commonly termed working memory), planning and adapting to the environment by utilizing the selected and retained information in animals are described below.

The behavioural assays used in this thesis have been highlighted in table 2 and the significance of the same will be explained in this section. However, a detailed
The description of the test itself will be explained in chapters 2, 3, 4, and 5.

Table 2: (Adapted from www.matrics.ucla.edu). Relevant animal and human tests measuring each of the cognitive domains affected in schizophrenia. Tests highlighted in red have been used in this programme of work.

<table>
<thead>
<tr>
<th>Cognitive domain</th>
<th>Animal Models/Tests</th>
<th>MATRICS Clinical Battery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual learning &amp; memory</td>
<td>Novel Object Recognition</td>
<td>NAB - Shape learning BVMT - Revised</td>
</tr>
<tr>
<td>Working memory</td>
<td>Operant or T-maze</td>
<td>BACS</td>
</tr>
<tr>
<td></td>
<td>Radial arm maze</td>
<td>WAIS-III Letter-Number sequence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>UoM Letter-Number span</td>
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<tr>
<td></td>
<td></td>
<td>Spatial Delayed Response Task</td>
</tr>
<tr>
<td>Social cognition</td>
<td>Social Interaction</td>
<td>MSCG - Managing emotions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MSCG - Perceiving emotions</td>
</tr>
<tr>
<td>Reasoning &amp; problem solving</td>
<td>Attentional set-shifting</td>
<td>WAIS-III Block Design</td>
</tr>
<tr>
<td></td>
<td>Serial Reversal Learning</td>
<td>NAB - Mazes</td>
</tr>
<tr>
<td></td>
<td>Maze tasks</td>
<td>BACS - Tower of London</td>
</tr>
<tr>
<td>Attention/aversion</td>
<td>5-Choice Serial Reaction Time Task</td>
<td>3-7 CPT</td>
</tr>
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<td></td>
<td>Time Task</td>
<td>Identical pairs CPT</td>
</tr>
<tr>
<td></td>
<td>PPI, auditory gating</td>
<td>PPI, auditory gating</td>
</tr>
<tr>
<td>Speed of processing</td>
<td>5-Choice Serial Reaction</td>
<td>Category Fluency</td>
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<tr>
<td></td>
<td>Simple Reaction time tasks</td>
<td>WAIS-III Digit-Symbol Coding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BACS - Symbol Coding</td>
</tr>
<tr>
<td>Verbal learning &amp; memory</td>
<td></td>
<td>NAB - Daily Living Memory</td>
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<tr>
<td></td>
<td></td>
<td>HVLT - Revised</td>
</tr>
</tbody>
</table>

**1.6.1. Prepulse Inhibition** (test used for selection of information) – Central to the cognitive deficits observed in schizophrenia is the disruption of information processing systems at the neuronal level. Sound information processing is critical for filtering and regulating extraneous stimuli and observations and distinguishing them from relevant information present in the environment, a phenomenon often referred to as sensory motor gating.

A standard technique used to measure this information processing is the startle response in subjects who have been subjected to stimulus (prepulse) prior to the
second stimulus after which the startle response is measured. Prepulse refers to the phenomena whereby a weak pre-stimulus presented 30 to 500 msec before a startling stimulus results in a decrease in the startle reflex (Perry et al., 2002). Prepulse inhibition of the startle reflex hence is an operational model of sensorimotor gating and cognitive dysfunction in humans (Braff 1993). Studies using this method have revealed that information processing mechanisms are impaired in schizophrenic patients. This model, first developed in human subjects is now widely used preclinically in rodents and nonhuman primates. Animals that have been administered DA agonists, such as amphetamine such that their ‘cortico-striato-pallido-thalamic circuitry’ has been pharmacologically altered exhibit inhibition of the startle response (Braff 1993). While there have been considerable reservations against the use of this model relating to utilisation of a relatively simple response to assess sensory motor gating, the test has now been corrected for level of stimulation and the subject’s attention span and ability and consequently this model is now of immense use in testing the efficacy of potential antipsychotic drugs.

1.6.2. Attentional set shifting (adaptation of behaviour model) – As with the previous tests the attentional set shifting test of cognitive dysfunction also attempts to replicate an impairment of certain aspects of the cognitive process in schizophrenia patients, in animals. The classical test to study attentional deficits in humans was the Wisconsin Card Sorting Test (WCST) (Grant 1948) where, based on the three perceptual dimensions (colour, shape and number), subjects are required to sort cards. The dysfunction is identified when ‘perseverative responding’ occurs, or in other words, when the subject fails to reject the first sorting rule after it has been changed (e.g. from colour to shape). This perseverative responding is what
is generally referred to as an impaired ability to shift attentional set. Primates have been shown to recognise and mediate attention between varied dimensions of complex stimuli, in a fashion similar to that observed in humans (Roberts et al., 1988). However, in rodents, this test was not validated until recently (Birrell & Brown 2000). The functional similarity of rats to primates in this paradigm was questioned on the basis that rodents do not possess complex behavioural patterns (which are thought to be mediated by frontal cortex) and hence would be an inappropriate subject to test complex cognitive functions. Studies by Birrell and Brown however, suggested otherwise, and resulted in the rodent model of attentional set shifting task. This test is now used in several studies (Colacicco et al., 2002; McLean et al., 2008; Rodefer et al., 2008; Broberg et al., 2008) to establish the neural/biochemical basis of complex behavioural patterns and also to screen potential antipsychotic drugs.

1.6.3. Reversal learning test (appropriate decision making test) – This is an experimental model based on contrived rewards or punishments for the subjects on performing a particular task. There are several versions of this model and a range of animals have been used as subjects for this test and the two major types of protocols in this model are the classical and operant models (Rescorla and Holland 1982). In the former, animals are trained to show a reflex action to an environmental stimulus that would not normally elicit that reflex. In operant conditioning, the subject is required to respond to stimulus and is rewarded for it. The reversal learning paradigm is an extension of the operant model, whereby the animal is trained to first respond to one stimuli (e.g. press one of the two levers for food) and then must learn a new rule in the same dimension (press the second lever to get food). This way, the
animal is offered two or more stimuli and is expected to make a choice response, with “correct” responses rewarded and “incorrect” responses not rewarded. This is a discrimination task and needs extensive training first to learn and then reverse the rule that they have learned. Animals like the squirrel monkey and rodents have proved to be very useful in such tests (Abdul-Monim et al., 2003). While this paradigm has been appropriate in the study of antipsychotic treatment, and has provided evidence of the differential effect of the typical and atypical antipsychotic drugs (Abdul-Monim et al., 2006), the main drawback of this task was the extensive training required prior to testing. Nonetheless it is an important preclinical model to study storage and retrieval of new information and learning patterns observed in schizophrenic patients.

1.6.4. The novel object recognition paradigm (NOR) (episodic memory test) – Certain preclinical tests allow the observation of relatively subtle cognitive deficits in the rat that resemble cognitive symptoms in subjects with schizophrenia. The cognitive deficits observed are seen in behaviours such as declarative memory deficits, which may be measured by recognition tasks such as NOR paradigm. A recognition memory task allows the comparison between presented stimuli and previously stored information. Developed originally by Berlyne (1950), the NOR was reintroduced in its present form by Ennaceur & Delacour (1988). The NOR test in rats is based on the differential exploration of familiar and new objects. It is a non-rewarded, ethologically relevant paradigm based on the spontaneous exploratory behaviour in rats, which measures episodic memory. Each session consists of two trials or phases. In the first trial, the rats are exposed two identical objects in the open field. During the second trial, rats are exposed to two dissimilar
objects, one familiar object from the first trial and one new object. Object recognition in rats can be measured as the difference in time spent exploring the familiar and the new (novel) object. Normal untreated rats have been shown to spend more time exploring the new object (Grayson et al., 2007). It was also found that rats were able to discriminate between the familiar and the novel object when the inter-trial interval was between 1 min and 3 hours, but not when it was greater than 24 hours, although this effect may be sex dependent in the rat (Sutcliffe et al., 2007). The duration of each trial is also important as a preference for the novel object lasts during the first 1 or 2 minutes, after which time preference diminishes as both objects become familiar and are explored equally. The NOR paradigm has been used extensively in this thesis and will be explained in detail in chapter 2. Behavioural tests have also been developed to assess negative symptoms of schizophrenia. Although several aspects such as alogia and affective flattening are impossible to measure in animals it is possible to assess some others such as those described below.

1.6.5. Social withdrawal (Interaction) – Social withdrawal is a negative symptom of schizophrenia and one of the few symptoms which manifest early in patients (Strauss et al., 1977). The condition has been modelled in both rats (Sams-Dodd 1998, 1999) and monkeys (Ellenbroek et al., 1996), which demonstrate a lack of willingness to interact with other animals. However, since speech is the chief form of interaction in man, this species-species difference limits the assessment of interaction in animals. Most studies focus on contact and non-contact behaviours of animals when attempting to model social withdrawal as a test for asociality. This test will be explained in detail in chapter 3 of this thesis.
Phencyclidine (PCP)

Phencyclidine commonly referred to as PCP or “angel dust”, is a synthetic arylcyclohexylamine of the chemical name phenylcyclohexylpiperidine (fig 1.2).

![Phencyclidine (C-HeN)](image)

Fig 1.2 Structure of phencycloxylpiperidine (PCP)

It is a dissociative anaesthetic that was found to induce psychosis in humans that closely resembled schizophrenia as early as the 1950s (Luby et al., 1959). Its use as an anaesthetic was discontinued a few years later due to adverse side effects which included delirium and confusion. However, a few years later it was brought back in use as a veterinary anaesthetic under the trade name Sernylan (Ferin et al., 1976).

PCP works primarily as an NMDA receptor (NMDAR) antagonist although it also has effects on a number of other binding sites (Itzhak and Khouri 1988; Rothman 1994). PCP displaces MK801 (a selective NMDAR antagonist, which is 10 times more potent than PCP as an NMDAR antagonist) binding in cortical brain slices in a manner correlated with its potency at the NMDA receptor (Wong et al., 1986). The glutamate hypothesis of schizophrenia is in fact supported by the observation that administration of PCP produced symptoms that mimic the core symptoms of schizophrenia, including positive and negative symptoms as well as impairment of
cognitive functions in humans (Javitt and Zukin, 1991; Silver et al., 2003). The action of PCP at the NMDAR is “use dependent”. It requires the ion channel of the NMDAR to be open (i.e., Mg blockade should be overcome by membrane depolarization) before PCP can bind to its site of action on the receptor. In order for the Mg blockade to be overcome, the membrane needs to be depolarized. The depolarization occurs when AMPA channels (which co-exist with NMDA receptors at many synapses) at the same and neighbouring synapses are activated. Since AMPA receptors are permeable to both Na\(^+\) and K\(^+\) ions even at resting potential (contrary to NMDA receptors) they can trigger depolarization of the nerve membranes and the consequent opening of the ion channel in the NMDA receptor. PCP antagonizes the action of the NMDA receptor by blocking this ion channel, which, results in the disturbance and cognitive dysfunction similar to that seen in schizophrenia. It is generally accepted that NMDAR antagonism is the underlying cause of majority of the behavioural effects of PCP administration in rats. When administered systemically potent NMDA antagonists enter the brain and reach maximal concentration within 10 minutes (Hucker et al., 1983), and are quick to block the NMDAR. Dissociation from the receptor complex is however, much slower (Foster & Wong et al., 1987). Figures 1.3a & 1.3b depict the simplified schematic sketch to represent the action of PCP at the NMDA receptor.
Non-NMDA receptors are selectively agonised by kainate and AMPA. The associated ion channels are more permeable to Na+, K+ and Ca2+ (Kandel et al., 1991). NMDA-gated channels are more permeable to Ca2+ than Na2+ ions (Kandel et al., 1991).

**1.7.1. Acute PCP administration**

PCP induces behavioural and neurochemical changes, after both acute and repeated administration in different dosing regimes. Acute exposure to PCP increases extracellular levels of DA, glutamate, noradrenaline and acetylcholine in the prefrontal cortex (Verma & Moghaddam 1996). Furthermore acute administration of PCP increases the firing rate of dopaminergic nucleus accumbens neurones (Svensson et al., 1995). The behavioural abnormalities associated with acute exposure range form increased locomotor activity and stereotypy (positive symptoms) to social withdrawal (negative symptoms) to deficits in attention and
working/episodic/spatial memory functions. Furthermore, these behavioural effects of PCP treatment are not limited to increased DA levels alone as they are not reversed by typical antipsychotic drugs. Indeed this effect distinguishes PCP based models from the traditional DA (amphetamine) based animal models.

However, acute treatment with PCP has limitations in modelling a chronic condition such as schizophrenia which shows enduring dysfunction in cognition and social behaviours. Repeated administration of PCP which can cause long-term neurochemical and behavioural changes that last for weeks even after withdrawing drug treatment, is proposed as a useful model, extremely relevant to negative and cognitive symptoms of schizophrenia (Jentsch et al., 1999). It is this underlying pharmacology that is now being used to model aspects of schizophrenia in animals.

1.7.2. Sub-chronic PCP treatment

It has been observed that repeated administration of PCP could model the symptoms of schizophrenia even in animals in a pattern similar to those seen in human volunteers and patients (Jentsch 2003).

Repeated treatment with PCP in rats and non-human primates models the frontal lobe deficits associated with schizophrenia, which results in the cognitive deficits (Jentsch 1997). Furthermore this model is not based on a hyperdopaminergic state and hence increased DA levels alone cannot be assumed to mediate most behavioural effects. This model can therefore be useful in distinguishing between typical and atypical antipsychotic compounds. It has been evidenced that the
phenotypic expression or behavioural patterns observed with repeated PCP administration in animals is accompanied by reductions in parvalbumin mRNA expression in GABAergic interneurons in parts of the prefrontal cortex and the hippocampus (Abdul-Monim et al., 2006) which is consistent with human post-mortem studies of patients which demonstrate the same loss (Zhang et al., 2002). Hypofrontality of schizophrenia measured by glucose utilisation in the prefrontal cortex after exposure to PCP is also similar in rodents and man (Wolkin et al., 1992). Deficits of N-acetylaspartate (NAA) and its precursor N-acetylaspartylglutamate (NAAG) in the temporal cortex and elevation of NAAG in the hippocampus observed in rodents treated with chronic PCP regime also mimic post-mortem findings on human reported in schizophrenia (Reynolds et al., 2005). These findings have progressively established the potential role of repeated PCP treatment as an important treatment regime to model schizophrenia in laboratory animals.

It has been demonstrated that sub-chronic treatment with PCP (2 mg/kg twice daily for seven days) induces a selective deficit in the NOR task in female rats (Grayson et al., 2007) and reversal learning (Abdul-Monim et al., 2006; McLean et al., 2008). In the attentional set shifting test male rats have been shown to be less sensitive to PCP-induced deficits in the extra-dimensional shift (EDS) stage of the test compared to female rats (McLean et al., 2008), which is also observed in NOR task albeit with acute PCP treatment (Grayson & Neill, unpublished findings). With respect to the NOR task, the deficit is only observed in the retention phase of the test, suggesting a specific and relatively subtle cognitive impairment. Both exploration in the acquisition phase and locomotor activity are unaffected by PCP
treatment. The effects of PCP in this paradigm may represent a selective deficit in episodic memory, which is known to be impaired in schizophrenia (Laws et al., 2006).

It has also been found that the atypical antipsychotic drug clozapine, but not the classical antipsychotic, haloperidol, can ameliorate and prevent (Idris et al., 2005b) the cognitive deficit induced by sub-chronic PCP (2 mg/kg i.p. twice a day for 7 days followed by 7 days drug-free period) in this paradigm (Grayson et al., 2007). Hence, this test has some predictive validity for the treatment of cognitive symptoms of schizophrenia and PCP treatment can be considered an effective means to model aspects of cognitive dysfunction in animals.

Although from the above description, it is evident that both acute and sub-chronic PCP dosing regime in adult animals could model the classic triad of positive, negative and cognitive symptoms of schizophrenia, not much is known about the effects of developmental treatment on adult behaviour and activity levels. This is precisely why this thesis is centred on neonatal NMDA antagonism. Few details on the various neonatal and prenatal treatment regimes are described beneath.

1.7.3. Perinatal NMDA antagonism

The concept of NMDA receptor hypofunction and the association of glutamate in developmental processes have given rise to several variations of animal models of schizophrenia, based on postnatal NMDA receptor blockade. Studies have shown that repeated neonatal PCP (10 mg/kg s.c. on PND 7, 9 and 11) administration leads
to some behavioural variations in adult animals, namely, reduction of baseline PPI of the acoustic startle response and retardation of acquisition of a spatial alternation task in female and male Sprague-Dawley rats (Wang et al., 2001). Studies have also shown deficits in cognitive flexibility, specifically in the rats’ ability to shift attentional set, similar to the impairment observed in schizophrenia patients, thereby supporting the validity of the early postnatal PCP regime as a disease-like model of the neurodevelopmental aspect of schizophrenia (Broberg et al., 2008). This study suggested a potential sex difference, wherein the male rats exhibited increased deficits in an attentional set shifting task when compared to the female rats following neonatal PCP 10 mg/kg. Studies have also shown increase in locomotor activity consistent with the effect observed in rats following a low dose of PCP (2 mg/kg s.c.) on PND 42 and D-amphetamine (2 mg/kg i.p.) in the adult stage (PND 60-108) (Depoortere et al., 2005). It was also shown by Depoortere et al. that the increase in locomotor activity in response to PCP and D-amphetamine was ameliorated by pre-treatment with olanzapine in female and male Wistar Han rats, following neonatal treatment on PNDs 7, 9 and 11 with PCP 10 mg/kg s.c. PCP at a lower dose (5 mg/kg) given once a day on PND 5-15 was shown to impair spatial reference memory in the Morris water maze test in adolescents (PND35) in both male and female adult Sprague-Dawley rats (PND 60) (Sircar 2003). Also, Sircar et al, 2003; Andersen & Pouzet, 2004 showed that male and female Sprague-Dawley rats when treated with either saline or PCP (8.7 mg/kg s.c.) induced an apoptotic effect in the cortical structures implicated in cognitive processing as reported by Wang et al, 2001. It was observed that the male PCP-treated rats in adult stage were less impaired during the spatial reference memory task in the Morris water maze, but severely impaired during the reversal and spatial working memory tasks, when
compared to the female adult rats, which were not affected by this treatment. This cognitive deficit was reversed by chronic treatment with D-serine (640 mg/kg and 1280 mg/kg s.c., given 30 min before the animals were tested and was maintained between the two learning tasks performed). See Table 3 for a brief review of behavioural and neurochemical effects of perinatal NMDA receptor antagonism.

Some studies have also shown impairments in working memory in adulthood in Sprague-Dawley rats with perinatal sub-chronic MK-801 administration (0.1 mg/kg for four days beginning from PND7, twice a day) (Stefani and Mogaddam, 2005) and disturbances in locomotor activity (Schiffelholz et al., 2004).

A single high dose of ketamine HCl (100 mg/kg i.p.) during late gestation stage (E18) has been shown to produce disturbances in learning and memory, in both
male and female Sprague-Dawley young adult rats (offspring) tested on PND81 (Mickley et al., 2004).

A competitive antagonist at the NMDA receptor, CGP 40116, when administered in the postnatal period (1.25 mg/kg on days 1, 3, 6 and 9; 2.5 mg/kg on days 12, 15, 18 and a final dose of 5 mg/kg on day 21), altered the structure of pyramidal neurones in the medial prefrontal cortex of adult male and female Wistar rats (Wedzony et al., 2005). This result agrees with the neurophil theory of schizophrenia (Goldman-Rakic, 1999) as the blockade of NMDA receptors in the postnatal period models morphological changes in the pyramidal neurones of the medial prefrontal cortex, as observed in certain cases of schizophrenia (Broadbelt et al., 2002). It is of note that postnatal blockade of NMDA receptors enhances locomotor activity, stimulated by amphetamine, suggesting the development of functional supersensitivity of subcortical dopaminergic systems, which models the positive symptoms of schizophrenia (Wedzony et al., 2005a).

In essence, several studies suggest that the neonatal NMDA receptor antagonist regime is a valuable model for studying the neurodevelopmental and NMDA receptor hypofunction hypotheses of schizophrenia. Neurochemical and behavioural changes indicative of those seen in schizophrenia are present long after cessation of drug administration, which suggests that a permanent change in structure and organization (of neural circuitry) has occurred during brain development. Thus, neonatal PCP treatment in the developing period induces long-lasting and significant schizophrenia-like behavioural changes in adulthood which has the clear advantage of incorporating a neurodevelopmental approach to the NMDA antagonist model.
1.8. Aims

1.8.1. General aim:
The main scope of this thesis is to study the nature of behavioural deficits in schizophrenia using a well established neonatal PCP treatment regime in male and female rats.

1.8.2. Specific aims:

1. To validate the effects of neonatal PCP treatment (measuring cognitive deficits) using both sexes using NOR, spatial memory task (SMT) and also measure locomotor activity both in the adolescent and adult stages.

2. To investigate the effects of neonatal PCP on a test for negative symptoms using social interaction test (SI).

3. To study the therapeutic effects of typical and atypical antipsychotic treatment following neonatal PCP treatment in both sexes using the NOR and SI tests.

4. Evaluating the long-term effects of neonatal PCP treatment, which is based on investigating the time of onset of cognitive deficits induced by neonatal PCP (deduced from the results of chapter 2) by testing the animals in the adolescent and young adult stages and testing after a year.

1.8.3. Significance:
PCP is used (acutely and sub-chronically) extensively as it is a pharmacological tool that is used to mimic cognitive and negative symptoms in various animal models, as to date, PCP treatment is considered one of the best pharmacological model of schizophrenia symptoms. Since not much is known about the effects of developmental treatment of PCP on adolescent and adult behaviour(s), the focus of work presented in this thesis is on neonatal NMDA antagonism using neonatal PCP dosing design. Neonatal PCP treatment produces long-term deficits in sensorimotor gating, and some cognitive functions and other schizophrenia-relevant behaviours, thus making it a good developmental model for the disorder. However, more work needs to be done to reveal the exact mechanisms underlying PCP-induced behaviour and neurochemical changes. This study will provide a thorough investigation into the effects of neonatal PCP administration on cognitive deficits and SI behaviour in the adolescent and adult life- a treatment based on the neurodevelopmental and NMDA receptor hypofunction hypotheses. It is hoped that this study will offer good construct and face validity for schizophrenia. Exploring the effects of neonatal PCP administration on developmental behaviour may provide valuable insights into pathological processes involved in schizophrenia and may identify new targets for future development of effective treatment for the disorder.
Chapter 2

Effect of neonatal PCP on cognition using NOR and SMT and locomotor activity (LMA) in adolescent and adult male and female rats
2. Introduction

It is becoming increasingly apparent that cognitive deficits in schizophrenia (see chapter 1 general introduction) will impair psychosocial functioning and reduce the integration of schizophrenic patients into society. One of the core features of schizophrenia is abnormal cognitive processing. The vast literature on cognitive functioning and schizophrenia has been relatively constant over the years but what has changed is the implication of the disorder with regards to schizophrenia. It may be noted that mere impairment in memory cannot always be regarded as a clear cognitive disorder. Hence, it is considered that cognitive deficits may be worsened by positive and negative symptoms as well as by current antipsychotic medication. A wide range of behavioural paradigms are available to model cognitive function and these paradigms may be chosen based on the particular aspect of cognition under study. Behavioural assays will be discussed in detail later in this chapter (section 2.3).

2.1. Neonatal PCP regime

Although several groups have established a neonatal PCP dosing regime (as discussed in chapter 1), they are all varied as they use different strains, gender and a different dosing regime. Although Sprague-Dawley is the most commonly used strain in most of the neonatal PCP protocols, hooded–Lister rats were used in all the studies in this thesis due to the fact that hooded-Lister rats have been tested extensively in our lab, following the success of a sub-chronic PCP treatment regime in adult rats in several cognitive tasks (Grayson et al., 2007; McLean et al., 2009; Idris et al 2009, Abdul-Monim et al., 2006). Time mated pregnant dams were purchased from Charles River UK (CRUK) and it was ensured that all the dams came from the same source for all the studies carried
out in this thesis. Along with investigating cognitive deficits, effects of neonatal PCP treatment in males and females were also investigated.

2.1.2. Gender differences in schizophrenia

Gender differences in schizophrenia have been investigated for many years (Halbreich and Kahn, 2003, Atalay et al., 2006). The incidence of schizophrenia has been found to be greater in men (Aleman et al., 2003, McGrath et al., 2004). The contrasting evidence showed that there was an overall increase in incidence in men being affected earlier in life and women showed an increased incidence later in life (Castle et al., 1993). Certainly it has been found that in general, women are less severely affected by the disease, necessitating fewer and shorter periods of hospitalization, more rapid remissions and better response to antipsychotic drugs (Szymanski et al., 1995, Salokangas, 2004). In animal studies, it has been demonstrated that acute and sub-chronic treatment with PCP (2 mg/kg twice daily for seven days) induces a selective deficit in the NOR (Grayson et al., 2007) and in reversal learning (Abdul-Monim et al., 2006; McLean et al., 2009) in female rats. In the attentional set shifting test male rats have been shown to be less sensitive to PCP-induced deficits in the extra-dimensional shift (EDS) stage of the test compared to female rats (McLean et al., 2008), which was also observed in NOR task albeit following acute PCP treatment (Grayson, unpublished findings).

It has been shown that PCP can cause dose-dependent morphological damage to neurones in the cerebral cortex of rats (Olney et al. 1991). While these effects were observed to be reversible after a single dose, effects after chronic doses have not been clearly demonstrated and it is likely that chronic use of the drug could be associated
with more permanent neurological damage. Also, female rats are twice as sensitive to these effects as male rats (Olney et al. 1989). The mechanisms underlying the sex differences in the pharmacological effects in rats are not well comprehended, but they have been found to be dose dependent and are found after single and chronic doses of the drug (Olney et al. 1989; Wessinger 1995). Additionally, it has been reported that females are much less efficient at metabolizing PCP than males (Lynch et al., 2002). Although sex differences of this type are not clearly understood in humans, this sexual dimorphism in the rat could be a useful tool for understanding the wide range of differences in effects and metabolism of the drug in humans. Most laboratories use male rats, and due to contradictory effects in various paradigms in males and females, this project aims to directly compare the effects of neonatal PCP in both sexes.

2.1.3. Behavioural tests

Each batch of the neonatal PCP treated rats during this project underwent several behavioural tests such as NOR, SMT and LMA (See table 1 for more details). The NOR task for rodents is a non-spatial memory test which involves the replacement of a familiar object with a novel object in the retention trial. In this project, the NOR task was adapted to assess spatial memory, where the identical objects were not replaced with a novel object, but were moved to a new position following varying inter-trial interval (Ennaceur et al., 1988). These tasks are ethologically relevant as they rely on the animal’s natural exploratory behavioural repertoire and avoid the confounding manipulation of reinforcement. Animals typically respond to environmental changes by preferential exploration of novel or moved objects over familiar or stationary objects; these preferences point towards the formation of memories regarding the location and identity of objects. Due to the relative simplicity and relevance of the task, it is
increasingly used as a powerful experimental tool to assess drug effects on cognition
and to investigate the neural mechanisms underlying spatial and episodic memory (De
Lima et al., 2005; Grayson et al., 2004; Sutcliffe et al., 2007). The NOR test has also
been listed under the TURNS initiative as a relevant and reliable test for studying visual
learning and memory deficits in schizophrenia (TURNS.ucla.edu). Due to its relevance
to deficits in schizophrenia, NOR and SMT paradigms have been predominantly used in
this chapter.

Also, this treatment has implications to the study of schizophrenia, which is
increasingly being viewed as a neurodevelopmental disorder; and behaviours produced
by this model, particularly deficits in memory are relevant to symptoms of the disorder.
Hence, the present chapter aimed to investigate whether neonatal PCP treatment would
affect object and spatial memory of adolescent and adult male and female rats using
NOR and SMT tests designed to assess object and spatial memory as well as locomotor
activity.
2.1.4. **Aims:** The aims of the experiments in this chapter were twofold.

a. To evaluate the cognitive deficits induced by neonatal treatment with PCP using the NOR paradigm and SMT.

b. To evaluate the time of onset of cognitive deficits induced by PCP by testing the animals in the adolescent (juvenile) stage and at adulthood.

Both male and female rats were used in this study and the working hypothesis was that neonatal PCP administered on PNDs 7, 9 and 11 would possibly act on several neurotransmitter receptors in several brain regions and affect behaviour long after treatment cessation. This treatment would also disrupt brain development at an early stage thereby causing long-lasting consequences which are of relevance to the study of psychiatric disorders such as schizophrenia, which is thought to be a neurodevelopmental disorder, involving NMDA receptor hypofunction.
2.2. Methods and materials

2.2.1. Animals and housing

Hooded-Lister rats were obtained from Charles River, UK. Five time-mated pregnant dams arrived 15 days prior to parturition and were housed individually in cages containing sawdust and paper bedding until weaning. All animals were housed under conditions of constant temperature (21 ± 1 °C) and humidity (50-58%) and a 12 hour light/dark cycle (lights on at 07:00 h) with free access to food and water. All experiments were conducted in accordance with the Animals Scientific Procedures Act, UK 1986 and were approved by the University of Bradford ethical review panel.

