# An Evaluation of Object-Place-Context Recognition as an Animal Model of Episodic Memory Impairment in Schizophrenia

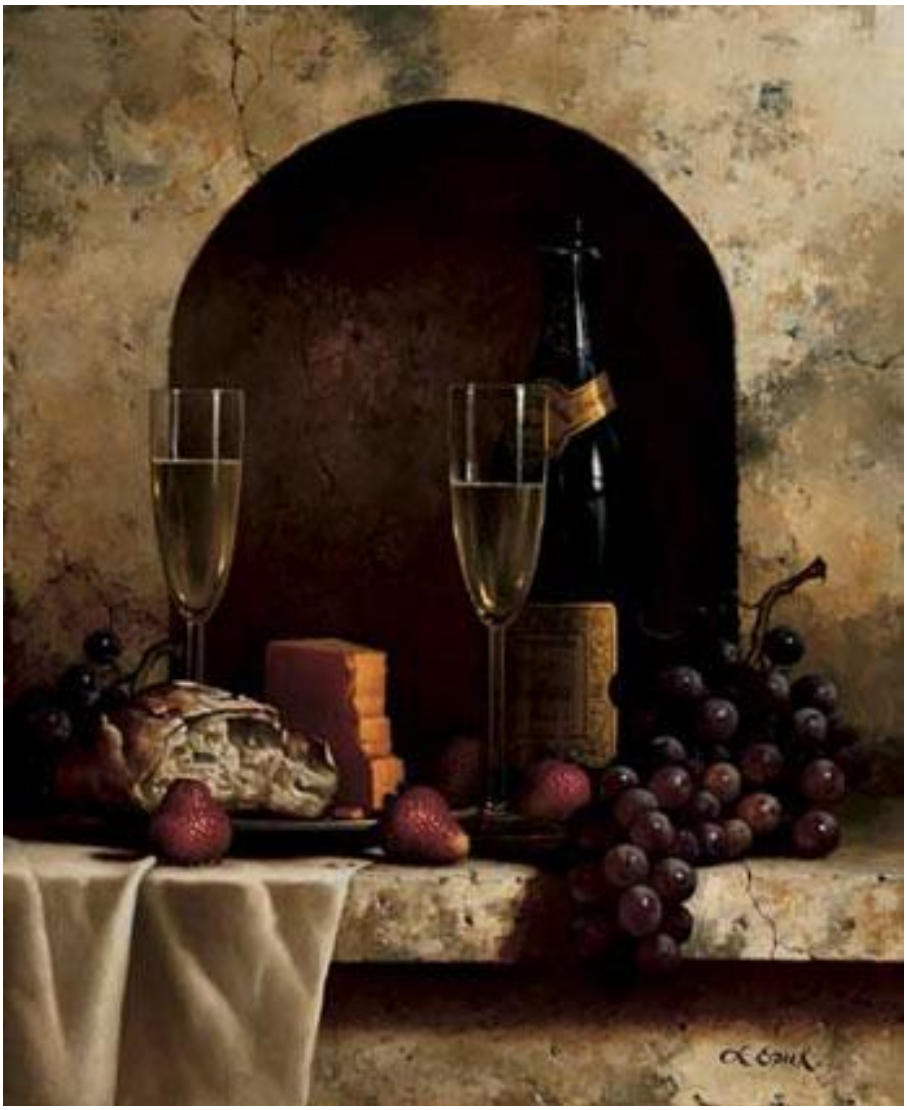
ROMAIN LE COZANNET, MSc.

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

JUNE 2010

*« If you talk to God, you are praying; if God talks to you, you have schizophrenia. If the dead talk to you, you are a spiritualist; if you talk to the dead, you are a schizophrenic. »  Thomas Szasz*

*Extract from: Schizophrenia: The Sacred Symbol of Psychiatry*



A date to remember by Loran Speck

# - ABSTRACT -

In schizophrenia impaired episodic memory is a debilitating cognitive symptom but is poorly controlled by currently available antipsychotic drugs. Episodic memory is the capacity to recall an event in time and place (what-where-when) and can be dissociated between remembering (autonoetic awareness) and knowing (noetic awareness). Withdrawal from subchronic treatment with the NMDA receptor antagonist phencyclidine (PCP) and rearing rats in isolation are widely used as animal models relevant to the pathophysiological origins of schizophrenia. Both PCP withdrawal and isolation rearing produce deficits in a variety of memory paradigms such as object recognition that are reversed by antipsychotic drugs. It is not known whether these treatments produce deficits in tasks that recapitulate the what-where-when aspects that characterise human episodic memory. In this thesis, it has been investigated whether withdrawal from subchronic PCP (5mg/kg i.p. twice daily for 7 days followed by 7 days withdrawal period) and isolation rearing disrupt memory in a task that requires simultaneous integration of memory for object (what), place (where) and context (when) (OPC recognition task). Rats learned to discriminate objects under specific spatial and contextual conditions (two sample phases). The effects of PCP withdrawal and isolation rearing were also examined in an object in place (OP) recognition task in which the context was kept constant (one sample phase). PCP

withdrawal but not isolation rearing rats impaired episodic-like memory in both recognition tasks. However, both PCP withdrawal and isolated rats unexpectedly showed impaired delay dependent reduction in total object exploration in the OPC task but not in the OP task, an index interpreted as being reflective of autonoetic awareness. On the basis of these experiments pharmacological studies with PCP withdrawal induced impairment in the OPC task had been investigated further. The antipsychotic drug clozapine (5 mg/kg) did not reverse PCP withdrawal effects in the OPC task. However the acetyl cholinesterase inhibitor (AChEI) donepezil (0.3 mg/kg), which has been shown to improve episodic memory in humans, did reverse the PCP withdrawal-induced impairment in OPC recognition, suggesting a potential role for AChEIs in treatment of memory impairment in schizophrenia. However, neither clozapine nor donepezil restored the total object exploration index of autonoetic awareness which could suggest that potential beneficial effects of AChEIs may be limited to noetic aspects of memory. In summary, these studies have shown that OPC recognition in rats may have some potential as a behavioural measure for animal models of episodic memory in schizophrenia.

# Acknowledgements

I would like to thank Dr. Paula Moran for accepting me as a PhD student at the University of Nottingham and for her support since the beginning. She has been an incredible supervisor. I also want to thank Professor Kevin Fone, who has always been there when I needed help and for his interesting feedbacks about my work. Thanks to Caitlin Jones who worked with the isolated rats before I used them.

I would like to thank Dr. Matthew O'Callaghan who helped me during my writing up period.

Many thanks to all the friends that I met during my PhD with whom I had a lot of fun during these 4 years in Nottingham with a special ovation to my two workmates who supported me daily during those years: Cecilie and Meghan.

I cannot finish this acknowledgement section without thanking my lovely wife to be and future Mrs Le Cozannet: Elodie Pillet. Many thanks to you my dear for your support and your love.

At last but not least many thanks to my family who has always been there to back me up when I needed.

Et Voila!

# CONTENTS

**CHAPTER 1: GENERAL INTRODUCTION ..... 8**

1.1 What is schizophrenia? ..... 8
1.1.1 Definition ..... 8
1.1.2 Symptomatology of schizophrenia ..... 9
1.1.3 Cause of schizophrenia ..... 10
1.1.4 Neurochemical dysfunction in schizophrenic patients ..... 11
1.1.4.1 Structural brain abnormalities ..... 11
1.1.4.2 Dopamine hypothesis ..... 12
1.1.4.3 Glutamatergic hypothesis ..... 18
1.1.4.3.1 The glutamate hypothesis: NMDA receptor antagonist studies ..... 20
1.1.4.3.2 The glutamate hypothesis: Post Mortem studies ..... 21
1.1.4.3.3 The glutamate hypothesis: Genetic studies ..... 22

1.2 Animal models relevant to schizophrenia ..... 22
1.2.1 Lesion models ..... 25
1.2.2 Developmental models ..... 25
1.2.2.1 Postweaning social isolation rearing ..... 26
1.2.3 Pharmacological animal models ..... 29
1.2.3.1 Glutamatergic Model ..... 30
1.2.3.1.1 Mk-801 model ..... 30
1.2.3.1.2 Ketamine model ..... 31
1.2.3.1.3 Subchronic PCP model ..... 31

1.3 Treatment of schizophrenia ..... 42
1.3.1 Antipsychotics ..... 44
1.3.2 Atypical antipsychotics ..... 45

1.4. Cognitive enhancers ..... 48
1.4.1 Cholinergic system ..... 48
1.4.1.1 Donepezil ..... 50

1.5 Cognitive impairment in schizophrenia ..... 53
1.5.1 Generality ..... 53
1.5.2 Memory ..... 54
1.5.2.1 Distinction between memories: explicit memory ..... 55

1.6. Episodic memory ..... 57
1.6.1 Definition ..... 57
1.6.2 Brain structures involved in episodic memory ..... 59
1.6.2.1 Temporal lobe ..... 59
1.6.2.2 Prefrontal Cortex ..... 60

1.7 Episodic memory deficit in schizophrenia ..... 62

1.8 Episodic memory paradigms ..... 65
1.8.1 Measuring episodic memory in humans ..... 65
1.8.2 Measuring episodic memory in animals ..... 68

1.9 The Object-Place-Context recognition task as a model of episodic memory in rodent? ..... 70

1.10 The aims of the thesis ..... 76

**CHAPTER 2: ESTABLISHEMENT OF THE OBJECT-PLACE-CONTEXT RECOGNITION TASK ..... 77**

2.1 Introduction ..... 77

2.2 Materials and Methods ..... 80
2.2.1 Animals ..... 80
2.2.2 Behavioural Testing ..... 80
2.2.2.1 Apparatus ..... 80
2.2.2.2 Habituation ..... 82
2.2.2.3 Object-Place-Context recognition task ..... 82
2.2.3 Data analysis ..... 86

2.3 Results ..... 87

2.4 Discussion ..... 90
2.4.1 Conclusion ..... 93

**CHAPTER 3: PCP WITHDRAWAL RELEVANT MODEL TO SCHIZOPHRENIA IN OPC AND OP RECOGNITION TASKS ..... 94**

3.1 Introduction ..... 94

3.2 Materials and Methods ..... 96
3.2.1 Animals ..... 96
3.2.2 Drug administration ..... 96
3.2.2.1 Subchronic PCP treatment ..... 96
3.2.3 Experiment 2: PCP withdrawal model relevant to schizophrenia in OPC recognition task ..... 97
3.2.3.1 Behavioural testing ..... 97
3.2.4 Experiment 3: PCP withdrawal model relevant to schizophrenia in OP recognition task ..... 100
3.2.4.1 Behavioural testing ..... 100
3.2.4.1.1 Habituation ..... 100
3.2.4.1.2 OP recognition task ..... 100
3.2.5 Data analysis ..... 103

3.3 Results ..... 105
3.3.1 Experiment 2: PCP withdrawal rats in OPC recognition task ..... 105
3.3.2 Experiment 3: PCP withdrawal rats in OP recognition task ..... 108

3.4 Discussion ..... 111
3.4.1 New component discrimination ..... 112
3.4.2 Total object exploration ..... 114
3.4.3 Conclusion ..... 115

**CHAPTER 4: ISOLATION REARING MODEL RELEVANT TO SCHIZOPHRENIA IN OPC AND OP RECOGNITION TASKS ..... 117**

4.1 Introduction ..... 117

4.2 Materials and Methods ..... 118
4.2.1 Animals ..... 118
4.2.2 Behavioural tasks before the OPC or the OP recognition task .. 119
4.2.3 Experiment 4 and 5: Isolation rearing model relevant to schizophrenia in OPC recognition task ..... 120
4.2.3.1 Behavioural testing ..... 120

4.3 Results ..... 121
4.3.1 Experiment 4: Isolated rats in OPC recognition task ..... 121
4.4.2 Experiment 5: Isolated rats in OP recognition task ..... 124

4.4 Discussion ..... 127
4.4.1 New component discrimination ..... 127
4.4.2 Total object exploration ..... 130
4.4.3 Conclusion ..... 131