2.2.2. Phencyclidine dosing regimens - Repeat administration to rat neonates.

The neonatal PCP dosing regime was adapted from Wang et al. 2001. Following birth (46 pups were born, 7-11 per litter, 14 females and 32 males), the pups were kept with their littermates and left undisturbed until the time of dosing. On PNDs 7, 9 and 11 mothers were removed from the home cage and held by an assistant. The male pups were removed and administered vehicle or PCP (10 mg/kg and 20 mg/kg via the subcutaneous route) and female pups were administered vehicle or PCP (10 mg/kg subcutaneous) into the loose skin at the back of the neck. The doses used were based on the long-term behavioural deficits induced by the doses observed in Wang et al., 2001 and the attentional set shifting deficits demonstrated by Broberg et al., 2008. Due to the small number of female pups in the litter, our study was limited to administration of one dose (10 mg/kg) only. At the time of dosing on PND 7, the pups were marked with a permanent tattoo code on the tails for identification purposes. Dosing was carried out in a separate room in an attempt to shield the mothers from pup vocalizations. Time away
from the litter was kept to a minimum. Pups were returned to the home cage immediately after dosing. Ambient noise in the holding room was minimised as far as possible and animals were not handled between the time of dosing and weaning. Pups were weaned between PNDs 22-23 and were housed 4-5 per home cage. All behavioural testing was carried out in the adolescent rats on PNDs 35-49 and in the adult rats on PNDs 60-96. See figure 2.1 & table 2.1(i) for the PCP treatment regime and timeline of behavioural testing in animals treated with PCP on PNDs 7, 9 and 11.

Figure 2.1. Neonatal PCP treatment, behavioural testing
<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>BATCH 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCP Dose PND</td>
<td>7, 9 and 11</td>
</tr>
<tr>
<td>PCP (mg/kg)</td>
<td>10 and 20 mg/kg-Males 10 mg/kg – Females</td>
</tr>
<tr>
<td>ID method</td>
<td>Tattoo</td>
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<tr>
<td>Cross-fostering</td>
<td>No</td>
</tr>
<tr>
<td>Weaning day</td>
<td>22-23</td>
</tr>
<tr>
<td>Group size (n) at PND35</td>
<td>8-11</td>
</tr>
<tr>
<td>PNDs 35-36</td>
<td>Novel Object Recognition</td>
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<tr>
<td>PNDs 46-48</td>
<td>Spatial Memory Task</td>
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<tr>
<td>PND 49</td>
<td>Locomotor Activity</td>
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<tr>
<td>PNDs 60-62</td>
<td>Novel Object Recognition</td>
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<td>Locomotor Activity</td>
</tr>
<tr>
<td>PNDs 82-83</td>
<td>Spatial Memory Task</td>
</tr>
</tbody>
</table>

Table 2.1(i). Neonatal Treatment regime and behavioural testing in the first batch of rats

Abbreviations: PND: Post Natal Day.

2.2.3. Behavioural testing

2.2.3(i). Novel Object Recognition

The NOR method was adapted from Grayson et al (2007).

2.2.3(i) a. Apparatus

The apparatus consisted of an open box made of Plexiglas (52 cm L; 52 cm W; 31 cm H) positioned 27 cm above the floor on a movable trolley (Figure. 2.2). The walls of the
box were black and the objects to be discriminated (in triplicate) were made of Plexiglas, plastic or metal (Figure 2.3). These objects were used as none of these were preferred one over the other as demonstrated previously by Grayson et al (2007). The heights of the objects were approximately the same (10 ± 2 cm). Objects were positioned 6 cm away from the walls of the box, in opposite corners. After each trial, the objects and the box were cleaned with 10% alcohol in an attempt to remove any persistent olfactory cues on the objects and the box. Care was also taken to ensure that the objects chosen for testing were not of any natural significance to the rats.

2.2.3(i) b. Habituation

The rats were given daily 30-min exploration periods in the NOR box for three days prior to the test day to ensure habituation to the empty apparatus and the test room environment.
2.2.3(i)c. Behavioural testing

A further 3-min habituation preceded the experimental trials on the day of testing. In the acquisition trial, each rat was placed into the box and exposed to two identical objects for a period of 3 min (fig 2.4). The rats were then returned to their home cage for a 1-min inter-trial interval (ITI). During this time, the entire box was cleaned thoroughly with 10% alcohol. The objects used in the acquisition trial were removed and one was replaced with an identical familiar copy and one with a novel object. Following the 1-min inter-trial interval, rats were returned to explore the familiar and novel object for 3-min in the retention trial (fig 2.5). The location of the novel object in the retention phase (i.e. where the objects were placed) was randomly assigned using a Gellerman schedule. The experiments were recorded onto videotape for subsequent behavioural analysis by an observer blind to treatments.

Object exploration was defined as the animal directing its nose to the object at a distance $\leq 2$ cm and/or touching it with its nose, turning around or sitting on the object was not considered as exploratory behaviour (Ennaceur et al., 2004). The exploration time (s) of each object in each trial was recorded manually using two stopwatches and the following were calculated: the total exploration time of both objects in the acquisition trial, total exploration time of both objects in the retention trial. The discrimination index represents the difference in exploration time expressed as a proportion of the total time spent exploring the two objects in the retention trial (i.e.) $(\text{novel} – \text{familiar})/(\text{novel} + \text{familiar})$. Locomotor activity in NOR task was recorded by counting the total number of lines crossed by the rats (the tip of their tails) during the acquisition and retention trials.
2.2.3(i)d. Statistical analysis

The data are expressed as mean ± S.E.M (n=8-11 per group). Exploration data were analysed by a repeated measures two-way ANOVA in males. A 3 x 2 mixed design was used, comparing pretreatment (vehicle, PCP 10 mg/kg and PCP 20 mg/kg) and object (left; right) for acquisition trial and object (novel; familiar) for retention trial. Any significant main effect of pretreatment was tested post hoc using student’s t-test, in order to examine whether the PCP treatments differed from vehicle treatment. In females, data were expressed as mean ± SEM. A 2 x 2 mixed design was used, comparing pretreatment (vehicle, PCP 10 mg/kg) and object (left; right) for acquisition trial and object (novel; familiar) for retention trial. Line crossings were expressed as mean ±S.E.M. of the total number of line crossed (tail crossings) during the acquisition and retention phases. Analysis of line crossings and discrimination index (DI) data was performed using one-way ANOVA followed by post-hoc Dunnett’s t-test.

2.2.4 (i) Spatial Memory task

The SMT was adapted from Sutcliffe et al (2007).

2.2. 4.(i)a Apparatus
The box used was the same as that in the NOR experiment described above (2.2.3(i)a) and shown in figures 2.4 & 2.5. The objects to be discriminated were available in quadruplicate and held no natural significance to the rats. They were made of a glass, plastic or metal. These objects were cleaned with 10% alcohol in between trials in an attempt to remove olfactory cues. For each subject, the position of the objects was counterbalanced and randomly altered to reduce any effects of object and place preference.

2.2.4.(i)b. Habituation

Animals were habituated to the test box arena for 3 days prior to the test day for a period of 30 min each time. The habituation phase was as for NOR (see 2.2.3(i) b).

As for NOR, the SMT is divided into three trials: acquisition trial, inter-trial interval and the retention trial.

Acquisition trial: In this trial, the animals explored two identical objects (A1 and A2) for a period of 3 min (Figure 2.6).

Inter-trial interval (ITI): During this trial, the animals were returned to their home cage for 1 min.

Retention trial: In this trial, copies of the same objects from the acquisition trial were presented to the animal; however, one object occupied a novel position (moved object) within the arena. (Figure 2.7).

Object exploration is the similar to the NOR test (see 2.2.3(i) b).
2.2.4.(i)c. Statistical analysis

The data are expressed as mean ± S.E.M (n=7-8 per group). Exploration data were analysed by a repeated measures two-way ANOVA in males. Exploration data were analysed by a repeated measures two-way ANOVA in males. A 3 x 2 mixed design was used, comparing pretreatment (vehicle, PCP 10 mg/kg and PCP 20 mg/kg) and object (left; right) for acquisition trial and object (novel position or moved object); familiar position or stationary object) for retention trial. Any significant main effect of pretreatment was tested post hoc using student’s t-test, in order to examine whether the PCP treatments differed from vehicle treatment. In females, data were expressed as mean ± SEM. A 2 x 2 mixed design was used, comparing pretreatment (vehicle, PCP 10 mg/kg) and object (left; right) for acquisition trial and object (novel position or moved object; familiar position or stationary object) for retention trial. Line crossings were expressed as mean ± S.E.M. of the total number of lines crossed during the acquisition and retention phases. Analysis of line crossings and discrimination index (DI) data was performed using one-way ANOVA followed by post-hoc Dunnett’s t-test.
2.2.5(i) Locomotor Activity

2.2.5(i)a. Apparatus

LMA was tested on PND 49 (adolescent stage) and PND 74 (adult stage). LMA response of the housing groups (n=8-11 per group in males and n=7 per group in females) was monitored using automated photocell cages (Figures 2.8 & 2.9) each fitted with two photocell units. The movement of each animal was monitored in a Plexiglas chamber (16 x 26 x 19 cm) covered with a wire mesh lid securely held with metal clips. Counts were recorded by means of photobeam interruptions within the box. LMA was monitored every 10 min over a 60 min period and summed to give a total count.

Fig 2.8 Locomotor activity test set up                  Fig 2.9 Locomotor activity box

2.2.5(i)b. Statistical analysis

For the locomotor activity data, the number of photobeam breaks generated every 10 minutes over a 60-min period was analysed using post hoc students t-test on the area under the curves (60 min with 10 min intervals, calculated by linear trapezoidal rule).
The area under the curve values were calculated for the locomotor activity measured during 0-60 min period with 10 min intervals.

2.3. Results

2.3.0 Effect of neonatal PCP treatment on NOR in adolescent and adult rats in both sexes.

Four sets of experiments were carried out in total in order to test the effect of neonatal PCP in both sexes at two different time points i.e. adolescent and adult stages. The effects that neonatal PCP had in this test at these time points in both the sexes will be described in detail in this section.

Overall two-way ANOVA in the acquisition trial (in each of the four sets of the experiments) consistently showed that drug treatment did not produce any significant effect on object recognition in this trial of the test and rats spent equal amount of time exploring both the objects; an effect that was observed for both vehicle and neonatal PCP treated rats in males. A similar effect was observed in females using paired t-test in the acquisition trial, which showed consistently no significant effect on object exploration in this trial, an effect observed for both vehicle and neonatal PCP treated animals.

In the retention trial of the test, two-way ANOVA (in each of the four sets of the experiments) showed a significant effect of vehicle treatment on object exploration in male rats. Post-hoc analysis then showed that vehicle treated rats spent significantly more time exploring the novel object compared to the familiar object. Rats that received neonatal PCP treatment however could not discriminate between the novel and familiar
object and spent similar time exploring the novel and the familiar object. A similar effect was observed in female rats using paired t-test in the retention trial, wherein the vehicle treated rats spent significantly more time exploring the novel object compared to the familiar object. Rats that received neonatal PCP treatment however could not discriminate between the novel and familiar object and spent either similar or more time exploring the novel object compared to the familiar object.
Experiment 2.3.1: The effect of neonatal PCP treatment (10 mg/kg & 20 mg/kg in males and 10 mg/kg in females) on episodic memory at two different time points- i.e. adolescents and adults in both males and females using NOR paradigm

2.3.1a. Effect of neonatal PCP treatment on acquisition trial in adolescents and adults in both males and females.

Adolescent Males & Females: In males, an overall two-way ANOVA revealed that neither vehicle nor drug treatment produce any significant effect on object exploration in the acquisition trial of the test (F2, 26 = 1.65, NS). In females, there was no significant effect on object exploration observed in this trial. Rats from all treatment groups spent almost equal times exploring both the objects (See figure 2.3.1a(i&ii))

Adult Males & Females: In males, an overall two-way ANOVA revealed that no treatment produced any significant effect on object exploration in the acquisition trial of the test (F2, 27 = 0.09, NS). A similar observation was made in the females, where paired t-test revealed no significant effect on object exploration in this trial (p<0.14). Rats from all treatment groups spent almost equal times exploring both the objects (See fig 2.3.1a(iii&iv)).
Figure 2.3.1a(i) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical objects in the 3-min acquisition trial in a novel object recognition task in male hooded-Lister rats tested in the adolescent stage. Data are shown as mean ± SEM of exploration time (s) n=8-11 per group.

Figure 2.3.1a(ii) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical objects in the 3-min acquisition trial in a novel object recognition task in female hooded-Lister rats tested in the adolescent stage. Data are shown as mean ± SEM of exploration time (s) n=7 per group.
Figure 2.3.1a(iii) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical objects in the 3-min acquisition trial in a novel object recognition task in male hooded-Lister rats tested in the adult stage. Data are shown as mean ± SEM of exploration time (s) (n=8-11 per group).

Figure 2.3.1a(iv) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical objects in the 3-min acquisition trial in a novel object recognition task in female hooded-Lister rats tested in the adult stage. Data are shown as mean ± SEM of exploration time (s) (n=7 per group).
2.3.1b. Effect of neonatal PCP treatment on retention trial in adolescents and adults in males and females.

Adolescent Males & Females: In males, an overall two-way ANOVA revealed a significant effect of treatment on object exploration time in the retention trial of the NOR task (F2, 26 = 25.5; p<0.001). Post-hoc analysis revealed the vehicle treated animals (control group) had a clear preference for the novel object compared to the familiar object – i.e. spent significantly more time exploring the novel versus familiar object (p<0.001). The effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both objects.

In females, a significant effect of treatment was observed on object exploration. Paired t-test revealed the vehicle treated animals (control group) had a clear preference for the novel object (p<0.001) (See figures 2.3.1b. i&ii). This effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both objects.

Adult Males and Females: In males, an overall two-way ANOVA revealed a significant effect of treatment on object exploration time in the retention trial of the NOR task ((F2, 27 = 5.79; p<0.01). Post-hoc analysis revealed the vehicle treated animals (control group) had a clear preference for the novel object compared to the familiar object – i.e. spent significantly more time exploring the novel versus familiar object (p<0.01). A similar effect was observed in females in this trial where there was significant effect of neonatal PCP on object exploration on paired t-test (p<0.01). (See figures 2.3.1b. iii&iv). This effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both objects.
Novel object recognition

Retention trial

Adolescent Male

![Bar chart showing the effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9, and 11) and vehicle (0.9% saline) on exploration of a novel and a familiar object in the 3-min retention trial in the NOR task in male hooded-Lister rats tested in the adolescent stage. Data are shown as mean ± SEM of exploration time (s), n=8-11 per group. Novel vs. familiar object exploration time ***p<0.001, Student’s t-test.]

Adolescent Female

![Bar chart showing the effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9, and 11) and vehicle (0.9% saline) on exploration of a novel and a familiar object in the 3-min retention trial in the NOR task in female hooded-Lister rats tested in the adolescent stage. Data are shown as mean ± SEM of exploration time (s), n=7 per group. Novel vs. familiar object exploration time ***p<0.001, Student’s t-test.]

Figure 2.3.1b(i) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9, and 11) and vehicle (0.9% saline) on exploration of a novel and a familiar object in the 3-min retention trial in the NOR task in male hooded-Lister rats tested in the adolescent stage. Data are shown as mean ± SEM of exploration time (s), n=8-11 per group. Novel vs. familiar object exploration time ***p<0.001, Student’s t-test.

Figure 2.3.1b(ii) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9, and 11) and vehicle (0.9% saline) on exploration of a novel and a familiar object in the 3-min retention trial in the NOR task in female hooded-Lister rats tested in the adolescent stage. Data are shown as mean ± SEM of exploration time (s), n=7 per group. Novel vs. familiar object exploration time ***p<0.001, Student’s t-test.
Novel object recognition

Retention trial

Adult Male

**Figure 2.3.1b(iii)** The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of a novel and a familiar object in the 3-min retention trial in the NOR task in male hooded-Lister rats tested in the adult stage. Data are shown as mean ± SEM of exploration time (s) (n=8-11 per group). Novel Vs familiar object exploration time **p<0.01, Student’s t-test.

Adult Female

**Figure 2.3.1b(iv)** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of a novel and a familiar object in the 3-min retention trial in the NOR task in female hooded-Lister rats tested in the adult stage. Data are shown as mean ± SEM of exploration time (s) n=7 per group. Novel Vs familiar object exploration time **p<0.01, Student’s t-test.
2.3.1c: Effect of neonatal PCP treatment on discrimination index (DI) in adolescents and adults in both males and females.

**Adolescent Males and Females:** Discrimination index is a measure of the exploratory preference of the animals for the novel or the familiar object in the retention trial in NOR calculated by dividing the difference between the duration of time spent exploring the novel and familiar objects by the total duration of exploration activity in the retention trial. A one-way ANOVA on the DI revealed a significant effect of neonatal PCP (10 mg/kg and 20 mg/kg) in the male rats (F2, 26 = 26.04; p<0.001). Post-hoc analysis showed that both the neonatal PCP groups displayed a significant reduction in DI (p<0.001) compared to the control rats (See figure 2.3.1c i). Paired t-test in females also showed significant effect of neonatal PCP (10 mg/kg) (p<0.001), where the neonatal PCP group displayed a significant reduction in DI when compared to the control rats (figure 2.3.1c ii).

**Adult Males and Females:** In males, an overall one-way ANOVA on the DI revealed a significant effect of neonatal PCP (F2, 27 = 6.04; p<0.01). Post-hoc analysis showed that the PCP 10 mg/kg group displayed a significant reduction in DI (p<0.01) compared to the control rats. Although PCP 20 mg/kg treatment showed a decrease in the DI, it failed to reach statistical significance (p = 0.12). In females, a paired t-test on the DI revealed a significant effect of neonatal PCP (10 mg/kg) treatment (p<0.01). The neonatal PCP group displayed a significant reduction in DI (p<0.01) compared to the control group (See figures 2.3.1c iii&iv).
Novel object recognition

Discrimination Index

Adolescent Males

Figure 2.3.1c (i) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the discrimination index, DI. Data are shown as mean ± SEM (n=8-11 per group). ***p<0.001 significant reduction compared to vehicle.

Adolescent Females

Figure 2.3.1c (ii) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the discrimination index, DI. Data are shown as mean ± SEM (n=7 per group). ***p<0.001 significant reduction compared to vehicle.
Novel object recognition

Discrimination Index

Adult Males

**Figure 2.3.1c (iii)** The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7,9 and 11) and vehicle (0.9% saline) on the discrimination index, DI. Data are shown as mean ± SEM (n=8-11 per group). **p<0.01 significant reduction compared to vehicle.

Adult Females

**Figure 2.3.1c (iv)** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7,9 and 11) and vehicle (0.9% saline) on the discrimination index, DI. Data are shown as mean ± SEM (n=7 per group). **p<0.01 significant reduction compared to vehicle.
2.3.1d: Effect of neonatal PCP treatment on Line Crossings in adolescents and adults in both males and females.

*Adolescent Males and Females:* In adolescent males, a one way ANOVA on the total line crossings during the acquisition plus retention trial of the test revealed a significant effect of neonatal PCP treatment on the line crossings of the animals (F2, 26 = 3.7; p<0.05). Further post-hoc analysis revealed that neonatal PCP 20 mg/kg group displayed a significant increase in line crossings (p<0.05) when compared to the control rats. However, the neonatal PCP 10 mg/kg treatment group did not show any significant effect on line crossings when compared to the control rats. Also, in adolescent females, paired t-test showed a significant increase in line crossings at 10 mg/kg of neonatal PCP treatment (p<0.05) when compared to the control group (See figures 2.3.1d i&ii).

*Adult Males and Females:* In adult males, an overall one way ANOVA on the total line crossings during the acquisition and retention trials of the test revealed no effect of neonatal PCP treatment on the line crossings of the animals (F2, 27 = 1.56; NS). Also, in females, paired t- test showed no significant effect of neonatal PCP treatment (10 mg/kg) on the line crossings of the animals when compared to the control group (p = 0.16) (See figures 2.3.1d iii&iv).
Figure 2.3.1d (i) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the total number of line crossings in the acquisition + retention trial in the novel object recognition task in males. Data are shown as mean ± SEM (n=8-11 per group). *p<0.05 significant increase in line crossings compared with vehicle, Dunnett’s t-test.

Figure 2.3.1d(ii) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the total number of line crossings in acquisition + retention trial in the novel object recognition task in females. Data are shown as mean ± SEM (n=7 per group). *p<0.05 significant increase in line crossings compared to vehicle, t-test.
Figure 2.3.1d(iii) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the total number of line crossings in an acquisition + retention trial in the novel object recognition task in adult males. Data are shown as mean ± SEM (n=8-11 per group).

Figure 2.3.1d(iv) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the total number of line crossings in an acquisition + retention trial in the novel object recognition task in adult females. Data are shown as mean ± SEM (n=7 per group).
Experiment 2.3.2: The effect of neonatal PCP treatment (10 mg/kg & 20 mg/kg in males and 10 mg/kg in females) on object location memory at two different time points—i.e. adolescents and adults in both males and females using SMT

Four sets of experiments were carried out in total in order to test the effect of neonatal PCP in both sexes at two different time points i.e. adolescent and adult stages. The effects that neonatal PCP had in this test at these time points in both the sexes will be described in detail in this section.

Overall two-way ANOVA in the acquisition trial (in each of the four sets of the experiments) consistently showed that drug treatment did not produce any significant effect on spatial memory in this trial of the test and rats spent equal amount of time exploring both the objects (A&B) an effect that was observed for both vehicle and neonatal PCP treated rats in males. A similar effect was observed in females using paired t-test in the acquisition trial, which showed consistently no significant effect on spatial memory in this trial, an effect observed for both vehicle and neonatal PCP treated animals.

In the retention trial of the test, two-way ANOVA (in each of the four sets of the experiments) showed a significant effect of vehicle treatment on spatial memory in male rats. Post-hoc analysis then showed that vehicle treated rats spent significantly more time exploring the moved object (novel position) compared to the stationary object (familiar position). Rats that received neonatal PCP treatment however could not discriminate between the stationary and moved objects and spent similar time exploring the stationary and moved objects. A similar effect was observed in female rats using paired t-test in the retention trial, wherein the vehicle treated rats spent significantly more time exploring the moved object compared to the stationary object. Rats that received neonatal PCP treatment however could not discriminate between the stationary
and moved objects and spent either similar or more time exploring the moved object compared to the stationary object.

2.3.2a. Effect of neonatal PCP treatment on the acquisition trial in adolescents and adults in both males and females using the SMT.

*Adolescent Males & Females:* In males, an overall two-way ANOVA revealed that no treatment produced any significant effect on spatial memory in the acquisition trial of the test (F2, 21 = 1.56; NS). Similarly, in females, paired t-test revealed no significant effect in this trial. Rats from all treatment groups spent almost equal times exploring both the objects (See figure 2.3.2a(i&ii))

*Adult Males & Females:* In males, an overall two-way ANOVA revealed that neither neonatal PCP (10 mg/kg and 20 mg/kg) nor vehicle (0.9% saline) produced any significant effect on object exploration in the acquisition trial of the test (F2, 21 = 1.57; NS). A similar observation was made in the females, where there was no significant effect on object exploration in this trial. Rats from all treatment groups spent almost equal times exploring both the objects (See fig 2.3.2a(iii&iv)).
**Spatial Memory Task**

**Acquisition trial**

**Adolescent Males**

![Bar chart showing exploration times for adolescent males with different doses of PCP and vehicle.](image)

**Figure 2.3.2a(i)** The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical objects (A & B) in the 3-min acquisition trial in a spatial memory task in male hooded-Lister rats tested in the adolescent stage. Data are shown as mean ± SEM of exploration time (s) (n=8 per group).

**Adolescent Females**

![Bar chart showing exploration times for adolescent females with different doses of PCP and vehicle.](image)

**Figure 2.3.2a(ii)** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical objects (A & B) in the 3-min acquisition trial in a spatial memory task in female hooded-Lister rats tested in the adolescent stage. Data are shown as mean ± SEM of exploration time (s) (n=7 per group).
Spatial Memory Task

Acquisition trial

Adult Males

![Graph showing the effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days 7, 9, and 11) and vehicle (0.9% saline) on exploration of two identical objects (A & B) in the 3-min acquisition trial in a spatial memory task in male hooded-Lister rats tested in the adult stage. Data are shown as mean ± SEM of exploration time (s) (n=8 per group).]

**Figure 2.3.2a(iii)** The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical objects (A & B) in the 3-min acquisition trial in a spatial memory task in male hooded-Lister rats tested in the adult stage. Data are shown as mean ± SEM of exploration time (s) (n=8 per group).

Adult Females

![Graph showing the effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9, and 11) and vehicle (0.9% saline) on exploration of two identical objects (A & B) in the 3-min acquisition trial in a spatial memory task in female hooded-Lister rats tested in the adult stage. Data are shown as mean ± SEM of exploration time (s) (n=7 per group).]

**Figure 2.3.2a(iv)** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical objects (A & B) in the 3-min acquisition trial in a spatial memory task in female hooded-Lister rats tested in the adult stage. Data are shown as mean ± SEM of exploration time (s) (n=7 per group).
2.3.1b. Effect of neonatal PCP treatment in the retention trial in adolescents and adults in both males and females using SMT.

*Adolescent Males & Females:* In males, an overall two-way ANOVA revealed a significant effect of treatment time in the retention trial of the SMT (F2, 21 = 22.06; p<0.001). Post-hoc analysis revealed the vehicle treated animals (control group) had a clear preference for the moved (displaced) object compared to the stationary object – i.e. spent significantly more time exploring the moved versus stationary (unmoved) object (p<0.001). This effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both objects.

A paired t-test in adolescent females also revealed a significant effect of neonatal PCP (10 mg/kg) on object exploration time in the retention trial of the SMT. The vehicle treated animals (control group) had a clear preference for the moved object compared to the stationary object – i.e. spent significantly more time exploring the moved versus stationary (unmoved) object (p<0.001) (See figures 2.3.2bi&ii), however the PCP treatment group showed no particular preference for either object. They spent similar amount of time exploring both the stationary and moved objects.

*Adult Males and Females:* In males, an overall 2-way ANOVA revealed a significant effect of treatment on object exploration time in the retention trial of the SMT (F2, 21 = 5.56; p<0.01). Post-hoc analysis revealed the vehicle treated animals (control group) had a clear preference for the moved (displaced) object compared to the stationary object – i.e. spent significantly more time exploring the moved versus stationary (unmoved) object (p<0.01). This effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both objects.

A paired t-test in adult females also revealed a significant effect of treatment on object exploration time in the retention trial of the SMT. The vehicle treated animals (control
group) had a clear preference for the moved object compared to the stationary object – i.e. spent significantly more time exploring the moved versus stationary (unmoved) object (p<0.05) (See figures 2.3.2biii&iv). This effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both objects.
Spatial Memory Task

Retention trial

Adolescent Male

**Figure 2.3.2b(i)** The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of a stationary and a moved object in the 3-min retention trial in a spatial memory task in male hooded-Lister rats tested in the adolescent stage. Data are shown as mean ± SEM of exploration time (s) (n=8 per group) of moved object Vs stationary object ***p<0.001, Student’s t-test.

Adolescent Female

**Figure 2.3.2b(ii)** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of a stationary and a moved object in the 3-min retention trial in a spatial memory task in female hooded-Lister rats tested in the adolescent stage. Data are shown as mean ± SEM of exploration time (s) (n=7 per group) of moved object Vs stationary object ***p<0.001, Student’s t-test.
Spatial Memory Task

Retention trial

Adult Male

Figure 2.3.2b(iii) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of a stationary and a moved object in the 3-min retention trial in a spatial memory task in male hooded -Lister rats tested in the adult stage. Data are shown as mean ± SEM of exploration time (s) (n=8 per group) of moved object Vs stationary object **p<0.01, Student’s t-test.

Adult Female

Figure 2.3.2b(iv) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of a stationary and a moved object in the 3-min retention trial in a spatial memory task in female hooded -Lister rats tested in the adult stage. Data are shown as mean ± SEM of exploration time (s) (n=7 per group) of moved object Vs stationary object *p<0.05, Student’s t-test.
2.3.2c: Effect of neonatal PCP treatment on the discrimination index (DI) in adolescents and adults in both males and females in a SMT.

Adolescent Males and Females: In males, one-way ANOVA on the DI revealed a significant effect of neonatal PCP (10 mg/kg and 20 mg/kg) on the DI (F2, 21 = 22.13; P<0.001). Post-hoc analysis showed that both the PCP groups displayed a significant reduction in the DI (p<0.001) compared to the control group.

In females, a paired t-test on the DI revealed a significant effect of neonatal PCP (10 mg/kg). The neonatal PCP group displayed a significant reduction in the DI (p<0.001) compared to the control group (See figures 2.3.2c i&ii).

Adult Males and Females: In males, a one-way ANOVA on the DI revealed a significant effect of treatment (F2, 21 = 11.01; p<0.001) Post-hoc analysis showed that the PCP groups displayed a significant reduction in the DI (p<0.001 (10 mg/kg) and p<0.01 (20 mg/kg)) compared to the control rats.

In females, a paired t-test on the DI revealed a significant effect of neonatal PCP (10 mg/kg). The neonatal PCP group displayed a significant reduction in the DI (p<0.001) compared to the control group (See figures 2.3.2c iii&iv).
Spatial Memory Task

Discrimination index

Adolescent Male

Figure 2.3.2c(i) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9, and 11) and vehicle (0.9% saline) on the discrimination index, DI in adolescent male rats. Data are shown as mean ± SEM (n=8 per group). One-way ANOVA followed by post-hoc Students t-test showed a significant reduction in DI in neonatal PCP group compared to control group ***p<0.001.

Adolescent Female

Figure 2.3.2c(ii) The effect of neonatal PCP (10mg/kg once a day on postnatal days (PND) 7,9 and 11) and vehicle (0.9% saline) on the discrimination index, DI in adolescent female rats. Data are shown as mean ± SEM (n=7 per group). ***p<0.001 significant reduction compared to vehicle.
Figure 2.3.2c(iii) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the discrimination index, DI in adult male rats. Data are shown as mean ± SEM (n=8 per group). One-way ANOVA followed by post-hoc Students t-test showed a significant reduction in DI in neonatal PCP groups compared to control group, ***p<0.001; **p<0.01.

Figure 2.3.2c(iv) The effect of neonatal PCP (10 mg/kg) or vehicle (0.9% saline) once a day on postnatal days (PND) 7, 9 and 11 on the discrimination index, DI in adult female rats. Data are shown as mean ± SEM (n=7 per group). ***p<0.001 significant reduction compared to vehicle.
2.3.2d: Effect of neonatal PCP treatment on Line Crossings in adolescents and adults in both males and females using SMT.

*Adolescent Males and Females:* In adolescent males, an overall one way ANOVA on the total line crossings during the acquisition plus retention trial of the test revealed a significant effect of neonatal PCP treatment on the line crossings of the animals ($F_{2, 21} = 4.7; p<0.05$). Further post-hoc analysis revealed that neonatal PCP 20 mg/kg group displayed a significant increase in line crossings ($p<0.05$) when compared to the control rats. However, the neonatal PCP 10 mg/kg treatment group did not show any significant effect on line crossings when compared to the control rats. Also, in adolescent females, paired t-test showed a significant increase in line crossings at 10 mg/kg of neonatal PCP treatment ($p<0.05$) when compared to the control group (See figures 2.3.2d i&ii).

*Adult Males and Females:* In adult males, an overall one way ANOVA on the total line crossings during the acquisition and retention trials of the test revealed no effect of neonatal PCP treatment on the line crossings of the animals ($F_{2, 21} = 1.08; NS$). Also, in females, paired t-test showed no significant effect of neonatal PCP treatment (10 mg/kg) on the line crossings of the animals when compared with the control group ($p = 0.12$) (See figures 2.3.2d iii&iv).
Spatial Memory Task

Line crossing

Adolescent Males

Figure 2.3.2d(i) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the total number of line crossings in acquisition and retention trials in the spatial memory task in adolescent male hooded-Lister rats. Data are shown as mean ± SEM (n=8 per group). *p<0.05 significant increase in line crossings compared with vehicle, Dunnett’s t-test.

Adolescent Females

Figure 2.3.2d(ii) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the total number of line crossings in acquisition and retention trials in the spatial memory recognition task in adolescent female hooded-Lister rats. Data are shown as mean ± SEM (n=7 per group). *p<0.05 significant increase in line crossings compared with vehicle, paired t-test.
Spatial Memory Task

Line crossing

Adult Male

![Graph showing line crossing for adult male hooded-Lister rats.]

**Figure 2.3.2d(iii).** The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the total number of line crossings in acquisition + retention trial in the spatial memory task in adult male hooded-Lister rats. Data are shown as mean ± SEM (n=8 per group).

Adult Female

![Graph showing line crossing for adult female hooded-Lister rats.]

**Figure 2.3.2d(iv)** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on total number of line crossings in acquisition + retention trial in the spatial memory recognition task in adult female hooded-Lister rats. Data are shown as mean ± SEM (n=7 per group).
Experiment 2.3.3: The effect of neonatal PCP treatment (10 mg/kg & 20 mg/kg in males and 10 mg/kg in females) on locomotor activity at two different time points—i.e. adolescents and adults in both males and females.

2.3.3a. Effect of neonatal PCP treatment on LMA in adolescents and adults in both males and females.

Adolescent Males and Females: In males, an overall one way ANOVA on locomotor activity revealed a significant increase in line crossings in neonatal PCP treated animals (F2, 27 = 2.83; p<0.05). Following post-hoc t-test, it was observed that the neonatal PCP 20 mg/kg group showed a significant increase in locomotor activity (p<0.05) when compared to neonatal PCP 10 mg/kg and control groups. Similarly, in females, neonatal PCP (10 mg/kg) treated animals displayed a significant increase in LMA (p<0.05) when compared with vehicle group (See figures 2.3.3 i&ii).

Adult Males & Females: In males, an overall one way ANOVA on LMA revealed no significant effect of neonatal PCP treatment (10 mg/kg and 20 mg/kg) (F2, 27 = 0.49; NS) when compared with vehicle. In females, paired t-test revealed no significant effect of neonatal PCP treatment (10 mg/kg) on LMA (p = 0.11) (See figures 2.3.3 iii & iv).

An overall two-way ANOVA used to analyse the adolescent Vs adult locomotor activity in males, did not reveal any significant effect in locomotor activity, although the adult males showed an overall reduction in locomotor activity when compared to the adolescent males in all treatment groups, this reduction failed to reach statistical significance. Similarly, paired t-test in females showed an overall reduction in locomotor activity in adults when compared to the adolescent females, failing to reach statistical significance.
Figure 2.3.3a(i) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on locomotor activity (LMA). Data are expressed as mean ± SEM activity counts every 10 minutes over a 60-min period (n=8-11 per group) and analysed using post-hoc Students t-test on the area under the curves. *p<0.05 significant increase in LMA, Student’s t-test.

Figure 2.3.3a(ii) The effect of neonatal PCP (10 mg/kg once a day on PNDs 7, 9 and 11) and vehicle (0.9% saline) on LMA. Data are expressed as mean ± SEM activity counts every 10 minutes over a 60-min period (n=7 per group); * p<0.05 significant increase in LMA, paired t-test.
Figure 2.3.3a(ii) The effect of neonatal PCP (10 and 20 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on locomotor activity (LMA). Data are expressed as mean ± SEM activity counts over a 60 min period (n=8 - 11 per group).

Figure 2.3.3a(iv) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on locomotor activity (LMA). Data are expressed as mean ± SEM activity counts over a 60 min period (n=7 per group).
2.4. Discussion

Results from this investigation clearly demonstrated that neonatal PCP treatment induces a disruption in object recognition and spatial memory. This treatment regime also led to an increase in locomotor activity in the adolescent male and female rats, but not in the adults.

In the early development of the central nervous system, stimulation of NMDA receptors play an important role in neuronal cell survival and establishing neuronal networks. The NMDA receptor stimulation may be important only during certain stages of the developmental period. Previous studies have shown that neonatal blockade of the NMDA receptor complex in animals replicate some neurodevelopmental aspects of symptoms of schizophrenia, which would lead to the early developmental disconnection of circuits between the hippocampus and frontal cortex (Deutsch et al., 1998). Several reasons could be attributed to the neurobiological effects of PCP. One possibility is linked to the observation that survival of NMDA receptor-bearing neurones is regulated by glutamatergic input (cellular level evidence), and when these neurones are deprived of this input for a sustained period of time, it may result in their degeneration (Ikonomidou et al., 1999; 2001). However, NMDA receptor dysfunction alone cannot cause degeneration. It must involve several other factors to bring about the cumulative effect. For this study, PCP 10 mg/kg was chosen as the low dose, to be administered once a day on PND 7, 9 and 11 as this been shown to produce significant, yet less widespread and mild insults. Administration of PCP 10 mg/kg three times a day mimicked the effects of 0.5 mg/kg MK-801 (given three times a day) administered on PND 7 (Ikonomidou et al., 1999) Also, it has been established by Wang et al (2005) that developmental factors along with unknown mechanisms of tolerance underlie the
apparent selective cortical neurotoxicity observed during neonatal PCP (10 mg/kg) 
administration in rat pups on PND 7, 9 and 11.

Recognition memory is integral to the present research into cognitive function/dysfunction. Several animal models of symptomatology of schizophrenia use familiarity/novelty discrimination, a behaviour displayed by all mammals and used by researchers to investigate potential mechanisms and therapies for the treatment of cognition in schizophrenia. With respect to the relevance of recognition memory in cognitive function/dysfunction, this study has revealed several significant findings based on the dosing regime used. The neonatal PCP regime showed significant deficits in object recognition and deficits in spatial memory, both in males and females, with the effects evident from as early as adolescence (juveniles) (Figures 2.3.1a(i&ii)- 2.3.2d (iii&iv). There was no significant sex-effect on NOR, spatial memory or on locomotor activity, although there was dose-specific response in a locomotor activity in adolescent male rats, where PCP (20 mg/kg) treated rats showed higher locomotor activity (p<0.05; table 2.1(ii)) when compared to PCP 10 mg/kg and vehicle treated rats. Interestingly, this dose-dependent effect in the adolescent male rats was not observed in the adult rats. Similarly, the neonatal PCP (10 mg/kg) treatment group showed a significant increase in locomotor activity (p<0.05; table 2.1 (ii)) when compared to the vehicle group in adolescent female rats. This increase in locomotor activity was not observed in the adult females. The differences in brain development and greater plasticity of the adolescent nervous system represent a possible explanation of the observed differential responsiveness to PCP between male and female adolescent and adult rats. This possible hypothesis has received support from previous studies examining age-dependent
differences in neurotransmission following administration of some of the drugs of abuse to adolescent versus adult rodents (Badanich et al., 2006; Frantz et al., 2007).

A literature review has shown that although not adequately outside the normal range to be diagnostically constructive, children that will eventually develop schizophrenia differ in a number of respects from other children, showing transitory neuromotor abnormalities (Walker et al., 1994), developmental delays (Jones et al., 1994) and being more anxious and socially withdrawn when compared to other children (Done et al., 1994; Jones et al., 1994). Yet, overt symptomatology of schizophrenia typically emerges only during adolescence. Evidence from lesion studies clearly demonstrates that deficits following excitotoxic lesions of the ventral hippocampus in infant rats become evident only during the adolescent period, effects that are more pronounced compared to effects or insults sustained in adulthood (Lipska et al., 1995, O'Donnell 2008). Conclusive studies have shown that relatively late maturational events occurring during puberty in the PFC, hippocampus or other limbic regions somehow trigger symptoms of early brain damage such as the onset of overt schizophrenia (Lipska et al., 1993). Although overall maturational events have been correlated, specific maturational events have not been linked clearly with the onset of symptoms. Stress has been postulated to play a significant role. Stressors have often been reported to worsen if not precipitate episodes of heightened schizophrenic symptomatology (Bogerts, 1989), with stress-precipitated neurochemical sensitization hypothesized to give rise to the pathophysiology of schizophrenia (Leiberman et al.1997). It has also been previously suggested that adolescence-associated maturational processes of the PFC, such as the enhancement of mesoprefrontal DA activity, may regulate the subcortical DA response to stress during this time period (Thompson et al., 2004). Early brain dysfunction may
predispose some individuals for the later development of schizophrenia by increasing their sensitivity to stressors during the vulnerable adolescent stage (Lipska et al., 1993). Among the neural regions that undergo developmental modifications during adolescence are stress-sensitive forebrain regions (Dunn 1988). This also includes the regions implicated in glucocorticoid negative feedback inhibition of stress-related hypothalamus-pituitary-adrenal axis (HPA) activation (Sapolsky et al., 1985) as well as those critical for the expression of sensitization to stressors and drugs (Pierce et al., 1997). So, in all, it could be speculated that the triggering of such interacting pathological processes could possibly lead to the potentiation of phasic DA responses that underlies the development of psychotic symptoms during late adolescence or early adulthood. This could in turn be attributed to the memory deficits observed in adolescent rats in our study using the neonatal PCP regime.

Previous in vivo studies have demonstrated that perinatal PCP administration resulted in profound behavioural abnormalities in the adolescent rat, which may be related to enhanced apoptotic cell death of neurones in the frontal cortex (Wang et al., 2003). These cortical deficits could lead to significant impact on the function of subcortical structures, such as the nucleus accumbens, which plays the role of an important regulatory centre by integrating the functions of the basal ganglia and the limbic system. The nucleus accumbens receives glutamatergic afferents from several brain regions, in particular the frontal cortex (Groenewegen et al., 1991; O’Donnell et al., 1995). Medium spiny neurones account for the majority of the neurostriatal cell population and correspond to a major synaptic target of dopaminergic input to the striatum (Graybiel et al., 1990). So, the treatment with neonatal PCP could have possibly led to the alteration
in the cortical input to these neurones during development and could have perhaps played a significant role in mediating the behavioural effects of neonatal PCP treatment.

2.4.1. Novel object recognition paradigm

To our knowledge, this is the first study to document a comparison between the sexes in object recognition in both adolescents and adults following neonatal PCP treatment using the NOR paradigm. The results obtained in the NOR task showed that the PND (days 7,9 and 11 with doses 10 mg/kg and 20 mg/kg) in males and (10 mg/kg) in females in hooded-Lister rats resulted in significant impairment of recognition memory both in adolescence (PND35-56) and in adulthood (PND >56) (Figures 2.3.1b(i,ii,iii,iv) 2.3.1c(i,ii,iii,iv). Both male and female rats were used in this study in order to compare the effect of neonatal PCP in both the sexes. Gender differences in many behavioural tasks have been observed in both humans and laboratory animals, although the influence of sexually dimorphic behaviours has not yet been clearly understood. Additionally, literature provides inconsistent and conflicting evidence on the difference between males and females in behavioural and cognitive tasks in animal studies (Voikar et al., 2001; Bowman et al., 2003; Conrad et al., 2004; Wilson et al., 2004). Hence, both male and female rats were used in this study in order to compare the effect of neonatal PCP in both the sexes. Also, from our study, it is evident that the total object exploration is similar in both males and females and there was no significant difference between the sexes, which further makes it interesting as there is no distinguishing feature between the sexes. There was no significant effect on exploration of the two identical objects in the acquisition trial in any treatment group. This indicates that the animals did not have any preference for either object following PCP or vehicle treatment.
In agreement to our findings, Harich et al (2008) showed that adult male Wistar rats treated with PCP on postnatal days 7,9 and 11 had reduced exploration of the novel juvenile rat when compared to the saline-treated rats in the social novelty discrimination task. Studies examining the effects of multiple doses of PCP on PND 7,9 and 11 have found long-lasting deficits in spatial working memory and SI in male mice (Pawlak et al., 2009). Similarly, Todorovic et al (2003) have demonstrated spatial learning and memory deficits in juvenile and adult male and female Sprague-Dawley rats following PND 7 treatment with NMDA antagonists and GABA mimetics (nitrous oxide, isoflurane and midazolam), similar to that used in human obstetric and pediatric anesthesia. Also, in agreement with our findings, Yuede et al (2010) observed that exposing female rat pups to PCP on three postnatal days 7,9 and 11 slowed acquisition of a delayed spatial alternation task when testing began on PND42, which is the adolescent stage. In effect this shows that the memory and learning deficits begin to present themselves in this model during adolescent stage, which is interesting, because in humans, the effects are normally observed in the late teens. Although PND35-56 is considered equivalent to teenage in human years, the observation of learning and memory deficits prior to late teens or early adulthood need to be analyzed. One way to look at this is that the emergence of behavioural changes in adolescence (both males and females) may possibly not be related to the surge of gonadal hormones during puberty because a similar temporal pattern of abnormalities was observed in animals deleted of gonadal hormones prior to puberty (Lipska & Weinberger 1994). Notably, removal of prefrontal neurones in adult animals with hippocampal lesions restored some of the behaviours (i.e. those that are modulated by, but not solely dependent on, prefrontal cortex), suggesting that aberrant development of prefrontal cortex in the context of early damage to the hippocampus (Lipska et al., 1998) may be a critical factor for the
expression of the behaviours, such as object and spatial memory deficits observed in our study in this chapter.

The object memory deficits seen in rats in our studies is analogous to the recent results where prenatal exposure to PCP produced impairment on recognition memory in the NOR task in postpubertal mice (Lu et al., 2010). It has been hypothesised that neuromaturational processes during postpubertal development (i.e. synaptic pruning) are adequate to precipitate the onset of schizophrenia in individuals with already compromised brain status (Weinberger, 1987, Olney et al., 1999, McGlashan and Hoffman, 2000). Although this fails to fully explain the later age of onset, it does help to elucidate that individuals with schizophrenia have poor adjustment during puberty, followed by sharp increases in symptoms during adolescence (Walker and Bollini, 2002) when pruning is in progress.

Our findings did not show any gender-specific changes in the NOR task. Similarly, neonatal ventral hippocampal lesion studies by Gomez et al (2003) observed deficits in spatial learning and memory using Morris water maze test in both sexes of the rats, although the males appeared to be affected to a greater extent than the females. Neuropathological studies observed that perinatal treatment of NMDA receptor antagonists induced an increase in apoptosis in studies using either male (Fredriksson et al., 2004) or female (Wang et al., 2004) rodents, and when using both genders there were no sex differences reported (Hansen et al., 2004; Ikonomidou et al., 1999, Monti and Contestabile, 2000).

The effect of neonatal PCP in NOR needs further investigation in terms of increasing the inter-trial interval. This study suggests that although the neonatal PCP regime
employed here impairs short-term object recognition memory, there may be other aspects of memory relating to long-term consolidated effects that remain unchanged. It would be of interest to explore this further to understand the nature of memory impairments in neonatal PCP treated rats.

2.4.2. Spatial Object Recognition Task

The results obtained in the SMT showed that neonatal PCP treatment in both males and females in hooded-Lister rats resulted in significant impairment of spatial memory both in adolescence (PND35-56) and in adulthood (PND >56) (Figures 2.3.2b(i,ii,iii,iv); 2.3.2c(i,ii,iii,iv).

There are two main strategies used by rats to learn locations of objects, and these are often complementary: an idiocentric strategy, based on the subject’s body position in space and allocentric strategy, which relies on the subject’s ability to learn spatial cues (Long and Kesner, 1996; Whishaw, 1985), with the first strategy being striatal specific and the second strategy being hippocampal specific (Packard & McGaugh, 1996; Devan et al., 1996). It could be assumed based on our results, that the PCP-treated rats (both adolescents and adults of both sexes) were impaired in their capacity to use the spatial cues (allocentric strategy) efficiently. On PNDs 7, 9 and 11 the dentate gyrus region of the hippocampus is in the developmental stage (Bayer et al., 1993). This structure is implicated in spatial learning (Bannerman et al., 1999; Moser et al., 1995) and allocentric orientation (Czéh et al., 2001). It could hence be speculated that an insult to the brain during the developmental stage of the dentate gyrus could have affected the allocentric orientation, thereby resulting in the spatial memory deficits observed in our studies. Similar studies using neonatal PCP (8.7 mg/kg) treatment in male Sprague-
Dawley rats on PND 7, 9 and 11 showed robust impairment in a SMT, although this effect was not fully observed in female rats (Andersen and Pouzet et al., 2004). The contrasting results could be attributed to a difference in animal strains, methodology and treatment dose.

2.4.3. Locomotor Activity:

The LMA results from our studies showed that the adolescent male rats with PCP 20 mg/kg treatment exhibited a significantly higher locomotor activity (p<0.05) (table 2.1(ii)) when compared with PCP 10 mg/kg and vehicle treated rats. Similarly, the female adolescent PCP (10 mg/kg) treated rats exhibited significantly higher locomotor activity when compared with the vehicle treated rats. This increase in locomotor activity in adolescent male and female rats were not observed in the adult rats in both sexes (table 2.1(ii)). The reason behind an increase in locomotor activity in the adolescent PCP 20 mg/kg and not in PCP 10 mg/kg treated males is unclear at present, although greater plasticity of the adolescent nervous system represents a possible explanation of the observed differential responsiveness to PCP between male and female adolescent and adults (Frantz et al., 2007). Also, in NOR and SMT, it was observed that there was a significant increase in line crossings in male adolescents treated with PCP at 20mg/kg and female adolescents treated with PCP at 10 mg/kg, the results were similar to what was seen in locomotor activity. Results for the adult male rats did not show any dose-effect between any of the drug treated groups unlike the adolescent rats. Akin to the memory deficits, this study did not reveal any sex-specific effect. The lack of effect of postnatal PCP treatment on adult PCP-induced locomotor behaviour in our present study is consistent with the fact that low-dose PCP (5 mg/kg) in postnatal rats
selectively alters some behaviours, possibly those associated with cortical and limbic NMDA receptors (Sircar et al., 2003).

Our data are in agreement with those reported by Gorter and de Bruin (1992), who reported that rats treated with MK-801 or vehicle from PND 8-19 showed no difference in open field activity in adulthood. However, the result from our study differs from some studies by Semba et al. (2001) that have shown stereotypic spontaneity in locomotor activity in male and female Sprague-Dawley rats following 5 and 10 mg/kg PCP daily from PND 1-14. They showed a dose-dependent reduction in stereotyped behaviour in adult rats. Studies have shown that acute PCP to rats leads to increased locomotor activity, ataxia, and stereotypy (Castellani and Adams 1981). PCP at 20 mg/kg and not 10 mg/kg - induced an increase in locomotor activity in our study in male and female adolescents, and is believed to be related to the positive clinical manifestations of schizophrenia, the result agreeing with the findings of Adams and Moghaddam, 1998. Neurotrophic molecules are considered significant in the development and maintenance of cortical neurones and synapses, e.g. brain-derived neurotrophic factor (BDNF) (Weickert et al., 1998). It plays an important and unique role in regulating a wide repertoire of functions at different developmental stages, including neuronal survival, migration, phenotypic differentiation, and axonal and dendritic growth in neonatal individuals (Huang et al., 2001; Lewin et al.,1996), as well as synaptic plasticity and behaviour in adulthood (Lu, 2003; Poo, 2001). Studies have shown an abnormal expression of BDNF during PND15 and 42 (i.e. adolescent stage in rats) may cause schizophrenia-like behaviour (Takahashi et al., 2006), which could possibly be attributed to a significant increase in locomotor activity following PCP treatment in male and female adolescents in our study.
Hence, it could only be speculated that there is a possibility that the significant locomotor activity observed in the adolescent male and female rats may be due to an abnormal expression of BDNF. However, the reason behind no drug-specific effect in adult male rats as against a drug-specific effect in the adolescent male rats is unclear at present.

Also, a recent finding observed that the blockade of NMDA receptors by phencyclidine has a down-regulatory effect on BDNF mRNA levels in the rodent brain which may relate to the behavioural deficits induced by PCP treatment. It was also observed that this effect was most pronounced and widespread in female, compared with male rats (Snigdha et al., 2011). Although our work did not see sexual dimorphic deficits following neonatal PCP treatment in object recognition and spatial object location memory, it is possible that neonatal PCP treatment has affected BDNF mRNA levels thereby resulting in cognitive deficits.
2.5. Conclusion

Several neonatal tests have been investigated in the pre-clinical stage extensively. This chapter investigated the effects of PCP on PND 7,9 and 11 on recognition and spatial memory as well and on locomotor activity. The next step in order to further support these findings (i.e. cognitive deficits of relevance to schizophrenia) would be to establish the effect of this neonatal PCP regimen on aspects of negative symptomatology. Since there is an increasing interest in the sex differences in metabolism of PCP in male and female rats (Shelnutt et al., 1999), we would further study the difference in effect in males and females in a SI paradigm of relevance to negative symptomatology in the neonatal PCP model of schizophrenia symptoms. Also, since there was no sex effect observed in object recognition or spatial memory, it would be interesting to see if the neonatal PCP dosing paradigm produces any sex effects in the SI task. This will improve our understanding of the correlation of the time of disruption of neuronal circuitry on negative symptoms and will help in attaining a clearer picture of the possible mechanism(s) involved. Hence, the next chapter will investigate if neonatal PCP treatment on PNDs 7,9 and 11 can influence the social behaviour in a putative model of negative symptomatology.
Chapter 3

To investigate the effects of neonatal PCP on SI in adolescent and adult male and female rats using the SI paradigm
3 Introduction

Deficits in social functioning characterises core negative symptoms in schizophrenia. It also focuses on disturbances in social behaviour, mainly, social withdrawal which presents an irrefutable “negative symptom” amenable to modelling in animals (Stahl & Buckley, 2007; Tandon et al., 2009). As described in the introduction and chapter 2, studies have shown that the NMDA/glutamate receptor antagonist, PCP, can mimic the complexity of schizophrenia symptoms, as it induces not just positive and cognitive symptoms, but also negative symptoms associated with the disease (Steinpreis, 1996). As seen in Burns’ review (2006), Bleuler clearly points out that the core features of schizophrenia include various cognitive and negative symptoms associated with the dysfunctional state. The negative symptoms consist of problems with motivation, reduced social behaviour and diminished affective responsiveness, which in turn result in poor functional outcome in individuals with schizophrenia.

The focus of the majority of work in this thesis has been in validating and characterizing neonatal PCP induced deficits in both sexes at two time points – in adolescents and adults in paradigms relevant to cognitive deficits observed in schizophrenia and LMA i.e. general activity levels. To this effect, chapter 2 focused on the cognitive deficits. However, schizophrenia is characterized by impaired cognitive functioning along with anxiety and depression-like symptoms (Schenkel et al., 2005). The latter form is usually referred to as negative symptoms of schizophrenia. See chapter 1 for full description of negative symptoms.

With the knowledge that cognitive dysfunction is a core feature of schizophrenia, there have been a remarkable rise in the number of attempts to understand and treat some or
most of the symptom clusters in the last few years. Example of programs to address this concern was seen in the creation of collaborations such as Treatment Units for Research on Neurocognition and Schizophrenia (TURNS) which was the second part of an earlier initiative called MATRICS- Measurement and Treatment Research to Improve Cognition in Schizophrenia (www.matrics.ucla.edu). The strong correlation between neurocognition, negative symptoms and functional outcome has led to the suggestion that the focus in treatment of schizophrenia needs pharmacological agents that improve cognition and reduce negative symptoms. Evidently, and as has been previously explained in this thesis with regards to modelling cognitive dysfunction, animal models which mimic negative symptoms would also be most useful in achieving this aim.

Social withdrawal is regarded as an important negative symptom of schizophrenia. Furthermore, measurements of SI in animals are relatively uncomplicated in comparison with other negative symptoms such as apathy. Several groups have been successful in replicating the inhibition of SI induced by NMDA-receptor antagonists in animals (Sams-Dodd 1995; Ellenbroek and Cools 2000). Given that rats display a rich array of social behaviours, and that they are a highly social species, SI tests provide an excellent basis for modelling these symptoms (Snigdha et al., 2008a; Jenkins et al., 2008).

Behaviourally, chronic PCP administration to male rats reduces the time spent by animals interacting with the control rats (Sams-Dodd 1995; Qiao et al., 2001). Interestingly, NR1 subunit knockdown mice also have significant social abnormalities, wherein the males maintain large distances from wild-type littermates and actively avoid social investigation in a resident-intruder paradigm (Mohn et al., 1999).
Interactions with peers during adolescence are considered to be imperative for social development in human adolescents with individuals spending more time interacting with peers during adolescence given more importance compared to any other developmental period (LaGraeca et al., 2001). Similarly, adolescent rats demonstrate higher levels of social behaviour than young adult or older animals, with these interactions being vital for developing the ability to communicate and comprehend species specific communication signals (Berg et al., 1999), essential for subsequent successful reproduction.