**CHAPTER 5: THE EFFECT OF ANTIPSYCHOTIC CLOZAPINE ON PCP WITHDRAWAL-INDUCED EPISODIC MEMORY DEFICIT IN RATS ..... 132**

5.1 Introduction ..... 132

5.2 Materials and Methods ..... 134
5.2.1 Animals ..... 134
5.2.2 Subchronic PCP treatment ..... 135
5.2.3 Experiment 6: Effect of clozapine on PCP withdrawal rats in OPC recognition task ..... 135
5.2.3.1 Clozapine ..... 135
5.2.4 Behavioural testing: OPC recognition ..... 136
5.2.5 Data analysis ..... 136

5.3 Results ..... 137

5.4 Discussion ..... 142
5.4.4 Conclusion ..... 143

**CHAPTER 6: THE EFFECT OF DONEPEZIL ON PCP WTHDRAWAL-INDUCED EPISODIC MEMORY DEFICIT IN RATS..... 144**

6.1 Introduction..... 144

6.2 Materials and Methods..... 145
6.2.1 Animals..... 145
6.2.2 Subchronic PCP treatment..... 146
6.2.3 Experiment 7: Effect of donepezil on PCP withdrawal rats in OPC recognition task..... 146
6.2.3.1 Donepezil..... 146
6.2.4 Behavioural testing: OPC recognition..... 147
6.2.5 Data analysis..... 147

6.3 Results..... 148

5.4 Discussion..... 153
5.4.4 Conclusion..... 155

**CHAPTER 7: LOCOMOTOR ACTIVITY TASKS IN PCP-WITHDRAWAL RATS..... 156**

7.1 Introduction..... 156

7.2 Materials and Methods..... 158
7.2.1 Animals..... 158
7.2.2 Apparatus..... 158
7.2.3 Drug administration..... 158
7.2.3.1 PCP..... 158
7.2.3.2 Clozapine..... 159
7.2.3.3 Donepezil..... 159
7.2.4 Behavioural testing..... 160
7.2.4.1 Experiment 8 and 9: PCP (3.2 mg/kg) challenge after OPC task with clozapine and with donepezil..... 161
7.2.4.2 Experiment 10-11: LMA after clozapine (5 mg/kg) and donepezil 0.3 mg/kg..... 162
7.2.5 Data analysis..... 163

7.3 Results..... 164
7.3.1 Experiment 8: PCP (3.2 mg/kg) challenge after OPC task under clozapine.....f.... 164
7.3.2 Experiment 9: PCP (3.2 mg/kg) challenge after OPC task under donepezil..... 166
7.3.3 Experiment 10: LMA after Clozapine (5 mg/kg)..... 168
7.3.4 Experiment 11: LMA after donepezil (0.3 mg/kg)..... 170

7.4 Discussion..... 171
7.4.1 Conclusion..... 172

**CHAPTER 8: GENERAL DISCUSSION.....173**

8.1 Differentiation between the effects of PCP withdrawal and isolation rearing in the OPC and OP recognition task ..... 174

8.2 Differentiation between OPC recognition task and OP recognition task ..... 177

8.3 Total object exploration time is delay dependent in the OPC task but not in the OP task ..... 179

8.4 Donepezil but not clozapine restore the PCP-induced deficit in episodic memory but not the delay-induced deficit in total object exploration..... 189

8.5 Long lasting impairment in locomotor activity induced by subchronic PCP treatment is not restored by chronic clozapine or donepezil injections ..... 193

8.6 Conclusion..... 195

**REFERENCES..... 197**

**APPENDIX.....226**

1.1 Material, method and results of the behavioural task that isolated rats performed before the OPC task and OP task. ..... 226
1.1.1 Appendix 1: Locomotion and Rearing Activity in a Novel Environment on PND 58..... 226
1.1.1.1 Data Analysis..... 227
1.1.1.2 Result ..... 228
1.1.2 Appendix 2: Recognition of a Novel Object in a Familiar Environment on PND 59..... 229
1.1.2.1 Data Analysis..... 231
1.1.2.2 Result ..... 232
1.1.3 Appendix 1.3: Prepulse Inhibition of the Acoustic Startle Response on PND 65 ..... 233
1.1.3.1 Data Analysis..... 235
1.1.3.2 Result ..... 236
1.1.4 Appendix 4: Conditioned Emotional Response Paradigm on PND 72 ..... 237
1.1.4.1 Data Analysis..... 239
1.1.4.2 Result ..... 239

2.1 Drug treatments by rats received from the subchronic PCP treatment, the OPC task and LMA task after clozapine and donepezil..... 241

# Abbreviations

| | |
|---|---|
| **5-HT** | 5-hydroxytryptamine |
| **AChEI** | acetylcholinesterase inhibitor |
| **AMI** | Autobiographical Memory Inventory |
| **CNTRICS** | Cognitive neuroscience treatment research to improve cognition in schizophrenia |
| **DAAO** | D-amino acid oxidase |
| **DAT** | Dopamine transporters |
| **dBB** | Diagonal band of Broca |
| **DI** | Discrimination Index |
| **DSPT** | Digit Span Distraction Test |
| **GABA** | Gamma-aminobutyric acid |
| **GCP II** | Glutamate carboxypeptidase II |
| **GMS** | Glycine modulatory site |
| **HPC** | Hippocampus |
| **LMA** | locomotor activity |
| **LTP** | Long term potentiation |
| **mACh receptors** | Muscarinic acetylcholine receptors |
| **MATRICS** | Measurement and treatment research to improve cognition in schizophrenia |
| **mPFC** | Medial PFC |
| **MK-801** | Dizocilpine maleate |

| | |
|---|---|
| **MS** | Medial septum |
| **NAA** | N-acetylaspartate |
| **NAAG** | N-acetyl-aspartyl glutamate |
| **nACh receptors** | Nicotinic acetylcholine receptors |
| **nBM** | Nucleus Basalis of Meynert |
| **NVH** | neonatal ventral hippocampal |
| **NMDA** | N-methyl-D-aspartate |
| **OP** | Object in place |
| **OPC** | Object-place-context |
| **PCP** | Phencyclidine |
| **PET** | Positron emission tomography |
| **PFC** | Prefrontal cortex |
| **PND** | Post natal day |
| **PPI** | Prepulse inhibition |
| **RAVLT** | Rey Auditory Verbal Learning Test |
| **R/K** | Remember/Know |
| **RBMT** | Rivermead Behavioural Memory Test |
| **ROC** | Receiver Operating Curve |
| **SPECT** | Single photon emission computed tomography |
| **WCST** | Wisconsin Card Sorting Test selective attention |

# - CHAPTER 1 -

# GENERAL INTRODUCTION

## 1.1 What is schizophrenia?

### *1.1.1 Definition*

Schizophrenia is a psychotic disorder with a prevalence around 0.7% of the adult population in the world. Though the annual incidence is low, between 0.016% and 0.042% in the world population, the prevalence is high due to chronicity (World Health Organisation 2009).