Several studies have clearly established social behaviour deficits following chronic and sub-chronic PCP treatment in male and female rats respectively (Sams Dodd et al., 1995; Snigdha et al., 2008a &b). Although there are a few studies that have investigated the sexual dimorphic nature of PCP treatment, this study is to our knowledge the first that aims at investigating the effect of neonatal PCP treatment in both male and female rats in both adolescent and adult stages. Also, in chapter 2 we have shown robust object recognition and spatial memory deficits following neonatal PCP treatment in both males and females; hence an important next step is to investigate the presence or absence of sex-specific deficits using the neonatal treatment regime, and if the sex-specific deficit does exist, when is it manifested i.e. the age of onset.
3.2 Aims
The experiments in this chapter have two main objectives:

1. To determine the effect of neonatal PCP (10 mg/kg) (PND 7, 9, 11) on social behaviours such as sniffing, following, avoiding, object exploration and fighting of male and female hooded-Lister rats in adolescents and adults. The objective is to investigate the time point at which the social withdrawal symptoms present themselves, as it was evident from the results of chapter 2 that the object and spatial memory deficits were robust in the adolescent stage and persisted until the adult stage in both males and females.

2. To determine if neonatal PCP treatment exhibits sex-specific deficits, therefore both males and females were tested.
3.3. Materials and methods

The study design was adapted from Sams-Dodd et al., 1996 and Snigdha & Neill 2008a; 2008b.

3.3.1. Animals and housing

Hooded-Lister rats were obtained from Charles River, UK. As seen in table 3.3.1a below, the second batch of five time-mated pregnant dams arrived 15 days prior to parturition and was housed individually in cages containing sawdust and paper bedding until weaning. All animals were housed under conditions of constant temperature (21 ± 1 °C) and humidity (50-58%) and a 12 hour light/dark cycle (lights on 07:00 h) with free access to food and water. All experiments were conducted in accordance with the Animals Scientific Procedures Act, UK 1986 and were approved by the University of Bradford ethical review panel.

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>BATCH 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCP (mg/kg)</td>
<td>10</td>
</tr>
<tr>
<td>PCP dosing (PND)</td>
<td>7, 9, 11</td>
</tr>
<tr>
<td>ID method</td>
<td>Tattoo</td>
</tr>
<tr>
<td>Weaning day (PND)</td>
<td>21-22</td>
</tr>
<tr>
<td>PNDs 35-56</td>
<td>Social Interaction</td>
</tr>
<tr>
<td>PNDs 56-72</td>
<td>Social Interaction</td>
</tr>
<tr>
<td>PND 84-105</td>
<td>NOR-Antipsychotic testing</td>
</tr>
<tr>
<td></td>
<td>SI-Antipsychotic testing</td>
</tr>
<tr>
<td>PND 180-190</td>
<td>NOR &amp; SMT</td>
</tr>
<tr>
<td>PND 350-390</td>
<td>NOR &amp; SMT</td>
</tr>
</tbody>
</table>

Table 3.3.1a: History of neonatal PCP treatment-batch 2.
3.3.2. Apparatus

The SI test was performed in an open-field in a NOR box (see 2.2.3(i) a chapter 2 for full description and picture of the apparatus).

The rats were weight matched (to within 15 g) and assigned to two treatment groups: in males n=10 pairs Vehicle (0.9% saline), n = 10 pairs PCP 10 mg/kg and two treatment groups in females n= 10 pairs vehicle (0.9% saline) and n= 10 pairs PCP 10 mg/kg. For more details pertaining to treatment and pairing of animals please refer to 3.3.3.

The rats were habituated to the test arena prior to the test day. Habituation consisted of placing all rats from one cage together in the empty test arena for 30 min, on the day before the test day.

3.3.3. Testing

Pairs of rats, unfamiliar to each other, receiving either the same treatment (vehicle and vehicle; n = 10 pairs) or different treatments (vehicle and PCP 10 mg/kg; n=10 pairs) in adolescent and adult males and the same treatment in adolescent and adult females (vehicle and vehicle; n = 10 pairs) or different treatments (vehicle and PCP 10 mg/kg; n= 10 pairs) were placed in the test arena together for 10 min as described in table 3.3.2a below.
A heavy object made of Plexiglas or metal was placed in the centre of the test arena to measure the differences in interaction of the test animal with an unfamiliar object and an unfamiliar animal at the same time. After the 10 min test phase, the object and the arena were cleaned with 10% alcohol in an attempt to remove traces of olfactory cues. Behaviour was recorded on video for subsequent blind scoring. A behavioural scoring program (Hindsight, Scientific Programming Services) was used to score the following parameters:

Following: The test rat follows (goes after) the con-specific rat around the arena (figure 3.3.3a).
Sniffing: The test rat sniffs the con-specific rat’s body, including the anogenital area (figure 3.3.3b).

Avoiding: The test rat avoids the con-specific rat and moves away from it when approached.

Object Exploration: The test rat explores the object that is placed in the centre of the test arena. (figure 3.3.3c).

Fighting: The test rat wrestles or fights with the con-specific rat when it tries to sniff or follow the test rat.

Figure 3.3.3 a: Following; 3.3.3b: Sniffing; 3.3.3 c: Object exploration
3.3.4. Statistical analysis

All data are expressed as mean ± S.E.M. ($n = 10$ pairs per group). Statistical analysis was done using independent t-test where appropriate, to compare individual group means.
3.4. Results

3.4.0. The effect of neonatal phencyclidine treatment (10 mg/kg) in adolescent and adult rats in both sexes

Four sets of experiments were carried out in total in order to test the effect of neonatal PCP in both sexes at two different time points i.e. adolescent and adult stages. The effects that neonatal PCP had in this test in these time points in both the sexes will be explained in detail in this section. The effect of neonatal PCP on social behaviours such as sniffing, following, avoiding, object exploration and fighting were analysed and each behaviour is explained in detail below.

The SI behaviours following vehicle treatment in adult male rats differed significantly from that of the adult female rats, the possible explanation for the significant difference in behaviours will be explained in detail in the discussion section (see 3.5). It was observed that the adult female rats (following vehicle treatment) spent significantly more time sniffing and following the conspecific animal and spent significantly less time avoiding and less (albeit not significant) time fighting the con-specific animal when compared with the adult male rats. They also spent significantly less time exploring the object in the middle in the presence of the conspecific animal (see table 3.4.0a).
<table>
<thead>
<tr>
<th>Social Interaction Behaviours</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sniffing</td>
<td>24868.5±3444.90</td>
<td>33497.5±5949.00 *↑</td>
</tr>
<tr>
<td>Following</td>
<td>4621.1±793.60</td>
<td>5391.2±1333.15</td>
</tr>
<tr>
<td>Avoiding</td>
<td>1434.7±348.10</td>
<td>309.7±260.00 *↓</td>
</tr>
<tr>
<td>Object Exploration</td>
<td>54191.6±4546.30</td>
<td>44490.3±6367.40 *↓</td>
</tr>
<tr>
<td>Fighting</td>
<td>281.2±80.22</td>
<td>50±12.00</td>
</tr>
</tbody>
</table>

Table 3.4.0a. Table showing adult male Vs female total time spent in msecs following vehicle treatment during SI behaviours such as sniffing, following, avoiding, object exploration and fighting.

*↓: Significant decrease in time spent in a particular social behaviour
*↑: Significant increase in time spent in a particular social behaviour
3.4.1: The effect of neonatal PCP treatment (10 mg/kg in males and females) on sniffing behaviour at two different time points-i.e. adolescents and adults in both males and females using the SI paradigm

Adolescent males & females: Administration of neonatal PCP (10 mg/kg once a day on PND 7, 9 and 11) in male adolescents showed a significant reduction in sniffing when compared to vehicle treated animals (figure 3.4.1a; t(9) = -1.71; p<0.05) and female adolescents also showed a significant reduction in sniffing behaviour (figure 3.4.1b; t(9) = -4.64; p<0.01) when compared to vehicle treated animals.

Adult males & females: Administration of neonatal PCP showed an overall increase in sniffing (although this effect did not reach statistical significance) in males when compared to vehicle treated animals (figure 3.4.1c; t(9) = -0.69; p=0.052). However, adult females demonstrated a significant and marked reduction in sniffing behaviour (figure 3.4.1d; t(9) = -4.73; p<0.001) when compared to vehicle treated animals.
**Figure 3.4.1a.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on sniffing behaviour in the 10-min social interaction test in adolescent male hooded-Lister rats. Data are shown as mean ± SEM of time (ms); n= 10 rats per group. *p<0.05 significant decrease in total time spent sniffing compared to vehicle, paired t-test.

**Figure 3.4.1b.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on sniffing behaviour in the 10-min social interaction test in adolescent female hooded-Lister rats. Data are shown as mean ± SEM of time (ms); n= 10 rats per group. **p<0.01 significant decrease in total time spent sniffing compared to vehicle, paired t-test.
Figure 3.4.1c. The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on sniffing behaviour in the 10-min social interaction test in adult male hooded-Lister rats. Data are shown as mean ± SEM of time (ms); n= 10 rats per group.

Figure 3.4.1d. The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on sniffing behaviour in the 10-min social interaction test in adult female hooded-Lister rats. Data are shown as mean ± SEM of time (ms); n= 10 rats per group. ***p<0.001 significant decrease in total time spent sniffing compared to vehicle, independent t-test.
3.4.2: The effect of neonatal PCP treatment (10 mg/kg in males and females) on following behaviour at two different time points—i.e. adolescents and adults in both males and females using the SI paradigm.

**Adolescent males & females:** Administration of neonatal PCP (10 mg/kg once a day on PND 7, 9, and 11) in male adolescents showed a significant increase in following when compared to vehicle treated animals (figure 3.4.2a; t(9) = -5.66; p<0.01). In contrast, female adolescents showed a significant reduction in following behaviour (figure 3.4.2b; t(9) = 2.78; p<0.01) when compared to vehicle treated animals.

**Adult males & females:** Administration of neonatal PCP showed a small increase (not reaching statistical significance) in following behaviour in adult males when compared to vehicle treated animals (figure 3.4.2c). Female adults showed a significant reduction in following behaviour (figure 3.4.2d; t(9) = -1.1; p<0.001) when compared to vehicle treated animals.
Following behaviour - Male adolescents

**Figure 3.4.2a.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on following behaviour in the 10-min social interaction test in adolescent male hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group. **p<0.01 significant increase in total time spent following compared to vehicle, independent t-test.

Following behaviour - Female adolescents

**Figure 3.4.2b.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on following behaviour in the 10-min social interaction test in adolescent female hooded-Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group. **p<0.01 significant decrease in total time spent following compared to vehicle, independent t-test.
Following behaviour - Male adults

Figure 3.4.2c. The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on following behaviour in the 10-min social interaction test in adult male hooded-Lister rats. Data are shown as mean ± SEM of time spent following (ms); n=10 rats per group.

Following behaviour - Female adults

Figure 3.4.2d. The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on following behaviour in the 10-min social interaction test in adult female hooded-Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group. ***p<0.001 significant decrease in total time spent following compared to vehicle, independent t-test.
3.4.3: The effect of neonatal PCP treatment (10 mg/kg in males and females) on avoiding behaviour at two different time points—i.e., adolescents and adults in both males and females using the SI paradigm

**Adolescent males & females:** Administration of neonatal PCP (10 mg/kg once a day on PND 7, 9, and 11) in male adolescents showed overall increase in avoiding behaviour when compared to vehicle treated animals (figure 3.4.3a) although this did not reach statistical significance. However, in female adolescents there was significant increase in avoiding behaviour (figure 3.4.3b; t(9) = -3.11; p<0.01) when compared to vehicle treated animals.

**Adult males & females:** Administration of neonatal PCP induced a small reduction in avoiding behaviour, without reaching statistical significance when compared to vehicle treated animals (figure 3.4.3c) when tested in male adults. However, female adults showed a significant increase in avoiding behaviour (figure 3.4.3d; t(9) = 2.01; p<0.01) when compared to vehicle treated animals.
Avoiding behaviour - Male adolescents

Figure 3.4.3a. The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on avoiding behaviour in the 10-min social interaction test in adolescent male hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group.

Avoiding behaviour - Female adolescents

Figure 3.4.3b. The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on avoiding behaviour in the 10-min social interaction test in adolescent female hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group. **p<0.01; significant increase in total time spent avoiding compared to vehicle, independent t-test.
Avoiding behaviour - Male adults

**Figure 3.4.3c.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on avoiding behaviour in the 10-min social interaction test in adult male hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group.

Avoiding behaviour - Female adults

**Figure 3.4.3d.** The effect of neonatal PCP treatment (10 mg/kg once a day on PNDs 7, 9 and 11) and vehicle (0.9% saline) on avoiding behaviour in the 10-min social interaction test in adult female hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group. **p<0.01; significant increase in total time spent avoiding compared to vehicle, independent t-test.
3.4.4: The effect of neonatal PCP treatment (10 mg/kg in males and females) on object exploration behaviour at two different time points—i.e. adolescents and adults in both males and females using the SI paradigm

**Adolescent males & females:** Administration of neonatal PCP (10 mg/kg once a day on PND 7, 9 and 11) in male adolescents induced significant increase in object exploration behaviour when compared to vehicle treated animals (figure 3.4.4a; \( t(9) = -1.10; \) \( p<0.05 \)). Also, in female adolescents there was significant increase in object exploration (figure 3.4.4b; \( t(9) = 1.02; \) \( p<0.05 \)) when compared to vehicle treated animals.

**Adult males & females:** Administration of neonatal PCP did not produce any effect on object exploration behaviour in the PCP treated animals when compared to vehicle treated animals (figure 3.4.4c) when tested in adult males. Similarly, in female adults there was no significant effect on object exploration when compared to vehicle treated animals (figure 3.4.4d; \( t(9) = 4.77; \) \( p=0.09 \)).
**Figure 3.4.4a.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on object exploration behaviour in the 10-min social interaction test in adolescent male hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group; *p<0.05 significant increase in total time spent exploring the object compared to vehicle, paired t-test.

**Figure 3.4.4b.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on object exploration behaviour in the 10-min social interaction test in adolescent female hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group; *p<0.05 significant increase in total time spent exploring the object compared to vehicle, paired t-test.
Object exploration behaviour - Male adults

![Graph showing the effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days 7, 9, and 11) and vehicle (0.9% saline) on object exploration behaviour in the 10-min social interaction test in adult male hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group.]

**Figure 3.4.4c.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on object exploration behaviour in the 10-min social interaction test in adult male hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group.

Object exploration behaviour - Female adults

![Graph showing the effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9, and 11) and vehicle (0.9% saline) on object exploration behaviour in the 10-min social interaction test in adult female hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group.]

**Figure 3.4.4d.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9, and 11) and vehicle (0.9% saline) on object exploration behaviour in the 10-min social interaction test in adult female hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group.
3.4.5: The effect of neonatal PCP treatment (10 mg/kg in males and females) on fighting behaviour at two different time points-i.e. adolescents and adults in both males and females using the SI paradigm

Adolescent males & females: Administration of neonatal PCP (10 mg/kg once a day on PND 7,9 and 11) in male adolescents induced a significant increase in fighting behaviour when compared to vehicle treated animals (figure 3.4.5a; p<0.01). In female adolescents there was an increase in fighting behaviour (figure 3.4.5b; p=0.1) when compared to vehicle treated animals, however this increase did not reach statistical significance.

Adult males & females: Administration of neonatal PCP showed small increase in fighting behaviour when compared to vehicle treated animals (figure 3.4.5c; p=0.12) however this effect did not reach statistical significance when tested in adult males. Neonatal PCP treatment in adult females induced an increase in fighting behaviour (figure 3.4.5d; p=0.053) when compared to vehicle treated animals, without reaching statistical significance.
**Fighting behaviour - Male adolescents**

![Bar chart showing the effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on fighting behaviour in the 10-min social interaction test in adolescent male hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group; **p<0.01 significant increase in total time spent fighting compared to the vehicle group, paired t-test.]

**Fighting behaviour - Female adolescents**

![Bar chart showing the effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on fighting behaviour in the 10-min social interaction test in adolescent female hooded Lister rats. Data are shown as mean ± SEM of time spent fighting (ms); n=10 rats per group.]

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**Figure 3.4.5a.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on fighting behaviour in the 10-min social interaction test in adolescent male hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group; **p<0.01 significant increase in total time spent fighting compared to the vehicle group, paired t-test.

**Figure 3.4.5b.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on fighting behaviour in the 10-min social interaction test in adolescent female hooded Lister rats. Data are shown as mean ± SEM of time spent fighting (ms); n=10 rats per group.
**Figure 3.4.5c.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on fighting behaviour in the 10-min social interaction test in adult male hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group.

**Figure 3.4.5d.** The effect of neonatal PCP treatment (10 mg/kg once a day on postnatal days (PNDs) 7, 9 and 11) and vehicle (0.9% saline) on fighting behaviour in the 10-min social interaction test in adult female hooded Lister rats. Data are shown as mean ± SEM of time (ms); n=10 rats per group.
3.5. Discussion

This study was designed in order to ascertain if the neonatal PCP treatment regime produces SI deficits in female and male rats, which may possibly replicate the negative symptoms observed in schizophrenia patients. Results from this study demonstrated two major findings. First, the adolescent females exhibited impairment in social behaviors following neonatal PCP treatment and the adolescent males, although they showed deficits in some behaviours such as sniffing and avoiding, did not exhibit robust social behaviour deficits. Second, the adult females, but not males showed social behavior deficits thereby demonstrating a sex-specific SI deficit (figures 3.4.1 - 3.4.5 c&d). For a summary of results, please see table 3.5.1.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adolescent</td>
<td>Adult</td>
</tr>
<tr>
<td>Sniffing</td>
<td>p&lt;0.05 (↑)</td>
<td>p=0.052 (↑)</td>
</tr>
<tr>
<td>Following</td>
<td>p&lt;0.01 (↑)</td>
<td>p=0.2 (NS)</td>
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<tr>
<td>Avoiding</td>
<td>NS</td>
<td>p=0.1 (NS)</td>
</tr>
<tr>
<td>Object exploration</td>
<td>p&lt;0.05 (↑)</td>
<td>NS</td>
</tr>
<tr>
<td>Fighting</td>
<td>p&lt;0.01 (↑)</td>
<td>NS</td>
</tr>
</tbody>
</table>

↑ - Increase in behaviour of the PCP treated animal when compared to vehicle treated animal
↓ - Decrease in behaviour of the PCP treated animal when compared to vehicle treated animal

Table 3.5.1: Summary of social interaction behaviour exhibited by adolescent and adult male and female hooded-Lister rats following neonatal PCP treatment. NS: Not significant.

Our findings show that the adult female control rats spent significantly more time sniffing and following the conspecific rats. They also spent significantly less time avoiding and fighting the conspecific rat, and spent significantly less time exploring the
object (placed in the centre) in the presence of the conspecific animal (table 3.4.0a), when compared with the adult male control rats. The female control rats spent significantly more time interacting with the conspecific animals when compared with the male rats.

The medial nucleus of the amygdala (MeA) plays a significant role in the social behaviour system in rodents (Scalia and Winans, 1975). Lesions to MeA produce severe deficits in social behaviour (Sheenan et al., 2001). Based on the role that MeA plays in social behaviour, it is sexually dimorphic in nature, i.e. structural and neurochemical sexual differences in adult MeA (Hines et al., 1992; Cooke et al., 1999). Studies have shown that posterodorsal subnuclei of the medial amygdala (MeApd) neurones in males are contacted by 80% more excitatory synapses than in females (Cooke & Wooley, 2005). These findings show that the prepubertal MeApd is sexually dimorphic and also show a sex difference in synaptic function that corresponds to a difference in synaptic structure. Taken together, it could be summarised that the significant differences in sniffing, following, avoiding and fighting behaviours between males and females in the control rats could be the result of differences in excitatory synapses between the sexes thereby leading to varying behaviours. This could in turn be linked to the levels of gonadal hormones. At puberty and during adulthood, changing levels of gonadal hormones act in many of the same parts of the brain to activate sexually dimorphic behaviors. The volume of the adult MeApd is greater in males than in females (Hines et al., 1992), and this appears to depend, at least in part, on circulating androgen levels. Thus, adult hormones may act in the MeA on an already sexually differentiated substrate.
Recent studies in mice show that the vomeronasal organ and the accessory olfactory bulb (AOB) are important in sex discrimination (Stowers et al., 2002; Luo et al., 2003). This suggests that the MeA, which receives input directly from the AOB, may be involved in forming higher-order representations from olfactory stimuli, namely the conspecific identity. This suggestion is supported by the finding that MeA lesions prevent males from avoiding individual conspecifics that defeated them in a recent fight (Luiten et al., 1985) and that oxytocin delivered to the MeA in oxytocin knock-out mice rescues their deficit in social recognition (Ferguson et al., 2001).

Yet another theory that could be considered to account for the male vs female differences in social behaviour observed in our findings is the MeA laterality (Cooke & Wooley, 2005; Xiao & Jordan, 2002). It is not clearly known as to how MeA laterality is established, and although speculative, if this concept is applicable to rats, the greater number of excitatory synapses in the left MeA of males may aid them in male-typical behaviors that require fine sensory discrimination and motor control, such as in male–male competition, modulating luteinizing hormone (LH) release. This could to an extent explain the increased fighting behaviour, increased avoiding of male counterparts, less sniffing and following and increased interest in the object against the conspecific animal (table 3.4.0a) when compared to the adult female rats.

Interactions with peers present a vital source of positive experience for human adolescents (Larson & Richards, 1991; LaGreca et al., 2001) and, in rats, SI has been shown to be more rewarding for adolescents than for their adult counterparts assessed using social conditioned place preference (CPP) in adolescent and adult male and female Sprague-Dawley rats (Douglas et al, 2004). It is apparent from our study that
following neonatal PCP treatment, female rats showed robust deficits in all social behaviors in the adolescent stage, i.e. sniffing, following, avoiding while in contrast, the male rats that received treatment exhibited social behavior deficits in some behaviors but not in all. The SI time of rats was closely related with their partner’s social activity during testing. Perhaps, PCP prompted differential interaction with the familiar rats in the home cage and this affected the social activity in the open field (White et al., 2009). However, the reason for robust SI deficits in female adolescent rats and not in male adolescent rats is elusive. An increase in SI was demonstrated in male but not female Wistar rats following isolation rearing which was more apparent when isolation began on PND21 compared to PND30 (Ferdman et al., 2007). Although this was observed in an isolation rearing model, the results from that study bear resemblance to the results from our study, where male adolescent and adult rats show increase in (not significant) social behaviours such as sniffing, following and reduction in avoiding behaviours following neonatal PCP (PND 7,9, 11) treatment (table 1). Neonatal ventral hippocampal lesion studies however showed that the lesions affected both male and female rats, with the males being affected to a greater extent when compared with females underscoring the influence of sex differences in the development of behaviours (Gomez et al., 2003).

The disruptive effects of PCP on social behavior in female rats reported in this study are consistent with previous investigations, albeit using a sub-chronic PCP dosing regime in adult females (Sams-Dodd et al., 1999; Lee et al., 2005; Snigdha & Neill 2008a and b). However, to our knowledge this is the first study to demonstrate SI deficits at two time points, adolescents and adults in animals that have been treated with neonatal PCP (PND 7,9, 11).
Several hypotheses have been put forward to explain the mechanisms of negative symptom-like behavioral changes induced by PCP. Although a recent study has contradicted this hypothesis (Keefe et al., 2007), the more widely accepted suggestion is that of a disturbance in DA and serotonin-mediated neurotransmission (Meltzer 1989). Furthermore, some data have shown a more active role of 5-HT₁A receptor activation in modulating glutamatergic dysfunction observed in schizophrenia and in animal models of the disease (Snigdha & Neill 2008b). Glutamate release is decreased by 5-HT₁A receptor activation (Mauler et al., 2001) and evidence of a 5-HT₁A/glutamatergic interaction has also been demonstrated at the neuroendocrine level (Bradbury et al., 2003). Evidence of the involvement of 5-HT₁A receptor activation in PCP-induced deficits in SI was shown in 2005 by Bruins –Slot and colleagues, who showed that 5-HT₁A receptor partial agonist antipsychotic drug attenuated PCP-induced disruptions in SI in male Sprague–Dawley rats. This was followed by studies which confirmed that compounds having combined 5-HT₁A receptor agonist/D₂ receptor antagonistic properties, but not selective D₂ antagonism reversed PCP-induced SIdficits in both male (Boulay et al., 2004; Depoortere et al., 2007) and female rats (Snigha & Neill 2008b).

Studies have also suggested that reduced brain-derived neurotrophic factor (BDNF) levels in rats exposed to PCP resulted in reduced action of dopaminergic neurones not only in the prefrontal cortex (PFC), but also in sub-cortical regions such as the amygdala, which can be associated with a depressive-like state (Larsen et al., 2010). It has been hypothesized that depressive symptomatology seen in both drug and non-drug-induced depressive conditions (Markou & Kenny, 2002) may be similar in construct to negative symptoms of schizophrenia, such as anhedonia and social
withdrawal. Although the pharmacology underlying the impairment in social behaviour deficits in our model warrants further investigation, this study confirms previous findings with respect to SI deficits in adult female rats following PCP treatment.

Our results are in contrast to the study by Latysheva & Rayvsky et al. 2003 and the contrasting findings may be the result of different drug treatments and testing protocols, i.e. in the study by Latysheva and Rayvsky, the pairing (of the con-specific and test animals) was between old and young male rats whereas in our study it was between animals of the same age group and similar size (~15g). Also, according to a review by File 2003 which agrees with our data, there are important sex differences in SI, which makes comparison between male and female data more interesting.

Pawlak et al 2009 showed that neonatal PCP (10 mg/kg) treatment on PNDs 7, 9 and 11 in male mice resulted in SI deficits in adults and they also showed a reduction in the number of gamma amino-butryic acid (GABAergic) interneurons and spine density in the frontal cortex and hippocampus. In our study however, we noticed that following neonatal PCP treatment, male adolescent animals showed significant deficits in social behaviours such as sniffing and an increase \textit{(not significant)} in avoiding. However, when tested in male adults, these deficits did not exist. It is interesting to note that in the past, limited rodent social behaviour studies have been conducted separately on males and females and those that investigated both sexes have yielded conflicting results (Bluthe et al., 1990 and Thor et al., 1982).

Studies have suggested that there are gender-specific differences in schizophrenia, influencing age of onset and prevalence of negative symptoms, as
evidenced by postmortem studies which showed a reduction in dopaminergic activity in patients with negative symptoms (Rao et al., 2003). Previous studies have revealed significant gender-dependent differences in schizophrenia patients. Negative symptoms and cognitive deficits more frequently affect male patients with severe structural brain and neurophysiological abnormalities (Thorup et al., 2007). These differences were thought to mainly result from the effect of sex hormones in conjunction with neurodevelopmental and psychosocial sex differences (Leung et al., 2000). Also, it has been shown that females with schizophrenia have lower levels of oestrogen than healthy females (Oades and Schepker, 1994; Riecher-Rossler et al., 1994; Goldstein, 2006). Diaz-Veliz et al (1999) suggested a modulatory influence of estrogen on behavior, possibly mediated by the activity of dopaminergic neurones, suggesting the basic differences between male and female rats with respect to behavior and neural organization. The gender differences in schizophrenia support the hypothesis of a mild protective effect of estrogens (Hafner et al 1998; Lindamer et al., 1997 and Seeman et al., 1997). Our results show that the females display significant SI deficits following neonatal PCP when compared with males, thereby questioning the possibility of compromise in the effects of oestrogen.

Ko et al. (2006) proposed that in schizophrenic women of reproductive age, lower levels of estrogen are associated with more severe negative symptomatology along with reduced cognitive performance. Estrogen is a pleiotropic hormone able to modulate a range of neuronal functions; however, no single mechanism of action has yet been attributed to its putative neuroprotective properties (Green and Simpkins, 2000). One proposed mechanism that has received considerable interest involves the dendritic spines of the hippocampal CA1 subfield, which have been shown to be sensitive to
oestrogen fluctuations (Birzniece et al., 2006). This effect may be mediated via glutamate receptors, specifically NMDARs (Woolley and McEwen, 1993). Oestrogen treatment has been shown to up-regulate NMDA receptor subunit-1 mRNA and protein expression (Gazzaley et al., 1996) and increase NMDA receptor number (Woolley et al., 1997). PCP acts via the NMDA receptor complex to cause NMDA receptor hypofunction (Rao and Kolsch, 2003) and attenuates long-term potentiation in the CA1 region and dentate gyrus of the hippocampal formation (Bourne et al., 1995). Hippocampal plasticity in the CA1 region has been shown to alter across the oestrous cycle of the rat in response to cyclic changes in oestrogen and progesterone. During the pro-oestrus phase, when steroid levels are at their peak, the number and density of CA1 pyramidal cell spine synapses reach a maximum; in contrast, during vaginal oestrous when hormones are at low levels, a 30% decline in synaptic density and number is observed (Woolley and McEwen, 1992, 1993; Woolley, 1998; Birzniece et al., 2006). This oestrogen-induced increase in spine density is positively correlated to increases in NMDA receptor binding and sensitivity (Daniel and Dohanich, 2001). Also, as per our findings females, following neonatal PCP treatment, exhibit significant SI deficits in their adolescent stage, that persists through adulthood. This could be attributed to the key relationship between oestrogen, NMDA receptors and the progression of schizophrenia-like SI behaviours.

However, this is a preliminary investigation and further studies with refined parameters are needed to clarify the sex-dependent deficits in social behavior in our model. This study shows sex-specific deficits in SI in hooded Lister rats. Evidently, methodological issues with regards to treatment regime and test design obscure direct comparisons with other studies. Furthermore, this is an investigative study employing
the neonatal PCP treatment paradigm which requires further investigation to clarify the sex-specific deficit. However, it remains to be determined if the sex-specific deficit is a long-term effect.

The ontogeny of fighting demonstrates an increase in fighting behaviour around adolescents especially in male adolescents which showed a significant increase in fighting behaviour. It could be presumed that different forms of social behaviour may be mediated by different neural systems, and, they may be differentially sensitive across ontogeny to individual behaviours. The variation in behaviour may depend on a variety of factors such as, age, sex, strain etc. It has been previously established that injection of vasopressin into the lateral septum or amygdyla of the brain evoked aggressive behaviour in rodents (Ingram et al., 1998). The expression of the local vasopressin mechanism is dependent on male sex steroids (De Vries et al., 1998), and the density of the vasopressin network of fibres in the lateral septum of the brain in male rats is inversely related to aggressiveness among individuals, perhaps reflecting greater vasopressin activity with aggressive behaviour (Stribley et al., 1999). So, it could be speculated that the increasing fighting behaviour exhibited by the adolescents could be the direct result of influence of vasopressin, the effect of which reduces considerably in adulthood as seen in 3.4.5c.

To summarise, this study clearly demonstrates that neonatal PCP induces robust deficits in social behaviors in adolescent and adult female rats. Recent reports have demonstrated long-lasting activation in half of the neurones exhibited an increase in firing rate during normal SI in the basolateral amygdala following treatment with PCP 10 mg/ kg in amygdala-lesioned monkeys. It was observed that they exhibited deficits in SI and were excluded from their social group (Katayma et al., 2009).
study that demonstrated the involvement of the amygdalar brain region with SI deficits was conducted by Matsuoka et al., (2008). They showed down-regulation in 23 genes and up-regulation in 16 genes, with the gene encoding arginine-vasopressin being most down-regulated, and that for transthyretin (Ttr) most up-regulated in the amygdala following MK-801 (0.13 mg/kg for 14 days) treatment in adult male rats. Future studies are required to further investigate the association between possible neural mechanisms involved in the origin and development of SI deficits in the neonatal PCP model. At present, most studies in the literature relate to the predictive validity of the SI task. This refers to the ability of a task to accurately estimate and predict the therapeutic value of drugs for the human condition.
3.6. Conclusion

In conclusion, this study provides evidence to show that neonatal PCP induces robust deficits in social behaviour in adult female rats with the effects presenting in the adolescent stage. The data from this study not only support the use of neonatal PCP as a valid animal model of negative and cognitive symptoms of schizophrenia but also demonstrate that a single neurotransmitter hypothesis is unlikely to fully describe or account for deficits observed in the schizophrenic brain. The study also draws attention to the fact that the deficits were observed in adolescent stage in females, but not in adolescent or adult males. This would indeed enable better extrapolation of data from preclinical to clinical studies and aid in improved understanding of the range of functionality of the model with regards to negative symptomotology.

Yet another observation from these findings pertaining to the social behaviours following vehicle treatment in adolescent and adult rats in both sexes is that there was no marked difference between the adolescent and adult rats in time spent during the various social behaviours such as sniffing, following, avoiding, object exploration or fighting, albeit with subtle increase or decrease, which did not reach statistical significance. Interestingly, the adolescent female control rats did not spend any time avoiding the con-specific animal, whereas the adult control female rats did spend a small amount of time avoiding the con-specific animal (did not reach statistical significance). Similarly, in fighting behaviour, it was observed that the adolescent female control animals spent more time fighting with the con-specific animal, whereas the adult female control rats spent less time fighting with the con-specific animal. Although, we do not have a clear explanation for the subtle differences between
adolescent and adult behaviour, it could only be speculated that there are differences in
the levels of electrical potentials which are the end results of coordinated firing of
groups of neurones. The varying levels of these electric potentials may reflect the ability
for synchronized firing of neurones across brain regions. The subtle differences
observed between adults and adolescents in our study could possibly be a reflection of
less mature, less efficient connections in the brain in adolescence

Given the heterogeneity of schizophrenia and its complex and unclear aetiology, it is
difficult to find animal models that completely mimic most or all of the symptoms
associated with the disorder. However, prediction of better therapeutic outcome in
schizophrenia is dependent on an understanding of the different symptom clusters that
are associated with the symptomatology. In light of the efficacy of neonatal PCP to
produce robust negative and cognitive deficits in rats (chapter 2), it appears that this
model may be a valid and useful tool to investigate the potential novel therapeutic
candidates. The next step is to investigate and assess the effect of classical and atypical
antipsychotics on the deficits induced by neonatal PCP treatment.
Chapter 4

Investigating the effects of antipsychotic agents on cognitive and negative symptomatology using NOR and SI paradigms in the neonatal PCP model
4.1 Introduction
The requirements of many patients with schizophrenia remain unmet with currently available antipsychotic drugs (Lieberman et al., 2005). In clinical practice, these drugs elicit only partial responses in the majority of patients with schizophrenia, and have virtually no effect on cognitive dimension, leading to frequent therapy-switching or the introduction of add-on therapies (Bernd 2008) such as lamotrigine augmentation in schizophrenia patients, D-serine, a selective full agonist at the glycine site of NMDA receptor (Levy et al., 2005) etc., given as an adjunct to atypical antipsychotics (Keefe et al., 2007).

The introduction of classical antipsychotics such as chlorpromazine revolutionized the treatment of schizophrenia in the 1950s (Meltzer, 1995). It was observed for the first time that chemical compounds could attenuate hallucinations and delusions, thereby facilitating patients to live outside of institutions. Unfortunately as with all the drugs, these substances produced unwanted side effects. Additionally, not all patients with schizophrenia showed positive responses to treatment with typical antipsychotic medications. However, the introduction of clozapine marked a turning point in antipsychotic therapy (Kane et al., 1988). This drug appeared to produce very few (if any) extrapyramidal side effects, and even turned out to be superior in therapeutic efficacy. It was effective in many patients who had been resistant to the typical antipsychotics and also appeared to be somewhat effective against the negative symptoms and showed some positive results in attenuating cognitive deficits of schizophrenia (Light et al., 2000). Although the atypical antipsychotics demonstrate a number of similarities, they also showed clear variations, e.g. in pharmacology.
Atypical antipsychotics are currently the most frequently prescribed class of drugs for schizophrenia (Harrington et al., 2006). Published evidence indicated that these agents showed increased efficacy with a lower risk of extrapyramidal symptoms (EPS) than typical antipsychotics (Haro et al., 2006). Although the use of atypical antipsychotics offered several benefits and reduced some of the aspects related to morbidity and mortality of schizophrenia, these drugs appeared to be linked with varying degrees of adverse metabolic effects, such as weight gain, impaired glucose metabolism and in some cases, more serious morbidity, such as cardiovascular disease (Newcomer et al., 2007; American Diabetes Association 2004).

The precise receptor binding profiles of atypical antipsychotics vary greatly. Each drug has unique pharmacological properties, including their mode of action at multiple receptors (Roth et al., 2004). This complex pharmacology leads to their interactions (of varying degree) with numerous histaminergic, serotonergic, dopaminergic, adrenergic and muscarinic acetylcholine receptors. The specific binding profile of different antipsychotic agents may help clarify the incidence of particular side effects associated with each drug. Thus, differences in receptor-affinity profiles may explain differences in side-effect profiles (as well as efficacy) between specific agents within this heterogeneous class of drugs (Nasrallah 2008).

The study of antipsychotic receptor binding is constantly evolving. Although the specific receptor-binding profile of various atypical agents is increasingly well understood, in some cases, the link between receptor binding and a specific clinical effect has not been definitively established.
4.1. 1. Receptor binding profiles

Functional selectivity is the term that best defines drugs that cause different signalling through a single receptor (e.g., full agonist at one pathway and antagonist at a second). Antipsychotic drugs have been grouped based on both pattern of clinical action and mechanism of action. The classical antipsychotic drugs such as chlorpromazine and haloperidol have been called typical or first generation, and have the ability to reduce positive symptoms of schizophrenia along with their tendency to produce side effects (extrapyramidal and endocrine) that are ascribed to their high affinity D2 receptor antagonism. Drugs such as clozapine, olanzapine, risperidone and others were then developed that were devoid of the neurological side effects and these are referred to as atypical or second generation antipsychotics (Ohlsen & Pilowsky, 2005). In general, atypical agents have an enhanced 5-HT$_{2A}$/D$_2$ affinity ratio, a characteristic that differentiates atypical from typical antipsychotics better than any other known pharmacologic feature and a characteristic that helps explain why typical and atypical agents may have different clinical effects (Meltzer, 1989). There is one approved third generation drug, aripiprazole, whose actions have been ascribed alternately to either D2 partial agonism or D2 functional selectivity. Conversely, the D2 functional selectivity hypothesis can accommodate all current data for aripiprazole, and also impacts on discovery of compounds that are not pure D2 antagonists e.g. mGluR2/3 agonist LY379268 (Mailman & Murthy, 2010).

Enhanced perspective of both the receptor-binding profiles of antipsychotic drugs and the (receptor mediated) associated side effects may help direct clinicians in tailoring
treatment to the needs of individual patients and encourage baseline screening and routine monitoring of patient weight, fasting plasma glucose and lipid profile.
4.1.2. Aim

It has been demonstrated in chapter 2 that neonatal PCP treatment produced robust object and spatial memory deficits in male and female rats both in the adolescent and in the adult stages, which was followed by the observation in chapter 3, that neonatal treatment produced impairment in SI behaviour in female adolescents, with the robust deficits also being seen in adulthood. One interesting finding was that there was a sex-specific deficit in the SI paradigm, wherein the males, although they exhibited deficits in some behaviour did not exhibit significant and robust deficits when compared with the females.

The aims of the experiments in this chapter were twofold

a. To evaluate the effects of antipsychotic agents on cognitive deficits induced by neonatal PCP treatment using the NOR paradigm.

b. To evaluate the effects of antipsychotic agents on social behaviour deficits induced by neonatal PCP treatment using the SI paradigm.

Both male and female rats were used in these paradigms, however in the experiment involving testing of antipsychotics using SI paradigm, only female rats were used based on the results obtained from chapter 3.
4.2. Methods and materials

4.2.1. Animals and housing

Hooded Lister rats were obtained from Charles River, UK. As shown in table 3.3.1a (chapter 3), the second batch of five time-mated pregnant dams arrived 15 days prior to parturition and were housed individually in cages containing sawdust and paper bedding until weaning. All animals were housed under conditions of constant temperature (21 ± 1 °C) and humidity (50-58%) and a 12 hour light/dark cycle (lights on 07:00 h) with free access to food and water. All experiments were conducted in accordance with the Animals Scientific Procedures Act, UK 1986 and were approved by the University of Bradford ethical review panel.

4.2.2a. Phencyclidine dosing regimens - Repeat administration to rat neonates.

The PCP dosing regime was adapted from Wang et al. 2001.

Following birth, the pups were kept along with their littermates and left undisturbed until the time of dosing. On postnatal days (PNDs) 7, 9 and 11 mothers were removed from the home cage and held by an assistant. The male pups were removed and administered vehicle or PCP (once a day; 10 mg/kg subcutaneous) and female pups were administered vehicle or PCP (10 mg/kg subcutaneous) into the loose skin at the back of the neck. The dose of PCP was chosen based on an extensive literature review as well as the results obtained from our previous studies in chapter 2. At the time of dosing on PND 7, the pups were marked with a permanent tattoo code on the tails for identification purposes. Dosing was carried out in a separate room in an attempt to shield the mothers from pup vocalizations. Time away from the litter was kept to a minimum. Pups were returned to the home cage immediately after dosing. Noise in the
holding room was minimised as far as possible and animals were not handled between the time of dosing and weaning. Pups were weaned between PNDs 21-22 and were housed as five females and four males per home cage based on their bodyweights. As females weighed lesser than males, we were able to house 5 animals in a homecage. However, males weighed more and four per cage was a comfortable set up for the male rats. All behavioural testing in this chapter was carried out in the adult rats on PND 84-105. (For the history of neonatal PCP and behavioural testing please refer to table in table3.3.1a chapter 3).

Figure 4.2.2a: Timeline for neonatal PCP treatment (PNDs 7,9,11 once a day)-both male and female hooded Lister rats

4.2.3. Behavioural testing

4.2.3 (i). Novel Object Recognition

The NOR method was adapted from Grayson et al (2007) and is described in detail in chapter 2.

4.2.3(i) a. Apparatus

The apparatus consisted of an open box made of Plexiglas (52 cm L; 52 cm W; 31 cm H) positioned 27 cm above the floor on a movable trolley (Figure 2.2 chapter 2). The walls of the box were black and the objects to be discriminated (in triplicate) were made of Plexiglas, plastic or metal (Figure 2.3, chapter 2). The heights of the objects were approximately the same (10 ± 2 cm). Objects were positioned 6 cm away from the walls of the box, in opposite corners. After each trial, the objects and the box were cleaned.
with 10% alcohol so as to remove any persistent olfactory cues on the objects and the box.

4.2.3(i) b. Habituation

The rats in each home cage were given daily 30-min exploration periods in the NOR box for three days prior to the test day to ensure habituation to the empty apparatus and the test room environment. Habituation aids in effective exploration of objects by the animals on the test day.

4.2.3(i)c. Behavioural testing

For a detailed explanation of behavioural testing paradigms – NOR and SI, please see Chapter 2 – section 2.2.2. and Chapter 3 – section 3.3.3.

4.2.3(i)d. Drug phase for NOR and SI

The male and female rats were randomly assigned to two treatment groups; n =10 animals were treated with vehicle and n=20 with neonatal PCP (see section 4.2.2a). The rats were left undisturbed until the day of weaning and were separated into males and females. They were tested in their adult stage (PND 84-105) using NOR and SI paradigms following treatment with one of the two antipsychotics or vehicle.

Rats were randomly assigned to receive either drug treatment (n=10 for haloperidol and risperidone) or vehicle. See table 4.2.3.(i)d for treatment received by the rats.
For the drug treatment, neonatal PCP 10 mg/kg group was sub-divided and re-used with one group receiving vehicle and the other group receiving the antipsychotic to be tested (haloperidol or risperidone in separate experiments, see table 4.2.3 (i)d). Haloperidol was prepared in distilled water. The dose of haloperidol was chosen on the basis of previous work showing that 0.05 mg/kg haloperidol significantly attenuated cognitive deficit induced by D-amphetamine in a reversal learning paradigm in female hooded Lister rats (Idris et al., 2005). Also, this dose of haloperidol has been shown to occupy 50% of D2 receptors (Kapur et al., 2003). Risperidone was dissolved in 0.9% saline. A dose of 0.2 mg/kg of risperidone was used, based on previous studies showing efficacy to reverse the object recognition memory deficits (Grayson et al., 2007) and deficits in set shifting (McLean et al., 2008) induced by sub-chronic PCP, although the dose chosen was slightly lower than the doses required for clinically comparable D2 receptor occupancy (i.e. 0.5-1.0 mg/kg (Kapur et al., 2003). Also, it has been demonstrated in previous operant studies in this lab that risperidone 0.2 mg/kg was efficacious against a sub-chronic and acute PCP-induced cognitive deficit (Neill et al., 2010 for review).
4.2.3(i)d. Statistical analysis

The data for NOR are expressed as mean ± S.E.M. Exploration data were analysed by a repeated measures two-way ANOVA. This compared pretreatment (vehicle, PCP 10 mg/kg and PCP+haloperidol or PCP+risperidone) and object explorations (left; right for acquisition trial and object novel and familiar for retention trial). Further analysis by post-hoc student’s t-test was carried out, if a significant effect was detected by the ANOVA, which compared the time spent exploring the novel and familiar object. Analysis of line crossings and discrimination index (DI) data was performed using one-way ANOVA followed by post-hoc Dunnett’s t-test, if a significant effect was observed.

The data for SI are expressed as mean ± S.E.M. (n = 10 pairs per group). Data were analysed by one-way ANOVA. This detected the effect of the drug treatment on each behaviour set observed during the test. Further analysis by post-hoc t-test was carried out, where appropriate, to compare individual group means.
4.3. Results

4.3.0 Effect of antipsychotic agents following neonatal PCP treatment using NOR paradigm in adult male and female rats.

Experiments were carried out in order to test the effect of acute typical (haloperidol) and acute atypical (risperidone) antipsychotic drugs following neonatal PCP and vehicle treatment in both male and female adult rats. The antipsychotic agents were tested in the adult stage only because of the small number of animals and these animals had to be re-used which affected the timeline of testing the adolescents. Also, the half-life of the drugs in adolescents is unclear and since the same animals were tested in the adult stage as well, we did not want any long-lasting drug effects, hence antipsychotic testing was limited to only the adult rats. The effect that each drug had in the test will be described in detail in this section. However, the effect of neonatal PCP to induce object recognition memory deficit in rats was observed consistently across all four experiments.

In all the tests, in the acquisition trial, it was consistently observed that drug treatment did not produce any significant effect on object exploration, i.e. rats spent equal amount of time exploring both the objects in this trial; an effect that was observed for both vehicle and neonatal PCP treated rats (detailed statistics in sections 4.3.1a and 4.3.2a). In the retention trial of the test, two-way ANOVA showed a significant effect of vehicle treatment on object exploration. Post-hoc analysis showed that vehicle treated rats spent more time exploring the novel object compared to the familiar object. Rats that received neonatal PCP treatment however did not discriminate between the novel and familiar object and spent almost similar time exploring the novel and the familiar objects.
Experiment 4.3.1: The effect of haloperidol following neonatal PCP treatment (10 mg/kg) on episodic memory using NOR paradigm in male and female rats.

4.3.1a Effect of drug treatment on acquisition trial

Adult Males & Females: An overall two-way ANOVA revealed that drug treatment did not produce any significant effect on object exploration in the acquisition trial of the test in males (F2, 21 = 0.85, NS) or in females (F2, 21 = 0.37, NS). Rats from all treatment groups spent equal time exploring both the objects (See fig 4.3.1a(i,ii)). There appeared to be a small reduction in total time spent exploring the objects in the group treated with 0.05 mg/kg of haloperidol in males only 4.3.1a(i). A one-way ANOVA on the total exploration times of both objects (A&B) did not show any effect on total exploration time in the acquisition trial in males following vehicle or PCP treatment. However, following acute haloperidol (0.05 m/kg) treatment, there was a small reduction in exploration in the acquisition trial, albeit not a significant reduction (F2, 46= 2.37, NS). In females, there was no significant effect in total exploration time in the acquisition trial following vehicle or PCP treatment (F2, 46= 0.93, NS). Although there was a very small increase in object exploration in the acquisition trial following acute haloperidol, it did not reach statistical significance (see table 4.3.0a).

4.3.1b Effect of drug treatment on retention trial

Adult Males and Females: A two-way ANOVA revealed a significant effect of drug treatment on object exploration time in the retention phase of the NOR task in males (F2, 21 = 25.47, p<0.001) and females (F2, 21 = 31.90, p<0.001) (figure 4.3.1b (i&ii)). Post-hoc analysis revealed that the control group (vehicle treated animals) had a clear preference for the novel object compared to the familiar object, i.e. spent significantly more time exploring the novel object compared to the familiar object in males.
(p<0.001) and in females (p<0.001). On the other hand, PCP group did not exhibit any preference for the novel or familiar object. They spent similar amount of time exploring both the objects (figure 4.3.1b (i&ii)).

Administration of the typical antipsychotic, haloperidol (0.05 mg/kg) did not reverse the deficit induced by neonatal PCP treatment in the males or in the females. A one-way ANOVA on the total exploration time of both objects (novel and familiar) in the retention trial showed an overall significant effect of haloperidol in reducing the total exploration time in males (F2, 46 = 8.37, p<0.01). Further post-hoc analysis revealed that there was a significant reduction in total exploration following acute haloperidol (0.05 mg/kg) in males (p<0.01) in the retention trial. However, in females, although animals treated with haloperidol showed a small increase in total exploration time in the retention trial, it failed to reach statistical significance (see table 4.3.0a).

4.3.1c: Effect of drug treatment on discrimination index (DI)

The DI is the measure of the exploratory preference of the animals for the novel or the familiar object in the retention trial of the NOR task. A one-way ANOVA on the DI revealed a significant effect of drug treatment on the discrimination ability of the rats (F2, 21 = 15.44, p<0.001) in males and (F2, 21 = 51.7, p<0.001) in females. Further post-hoc analysis on the DI demonstrated that the neonatal PCP treatment group and PCP+ haloperidol group displayed a significant reduction in DI in males (p<0.001) and in females (p<0.001) compared to the control animals demonstrating no effect of haloperidol to reverse the deficit.
4.3.1d Effect of drug treatment on line crossings

Figure 4.3.1d (i&ii) shows the effect of drug treatment (PCP and haloperidol) on line crossing in acquisition and retention trial of the rats. No significant effect of drug treatment on line crossings in the acquisition and retention trial was observed either in males (F2, 21 = 2.2, NS) or in females (F2, 21 = 1.74, NS).
**Adult Males-Acquisition Trial**

![Bar graph](image)

Figure 4.3.1a (i). Mean exploration time(s) of 2 identical objects in the acquisition trial following vehicle, neonatal PCP 10 mg/kg and haloperidol 0.05 mg/kg in adult male hooded Lister rats. Data are expressed as mean ±SEM (n=8 per group).

**Adult Female Acquisition Trial**

![Bar graph](image)

Figure 4.3.1a (ii). Mean exploration time(s) of 2 identical objects in the acquisition trial following vehicle, neonatal PCP 10 mg/kg and haloperidol 0.05 mg/kg in adult female hooded Lister rats. Data are expressed as mean ±SEM (n=8 per group).
**Figure 4.3.1b (i)**. Mean exploration time(s) of a novel and a familiar object following vehicle, neonatal PCP 10 mg/kg and haloperidol 0.05 mg/kg in adult male hooded Lister rats. Data are expressed as mean ±SEM (n=8 per group respectively). Significant difference between time spent exploring the familiar and novel object ***p<0.001, paired t-test.

**Figure 4.3.1b (ii)**. Mean exploration time(s) of a novel and a familiar object following vehicle, neonatal PCP 10 mg/kg and haloperidol 0.05 mg/kg in adult female hooded Lister rats. Data are expressed as mean ±SEM (n=8 per group respectively). Significant difference between time spent exploring the familiar and novel object ***p<0.001, paired t-test.
Figure 4.3.1c (i). Discrimination index (DI) following vehicle, neonatal PCP 10 mg/kg and haloperidol (0.05 mg/kg) in adult male hooded Lister rats. Data are expressed as mean ± SEM (n= 8 per group). Significant reduction compared to vehicle***p<0.001, Dunnett’s t-test.

Figure 4.3.1c (ii). Discrimination index (DI) following vehicle, neonatal PCP 10 mg/kg and haloperidol (0.05 mg/kg) in adult female hooded Lister rats. Data are expressed as mean ± SEM (n= 8 per group). Significant reduction compared to vehicle***p<0.001, Dunnett’s t-test.
**Adult Males- Line Crossings**

![Bar chart showing the effect of haloperidol (0.05 mg/kg) and neonatal PCP treatment on the total number of line crossings in adult male rats in the novel object recognition task. Data are expressed as mean ± SEM (n= 8 per group).](image)

**Figure 4.3.1d (i).** The effect of haloperidol (0.05 mg/kg) and neonatal PCP treatment on the total number of line crossings in adult male rats in the novel object recognition task. Data are expressed as mean ± SEM (n= 8 per group).

**Adult Females - Line Crossings**

![Bar chart showing the effect of haloperidol (0.05 mg/kg) and neonatal PCP treatment on the total number of line crossings in adult female rats in the novel object recognition task. Data are expressed as mean ± SEM (n= 8 per group).](image)

**Figure 4.3.1d (ii).** The effect of haloperidol (0.05 mg/kg) and neonatal PCP treatment on the total number of line crossings, in adult female rats in the novel object recognition task. Data are expressed as mean ± SEM (n= 8 per group).
### Table 4.3.0a
Table showing total exploration time in seconds in both trials of the NOR test following acute haloperidol (0.05 mg/kg).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MALES</th>
<th>FEMALES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MALES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>44.56±3.56</td>
<td>49.62±3.04</td>
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<tr>
<td>PCP 10 mg/kg</td>
<td>45.5±2.25</td>
<td>51.75±4.11</td>
</tr>
<tr>
<td>PCP + Haloperidol (0.05 mg/kg)</td>
<td>38.75±2.9</td>
<td>50.75±2.7</td>
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<tr>
<td><strong>FEMALES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>51.37±3.13</td>
<td>39.0±2.70</td>
</tr>
<tr>
<td>PCP 10 mg/kg</td>
<td>40.87±2.9</td>
<td>42.50±2.93</td>
</tr>
<tr>
<td>PCP + Haloperidol (0.05 mg/kg)</td>
<td>39.12±3.85</td>
<td>48.0±3.21</td>
</tr>
</tbody>
</table>

↑: Increase, but did not reach statistical significance compared to vehicle.
**: Significant reduction in exploration time when compared to vehicle group.

A: Acquisition trial
R: Retention trial
Total: Acquisition+retention trials
Experiment 4.3.2: The effect of risperidone on the neonatal PCP induced deficit on episodic memory using the NOR paradigm in male and female rats.

4.3.2a Effect of drug treatment on acquisition trial

Adult Males & Females: An overall two-way ANOVA revealed that drug treatment did not produce any significant effect on object exploration in the acquisition trial of the test in males (F2, 20 = 1.28, NS) or in females (F2, 20 = 0.72, NS). Rats from all treatment groups spent equal time exploring both the objects (See fig 4.3.2a(i,ii)). There was a reduction in total time spent exploring the objects in the group treated with 0.2 mg/kg of risperidone. A one-way ANOVA on the total exploration times of both objects showed that risperidone had a significant effect to reduce total exploration time in males (F2, 45 = 15.32, p<0.001) and (F2, 45 = 11.72, p<0.01) in females respectively. Further post-hoc analysis revealed that the reduction in total exploration activity was significant at a dose of 0.2 mg/kg risperidone in males (p<0.001) and in females (p<0.01) (see table 4.3.0b).

4.3.2b Effect of drug treatment on retention trial

Adult Males and Females: A two-way ANOVA revealed a significant effect of drug treatment on object exploration time in the retention trial of the NOR task in males (F2, 20 = 9.01, p<0.01) and females (F2, 20 = 3.96, p<0.05) (figure 4.3.2b (i&ii)). Post-hoc analysis revealed that the control group (vehicle treated animals) had a clear preference for the novel object compared to the familiar object, i.e. spent significantly more time exploring the novel object compared to the familiar object in males (p<0.01) and in females (p<0.05). On the other hand, PCP group did not exhibit any preference for the novel or familiar object. They spent similar amount of time exploring both the objects (figure 4.3.2b (i&ii)).
Administration of the atypical antipsychotic, risperidone (0.2 mg/kg) reversed the deficit induced by neonatal PCP treatment in males and in females (p<0.05), wherein the acute risperidone treatment restored the animal’s preference for the novel object, as observed in the control group. A one-way ANOVA on the total exploration time of both objects in this trial once again showed an overall significant effect of risperidone in reducing the total exploration time in males (F2, 45 = 10.43, p<0.001) and in females (F2, 45 = 6.36, p<0.01). Post-hoc analysis revealed that, at a dose of 0.2 mg/kg of risperidone the exploration time during the retention trial was significantly reduced in males (p<0.001) and in females (p<0.01) (See table 4.3.0b).

4.3.2c: Effect of drug treatment on discrimination index (DI)

The DI is the measure of the exploratory preference of the animals for the novel or the familiar object in the retention trial of the NOR task. A one-way ANOVA on the DI revealed a significant effect of drug treatment on the discrimination ability of the rats (F2, 20 = 4.6, p<0.05) in males and (F2, 20 = 6.93, p<0.01) in females. Further post-hoc analysis on the DI demonstrated that the neonatal PCP treatment group displayed a significant reduction in DI in males (p<0.05) and in females (p<0.01) compared to the control animals, which was significantly attenuated by risperidone at 0.2 mg/kg in males (p<0.05) and in females (p<0.01, fig 4.3.2c (i&ii) ).

4.3.2d Effect of drug treatment on line crossings

Figure 4.3.2d (i&ii) shows the effect of drug treatment (PCP and risperidone) on line crossings of the rats. No significant effect of drug treatment on line crossings in the acquisition plus retention trial was observed (F2, 20 = 1.72, NS) in males and in females.
**Figure 4.3.2a (i).** Mean exploration time(s) of identical objects in the acquisition trial following vehicle, neonatal PCP 10 mg/kg and risperidone 0.2 mg/kg in adult male hooded Lister rats. Data are expressed as mean ±SEM (n=8 per group respectively).