Schizophrenia was first described as dementia praecox (early dementia) by Emil Kraepelin but the term schizophrenia was used by a Swiss psychiatrist, Eugen Bleuler. It is derived from the Greek terminology: *"skhizein"* which means split and *"phrên"* meaning mind. The mind split in schizophrenia refers to a division between thinking and affect resulting in an inappropriate expression of affect.

### *1.1.2 Symptomatology of schizophrenia*

The hallmark features of this mental illness are hallucinations and delusions which induce impaired judgement and loss of contact with reality. Symptoms are typically manifest between the ages of 16 and 30. It is found more frequently and more severely in men (Nicole et al., 1992, Post, 2001). Men also develop schizophrenia slightly earlier than women around 18 years of age in men and around 25 years of age in women. However, women have a higher incidence after 30 (Sham et al., 1994).

Symptoms can be divided into three main categories: psychotic or “positive” symptoms, deficit or “negative” symptoms and cognitive impairments (Crow, 1980, Andreasen, 1982 , Liddle, 2002).

- *Positive symptoms* are additional behaviours and thoughts not seen in the general population resulting in hallucination, delusions, bizarre behaviour and positive formal thought disorder (Kay, 1990, Mitchell et al., 2001).

- *Negative symptoms* are lack of drive and behaviour seen in the general population such as affective flattening, alogia, avolition-apathy and anhedonia asociality (Kay, 1990, Ananth et al., 1991, Mitchell et al., 2001).

- *Cognitive dysfunctions* in attention, language, memory and executive functions have been reported (Gold et al., 1992, Clare et al., 1993, Milner et al., 1998). These cognitive impairments are of particular

significance for the disease as they are more closely associated with poor outcome in patients than are the other symptoms such as hallucinations or delusions (Green et al., 2004, Berenbaum et al., 2008).

All these deficits have a strong impact on daily social life, education and employment. The negative and cognitive symptoms tend to be more persistent and chronic (Breier et al., 1991), However, although the psychotic symptoms have an episodic pattern they are usually the impetus for hospitalization when active (Andreasen, 1995).

### *1.1.3 Cause of schizophrenia*

The cause is still unknown. Although, hypotheses suggest that genetic (Guidry and Kent, 1999), viral such as influenza virus (Pearce, 2001) or pre-natal environmental (Weissman et al., 2006) factors are important. Alternatively, these factors could create a vulnerability to develop schizophrenia in adulthood. Exogenous factors (e.g. drugs, social isolation, stress…) may increase vulnerability and could be cause of schizophrenic symptom expression which appears when young adults are at their most vulnerable (beginning of independence) and finding their identity (Asarnow and Goldstein, 1986, Harris et al., 1986) (see Figure 1.1).

It is also generally accepted by researchers that schizophrenic symptoms can be induced by disturbance in neurochemical and/or structural brain architecture.

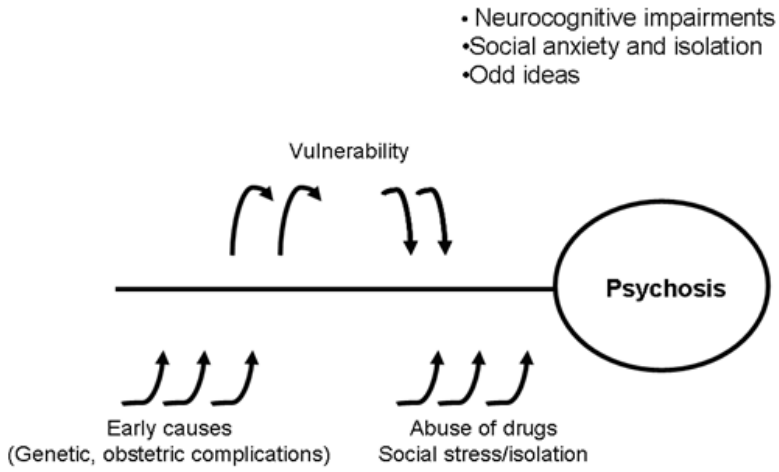



**Figure 1.1: The developmental origins of schizophrenia (From Howes et al., 2004).**

### *1.1.4 Neurochemical dysfunction in schizophrenic patients*

#### 1.1.4.1 Structural brain abnormalities

A number of studies have shown abnormal cerebral morphologies in schizophrenic patients (Ward et al., 1996, Velakoulis et al., 2000). Schizophrenic patients have been shown to have a significant reduction of brain and intracranial size compared to a control group. These have been suggested to result from genetic and environmental factors which can be progressive (Ward et al., 1996, Velakoulis et al., 2000). Furthermore there is ventricular enlargement, notably the third ventricle and lateral ventricles which affect the adjacent brain regions such as

temporal cortex and HPC (hippocampus) (Weinberger et al., 1979, Weinberger et al., 1980, Shenton et al., 2001). The prefrontal cortex (PFC) of schizophrenic patients, which is implicated in organisation of action and memory, shows a reduced volume and a progressive decrease of gray matter volume (Gur et al., 2000, Thompson et al., 2001).

#### 1.1.4.2 Dopamine hypothesis

The dopaminergic system consists of four main pathways, dopaminergic neurons project from the ventral tegmental area (VTA) to the limbic system (mesolimbic pathway) and to the PFC (mesocortical pathway), the nigrostriatal pathway involved in the control of motivity and which is depleted in Parkinson's disease (Kim et al., 2003), and the tuberoinfundibular pathway which transmits dopamine from the hypothalamus to the pituitary gland and influences the secretion of the hormone prolactine (Gonzalez et al. 2004).

The effect of dopamine is mediated through activation of dopamine receptors. There are two main families of dopamine receptors: D1-like (D1 and D5) and D2-like (D2, D3 and D4). The division is based on their inhibitory or excitatory function effect on adenyl cyclase. D1-like (D1 and D5) linked to Gs-protein (s=stimulant) and D2-like (D2, D3 and D4) linked to Gi-protein (i=inhibitory) (Contreras et al., 2002, Nieoullon, 2002).

Three pharmacological lines of evidence support the dopamine hypothesis:

The first line of evidence comes from amphetamine users and postulates an abnormal release of dopamine by dopaminergic neurons. Amphetamine is an indirect dopamine agonist that induces an increased release of dopamine by an action through the dopamine transporter (DAT) (Schmitz et al., 2001) and inhibits dopamine reuptake. Amphetamine, also disrupts vesicular monoamine transporter inducing an increasing released of dopamine into the neuronal cytoplasm (Feldman et al., 1997) Amphetamine abuse induces and exacerbates schizophrenic symptoms respectively of non-psychotic individuals and schizophrenic patients (Bell, 1973, Crow, 1980).

The second line of evidence comes from the efficacy, on the positive symptoms of schizophrenia, of traditional antipsychotic drugs such as haloperidol and chlorpromazine which are dopamine D2 receptor antagonists (Carllson and Lindqvist, 1963, Carlsson, 1978, Seeman et al., 1987, Talbot and Laruelle, 2002). The dopamine D2 receptor antagonist effect of antipsychotics and their efficacy in regulating symptoms suggests dopaminergic hyperactivity in schizophrenia (Carlsson, 1978).

Third line of evidence comes from post mortem studies which describe an abnormal elevated level of dopamine D2 receptors in schizophrenic brain striatum (Seeman et al., 1987). Neuroimaging studies in psychotic patient (e.g. positron emission tomography (PET) and single photon emission computed tomography (SPECT))

highlighted an abnormal level of dopamine release with an exacerbation during psychosis (Abi-Dargham et al., 1998, Abi Dargham, 2002).

According to the dopaminergic hypothesis the dopamine hyperactivity in mesolimbic regions of the brain is leading to the positive symptoms of the disease (treated by antipsychotic) while hypoactivity in prefrontal cortical regions is leading to negative and or cognitive symptoms of the disease (which are antipsychotics resistant) (Weinberger, 1988, Ananth et al., 1991, Gold and Weinberger, 1995).

An analysis of dopamine receptors in schizophrenic brain using PET and SPECT has shown less dopamine D1 receptor expression in PFC in schizophrenia confirming the dopamine hypoactivity in PFC (Okubo et al., 1997). On the other hand, the majority of the studies fail to show a difference in dopamine D2 receptor density in schizophrenic patients in PFC (Martinot et al., 1990, Hietala et al., 1994, Nordstrom et al., 1995). However, higher occupancy of dopamine D2 receptor is observed in patients with schizophrenia experiencing an episode of illness exacerbation compared with healthy controls. This could be a consequence of higher levels of free dopamine in the vicinity of D2 receptors or/and higher affinity of D2 receptors for dopamine (Abi-Dargham et al., 2000). A complementary study by Laruelle et al. (2000) has focused on the role of dopamine transporter (DAT) which controls dopamine concentration in the synaptic cleft (Laruelle et al.,

2000). There is no increase of DAT density between schizophrenic patients and control. Furthermore, there is no relationship between amphetamine induced dopamine release and DAT density suggesting dopamine hyperactivity is associated with a deregulation of dopamine neurons rather than an increase in the number of these neurons (Laruelle et al., 2000).

Further evidence suggests that the dopaminergic deregulation observed in schizophrenia could be the result of the dysconnectivity of cortico-subcortical and intracortical networks, with a main deregulation in PFC (Nieoullon, 2002). This postulate suggests a subcortical hyperactivity of dopaminergic neurons to compensate for cortical hypoactive dopaminergic neurons (Weinberger et al., 1994, Marsden, 2006) (Figure 1.2).

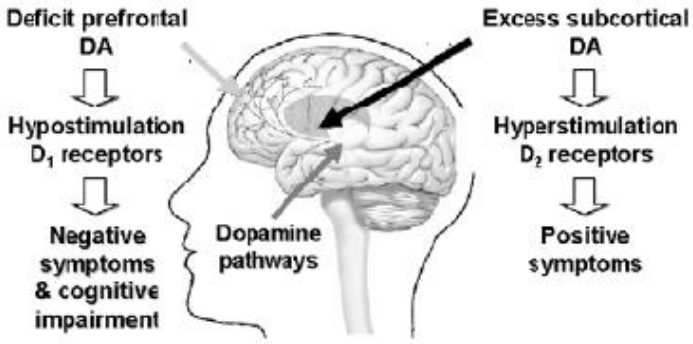



**Figure 1.2: Dopaminergic imbalance in schizophrenia. Alteration in cortical DA transmission may contribute to a reactional process increasing striatal DA transmission. Such hyperactivity of subcortical DA transmission could likely compensate for deficits related to cortical DA depletion and consequent frontal hypoactivity, but could also contribute to behavioural impairment (From Abi-Dargham, 2002).**

The activity of midbrain dopaminergic neurons is under the control of PFC, via an activating pathway involving direct glutamatergic projections into dopaminergic cells and an indirect inhibitory pathway involving γ-aminobutyric acid (GABA)ergic interneurons via the striatum. The indirect inhibitory GABAergic pathway is also under the control of the glutamatergic system from the PFC (Marsden, 2006). After amphetamine administration, dopaminergic cells are inhibited via the inhibitory pathway and by their presynaptic D2 autoreceptors, to reduce the dopamine released. Ketamine, an N-methyl-D-aspartate receptor (NMDA receptor) antagonist, potentiates the DA release produced by amphetamine showing similar response seen in schizophrenic patients (Kegeles et al., 2000). These data indicate that dopaminergic dysfunction in schizophrenia could result from a disruption of the glutamatergic and GABAergic neuronal systems (Figure 1.3).