**Figure 4.3.2a (ii).** Mean exploration time(s) of identical objects in the acquisition trial following vehicle, neonatal PCP 10 mg/kg and risperidone 0.2 mg/kg in adult female hooded Lister rats. Data are expressed as mean ±SEM (n=8 per group respectively).
**Figure 4.3.2b (i).** Mean exploration time(s) of a novel and a familiar object following vehicle, neonatal PCP 10 mg/kg and risperidone 0.2 mg/kg in adult male hooded Lister rats. Data are expressed as mean ±SEM (n=8 per group respectively). **p<0.01; *p<0.05 significant increase in time spent exploring the novel compared to familiar object, paired t-test.

**Figure 4.3.2b (ii).** Mean exploration time(s) of a novel and a familiar object following vehicle, neonatal PCP 10 mg/kg and risperidone 0.2 mg/kg in adult female hooded Lister rats. Data are expressed as mean ±SEM (n=8 per group respectively). *p<0.05 significant increase in time spent exploring the novel compared to familiar object, paired t-test.
Figure 4.3.2c(i). The effect of risperidone (0.2 mg/kg) and neonatal PCP treatment on the discrimination index, DI. Data are expressed as mean ±SEM (n=8 per group) in adult male hooded Lister rats. *p<0.05 significant reduction in DI compared to vehicle; #p<0.05 significant increase in DI compared to the PCP group, Dunnett’s t-test.

Figure 4.3.2c(ii). The effect of risperidone (0.2 mg/kg) and neonatal PCP treatment on the discrimination index, DI. Data are expressed as mean ±SEM (n=8 per group) in adult female hooded Lister rats. **p<0.01 significant reduction in DI compared to vehicle; #p<0.01 significant increase in DI compared to the PCP group, Dunnett’s t-test.
**Figure 4.3.2d(i).** The effect of risperidone (0.2 mg/kg) and neonatal PCP treatment on line crossings. Data are expressed as mean ±SEM (n=8 per group) in adult male hooded Lister rats.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Line Crossings (s)</th>
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</thead>
<tbody>
<tr>
<td>Vehicle</td>
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<tr>
<td>PCP</td>
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</tr>
<tr>
<td>PCP+Risp</td>
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</tr>
</tbody>
</table>

**Figure 4.3.2d(ii).** The effect of risperidone (0.2 mg/kg) and neonatal PCP treatment on the line crossings. Data are expressed as mean ±SEM (n=8 per group) in adult female hooded Lister rats.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Line Crossings (s)</th>
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<tbody>
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<td>Vehicle</td>
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<tr>
<td>PCP 10 mg/kg</td>
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</tr>
<tr>
<td>PCP+Risp 0.2 mg/kg</td>
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</tr>
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</table>
Table: 4.3.0.b. Table showing total exploration time in seconds in both trials of the NOR test following acute risperidone (0.2 mg/kg).

** - ***: Significant reduction in exploration time when compared to vehicle group.
A: Acquisition trial
R: Retention trial
Total: Acquisition + retention trials

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<th>R</th>
<th>TOTAL</th>
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<td>Vehicle</td>
<td>52.12±4.70</td>
<td>37.75±3.26</td>
<td>88.62±6.34</td>
</tr>
<tr>
<td>PCP 10 mg/kg</td>
<td>63.25±3.03</td>
<td>34.75±6.73</td>
<td>98.0±8.40</td>
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<tr>
<td>PCP + Risperidone (0.2 mg/kg)</td>
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<td>19.57±4.53 ***</td>
<td>43.14±6.27***</td>
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<td>94.37±4.58</td>
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<tr>
<td>PCP + Risperidone (0.2 mg/kg)</td>
<td>34.14±7.11**</td>
<td>29.14±7.42 **</td>
<td>43.28±11.20***</td>
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4.3.3.0. The effect of haloperidol (0.05 mg/kg) and risperidone (0.2 mg/kg) on neonatal PCP-induced deficits in SI behaviours in adult female rats

4.3.3.1a(i) Effect of haloperidol (0.05 mg/kg) and risperidone (0.2 mg/kg) on neonatal PCP-induced deficits on sniffing behaviour in adult female rats in the SI paradigm.

Administration of neonatal PCP induced an overall significant reduction in time spent engaged in social behaviour such as sniffing the conspecific animal (fig 4.3.3.1a(i)). Overall one-way ANOVA revealed a significant effect of drug treatment on sniffing (F3, 27 = 14.32, p<0.001). Further analysis using post-hoc paired t-test demonstrated that haloperidol (0.05 mg/kg) did not reverse the neonatal PCP-induced deficit in sniffing behaviour. However, risperidone markedly influenced the sniffing behaviour that was affected by PCP and significantly increased investigative sniffing (p<0.001) compared with the PCP alone group (fig 4.3.3.1a(i)).

4.3.3.1a(ii) Effect of haloperidol (0.05 mg/kg) and risperidone (0.2 mg/kg) on neonatal PCP-induced deficits on following behaviour in adult female rats in the SI paradigm.

Administration of neonatal PCP induced an overall significant reduction in time spent engaged in social behaviour such as following the conspecific animal (fig 4.3.3.1a(ii)). An overall one-way ANOVA revealed a significant effect of drug treatment on following (F3, 27 = 3.32, p<0.05). Further analysis using post-hoc paired t-test
demonstrated that PCP induced a significant reduction in following (p<0.05). Acute treatment of haloperidol (0.05 mg/kg) did not reverse the neonatal PCP-induced deficit in following behaviour. Although risperidone markedly increased the following behaviour that was reduced by PCP, the increase just failed to reach statistical significance (p=0.052) when compared with the PCP alone group (fig 4.3.3.1a(ii)).

4.3.3.1a(iii) Effect of haloperidol (0.05 mg/kg) and risperidone (0.2 mg/kg) on neonatal PCP-induced deficits on avoiding behaviour in adult female rats using SI paradigm.

Administration of neonatal PCP induced an overall significant increase in time spent avoiding the conspecific animal (fig 4.3.3.1a(iii)). An overall one-way ANOVA revealed a significant effect of drug treatment on avoiding behaviour (F3, 27 = 19.1, p<0.001). Further analysis using post-hoc paired t-test demonstrated that PCP treated rats showed a significantly increased avoiding behaviour (p<0.001). Although haloperidol (0.05 mg/kg) reduced the increase in avoiding behaviour induced by PCP, it did not significantly reverse the neonatal PCP-induced deficit in avoiding behaviour. However, risperidone significantly decreased the increase in avoiding behaviour induced by PCP, (p<0.001) when compared with the PCP alone group (fig 4.3.3.1a(iii)).
4.3.3.1b(i) Effect of haloperidol (0.05 mg/kg) and risperidone (0.2 mg/kg) on neonatal PCP-induced object exploration in adult female rats using the SI paradigm.

Administration of neonatal PCP induced a small increase in time spent exploring and interacting with the inanimate object (fig 4.3.3.1b(i)), however this failed to reach statistical significance compared to vehicle. An overall one-way ANOVA did not reveal any significant effect of drug treatment on object exploration (F3, 27 = 1.08, NS). Also, there was no effect or change of object exploration or interaction following haloperidol or risperidone treatment when compared with PCP alone group (fig 4.3.3.1b(i)).

4.3.3.1b(ii) Effect of haloperidol (0.05 mg/kg) and risperidone (0.2 mg/kg) on neonatal PCP-induced deficits on fighting behaviour in adult female rats using SI paradigm.

Administration of neonatal PCP induced a slight increase in time spent fighting with the conspecific animal (fig 4.3.3.1b(ii)), however this failed to reach statistical significance. An overall one-way ANOVA did not reveal any significant effect of drug treatment on fighting behaviour (F3, 27 = 1.01, NS). Also, there was no effect on fighting behaviour following haloperidol or risperidone treatment when compared with PCP alone group (fig 4.3.3.1b(ii)).
Figure 4.3.3.1a(i). The effect of haloperidol (H) (0.05 mg/kg, i.p.), risperidone (R) (0.2 mg/kg) on neonatal PCP (10 mg/kg; PND 7,9,11) induced deficits in sniffing in a 10 min social interaction test. Data were analysed by one-way ANOVA and are shown as mean ±SEM of time (msec). N= 8 rats per group. ***p<0.001 significant decrease in total time spent sniffing compared to vehicle (V); ###p<0.001 significant increase in time spent sniffing compared to neonatal PCP alone, Dunnett’s t-test.

Figure 4.3.3.1a (ii). The effect of haloperidol (H) (0.05 mg/kg, i.p.), risperidone (R) (0.2 mg/kg) on neonatal PCP (10 mg/kg; PND 7,9,11) induced deficits in following in a 10 min social interaction test. Data were analysed by one-way ANOVA and are shown as mean ±SEM of time (msec). N= 8 rats per group. *p<0.05 significant decrease in total time spent following compared to vehicle (V); p=0.052 increase in time spent following compared to neonatal PCP alone, Dunnett’s t-test.
Social Interaction - Avoiding Behaviour in Adult Females

Figure 4.3.3.1a(iii). The effect of haloperidol (H) (0.05 mg/kg, i.p.), risperidone (R) (0.2 mg/kg) on neonatal PCP (10 mg/kg; PND 7,9,11) induced deficits in avoiding in a 10 min social interaction test. Data were analysed by one-way ANOVA and are shown as mean ±SEM of time (msec). N= 8 rats per group. ***p<0.001 significant increase in time spent avoiding the target conspecific animal compared with vehicle; ###p<0.001 significant decrease in total time spent avoiding the target conspecific animal compared to the PCP group, Dunnett’s t-test.

Figure 4.3.3. 1b(i). The effect of haloperidol (H) (0.05 mg/kg, i.p.), risperidone (R) (0.2 mg/kg) on neonatal PCP (10 mg/kg; PND 7,9,11) induced object exploration in a 10 min social interaction test. Data were analysed by one-way ANOVA and are shown as mean ±SEM of time (msec). N= 8 rats per group.
Figure 4.3.3. lb(ii). The effect of haloperidol (H) (0.05 mg/kg, i.p.), risperidone (R) (0.2 mg/kg) and neonatal PCP (10 mg/kg; PND 7,9,11) on fighting behaviour in a 10 min social interaction test. Data were analysed by one-way ANOVA and are shown as mean ±SEM of time (msec). N= 8 rats per group.
4.4. Discussion

4.4.1. Attenuation of neonatal PCP induced deficits in the NOR paradigm

Antipsychotic agents have long been the primary component of effective treatment for schizophrenia and are indeed vital for patients’ integration into society. Recent drug discovery approaches for schizophrenia have consistently been attempting to target cognitive dysfunction, which is documented as the most pervasive symptom cluster in patients, although there is an increasing need for new therapeutic agents to treat negative symptomatology as well.

In this study, acute treatment with risperidone demonstrated efficacy at a dose of 0.2 mg/kg, while acute treatment with haloperidol at a dose of 0.05 mg/kg did not attenuate the neonatal PCP induced episodic memory deficits tested using the NOR paradigm. It was also observed that acute treatment with haloperidol (0.05 mg/kg) and risperidone (0.2 mg/kg) reduced object exploration, but not line crossings in the NOR task. The possible explanation could be that the doses used produced significant sedative effect, which is similar to the findings of Redrobe et al., 2010. However, the reduction in activity effect of risperidone did not impair the rat’s capacity to discriminate the objects.

The NOR paradigm used in our work was based on impairment of object recognition by administration of neonatal PCP in both male and female rats in order to mimic the impairment of episodic memory seen in schizophrenic patients. The results obtained from this study further agree with the results obtained using the sub-chronic PCP regime in adult females (see Neill et al., 2010 for review). The effect of neonatal PCP shown in
this chapter is consistent with the findings of chapter 2. Our ability to replicate this effect provides a solid basis to use this model to test potential antipsychotic agents. The effects of antipsychotic drugs were tested only using NOR and not SMT in the experiments presented in this chapter due to time constraints and limitations in the number of animals available. The experiments were planned between PND 84-105 and the experiments were designed in such a way that there was sufficient (one week) wash-out period to render the animals drug free before starting the next set of experiments. Since the animals were re-used, there was not enough time or animals available to carry out antipsychotic testing using a variety of drugs.

There have been several theories attempting to explain the mechanism underlying PCP-induced disruption of recognition memory. In light of its use as a paradigm of significance for testing novel antipsychotic agents, elucidation of the mechanism involved in the disruptive effect becomes even more important.

It is apparent from our findings that the neonatal PCP-induced cognitive deficit in adults was reversed by the atypical antipsychotic risperidone (0.2 mg/kg), but not by the classical agent haloperidol. Previous studies using the sub-chronic PCP treatment regime in adults showed that risperidone, but not haloperidol reversed the PCP-induced object recognition deficit (Grayson et al., 2007). Similar results were obtained using an attentional set shifting paradigm, where the PCP-induced deficits were reversed by risperidone and not by haloperidol (McLean et al., 2008). In agreement to our current findings, a recent study by Hashimoto et al (2005) showed that repeated administration of PCP (10 mg/kg/day for 10 days) caused cognitive deficits in adult mice for more than 6 weeks after the final administration of PCP, and that the PCP-induced cognitive
deficits could not be improved by sub-chronic administration of haloperidol. Also, risperidone reversed the PCP-induced (5 mg/kg once a day for 5 days) deficit in working memory using hole board learning paradigm (Schroeder et al., 2000). Also, a study investigating the effect of risperidone in schizophrenia patients on working memory has revealed positive effects (Meltzer et al., 1999), and improved performance on the Wisconsin Card Sort Test (WCST) (Rossi et al., 1997).

Some clinical studies provide further support for our results that the atypical antipsychotic agent, risperidone has shown promise in treating neurocognitive deficits in humans, whereas the typical antipsychotic agent haloperidol failed to improve the various domains of cognitive function (Harvey et al., 2001). The inability of haloperidol to reverse the object recognition deficit induced by neonatal PCP may be due to its high affinity for D₂ receptors and minimal 5-HT₂A receptor affinity (Siegfried et al., 2001). However, risperidone on the other hand was highly effective and it has both high D₂ and 5-HT₂A receptor affinity (Leysen et al., 2005). Also, risperidone is suggested to be one of the most effective atypical antipsychotics to improve memory in the clinic (Meltzer et al, 1999).

In conclusion, reversal of neonatal PCP-induced cognitive deficits, using the NOR test, may be a potential animal model by which to investigate atypical antipsychotic ability to ameliorate cognitive deficits in schizophrenia.
4.4.2. Attenuation of neonatal PCP induced deficits in the SI paradigm

The key finding of this investigation is that neonatal PCP treatment induced robust deficits in SI in adult female rats and the deficits were attenuated by the atypical antipsychotic, risperidone (0.2 mg/kg) but not by the typical antipsychotic, haloperidol (0.05 mg/kg). This result is consistent with previous findings where a sub-chronic PCP-induced deficit was ameliorated following acute treatment with risperidone but not with haloperidol using SI paradigm (Snigdha et al., 2008a).

Previous studies using sub-chronic paradigms have reported the induction of PCP-induced deficits in social behaviours in adult male rats. However, as detailed in chapter 3, the neonatal PCP paradigm did not induce robust deficits in social behaviours in male rats neither in the adolescent nor in the adult stage. On the other hand the female rats showed robust deficits both in the adolescent and adult stages. This is the reason behind using female rats to investigate the effect of antipsychotics in this chapter. Results from our investigation showed that acute treatment with risperidone significantly reversed the SI deficits produced by neonatal PCP. Haloperidol (from our results) did not attenuate the social behavioural deficits induced by neonatal PCP. Haloperidol owes its antipsychotic effect primarily to its antagonistic activity on dopaminergic D₂ receptors and binding to other receptors such as histamine and serotonin is not significant (Granger et al., 2005).

Risperidone targets multi receptor systems with different affinities (Schotte et al., 1996). Similar to other atypical antipsychotic agents, risperidone displays a greater affinity for serotonin 5-HT₂A than D₂ receptors. It also has affinity for D₃ and D₄
receptors, histamine H1 receptors and adrenergic α1 and α2 receptors (Schotte et al., 1996). Numerous studies have reported the behavioural, neurochemical and pharmacological properties of risperidone (reviewed in Arnt and Skarsfeldt, 1998; Waddington and Casey, 2000). Tarazi et al., 2002 quantified the long-term effects of risperidone and other antipsychotic drugs on 5-HT receptor subtypes in adult rat brain and reported varying effects on cortical 5-HT1A vs. 5-HT2A receptors, although the repeated effects of risperidone on 5-HT receptors in developing rat brain, i.e. our model warrants investigation so as to elucidate the developmental effects of risperidone and its influence on brain maturation. Microdialysis studies in adult animals demonstrated that risperidone-induced increases in cortical DA release resulted from a combined blockade of 5-HT2A and D2 receptors through a 5-HT1A-sensitive stimulatory mechanism and not from direct 5-HT1A receptor activation by risperidone (Ichikawa et al., 2001). It could be speculated that such interactions probably could be observed in juvenile animals and contribute to risperidone-induced increases in cortical 5-HT1A receptors. Most recently, risperidone was tested for affinity at the 5-HT1A and 5-HT2A receptors in juvenile (PND 30) and adult (PND 90) animals using whole Sprague-Dawley rat brain preparations. Recent studies have also shown differential effects of riseridone on 5-HT systems (1A & 2A) receptors in juvenile and adult rats (Choi et al., 2010). So, it could be speculated that the positive cognitive and social behavioural effects of risperidone observed in our study could indirectly involve 5-HT1A receptors along with a combined blockade of 5-HT2A/D2 receptors. The disruptive effects of neonatal PCP on social behaviour reported in this study has been discussed at length in chapter 3 and the studies are consistent with previous investigations albeit different treatment paradigms (Sams-Dodd et al., 2006; Snigdha et al., 2008a&b). A novel criterion introduced in this work is the neonatal PCP model (i.e., PCP 10 mg/kg PND 7,9 and 11 once a day, sub cut) tested on both males
and females both in the adolescent and in the adult stages (chapter 3). In conjunction with prior studies, it appears that the significant deficits in interactive behaviours such as sniffing and following (fig 4.3.3.1a) are relevant to the social context, since object interaction (exploration) remains fairly unaffected following treatment with neonatal PCP (fig 4.3.3.1b). Several hypotheses have been put forward to explain the mechanisms of negative symptom-like behavioural changes induced by PCP. One of the widely accepted propositions is that of a disturbance in DA and serotonin-mediated neurotransmission (Meltzer 1989). The main evidence for this theory is derived from the efficacy of 5-HT$_{2A}$-D$_2$ antagonists such as risperidone to improve negative symptoms. Further investigation is warranted to understand the pharmacology involved in the attenuation of social behaviour deficits induced by neonatal PCP in our study.

Findings from this study indicate that acute treatment with the typical antipsychotic haloperidol did not show efficacy to improve the disruptive effect of neonatal PCP on social behaviours. This appears consistent with previous reports that haloperidol was ineffective in reversing PCP-induced SI deficits in female (Snigdha et al., 2008a) and male rats (Sams-Dodd 1996) using the sub-chronic PCP treatment regime at adulthood. Risperidone and other atypical antipsychotics appear to promote a higher quality of social life and everyday life (Franz et al, 1997). In one evaluation, compared to haloperidol, a conventional antipsychotic, risperidone-treated patients obtained more than twice as many quality-adjusted years as haloperidol treated patients (Chouinard & Albright, 1997). Risperidone was also shown to be effective in improving hyperactivity, unstable mood, aggression and self-injurious behaviours in children with pervasive developmental disorders (Perry et al., 1997; Barnard et al., 2002; McCracken et al.,
2002; Erickson et al., 2005), and in reducing the severity of motor and vocal tics in patients with Tourette’s syndrome (Bruggeman et al., 2001).
4.5. Conclusion

In conclusion, this study provides evidence to show that the robust cognitive deficits in male and female rats and deficits in social behaviour in female rats induced by neonatal PCP treatment can be reversed by risperidone, but not by haloperidol. The effect also draws attention to the diversity in the mechanism of action of the present generation of drugs and highlights the need to further explore these differences. These data not only support neonatal PCP as a valid animal model of cognitive and negative symptoms but also demonstrates that a single neurotransmitter hypothesis is unlikely to fully describe or account for deficits observed in the schizophrenic brain. Having replicated the deficits induced by neonatal PCP treatment, and the attenuation of the deficits using risperidone, the next experiment in chapter 5 aims to investigate the working hypothesis that the neonatal PCP treatment during a critical period of development would result in long-lasting deficits in episodic and spatial memory.
Chapter 5
Long-term effects of neonatal PCP administration on NOR and SMT in male and female rats tested on PND 190 days and PND 365
5. Introduction

The main pharmacological property of PCP is non-competitive antagonism of NMDAR. However, PCP also has affinity for e.g. the D2R, the 5-HT2R, the sigma receptor, as well as potassium channels (Rothman, 1994, Kapur and Seeman, 2002).

Acute PCP as a schizophrenia model has its limitations because the neuroanatomical and imaging evidence supporting the developmental or neurodegenerative aspect of schizophrenia and the chronic nature of the disease is not well characterized with one acute dose. The use of sub-chronic PCP administration as a model also can be challenged on some levels too. The mechanisms underlying cortical dysfunction in schizophrenia and the PCP-induced behavioural deficits observed with the neonatal model are of different origin, the similarities in the overall functional deficits may be of importance in designing novel pharmacological approaches that are targeted at improving cortical function.

Transient inhibition of NMDA receptors during a critical period of development can produce selective cognitive and social behavioural deficits and some of these effects were reversed by risperidone (chapters 2,3 and 4). Also, NMDA receptors are involved in long-term potentiation (LTP), which is considered to be the physiological base for learning and/or memory (Morris et al., 1989; Harris et al., 1984; Stringer et al., 1983). Several studies have shown that brief exposure to NMDA antagonists (following postnatal treatment) such as PCP induce enduring learning and/or memory impairments (Kawabe et al., 1998a;b; Yoshihara and Ichitani 2004; White et al., 2009). Although there is no conclusive evidence as to when a critical period begins or ends, it could only be implied that there exists a specific time-window when the nervous system may be
vulnerable to drug-induced changes or neurotoxicity, leading to enduring behavioural
deficits (White et al., 2009). It is of note that the actions of NMDA receptor antagonists
are upstream in regions that project to areas vulnerable to neurotoxic insult such as
apoptosis and cell death. While significant progress has been made in describing the
circuitry involved in adult-type excitotoxicity (Olney 2002), modifications in the
upstream regulatory circuits by neonatal antagonism remain largely unknown. The
finding that developmental insult (i.e. neonatal NMDA treatment) may alter/influence
cognitive abilities and social behaviours (chapters 2, 3 and 4) argues for further
investigations to analyse the long-term effect of these insults, particularly since
schizophrenia patients have a similar developmental profile and symptomatology,
which is the reason behind the studies described in this chapter.
5.1 Aim

It has already been established in chapters 2, 3 and 4 that the neonatal PCP treatment on PNDs 7, 9 and 11 produces robust object and spatial memory and social behaviour deficits and that some of these deficits could be reversed by risperidone but not by haloperidol. In order to further investigate whether a brief disruption of NMDA receptor function during a critical stage of development, i.e. PND 7, 9 and 11 is sufficient to produce long-lasting object and spatial memory deficits, the animals were tested again on PND 180-190 and PND 350-365 using NOR and SMTs.
5.2. Methods and materials

5.2.1. Animals and housing

Hooded Lister rats were obtained from Charles River, UK. As seen in table 3.3.1a (chapter 3), the second batch of five time-mated pregnant dams arrived 15 days prior to parturition and were housed individually in cages containing sawdust and paper bedding until weaning. All animals were housed under conditions of constant temperature (21 ± 1 ºC) and humidity (50-58%) and a 12 hour light/dark cycle (lights on 07:00 h) with free access to food and water. All experiments were conducted in accordance with the Animals Scientific Procedures Act, UK 1986 and were approved by the University of Bradford ethical review panel.

5.2.2a. Phencyclidine dosing regimens - Repeat administration to rat neonates.

The PCP dosing regime was adapted from Wang et al. 2001.

Following birth, the pups were kept along with their littermates and left undisturbed until the time of dosing. On PNDs 7, 9 and 11 mothers were removed from the home cage and held by an assistant. The male pups were removed and administered vehicle or PCP (once a day; 10 mg/kg subcutaneous) and female pups were administered vehicle or PCP (10 mg/kg subcutaneous) into the loose skin at the back of the neck. At the time of dosing on PND 7, the pups were marked with a permanent tattoo code on the tails for identification purposes. Dosing was carried out in a separate room in an attempt to shield the mothers from pup vocalizations. Time away from the litter was kept to a minimum. Pups were returned to the home cage immediately after dosing. Noise in the holding room was minimised as far as possible and animals were not handled between the time of dosing and weaning. Pups were weaned between PNDs 21-
22 and were housed as five females per home cage and four males per home cage. All behavioural testing was carried out in the adult rats on PND 180-190 and PND 350-365. (For the history of neonatal PCP and behavioural testing regime, please refer to figure 5.2.2a).

![Timeline for neonatal PCP treatment (PNDs 7,9,11 once a day)-both male and female hooded Lister rats](image)

**Figure 5.2.2a:** Timeline for neonatal PCP treatment (PNDs 7,9,11 once a day)-both male and female hooded Lister rats

### 52.3. Behavioural testing

#### 5.2.3 (i). NOR and SMT

The NOR method was adapted from Grayson et al (2007) and SMT was adapted from Sutcliffe et al., 2007. For details regarding apparatus, habituation, behavioural testing—both NOR and SMT, please refer to chapter 2.

#### 5.2.3(i)a. Drug phase

The male and female rats were randomly assigned to two treatment groups; 10 animals were treated with vehicle and 10 with PCP 10 mg/kg (see section 5.2.2a; table 3.3.1a-chapter3). The rats were left undisturbed until the weaning day and were separated into males and females. They were tested in their adult stage PND 190 and PND 365 using NOR and SMT. The animals were not challenged with PCP again prior to behavioural testing. They did not have any drug treatment when they were tested on PND 180-190 and PND 350-365.
5.2.3(i)b. Statistical analysis

The data are expressed as mean ± S.E.M (n=10 per group). Student’s paired \( t \)-test was performed to compare the effect of treatment on time spent exploring the familiar versus the novel object in the NOR paradigm and stationary versus moved objects using SMT. Line crossings were expressed as mean ± S.E.M. of the total number of line crossed during the acquisition and retention phases. Analysis of line crossings and discrimination index (DI) data was performed using one-way ANOVA followed by post-hoc Dunnett’s \( t \)-test.
5.3. Results

5.3.0 Effect of neonatal PCP treatment (10 mg/kg) on object recognition in adult rats (PND 190 and 365) in both sexes.

Four sets of experiments were carried out in total in order to test the effect of neonatal PCP in both sexes at two different time points i.e. PND 190 and PND 365. The effects that neonatal PCP had in this test at these time points in both the sexes will be explained in detail in this section. It is important to note that there was no drug treatment during the test phase. The animals were treated with PCP 10 mg/kg on PNDs 7,9 and 11 and since then they did not receive a challenge dose of PCP prior to the test phase.

Students paired t-test in the acquisition trial (in each of the four sets of the experiments) consistently showed that drug treatment did not produce any significant effect on object exploration in this trial and rats spent an approximately equal amount of time exploring both the objects in this phase; an effect that was observed for both vehicle and neonatal PCP treated rats.

In the retention trial of the test, (in each of the four sets of the experiments) the animals showed a significant effect of vehicle treatment on object exploration. Paired t-test showed that vehicle treated rats spent more time exploring the novel object compared with the familiar object. Rats that received neonatal PCP treatment however could not discriminate between the novel and familiar object and spent almost similar time exploring both the objects, at any time point.
Experiment 5.3.1: The effect of neonatal PCP treatment (10 mg/kg in males and females) on episodic memory at two different time points -i.e. PND 190 and PND 365 in both males and females using NOR paradigm

5.3.1a. Effect of neonatal PCP treatment in the acquisition trial in male and female animals on PND 190 and PND 365.

*Adult PND 190 Males& Females:* In males, paired t-test revealed that neither neonatal PCP (10 mg/kg) nor vehicle (0.9% saline) produced any significant effect on object exploration in the acquisition trial of the test. Also, in females, there was no significant effect on object exploration observed in this trial. Rats from both treatment groups spent almost equal times exploring both the objects (See figure 5.3.1a(i))

*Adult PND 365 Males & Females:* In males, paired t-test revealed that neither neonatal PCP (10 mg/kg) nor vehicle (0.9% saline) produced any significant effect on object exploration in the acquisition trial of the test. A similar observation was made in the females, where there was no significant effect on object exploration in this trial, i.e. rats from both treatment groups spent almost equal times exploring both the objects (See fig 5.3.1a(ii)).
Adult PND 190 - Novel object recognition task (Acquisition trial)

Figure 5.3.1a (i) The effect of neonatal PCP (10 mg/kg once a day on PNDs 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical objects in the 3-min acquisition trial in a novel object recognition task in male and female hooded Lister rats tested at PND 190. Data are shown as mean ± SEM of exploration time (s) (n=10 per group).

Adult PND 365 - Novel object recognition task (Acquisition trial)

Figure 5.3.1a (ii) The effect of neonatal PCP (10 mg/kg once a day on PNDs 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical objects in the 3-min acquisition trial in a novel object recognition task in male and female hooded Lister rats tested at PND 365. Data are shown as mean ± SEM of exploration time (s) (n=10 per group).
5.3.1b. Effect of neonatal PCP treatment in the retention trial in male and female animals on PND 190 and PND 365.

Adult PND 190 Males & Females: In males, a significant effect of treatment was observed on object exploration. Paired t-test revealed that the vehicle treated animals (control group) had a clear preference for the novel object compared to the familiar object – i.e. spent significantly more time exploring the novel versus familiar object (p<0.001) (See figure 5.3.1b(i)). This effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both objects.

In females, a significant effect of treatment was observed on object exploration. Paired t-test revealed the vehicle treated animals (control group) had a clear preference for the novel object (p<0.01) (See figure 5.3.1b.(i)). This effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both objects.

Adult PND 365 Males and Females: In males, a significant effect of treatment was observed on object exploration. Paired t-test revealed that the vehicle treated animals (control group) had a clear preference for the novel object compared to the familiar object – i.e. spent significantly more time exploring the novel versus familiar object (p<0.001) (See figure 5.3.1b(ii)). This effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both objects.

A similar effect was observed in females, wherein a significant effect of treatment was observed on object exploration. Paired t-test revealed the vehicle treated animals (control group) had a clear preference for the novel object (p<0.01) (See figure 5.3.1b.(ii)). This effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both objects.
Adult PND 190 - Novel object recognition task (Retention trial)

**Figure 5.3.1b (i)** The effect of neonatal PCP (10 mg/kg once a day on PNDs 7, 9 and 11) and vehicle (0.9% saline) on exploration of familiar and novel objects in the 3-min retention trial in a novel object recognition task in male and female hooded Lister rats tested at PND 190. Data are shown as mean ± SEM of exploration time (s) (n=10 per group). ***p<0.001; **p<0.01 significant increase in exploration of novel versus familiar object; students t-test.

Adult PND 365 - Novel object recognition task (Retention trial)

**Figure 5.3.1b (ii)** The effect of neonatal PCP (10 mg/kg once a day on PNDs 7, 9 and 11) and vehicle (0.9% saline) on exploration of familiar and novel objects in the 3-min retention trial in a novel object recognition task in male and female hooded Lister rats tested at PND 365. Data are shown as mean ± SEM of exploration time (s) (n=10 per group). ***p<0.001, **p<0.01; significant increase in exploration of novel versus familiar object; students t-test.
5.3.1c: Effect of neonatal PCP treatment on the discrimination index in male and female animals on PND 190 and PND 365.

*Adult PND 190 Males and Females:* Discrimination index is a measure of exploratory preference of animals for novel or familiar object in the retention trial in the NOR paradigm calculated by dividing the difference between the duration of time spent exploring the novel and familiar objects by the total duration of exploration activity in the retention trial. Paired t-test in both males and females showed a significant effect of neonatal PCP (10 mg/kg) in the rats, where both males and females treated with neonatal PCP showed significant reduction in DI when compared with the vehicle rats (p<0.001, males; p<0.05, females). (See figures 5.3.1c (i)).

*Adult PND 365 Males and Females:* Paired t-test in both males and females showed a significant effect of neonatal PCP (10 mg/kg) in the rats, where both males and females treated with neonatal PCP showed significant reduction in DI when compared with the vehicle rats (p<0.001, males; p<0.001, females). (See figures 5.3.1c (ii)).
Adult PND 190 - Novel object recognition task (Discrimination Index)

![Bar chart showing discrimination index for males and females at PND 190.](image)

**Figure 5.3.1c (i)** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the discrimination index, DI in both male and female rats at PND 190. Data are shown as mean ± SEM (n=10 per group). ***p<0.001; *p<0.05 significant reduction compared to vehicle; students t-test.

Adult PND 365 - Novel object recognition task (Discrimination Index)

![Bar chart showing discrimination index for males and females at PND 365.](image)

**Figure 5.3.1c (ii)** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the discrimination index, DI in both male and female rats at PND 365. Data are shown as mean ± SEM (n=10 per group). ***p<0.001; significant reduction compared to vehicle, students t-test.
5.3.1d. Effect of neonatal PCP treatment on line crossings in male and female animals on PND 190 and PND 365.

*PND 190 & PND 365 Adult Males and Females:* Students paired t-test on line crossings (in each of the four sets of the experiments) consistently showed that drug treatment did not produce any significant effect on line crossings; an effect that was observed for both vehicle and neonatal PCP treated rats in both sexes. (See figures 5.3.1d (i&ii)).
Adult PND 190 - Novel object recognition task (Line crossings)

![Bar chart showing the effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9, and 11) and vehicle (0.9% saline) on the total number of line crossings in acquisition plus retention trial in the novel object recognition task on adult PND 190 in both male and female hooded Lister rats. Data are shown as mean ± SEM (n=10 per group).]

**Figure 5.3.1 d(i)** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9, and 11) and vehicle (0.9% saline) on the total number of line crossings in acquisition plus retention trial in the novel object recognition task on adult PND 190 in both male and female hooded Lister rats. Data are shown as mean ± SEM (n=10 per group).

Adult PND 365 - Novel object recognition task (Line crossings)

![Bar chart showing the effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9, and 11) and vehicle (0.9% saline) on the total number of line crossings in acquisition plus retention trial in the novel object recognition task on adult PND 365 in both male and female hooded Lister rats. Data are shown as mean ± SEM (n=10 per group).]

**Figure 5.3.1 d(ii)** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9, and 11) and vehicle (0.9% saline) on the total number of line crossings in acquisition plus retention trial in the novel object recognition task on adult PND 365 in both male and female hooded Lister rats. Data are shown as mean ± SEM (n=10 per group).
Experiment 5.3.2: The effect of neonatal PCP treatment (10 mg/kg in males and females) on spatial memory at two different time points—i.e. PND 190 and PND 365 in both males and females using SMT.

Four sets of experiments were carried out in total in order to test the effect of neonatal PCP in both sexes at two different time points i.e. PND 190 and PND 365. The effects that neonatal PCP had in this test at these time points in both the sexes will be explained in detail in this section. It is of note that there was no drug treatment during the test phase. The animals were treated with PCP 10 mg/kg on PNDs 7, 9 and 11 and since then they did not receive a challenge dose of PCP prior to the test phase.

Students paired t-test in the acquisition trial (in each of the four sets of the experiments) consistently showed that drug treatment did not produce any significant effect on spatial memory in this trial of the test. Rats spent equal amount of time exploring both the stationary and moved objects in this trial; an effect that was observed in both vehicle and neonatal PCP treated rats in both sexes. In the retention trial, paired t-test showed that vehicle treated rats spent more time exploring the moved object when compared to the stationary object. Rats that received neonatal PCP treatment however could not discriminate between the moved and the stationary objects and spent almost either similar or more time exploring both the objects, an effect that was observed in both the sexes. This will be explained in detailed sections below.
5.3.2a. Effect of neonatal PCP treatment in the acquisition trial in male and female animals on PND 190 and PND 365 in a SMT.

*Adult PND 190 Males & Females:* In males, paired t-test revealed that neither neonatal PCP (10 mg/kg) nor the vehicle (0.9% saline) treated group produce any significant effect on object exploration in the acquisition trial of the test. A similar observation was made in females where there was no significant effect on object exploration in this trial, i.e. rats from both treatment groups spent almost equal times exploring both the objects (A & B) (See figure 5.3.2a(i))

*Adult PND 365 Males & Females:* In males, paired t-test revealed that neither neonatal PCP (10 mg/kg) nor the vehicle (0.9% saline) treated group produce any significant effect on object exploration in the acquisition trial of the test. A similar observation was made in females where there was no significant effect on object exploration in this trial, i.e. rats from both treatment groups spent almost equal times exploring both the objects (A&B)(See figure 5.3.2a(ii))
Adult PND 190 – Spatial memory task (Acquisition trial)

Figure 5.3.2.a(i) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical (stationary) objects (A & B) in the 3-min acquisition trial in a spatial memory task in adult male and female hooded Lister rats tested on PND 190. Data are shown as mean ± SEM of exploration time (s) (n=10 per group).

Adult PND 365 – Spatial memory task (Acquisition trial)

Figure 5.3.2.a(ii) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of two identical (stationary) objects (A & B) in the 3-min acquisition trial in a spatial memory task in adult male and female hooded Lister rats tested on PND 365. Data are shown as mean ± SEM of exploration time (s) (n=10 per group).
5.3.2b. Effect of neonatal PCP treatment in the retention trial in male and female animals on PND 190 and PND 365 in SMT.

Adult PND 190 days Males & Females:

In males, paired t-test revealed a significant effect of neonatal PCP (10 mg/kg) on object exploration time in the retention trial of the SMT. The vehicle treated animals (control group) had a clear preference for the moved object (i.e. novel position) compared to the stationary object (familiar position) – i.e. spent significantly more time exploring the moved object compared to the stationary object (p<0.001). However, the PCP treated animals spent similar amount of time exploring both the moved and the stationary objects.

In females, a significant effect of neonatal PCP (10 mg/kg) was observed on object exploration in SMT. The vehicle treated animals (control group) had a clear preference for the moved object (p<0.05) (See figures 5.3.2b. (i)). The PCP treated animals however spent similar amount of time exploring both the moved and the stationary objects.

Adult PND 365 Males and Females:

In males, paired t-test revealed a significant effect of neonatal PCP (10 mg/kg) on object exploration time in the retention trial of the SMT. The vehicle treated animals (control group) had a clear preference for the moved object compared to the stationary object – i.e. spent significantly more time exploring the moved versus stationary object (p<0.01).

A similar effect was observed in females in this trial where there was significant effect (p<0.01) on object exploration in the SMT in vehicle rats (See figures 5.3.2b. (ii)). This effect was abolished in rats that had been treated with neonatal PCP i.e. these rats spent a similar amount of time exploring both moved and stationary objects.
Adult PND 190 – Spatial memory task (Retention trial)

Figure 5.3.2b(i) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of a stationary and a moved object in the 3-min retention trial in a spatial memory task in male and female hooded Lister rats tested in the adult stage on PND 190. Data are shown as mean ± SEM of exploration time (s) (n=10 per group). ***p<0.001; *p<0.05 significant increase in object exploration of moved versus stationary object, students t-test.

Adult PND 365 – Spatial memory task (Retention trial)

Figure 5.3.2b(ii) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on exploration of a stationary and a moved object in the 3-min retention trial in a spatial memory task in male and female hooded Lister rats tested in the adult stage on PND 365. Data are shown as mean ± SEM of exploration time (s) (n=10 per group). **p<0.01 significant increase in exploration of moved versus stationary object, students t-test.
5.3.2c: Effect of neonatal PCP treatment on the discrimination index in male and female animals on PND 190 and PND 365 in a SMT.

*Adult PND 190 Males and Females:* Discrimination index is a measure of exploratory preference of animals for the novel position (moved object) or the stationary position (familiar position) in the retention trial in the SMT calculated by dividing the difference between the duration of time spent exploring the stationary and moved positions by the total duration of exploration activity in both the positions in the retention trial. Paired t-test in both males and females showed a significant effect of neonatal PCP (10 mg/kg) in the rats, where both males and females treated with neonatal PCP showed significant reduction in DI when compared with the vehicle rats (p<0.01, males; p<0.05, females). (See figures 5.3.2c (i)).

*Adult PND 365 old Males and Females:* In males, DI revealed a significant effect of neonatal PCP (10 mg/kg) on the rats, i.e. the PCP group displayed a significant reduction in DI (p<0.01) compared to the control rats. In females, analysis of the DI revealed a significant effect of neonatal PCP (10 mg/kg) of the rats, i.e, the neonatal PCP group displayed a significant reduction in DI (p<0.01) compared to the control rats (See figures 5.3.2c (ii)).
Adult PND 190 – Spatial memory task (Discrimination Index)

Figure 5.3.2c(i) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the discrimination index, DI in adult male and female hooded Lister rats on PND 190. Data are shown as mean ± SEM (n=10 per group). **p<0.01; *p<0.05 significant reduction compared to vehicle, students t-test.

Adult PND 365 – Spatial memory task (Discrimination Index)

Figure 5.3.2c(ii) The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the discrimination index, DI in male and female hooded Lister rats at PND 365. Data are shown as mean ± SEM (n=10 per group). **p<0.01; significant reduction compared to vehicle, students t-test.
5.3.2d. Effect of neonatal PCP treatment on line crossings in male and female animals on PND 190 and PND 365 in a SMT.

PND 190 and PND 365 Adult Males and Females: Students paired t-test on the line crossings (in each of the four sets of the experiments) consistently showed that drug treatment did not produce any significant effect on line crossings; an effect that was observed for both vehicle and neonatal PCP treated rats. (See figures 5.3.2d (i&ii)).
**Figure 5.3.2d(i).** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the total number of line crossings in the acquisition + retention trial in the spatial memory task in male and female hooded Lister rats on PND 190. Data are shown as mean ± SEM (n=10 per group).

**Figure 5.3.2d(ii).** The effect of neonatal PCP (10 mg/kg once a day on postnatal days (PND) 7, 9 and 11) and vehicle (0.9% saline) on the total number of line crossings in the acquisition + retention trial in the spatial memory task in male and female hooded Lister PND 365 rats. Data are shown as mean ± SEM (n=10 per group).
5.4. Discussion

The key finding of this investigation is that neonatal PCP treatment induces robust and enduring deficits in NOR and SMTs in both male and female adult rats that lasts up to PND 190 and even a year. To our knowledge, this is the first study wherein the male and female rats were tested on PND 190 and 1 year following neonatal treatment on PND 7,9 and 11 with no drug challenge prior to the test stage. We have already seen robust deficits in episodic and spatial memory in both adolescents and adults in both sexes following neonatal PCP treatment in chapter 2. Chapter 3 showed clear deficits in SI in female rats. Although the previous chapters (2 and 4) provide evidence for the robust effect of the neonatal PCP model, the long-term (enduring) effects of this model remained to be investigated and understood. In that respect, this chapter aimed at investigating the lasting effects of neonatal PCP, which is the reason behind testing the animals after 180 days and a year of treatment with neonatal PCP.

Although some information has been made available with regards to the pharmacological effects of neonatal PCP on the central nervous system (CNS), the same cannot be said about the knowledge and understanding of their long-lasting effects. Findings in nonhuman primates treated with amphetamine analogues, such as ±3,4-methylenedioxymethamphetamine (MDMA), indicate that these drugs can produce long-lasting, probably permanent, changes in brain serotonergic innervation and animals treated with PCP as well as related drugs develop neurodegenerative changes in selected brain regions (McCann et al., 1997). The aim of the experiments reported in this chapter is to investigate the long-lasting effect of neonatal PCP treatment, the treatment
paradigm that has been shown to elicit widespread apoptosis in rodents when given during the perinatal period (Ikonomidou et al., 1999).

Studies have shown that blockade of NMDA receptors by sub-chronic PCP administration has a long-lasting down-regulatory effect on BDNF mRNA expression in the female rat brain which may underlie some of the behavioural deficits observed post PCP administration (Snigdha et al., 2010). Interestingly, it has been consistently shown that PCP triggers apoptotic neurodegeneration in the developing brain of rodents (Ikonomiou et al., 1999), while causing excitotoxic neurodegeneration in the mature brain Olney et al.,(1991). This is accompanied by a range of behavioural and neurochemical alterations, observed both immediately following treatment, and as a long-term result of perinatal treatment, thereby providing more evidence for the role of NMDA receptor hypofunction in the disorder (Olney 2002).

The NMDA receptor plays a key role in the acquisition and storage of new information (Baker et al., 2002; Nakazawa et al., 2004). A large amount of studies indicate a direct involvement of hippocampal-dependent spatial memory (Latysheva et al., 2003; Kesslak et al., 2003; Sircar 2003). However, there are only a few studies in rodents investigating performance in the hippocampus-independent NOR paradigm (Baker et al., 2002; Karasawa et al., 2008; Abe et al., 2004; Rampon et al., 2000), a test based on the natural tendency to spend more time exploring the novel objects than familiar ones. Results from this study showed that early postnatal treatment with an NMDA receptor antagonist led to the disturbance of NOR and spatial memory in PND>180 and 1 year old rats.
Repeated PCP treatment on PND5-15 impaired spatial learning after 3-6 weeks of withdrawal (Sircar 2003) and it also impaired reversal learning in adulthood (Andersen & Pouzet, 2004). These studies suggest that in developing rats, administration of PCP impairs learning during extended withdrawal, and that such impairment depends on the specific time of exposure. Most of the developmental studies have employed repeated treatment for 4-10 days during early development or, as in this study, on PNDs 7, 9 and 11. Results from our study are in agreement with these studies that insults during critical periods of neuronal development lead to memory deficits and that the deficits are long-lasting. According to the results from our study, the deficits will exist a year after this neuronal insult thereby an implication is that this may possibly be the time-window when the nervous system may be vulnerable to drug-induced changes or neurotoxicity, leading to enduring behavioural deficits.

According to a recent study, brief exposure to PCP (9 mg/kg) on PND 50-51 selectively impaired reversal learning in adulthood in a reversal learning task (White et al., 2009). Our studies showed that a brief exposure on PNDs 7,9 and 11 showed long lasting and enduring episodic and spatial memory deficits at PND> 1 year. It is apparent that our study does provide the evidence that even a brief exposure to drugs at a vulnerable timeframe of a developing brain is likely to produce enduring cognitive deficits.

The role of the prefrontal cortex (PFC) in object recognition has not been fully elucidated yet. While some findings suggested the involvement of PFC in object recognition memory (Bachevalier et al., 1986; Meunier el., 1997), others suggested that the PFC does not play a role in the retrieval or use of information necessary for discriminating object familiarity (Hannesson et al., 2004). Indeed, it has been suggested
that circuitry linking the perirhinal cortex (Prh), mediodorsal thalamus, and ventrolateral PFC in primates is essential for object recognition memory (Meunier et al., 1997; Parker et al., 1998). Interestingly, it has been observed that in non-human primates, early postnatal damage to the hippocampal region also hampers the development of the dorsal prefrontal cortex and the mechanisms whereby the dorsal prefrontal cortex regulates subcortical DA function are akin to those seen in patients with schizophrenia (Bertolino et al., 1997; Saunders et al., 1998). Perinatal administration of PCP resulted in apoptotic changes in the frontal cortex (Wang et al., 2001; Hansen et al., 2004). The postnatal treatment of rats with PCP on PNDs 7, 9 and 11 was proposed as a neurodevelopmental model of schizophrenia (Wang et al., 2001). This treatment regime produced widespread neurodegeneration in brain areas relevant to the cognitive deficits observed in the schizophrenic patients, namely hippocampus and frontal cortex (Contestabile 2000). Western blot analysis of the cortex revealed a persistently increased amount of NMDA-R1 receptor protein in MK-801-treated rats. This finding supports previous studies in which perinatal administration of PCP also led to increased cortical NMDA-R1 receptor protein (Wang et al., 2001; Anastasio et al., 2008). Furthermore, post mortem studies in human schizophrenia found a comparable up-regulation of the NMDA-R1 within different areas of the cortex (Nudmamud-Thanoi et al., 2004; Dracheva 2001). This upregulation in the key subunit of the NMDA receptor may be interpreted as a compensatory response to glutamatergic deficits and may have implications for the pathological changes in behaviour that were found in the recent studies in rodent and in humans (Wang et al., 2001; Latysheva et al., 2003; Nudmamud-Thanoi et al., 2004; Dracheva 2001).
Spatial learning and memory deficits have been linked to hippocampal dysfunction. However, there is substantial evidence that an extended extra-hippocampal circuit plays a vital role in mediating spatial learning and memory functions (Aggleton et al., 1999; Mitchell et al., 2002). This circuit includes, in addition to the hippocampal formation, the retrosplenial cortex, anterior thalamic nuclei and mammillary bodies (Aggleton et al., 1999). According to a recent study by Yuede et al., (2010) assessing the density of AC3-positive profiles showed severe damage of retrosplenial cortex, caudate/putamen, anterior thalamic nuclei (which are part of hippocampal circuitry) in mice treated with PCP on PNDs 2 & 7, the damage being severe in mice treated on PND 7. Similarly, our study, showed robust long-lasting deficits in spatial and recognition memory deficits following PCP treatment on PND7, 9 and 11. It could therefore be suggested that neuronal insult at an early stage may have resulted in severe damage of the hippocampal circuitry, thereby causing spatial and object recognition memory deficits.

A vital determinant of apoptosis in rodents is a varying degree of sensitivity of diverse neuronal populations to the apoptogenic effects of NMDA antagonists (such as PCP) and GABA mimetic agents, which is highly dependent upon the age of exposure i.e., the hippocampus, subiculum and caudate/putamen have been observed to be relatively more sensitive during the early periods of synaptogenesis, while other areas, particularly the cortical regions and some thalamic nuclei are found to be more sensitive in the mid to later periods (Ikonomidou et al., 2000). To date, reports on the long-term behavioural effects of postnatal PCP exposure have focused on PND7 or later, which corresponds to the mid to late period of synaptogenesis. Hence, it could be suggested that, since the treatment regime in our study was on PND 7, 9 and 11, it could have possibly led to disruptions in the process of synaptogenesis, thereby resulting in long-term cognitive
deficits as seen in our study. In agreement with our result, findings from Yuede et al (2010) indicate that exposing male and female mice to PCP (single dose of 35 mg/kg on PND 2 and 50 mg/kg on PND 7 at 10 µl/g) resulted in severe, long-term impairment in spatial learning and memory (using the Morris water maze task) and in contextual fear conditioning tested on PND 170, and these impairments were found to be most likely permanent in nature, which again agrees with our findings which shows robust deficits in spatial learning and in object memory task on PND 190 with no drug treatment during the testing phase.
5.5 Conclusion

In conclusion this study provides evidence to show that neonatal PCP treatment induces robust and long-lasting deficits in NOR and SMTs, when tested on PND>180 and 1 year old male and female rats.

Since PND 7, 9 and 11 correspond to the mid-to-late synaptogenesis, we also studied the potential long-term cognitive deficits in male and female rats exposed to PCP during this period of vulnerability. The study draws attention to the fact that a pharmacological insult during neonatal days 7,9 and 11 could possibly result in a damage to the process of synaptogenesis thereby resulting in significant cognitive deficits seen even in the absence of the drug up to 1 year post dosing. This hypothesis needs testing, which will be discussed in future work (chapter 7).

In summary, these data not only support the use of neonatal PCP as a valid model of cognitive deficit and negative symptoms of schizophrenia but also reiterates the importance of PNDs 7,9 and 11 during neuronal development. The next and final chapter will recapitulate all findings reported in this thesis thus far and will attempt to reconcile the behavioural findings in a conceptual model that will be useful for designing future experiments in this area of work.
Chapter 6

General discussion
6.1 General Discussion

The work reported in this thesis forms part of a larger effort by researchers working on schizophrenia to establish an animal model that not only mimics behavioural aspects of the disorder but also addresses pathophysiology. The model studied here is the neonatal PCP model, which is a neurodevelopmental model. Work presented in this thesis shows that this model induced disruption in behavioural tasks of significance to schizophrenia. The majority of work in this area has been done using sub-chronic or acute or chronic NMDA receptor antagonist treatment regimes in adult animals. Some however have focused on neonatal pharmacological insults in mostly in male (fewer studies in females) rodents using varying behavioural paradigms. Hence our study focussed on validating this model in both male and female rats using behavioural tests that have been used successfully in sub-chronic, acute and chronic paradigms and to investigate sex differences following neonatal PCP treatment. It was important to validate the effect of the treatment regime and also to confirm if the nervous system is susceptible to drug-induced changes, leading to behavioural deficits, which is the basis for testing the animals at four different time points (i.e. PND 35, PND 56, PND 190 and PND 365). The data summarized here describes the robust effect of neonatal PCP to impair aspects of cognitive function in male and female rats and social function in female rats and also provides a conceptual hypothesis that attempts to explain the underlying neurobiology of this deficit.

The chief findings of this thesis are summarized below:

1. Neonatal PCP treatment (10 & 20 mg/kg in males and 10 mg/kg in females; once a day for 3 days on PND 7,9 and 11) causes object recognition and spatial memory
impairment in male and female rats both in the adolescent (PND35-56) and in the adult stages (PND>56) (chapter 2). Also, there was a significant increase in LMA following PCP 20 mg/kg treatment in adolescent males and a significant increase in LMA following PCP 10 mg/kg treatment in adolescent females. These effects were not observed in the adult rats, either in males and females, thereby giving some evidence for a clear time course effect.

2. Based on the results obtained in chapter 2, PCP 10 mg/kg (both in males and females; once a day for 3 days on PND 7,9 and 11) was tested for effects in a test of relevance to negative symptomatology in schizophrenia. Results showed that female rats showed impairment in SI in the adolescent stage and these deficits persisted into adulthood. However in the male rats, although there was a slight trend towards PCP-induced SI deficits in the adolescent stage, it was not seen in the adult stage. This result is clearly the first evidence of male versus female differences in our study.

3. Object recognition memory deficits (in males and females) and SI deficits (in females) were challenged with an acute dose of classical haloperidol and atypical risperidone, an acute dose of haloperidol failed to reverse the object recognition memory deficits induced by neonatal PCP treatment in both males and females. Risperidone in contrast reversed the object recognition (males and females) and SI deficits (females).

4. The next step was to investigate the temporal profile of this treatment regime, and hence the animals were tested on PND 190 and PND 365. The animals did not have any challenge dose of PCP during the test stage. The result showed that there was a significant deficit in object and spatial recognition memory in both male and female animals that persisted for a year following neonatal treatment.
Despite increasing focus on the significance of cognitive impairment in schizophrenia, the development of novel treatments has been slow. Hyman and Fenton's (2003) identification of a “translational gap” between preclinical and clinical science underscores the need to revise preclinical, clinical, and regulatory practice. A review of the clinical literature identifies evidence for some cognitive benefits with current antipsychotics (Mailman & Murthy, 2010). The magnitude of these effects may, in some cases, be too small to be functionally relevant, but the data might nevertheless allow translational links to be identified between clinical and preclinical studies. Effective closure of the translational gap for cognitive deficits in schizophrenia will require the design of a coherent preclinical strategy, and our study could be considered a small step to achieving that goal.

A remarkable conceptual shift in opinion about the neurobiology of schizophrenia has been taking place for a long while now after several decades of speculation that schizophrenia occurs because of cerebral pathological events that ensue or are expressed around early adult life. It has also been referred to as a neurodevelopmental disorder in which the chief cerebral insult or pathological process arises during brain development long before the illness clinically manifests (Bogerts et al., 1989; Crow et al., 1989; Murray et al., 1992; Weinberger 1987). An association between putative abnormalities in intrauterine development (Brown et al., 2001) and schizophrenia has been consistently reported and the evidence ranges from highly circumstantial and weak (i.e.) slight over expression of minor physical anomalies (Schiffman et al., 2002) to compelling (e.g. replicated cytoarchitectural anomalies) (Mirmics et al., 2001; Benes & Berretta, 2001). The facts may be interpreted to interlink a rational story of developmental abnormalities; however, individual findings show one
too many inconsistencies and methodological problems. The most important research questions at present are whether substantiation of cytoarchitectural deviations can be more broadly replicated and whether the methodological uncertainties can be resolved.

The orientation of neurones and their internal connections (links) are predetermined during the second trimester of birth (Zaidel et al., 1997). There is also a general assumption that such links and patterns do not change during life, even if cells are lost or if secondary pathological conditions arise, and if this postulation is considered, then abnormalities of cytoarchitecture would strongly implicate pathological development (Weinberger, 1999). In essence, if one looks at the brain abnormalities in schizophrenia as a whole, the most logical explanation is that of anatomical deviations that predate the onset of the illness, which in turn is consistent with the notion of a developmental defect, and that may implicate a failure of second-trimester neuronal migration leading to cortical maldevelopment (Arnold et al., 1991), the support for which comes from cortical cytoarchitecture studies by Akbar et al., (1996). Therefore, the possibility of cortical maldevelopment in the second trimester is also critical to a discussion of the additional speculation about neurodevelopmental factors and models that have been investigated in this thesis.