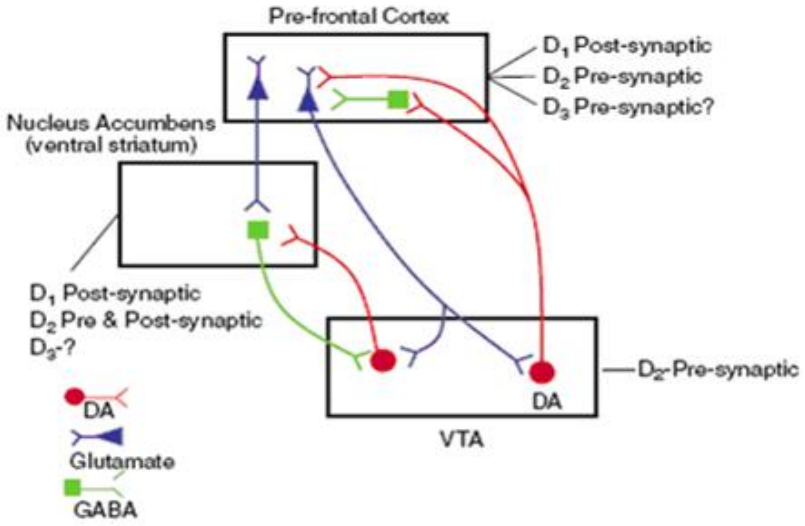



**Figure 1.3: Simplified diagram showing the neural pathways linking the prefrontal cortex, the nucleus acumens and the ventral tegmental area (VTA) (From Marsden, 2006).**

As previously mentioned D2 receptor antagonists can reduce the positive symptoms of schizophrenia (Abi-Dargham et al., 2000, Abi Dargham, 2002), while the negative symptoms are associated with reduced dopamine function in the PFC (Brozoski et al., 1979, Lynch, 1992). It has been shown that the D1 receptor agonist SKF38393 reduces extracellular concentration of glutamate and GABA in mPFC which can be reversed by the D1 receptors antagonist SCH23390 (Abekawa et al., 2007) and that D2/D3 antagonism increases glutamate function via an inhibition of the GABAergic system (Hatzipetros and Yamamoto, 2006).

### 1.1.4.3 Glutamatergic hypothesis

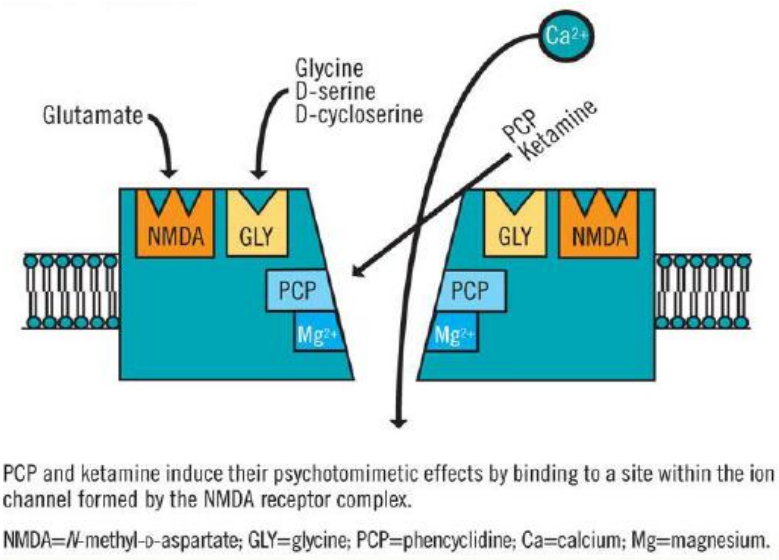
Glutamate is one of principal excitatory neurotransmitters of the central nervous system (CNS). Two groups of receptors mediate glutamatergic transmission: ligand-gated ion channels (NMDA, AMPA and kainate) and G-protein linked receptors (mGluRs) (Pin and Duvoisin, 1995).

At present, eight mGluR subtypes have been identified by molecular cloning (mGluR1-8) (Pin and Duvoisin, 1995). They are classified into three groups according to their sequence homology, mechanisms of signal transduction and pharmacology. MGluRs regulate glutamate activity. Glutamate is implicated in diverse processes such as: synaptic plasticity, neuronal death or control of cardiac activity (Pin and Duvoisin, 1995).

The NMDA receptor is responsible for the early and the late phases of Long Term Potentiation (LTP) that define the adaptive plasticity. NMDA receptor can also contribute to the excitatory postsynaptic currents (EPSCs) and dendritic spikes (Squire, 1992, Coyle, 2006). It has been consistently demonstrated that glutamatergic hypoactivity induced by glutamatergic antagonists mimic schizophrenic's symptoms (Luby et al., 1959, Javitt and Zukin, 1991, Coyle and Tsai, 2004, Coyle, 2006, Javitt, 2007).

The NMDA receptor is a tetramer containing two subunits: NR1, which is required for channel function and NR2A-D, which affects the biophysical and pharmacological characteristics of the NMDA receptor. To be activated, the NMDA receptor requires both glutamate and

glycine binding at two distinct sites, the NR2A-D and “glycine modulatory site” (GMS) on the NR1 subunit (Coyle et al., 2002). This co-binding will release $Mg^{2+}$ from the channel and allow the $Ca^{2+}$ entry into in the post-synaptic neuron (Lynch and Guttmann, 2001) (Figure 1.4.).




PCP and ketamine induce their psychotomimetic effects by binding to a site within the ion channel formed by the NMDA receptor complex.

NMDA=*N*-methyl-D-aspartate; GLY=glycine; PCP=phencyclidine; Ca=calcium; Mg=magnesium.

**Figure 1.4: Schematic model of the NMDA receptor complex showing the binding sites for glutamate, glycine and D-serine (From Javitt et al., 2007).**

Synaptic glutamate is synthesized from glutamine supplied by astrocytes. Another process to synthesize glutamate involves N-acetyl-aspartyl glutamate (NAAG), an abundant neuropeptide in the mammalian nervous system. NAAG is catabolised to N-acetylaspartate (NAA) and glutamate by glutamate carboxypeptidase II (GCP II) and possesses a glycine reversible antagonist effect on the NMDA receptor as well as an agonist effect at the mGluR3 receptor which both induce negative NMDA receptor modulation (Bergeron et al., 2005).

To regulate this system, the astrocytes play a central role by providing lactate for oxidative metabolism and glutamine. They are involved in the control of NMDA receptor activity by expressing glutamate (excitatory amino acid transporters: EAAT 1 and 2) and glycine transporters (GlyT1); by synthesizing and / or catabolising D-serine via two enzymes located in the astrocyte racemase and D-amino acid oxidase (DAAO) respectively (Coyle, 2006); and by expressing GCP II which controls the NAAG concentration (Bergeron et al., 2005).

The NMDA receptor hypofunction hypothesis for schizophrenia comes from three main different types of investigation: NMDA receptor antagonist studies, post-mortem studies and genetic studies with schizophrenic patients compared to control groups.

*1.1.4.3.1 The glutamate hypothesis: NMDA receptor antagonist studies*

Phencyclidine (PCP) was first synthesised in 1926. In mid 1950s the pharmaceutical organisation Parke-Davis developed PCP (sernyl, name which derived from “serenity”) as a human anaesthetic. In 1957, there were problems during the first test on human subjects, when studies showed PCP could induce psychotic effects which mimic schizophrenic symptoms (Luby et al., 1959).

The emerging pharmacology understanding of NMDA receptors revealed that PCP was an NMDA antagonist thereby suggesting an endogenous dysfunction of NMDA receptor in psychosis (Javitt and

Zukin, 1991). Ketamine, another NMDA receptor antagonist, in healthy humans and schizophrenic patient also consistently evokes negative symptoms and cognitive symptoms of schizophrenia, as well as positive symptoms (Krystal et al., 1994, Newcomer et al., 1999, Curran and Morgan, 2000, Krystal et al., 2005, Javitt, 2007). This idea suggests that dopaminergic deregulation observed in striatal and prefrontal systems in schizophrenia results from a NMDA receptor dysfunction (Javitt, 2007).

*1.1.4.3.2 The glutamate hypothesis: Post Mortem studies*

Post mortem studies indicate significant changes in glutamate receptor binding, transcription and subunit protein expression in the PFC, thalamus and HPC of subjects with schizophrenia. There is a decrease of NR1 subunits of the NMDA receptor in HPC and PFC (Bergeron et al., 2005, Lewis and Moghaddam, 2006). In schizophrenic subjects, GCP II, which catabolises NAAG into glutamate and NAA, is reduced in frontal cortex, HPC and temporal cortex in a comparison of to suitable controls (Coyle, 2006). The NAAG over expression implicates a hypo-glutamatergic activity via its action of MGluR3 agonist and glycine reversible antagonist of the NMDA receptor. It was additionally show that there is an over expression of the glutamate transporters EAAT 1 and 2 in thalamus which compromises glutamatergic transmission (Meador-Woodruff et al., 2003).

*1.1.4.3.3 The glutamate hypothesis: Genetic studies*

Several genetic studies demonstrate an association with different genes and the risk for schizophrenia. The gene G72 is over expressed in PFC (Coyle and Tsai, 2004, Coyle, 2006). G72 gene codes for a protein that activates DAAO, the enzyme that catabolises D-serine. A reduction of D-serine would lead to NMDA receptor hypofunction (Stevens et al., 2003). The genes GRM3 or 7 which code for metabotropic glutamate receptorsb (Fuji et al., 2003; Ganda et al., 2009), as well as SLC1A1, A2 and A3 which code for glutamate transporters (EAAT1, 2 and 3) (Deng et al., 2004; Deng et al., 2007) have been shown to be linked to schizophrenia.

To conclude, the glutamatergic hypofunctionality in schizophrenia is thought to cause an indirect down regulation of GABAergic function, of mesolimbic dopamine hyperactivity and via a direct pathway of cortical dopaminergic hypoactivity (Kegeles et al., 2000, Lewis and Moghaddam, 2006, Marsden, 2006).

## 1.2 Animal models relevant to schizophrenia

Animal models are important both for the identification of novel therapeutic strategies and for the understanding the pathophysiology of symptoms. At the present time, there are no animal models that

capture all the features of schizophrenia, or indeed any psychiatric illness (Geyer and Moghaddam, 2002, Wong and Van Tol, 2003).

The validity of a model consists of three levels of validity: predictive, face and construct validity (Willner, 1991). Predictive validity concerns predictions of pharmacological manipulations, these should replicate clinical findings. A good predictive model should include no false positive or false negative results. For example animal model relevant to schizophrenia should respond positively toward typical (e.g. haloperidol) and atypical (e.g. clozapine) antipsychotic on behavioural tasks that assess the positive symptoms and negative symptoms but not the cognitive symptoms. Face validity concerns the phenomenological similarities between the model and the disease such as similarities of symptoms. There should be as many similarities between the disease and the model with a minimum of dissimilarities. Construct validity is considered the highest level of validity and refers to the underlying pathological state of the disease. In the sense that biochemical changes should reflect those observed in the disease and induce a change in behavioural experiments. The combination of all different models of schizophrenia allows the understanding of pathophysiological mechanisms and neurobiology of the disease (Willner, 1991). In rats there are three main types of model: Lesion, developmental and pharmacological models. Table 1.1 below recapitulates the advantages and the disadvantages of those models.

| Type | Model in rats | Positive aspect | Negative aspect |
|---|---|---|---|
| *Pharmacological model* | PCP model | Based on the glutamatergic hypothesis | different routes and dosages induce different symptoms |
| | Amphetamine model | Based on the dopaminergic hypothesis | |
| *Developmental model* | Isolation-rearing | Developmental model rather drug administration or lesion | reversibility of re-socialization<br><br>long time before using this model |
| *Lesion models* | NVH | Particular interest because of the relation between striatum and HPC DA innervations and the core feature of schizophrenia | Size of the lesion and invasive effect can induce different results |
| | mPFC | Represent the structural abnormalities seen in schizophrenia and disturb striatal DA regulation | |

**Table 1.1: Overview of advantages and disadvantages of the three animal models relevant to schizophrenia in rats: pharmacological, developmental and lesion model.**

### *1.2.1 Lesion models*

Historically, lesion studies have contributed to our understanding of the pathophysiology of various brain regions in different diseases, and this is also the case in schizophrenia (Geyer and Moghaddam, 2002, Wong and Van Tol, 2003).