It has been demonstrated that male rats respond best (i.e. demonstrate deficits in cognitive tasks) to an acute dose of PCP $\geq$5 mg/kg (Olney et al., 1999). This is in contrast to female rats, which demonstrate significant deficits in behavioural tasks following acute doses and a chronic dosing regimen of 2 mg/kg (Neill et al., 2010 for review). However, there has always been contradiction with regards to the effect of neonatal treatment of PCP (and other NMDA receptor antagonists), wherein some
reports show robust deficits in males and some in females (see table 3, chapter 1). This was the basis of the decision to test both male and female rats and at doses based on the robust deficits adapted from Wang et al., 2003, 2008 in order to validate and investigate further a model of the complex dysfunction as seen in schizophrenia. Also, it has been established that treatment with neonatal NMDA antagonists reduced the number of parvalbumin (PV) interneurons, which in turn was the result of apoptosis (Wang et al., 2003, 2008).

According to the neurodevelopmental theory of schizophrenia the late second trimester of pregnancy is the period of development of foetal central nervous system (CNS) during which exposure to any form of insult (viral, environmental etc) would lead to an increase in the possibility of subsequent development of schizophrenia as an adult (Beckmann et al., 1999; Bunney et al., 1995). This period corresponds to the first 2 weeks of postnatal life in the rat, in terms of similar neurodevelopmental changes (Bayer et al., 1993; Clancy et al., 2001). One way to induce such a neurodevelopmental abnormality is the administration of NMDA receptor antagonists. This treatment using varying treatment regimes during the development of CNS structures in animals has previously been shown to induce long-term effects on NMDA receptor activity (Gorter et al., 1992), and on the development of the CNS and synaptogenesis (Bellinger et al., 2002). So, in order to inhibit the neurodevelopmental processes, the rats should be treated during these weeks. Based on this theory and the dosing regimen adapted from Wang et al., 2001, we decided to dose the rat pups on PNDs 7, 9 and 11 for the work reported in this thesis. The 7–11 day window appears to be critical, with even brief exposure to NMDA receptor antagonists resulting in deleterious effects on CNS development and function (Haberny et al., 2002). In a recent study by Pawlak et al.,
2009 to examine whether SI and SMT deficits induced by neonatal PCP treatment are
due to the PCP treatment during neurodevelopment, adult mice were treated with PCP
(10 mg/kg, s.c.) 3 times every second day and subjected to the SI test on postnatal week
16 (i.e. tested in adulthood). No impairment of SI behaviours were observed in mice
that had been treated with PCP in adulthood. This study further confirms that the results
reported in our study are in fact the result of PCP treatment at the neonatal stage.

Although there is no conclusive evidence of involvement of one particular
neurotransmitter system in the downstream effects of NMDA receptor blockade
observed in our studies, it is possible that neonatal PCP may have increased NMDA and
GABA\(_A\) receptor effects during the course of brain development (Du Bois et al., 2008;
2009). It has been shown that M\(_{1/4}\) receptor binding levels were also significantly
increased following PCP 10 mg/kg at adolescence in a study which examined M\(_{1/4}\)
receptor binding levels at several time points (1 day, 1 week, 3 weeks and 10 weeks)
following PCP treatment (10 mg/kg, s.c) on postnatal days 7, 9 and 11 (Du Bois et al.,
2009). As mentioned above, with regards to a possible increase in NMDA receptor
density in PCP-treated rats, the reason for the increase could be either through a loss or
due to improper wiring of glutamatergic neurones which could lead to a decrease in
glutamate-NMDA receptor interaction, thereby leading to a compensatory upregulation
of NMDA receptors (figure 6.1.b).

Numerous reports have linked alterations in neurotrophic factors to
schizophrenia (Guillon et al., 2007; Durany et al., 2001) and subsequent induction of
deficits akin to schizophrenia in animal models (Fumagalli et al., 2004). Of particular
interest is BDNF which is expressed in abundance in regions involved in learning and
memory of both the rat and human brain (Choi et al., 2010). It could be suggested that
the blockade of NMDA receptors by neonatal PCP had a down-regulatory effect on
BDNF mRNA levels in rodent brain which could relate to the object recognition and
spatial memory deficits induced by neonatal PCP reported in chapters 2, 4 and 5. Due to
contrasting effects of PCP treatment and varying dosing regimes on BDNF between
males (Takahashi et al., 2006; Semba et al, 2006; Harte et al., 2007) and females
(Snigdha et al., 2011) the effect of neonatal PCP on BDNF regulation in males and
females needs to be investigated further.

It was evident from chapter 2, that locomotor activity in adolescent males and
females following neonatal PCP (20 mg/kg and 10mg/kg respectively) treatment was
significantly increased (p<0.05) when compared to the control animals. These data are
to an extent in agreement with reported increases in novelty-induced hyperactivity in
female rats (Harris et al., 2003) and in rats that had exposure to neonatal NMDA
receptor antagonists on PND 7,9 and 11 (du Bois et al., 2008). However, in our study,
we observed that the significant increase in locomotor activity was observed only in the
adolescent rats and not in the adults. It could possibly be due to the fact that the
transient exposure to PCP in our study may have been insufficient to produce a long-
term effect on locomotor activity (chapter 2), although there was significant effect on
cognition observed.

Previous studies have shown that differences in locomotor response to neonatal
PCP 3 mg/kg treatment (to male Sprague Dawley rats on PND 10, 12, 21, 37, 60 or 90)
depended on the age of the animal, and animals on PND21 and PND37 (just weaned
and adolescent animals) displayed an increase in locomotor responses when compared
to the adult rats (Jacobs et al. 2000). Our result is in agreement with this in that the adolescent rats, but not the adults showed a significant increase in locomotor activity in automated chambers and line crossings in NOR following neonatal PCP (chapter 2). The significant increase in locomotion seen in our study (chapter 2) in the adolescent rats could possibly be attributed to modification in the developmental expression of neurotransmitter systems such as the dopaminergic system. Du Bois et al., 2008 observed that D_2 receptor binding was reduced in the caudate putamen (CPu) and nucleus accumbens (Acb), along with increased tyrosine hydroxylase mRNA (TH mRNA) expression in the ventral tegmental area (VTA) of neonatal PCP-treated rats. It was suggested that the VTA caused an increase in DA output resulting in over-stimulation of striatal D_2 receptors, thereby resulting in the hyperactivity seen in these adolescent rats. As mentioned above, locomotor activity was significantly increased when compared to vehicle (control) rats only in the adolescent males treated with PCP 20 mg/kg and this effect was not observed in the adults. Some studies have shown that corticosterone can interfere with NMDA receptor mediated signals (Sato et al. 2004). This interference can lead to modification in locomotor activity (Tenk et al. 2006), giving rise to variable results. This may possibly explain why only a small response was observed (i.e only in the adolescents) in our study.

In chapter 3, we observed that the neonatal PCP regime induced robust SI deficits in the adolescent females and to some extent in certain social behaviours in adolescent males, although this effect was not as robust as in females. However, the increasing trend of deficits (although not reaching statistical significance) in male rats in some behaviours did not exist in the adult rats, whereas the deficits persisted in the adult female rats. Neonatal administration of 0.6-5 mg/kg/day of NMDA receptor antagonist,
CGP 43487 was found to cause a dose-dependent reduction in SI (Gould and Cameron 1997) in male rats. Yet another study by Du Bois et al., 2008 showed no effect on SI using female rats following 10 mg/kg PCP on PND 7,9 and 11. This is in direct contrast to our finding wherein the female adolescent and adult rats showed robust SI deficits. The findings could be attributed to a difference in testing protocols. Our study used an inanimate object, which also was part of the social behaviour, where it was noticed that the control rats (con-specific) and the test rat sniffed together, and the PCP treated animal explored the object only when the con-specific rat was not at the object. This also added another dimension to the social behaviour. Our study took several behaviours into consideration, i.e. sniffing, following, avoiding, object exploration and fighting and there is no available study for direct comparison of all these behaviours.

Longitudinal studies of schizophrenia patients indicate that in many cases, (in the prodromal stage), they demonstrate difficulty establishing normal relationships with other people and many avoid any form of contact with peers at school (Auerbach et al., 1993; Jones et al., 1994). This has been interpreted as an early sign of negative symptoms and it has been suggested that these group of symptoms may present themselves very early on in schizophrenia patients. For developmental models of schizophrenia, (like this model), it is therefore reasonable to expect deficits in social behaviour to be present before or after puberty (in our study, these deficits were observed in the female adolescents and to an extent in male adolescents). In agreement with our findings in this neonatal PCP model, previous studies have shown that neonatal ventral hippocampal lesioned rats demonstrated deficits in their SI at both PND 35 & 65 (Sams-Dodd et al., 1997).
In summary, the time points at which the different behaviours corresponding to both cognition (chapter 2) and SI (chapter 3) emerged in this model correlated with clinical findings, and very importantly demonstrated that some of the consequences of an abnormal developmental process may present themselves only in the adolescent stage (i.e. increasing trend of SI deficits in male adolescents and absent in the adults in our study) and some may be present from the adolescent stage and persist throughout adulthood and may be still present even after a year (object recognition memory deficits in chapter 5) and this may depend on the maturational processes around puberty.

In chapter 4, acute risperidone administration, 30 min prior to testing, was effective in attenuating the neonatal PCP-induced deficits in NOR in both male and female adult rats and SI deficits in female rats. The pro-cognitive effect of risperidone in this model is in agreement with the findings following sub-chronic PCP treatment in adult animals using NOR, reversal-learning and SI tests (Becker & Grecksch 2004; Grayson et al., 2007; Abdul-Monim et al., 2006; McLean et al., 2010).

When patients on typical antipsychotics were switched to risperidone and monitored for four weeks, it was observed that only risperidone improved verbal working memory (Mori et al., 2004). Significant improvements in immediate memory were reported following risperidone. Furthermore, in a comparison of risperidone and haloperidol in stable schizophrenics using a double-blind assessment of fixed and flexible doses, risperidone-treated patients showed greater improvement in verbal learning (Kerns et al., 1999). The mechanism for the pro-cognitive effect of acutely administered risperidone is unclear. In the context of the neurodevelopmental disconnection hypothesis of schizophrenia, atypical antipsychotics (in contrast to conventional antipsychotics) induce neuronal plasticity (i.e. the ability of the nervous
system to adapt to environmental changes) and synaptic remodelling, not only in the striatum but also in the prefrontal cortex and hippocampus (Jiri et al., 2006), suggesting remodelling of neuronal circuits required for this effect rather than mere receptor blockade or changes in neurotransmitter levels. Previous studies have speculated that the cognitive impairments in patients with schizophrenia could possibly be the result of decreased frontal cortical dopaminergic activity, which may be further exacerbated by typical neuroleptic-induced blockade of cortical D₂ receptors. Hence, researchers suggested the use of risperidone to increase cortical dopaminergic activity, possibly due to its ability to block 5-HT₂A receptors (Honey et al., 1999). This increase in dopaminergic prefrontal cortical activity by risperidone has been observed not only in the rat, but also in monkeys (Youngren et al., 1999).

So, to summarise, serotonergic modulation is associated with an increase in striatal DA release and the effects on the negative and cognitive symptoms of schizophrenia relate to DA release in the prefrontal cortex; which can be modulated by D₂/5-HT₂A receptor antagonism (i.e. risperidone), partial D₂ receptor antagonism or the preferential blockade of inhibitory DA autoreceptors (Horacek et al., 2006). In the context of the neurodevelopmental hypothesis of schizophrenia, risperidone, in contrast to haloperidol, induces neuronal plasticity and synaptic remodelling, not only in the striatum but also in other brain areas such as the prefrontal cortex and hippocampus, thereby possibly normalising glutamatergic dysfunction and structural abnormalities and affect the core pathophysiological substrates for schizophrenia.

Neither the mechanisms underlying PCP-induced alterations in behaviour nor NMDA receptor upregulation nor those responsible for the ability of risperidone to alleviate these alterations is clear, although we tried to derive a conceptual mechanism
of action of neonatal PCP and the effect of antipsychotic drugs. Although, further work is required to determine the effects of neonatal PCP administration on the other cognitive domains affected in schizophrenia, the existing data suggest that the model may be useful for identifying agents or mechanisms with the potential to improve cognitive performance in schizophrenia.

6.1.a. Mechanism of action of neonatal PCP treatment – A possible explanation

Phencyclidine has a rich pharmacology; its major action is as a non-competitive antagonist of the NMDA class of glutamate receptor and it also has some effect on several other neurotransmitter systems, namely cholinergic, serotonergic, dopaminergic and GABAergic (Yonezawa et al., 1998). Furthermore, PCP has affinity for the D_2R and the 5-HT_2R and hence there is a strong possibility that the behaviour disrupting effects of PCP are mediated via direct or indirect interactions with all or some of these receptors (Kapur and Seeman 2002). The behavioural data reported in this study is a step towards validating and understanding the neonatal PCP model of cognitive and negative symptoms of schizophrenia, although a clearer understanding of the cellular and molecular mechanisms underlying these deficits is required to further understand the model. However, it is possible to provide a tentative explanation for the observations in this thesis. An attempt to understand the mechanism of action of the NMDA antagonist, PCP and the effect of its neonatal treatment is shown in fig 6.1.
Figure 6.1.b Possible mechanism of action of neonatal PCP

- **NMDA antagonist, PCP**
- **Glutamate**

1. Represents the normal voltage-dependent activation of the NMDA receptor by glutamate, which results in the opening of Ca\(^{2+}\) channels.

2. Non-competitive inhibition of the NMDA receptor by neonatal PCP, which blocks the channel, thereby inhibiting the entry of Ca\(^{2+}\) ions into the cell.

3. As a result of inhibition of the NMDA receptors by PCP, compensatory upregulation of the NMDA receptor allows for the accumulation of toxic levels of intracellular Ca\(^{2+}\) ions, which in turn causes cell death by apoptosis.

4. Neurodevelopmental insult resulting in NOR and social interaction deficits

This figure shows that the exposure of developing brains to NMDA antagonists (such as phencyclidine) causes a compensatory upregulation of NMDA receptors, making cells bearing these receptors more vulnerable to the excitotoxic effects of glutamate, thereby resulting in neuronal cell death via apoptosis.
One of the major hypotheses regarding the pathogenesis of schizophrenia is a neurodevelopmental abnormality. However, the mechanism of onset of schizophrenia symptomatology, in which increased dopaminergic activity in mesolimbic and decreased dopaminergic activity in mesocortical DA systems plays a pathological role is not well understood. However, it has been previously shown that the limbic regions of rodents (one of the major dopaminergic pathways), which includes hippocampus, are developed postnatally (Diamond et al., 1990). Hence, any insult to a part of the limbic system, e.g. the hippocampus or the amygdala during its early developmental stages, could lead to a disturbance in the limbic system, thereby affecting the dopaminergic pathway, which could be speculated to cause cognitive and SI deficits early in life, which persist into adulthood i.e. in our study, the deficits (cognitive and social behaviours) were observed in adolescence and at adulthood. The increase in locomotor activity in the male adolescents (following neonatal PCP 20 mg/kg treatment) and female adolescents (following neonatal PCP 10 mg/kg treatment) (see chapter 2) and not in adults provides evidence to the effect of neonatal PCP treatment on the dopaminergic pathway that influences locomotor activity. The process of neuronal development during early life is well orchestrated. After an initial period of neurogenesis, early prenatal brain development is characterised by cell differentiation, neuronal migration and axon and dendritic branching to form connections to various regions of the brain (Andersen 2003; Bear et al. 2007). These key processes are regulated by radial glia and neurotrophic factors. Neurotrophins, such as nerve growth factor, not only regulate proliferation, differentiation and cell survival, but also control synaptic morphology and plasticity. In the central nervous system, neurotrophins are synthesised predominantly by neurones in an activity dependent manner (Cirulli et al. 2003). Synaptogenesis is prolific during the growth spurt period of brain development,
which is characterised by cell differentiation, growth and elimination, neuronal migration and axon and dendritic branching to form connections with various regions of the brain (Andersen 2003). The immature brain contains an excessive number of neurones, dendrites, axons and synaptic connections. During the brain growth spurt period, elimination of redundant structures (via apoptosis) and rearrangement of connections also occur (Huttenlocher 1984). Glutamate stimulates developmental neuronal outgrowth primarily through NMDA receptors, which are essential for neuronal differentiation and establishment (Pearce et al. 1987). During brain growth spurt periods, neurones containing NMDA receptors are sensitive to both over and under stimulation (Ikonomidou et al. 1989, 1999). Furthermore, developing neurones rely on appropriate glutamate stimulation of NMDA receptors for survival. Interference with or deprivation of NMDA receptor stimulation during the synaptogenesis period can cause apoptosis of neurones that would normally not have been deleted (Ikonomidou et al. 2001). Studies have shown that rats treated with PCP on postnatal day 7 (10 mg/kg subcutaneously) triggers widespread apoptosis in areas such as the frontal, cingulate and retrosplenial cortices (Ikonomidou et al. 1999); of note these areas have all been reported as having altered neurotransmitter receptor expression in schizophrenia (Du Bois & Huang 2009). Therefore, it could be speculated that this alteration in neuronal circuitry in all these areas could be a major contributing factor for the robust memory and SI deficits observed in our study (chapters 2,3, 4,5). Synapse and receptor density continues to increase during development until it reaches a peak at preadolescence, when synaptic pruning gradually reduces levels to that of the adult. This pattern of synapse/receptor development has been observed in humans (Giedd et al.1999), non-human primates (Lidow et al. 1991) and rodents (Andersen et al. 1997;Andersen et al. 2000). The maturation of cognition is related to synaptic remodelling and enhanced
connectivity during this stage of brain development (Andersen 2003). It has been suggested that a fault in synaptic pruning may contribute to the development of schizophrenia (Feinberg 1982; Hoffman and Dobscha 1989; Keshavan et al. 1994). On the other hand, synaptic pruning may simply unmask an already compromised brain by causing a critical number of synaptic connections to be lost (Konradi and Heckers 2003). The symptoms of schizophrenia typically emerge during late adolescence to early adulthood, an age which coincides with synaptic pruning in the brain, as observed in our studies where the memory deficits and social deficits started presenting in the adolescent stage (i.e. PND35-56). In rats, peak expression of NMDA receptors and brain developmental synaptogenesis occur mainly within the first 2 weeks after birth. Accordingly, during this period the brain is vulnerable to NMDA receptor hypofunction-induced apoptosis in the cortex and limbic system (Lee & Choi 1992). It has been shown in rodents that blockade of other major excitatory neurotransmitter systems during developmental brain synaptogenesis does not reproduce such apoptosis. This includes the use of muscarinic receptor, non-NMDA glutamatergic receptor or Ca²⁺-channel blockers, or blockade of a major inhibitory system (Olney 2002). However, GABA AA receptor agonists and sodium channel blockers can also induce apoptosis during the developmental brain synaptogenesis period. It was first thought that NMDA receptor antagonists had no adverse effects on the developing brain. In animals, the excitotoxic vacuole reaction caused by relatively high doses of NMDA receptor antagonists in adults was absent in the very young (Farber et al. 1995a). It has now been consistently shown that when NMDA receptor antagonists like PCP and MK-801 are administered during the first 2 postnatal weeks, which coincides with the brain’s growth spurt period of synaptogenesis, they trigger apoptotic neurodegeneration (Hwang et al. 1999; Ikonomidou et al. 1999; Monti and Contestabile 2000; Wang et al.
2001; Harris et al. 2003; Wang et al. 2003; Hansen et al. 2004; Wang et al. 2004; Wang et al. 2005; Wang and Johnson 2005). Hence, the neonatal PCP dosing regime in our study has altered key pathways via NMDA receptor antagonism as the brain circuitry is affected as a result of the insult at an early age. Also, since the dosing regime was followed for 3 days (PND 7, 9 and 11), i.e. during the critical period of neuronal development, it could have possibly resulted in the induction of immediate early genes (IEGs), thereby increasing the transcription of downstream genes, which have been previously documented to give rise to long-lasting changes in the relevant circuit connections (Grace 2000) thereby causing cognitive and SI deficits from the adolescent stage.

The influence of DA on cortical excitability has been shown to differ between pre- and postpubertal rats (Tseng and O’Donnell 2007). Moreover, the maturation of this response was altered in another developmental model for schizophrenia i.e. neonatal ventral hippocampal lesioned rats (O’Donnell et al. 2002). Neonatal PCP-treated rats have altered D2 receptor expression throughout development which may influence NMDA and GABA receptors at adolescence; however the exact contribution of the dopaminergic system is unclear at this point. Studies have also shown that the adolescent age showed the greatest magnitude of modifications in receptor density in the greatest number of areas (du Bois 2008). This has relevance to the age of onset of schizophrenia, which typically occurs around adolescence or early adulthood. This could be related to the results from our studies, where neonatal PCP treatment has probably affected receptor density in the adolescent stage, thereby altering it to the maximum magnitude giving rise to long-lasting deficits in cognitive and social behaviours (chapters 2, 3, 5). If early life events lead to changes in neurotransmission...
which peak at adolescence, this may explain the vulnerability of this time to environmental stressors that can trigger psychosis. Longitudinal imaging studies in individuals with a high risk of developing psychosis may reveal the relationship between changes in neurotransmission and onset of schizophrenia and other disorders. This is in agreement with the results seen in our study, wherein the neonatal PCP treated rats had robust deficits from the adolescent time point and deficits being persistent long term, i.e. a year after the neonatal PCP treatment. One explanation for the robust deficit due to the blockade of NMDA receptors during the critical period of receptor sensitivity could be either via the induction of IEGs or some unknown mechanism, which resulted in an upregulation of NMDA receptors which led to the permanent changes in the neuronal connections. This blockade could also selectively destroy specific GABAergic neuronal populations, and therefore a decrease in the number of GABA-producing cells may lead to an upregulation of GABAA receptors. In support of this, Wang et al. (2008) established that perinatal PCP treatment resulted in the selective loss of parvalbumin-containing interneurons in cortical brain regions in early adulthood. A loss of or decreased stimulation of GABAergic cells would presumably lead to disinhibition and over-excitation of pyramidal neurones. In the PFC, a reduction in GABAergic transmission would reduce inhibitory inputs thereby leading to cognitive deficits seen in this model (Andersen and Pouzet 2004; Wang et al. 2001; Wiley et al. 2003, chapters 2,4,5). Hence, the suggestion that perinatal blockade of NMDA receptors leads to long-term deficits in glutamatergic and GABAergic neurotransmission has relevance to schizophrenia pathology and treatment.

Although muscarinic compounds were not tested in this study, these receptors are important for cognitive function, and damage to cholinergic pathways or antagonism
of muscarinic receptors produces cognitive deficits in both animals and humans (Moreira et al. 2005). One of the main reasons why muscarinic receptors are vital is because of their interaction with the NMDA receptors in the hippocampus and that they may be particularly important for memory and learning (Markram and Segal 1990). Due to the insult to the neonatal brain via PCP treatment on PND 7, 9 and 11 it could be speculated that probably the interconnectivity between the NMDA and muscarinic receptors has been interrupted thereby possibly resulting in deficits observed in spatial learning and object recognition memory deficits in our study. Furthermore, it has been evidenced that the ability of antipsychotic drugs to improve cognitive symptoms relates, in part, to their ability to augment acetylcholine and DA in the PFC (Li et al. 2007), which probably also reflects deficits in cholinergic transmission, which may in part contribute to the cognitive deficits in this model. Further studies are needed to reveal the exact mechanisms underlying the pathological changes to the cholinergic system in schizophrenia and following neonatal PCP exposure. So, in essence, based on previous studies, it could be deduced that neonatal PCP treatment probably alters the expression of muscarinic receptors, particularly M₁ receptors, by interfering with NMDA, dopaminergic and glutamatergic transmission in the PFC thereby resulting in robust cognitive deficits seen in our study.

Adult PCP treatment was observed to affect the dopaminergic system, by increasing D₂ receptor binding density in the caudate putamen and nucleus accumbens, and also by escalating the DA transporter (DAT) binding density in the ventral tegmental area and caudate putamen (Du Bois et al., 2008). In the developmental model, the neonatal PCP treatment produced widespread changes in the expression of several neurotransmitter receptors (Du Bois et al., 2008). The influence of DA on cortical excitability has been shown to differ between pre- and postpubertal rats (Tseng and O’Donnell 2007). PCP-
treated rats have altered D$_2$ receptor expression throughout development (du Bois et al. 2008), which may influence NMDA and GABA$_A$ receptors at adolescence; however the exact contribution of the dopaminergic system is unclear. One possible explanation could be that the blockade of NMDA receptors during the critical period of receptor sensitivity could by some unknown mechanism, cause an upregulation of NMDA receptors which never returns to normal. This blockade could also selectively destroy specific GABAergic neuronal populations, and therefore a decrease in the number of GABA-producing cells may lead to an upregulation of GABA$_A$ receptors. In support of this, Wang et al. (2008) found that perinatal PCP treatment resulted in the selective loss of parvalbumin-containing interneurons in cortical brain regions in early adulthood. Perhaps a combination of both deficits in glutamatergic transmission and loss of specific neuronal populations contribute to increased binding density of both NMDA and GABA$_A$ receptors.

In summary, it has been demonstrated that neonatal PCP treatment induces cognitive and SI deficits, which present themselves in the adolescent stage and persist long after treatment cessation for up to one year. It has also been demonstrated that risperidone, but not haloperidol was able to reverse the cognitive and SI deficits induced by neonatal PCP. Also, it has been established that female rats showed clear SI deficits when compared to male rats thereby establishing a sex difference in SI deficits following neonatal PCP treatment. Overall, these findings demonstrate that disrupting brain development at an early stage can have long-lasting consequences which are relevant to the study of psychiatric disorders such as schizophrenia, which is thought to be a neurodevelopmental disorder, involving NMDA receptor hypofunction.
7. Further studies

Schizophrenia is a polygenetic disorder with several genes each conferring a small level of risk. These genetic effects combine with early life environmental occurrences to increase the risk of developing schizophrenia later in life. It would therefore be interesting to investigate the effects of a manipulation such as neonatal PCP treatment in an animal with a high genetic loading for schizophrenia. Most gene manipulations, such as knockouts, are performed in mice. Most of the behavioural tests (some of which are used in this thesis) can also be carried out in mice (NOR, SMT, SI, PPI) (Yuede et al., 2010). Many of the schizophrenia risk genes have been manipulated in mice and these transgenic animals would seem to be a next step in animal modelling of schizophrenia in combination with the neonatal PCP model.

The effect of neonatal PCP treatment on brain regions and correlations between region size and behavioural effects requires further investigation, but further work on this could shed light on structural changes in the brains of pre-adolescent, adolescent, adult and aged rats. Functional MRI (fMRI) can be used to investigate regional changes in brain activation following drug administration which could elucidate differences in responses of deficits induced by neonatal PCP.

Our findings in chapter 3 demonstrated sexual dimorphic effects following neonatal PCP treatment, wherein the adult female rats showed significant deficits in SI following neonatal PCP treatment. Findings also showed significant sex difference in the control rats in behaviours such as sniffing, following, avoiding, fighting and object exploration. Studies could be done to determine whether the MeApd synaptic organisation is
sexually dimorphic before puberty following neonatal PCP treatment using whole-cell voltage-clamp recording and quantitative electron microscopy to evaluate synaptic currents and synapse numbers in the prepubertal, adolescent, adult and aged MeApd. Studies could also be done to address the lateralised gonadal steroid receptor expression in rats (i.e. estrogen and androgen receptors both in the control and in the neonatal PCP-induced rats.

In order to better understand the possible alterations in the levels of neurotransmitters, examination of levels of neurotransmitters at different time-points (i.e. adolescents, adults and a year old) following neonatal PCP treatment using in vivo microdialysis would provide further information on the mechanisms underlying receptor changes seen in this model.

A study could be done to examine the efflux of glutamate and serotonin in control and neonatal PCP treated rats performing the NOR task using in-vivo microdialysis. A detailed study could also be carried out to perform in-vivo microdialysis to look at the DA levels since DA levels have been implicated in schizophrenia. Since we also observed a robust SI deficit in female rats, an in-vivo microdialysis experiment performing SI paradigm probing the amygdala region could be done. We have clearly demonstrated object and spatial memory deficits in both sexes following neonatal PCP treatment. It has already been established that object recognition ability depends on hippocampal regions. However, it has also been shown that this recognition ability also involves perirhinal cortex of the brain. It will be interesting to explore DA levels in these regions following neonatal PCP in the object and spatial recognition memory
tasks. This will help us in understanding the mechanism of action and interaction following PCP treatment and the mechanism of object and spatial memory.

It is noteworthy that the neonatal PCP model described here accounts for the developmental aspects of schizophrenia. Given the need to have a model that resembles all aspects of schizophrenia as closely as possible, this appears to be a reasonable model to undertake studies focussing on behavioural outcomes of early PCP neuronal insult.

To summarise, the behavioural data presented here strongly argue for substantial validity of neonatal PCP administration in male and female rats as an effective animal model of aspects of schizophrenia symptomatology.
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