Psychosis lesion models include: neonatal ventral hippocampal (NVH) or medial PFC (mPFC) lesions. There is a close structural interconnection between these two structures (e.g. glutamatergic pathways from the HPC to the PCF), lesions of one region affects function of the other one (Jay et al., 1992). This must be taken into consideration in lesion studies (Lipska, 2004). However, Neonatal ventral hippocampal lesion results in prepulse inhibition (Béracochéa et al.), latent inhibition, delayed alternation and social contact deficit which are representative tasks of schizophrenic positive and negative symptoms in animals (Lipska and Weinberger, 2000).

### *1.2.2 Developmental models*

As previously mentioned, early life events influence brain development and subsequent adult behaviour and play an important role in the causation of certain psychiatric disorders including schizophrenia. With the aim of understanding these early environmental factors, some developmental based models of central nervous system disorders in rat have been proposed. These models focus on environmental

manipulations like isolation rearing, maternal malnutrition or hypoxia, which induce psychotic like disorder and furthermore are associated with neurochemical changes in the dopamine system (Geyer and Moghaddam, 2002, Wong and Van Tol, 2003).

Geyer (Geyer et al., 1993) was the first to show PPI impairment in the developmental model of rat pups reared in isolation when tested in adulthood (Geyer et al., 1993). Furthermore, the main disadvantage of the developmental model is the difficulty to reproduce the model and the long delay before use; however the etiological validity of the model is not negligible (Geyer et al., 2001). The postweaning social isolation rearing model is used in the Chapter 4 of the thesis.

#### 1.2.2.1 Postweaning social isolation rearing

Isolation rearing model in rats is a neurodevelopmental model of schizophrenia. Early social isolation mimics the early environmental stress which will induces an alteration of brain development resulting in behavioural deficits. Isolated rats develop long-term changes relevant to schizophrenia symptoms and it is used as a model relevant to schizophrenia in rats (Lapiz et al., 2003, Day-Wilson et al., 2006, Fone and Porkess, 2008).

Isolation rearing induces long term neurobiological, neurochemical and behavioural changes in rats. Isolated rats show a hyperfunction of mesolimbic dopaminergic systems (Jones et al., 1992, Hall, 1998), a

hypofunction of mesocortical dopaminergic systems (Heidbreder et al., 2000, Peters and O'Donnell, 2005), and glutamatergic hypofunction in the PFC (Melendez et al., 2004, Levine et al., 2007) as well as a decrease of dopamine turnover in the mPFC (Heidbreder et al., 2000), which all mimic neurobiological dysfunctions observed in schizophrenia. Post mortem analysis of isolated rats compared with group housed rats revealed alterations in dopamine level, 5-hydroxytryptamine (5-HT) in the nucleus accumbens and striatum (Jones et al., 1992, Leng et al., 2004). These abnormalities lead to behavioural impairment such as hyper-reactivity to novel environment (Gentsch et al., 1982), reduced PPI of startle (Geyer et al., 1993) and some cognitive deficits such as object recognition (Bianchi et al., 2006, McLean et al., 2008). The schematic overview designed by Fone and Porkess (Fone and Porkess, 2008) represents the behavioural and neurochemical changes after social isolation compared to group housed rats (Figure 1.5).

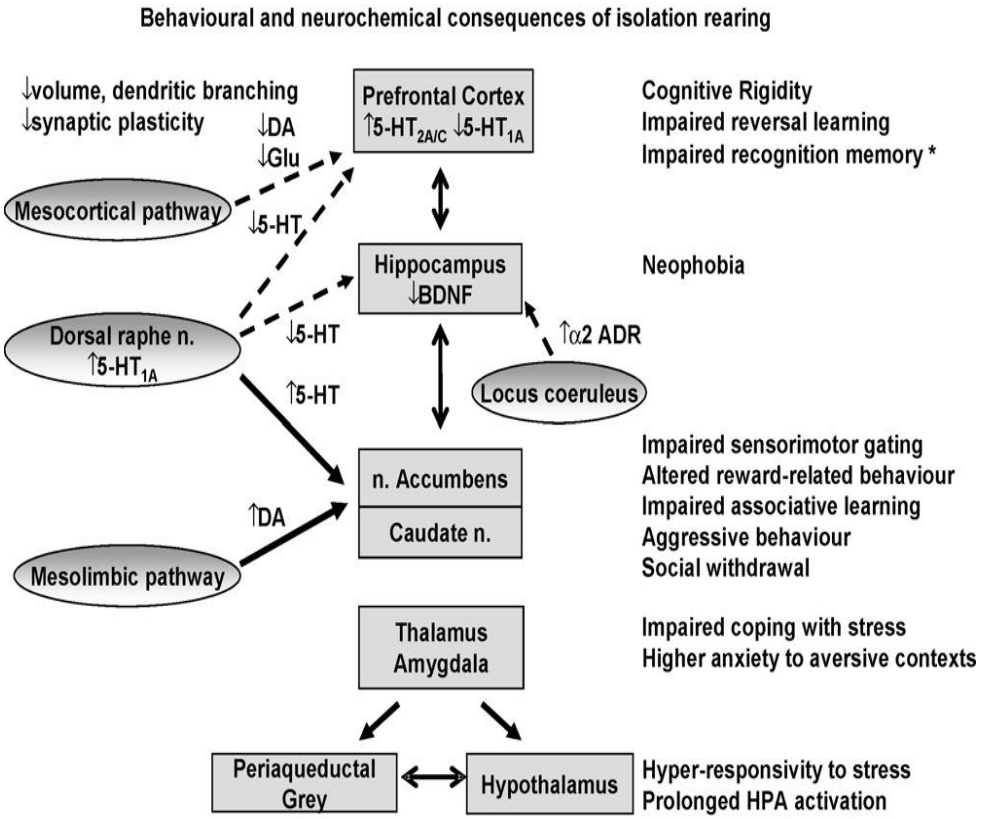



**Figure 1.5: Schematic overview designed by Fone and Porkess (Fone and Porkess, 2008) of behavioural and neurochemical changes after social isolation compared to group housed rats. Single headed lines reflect a decrease (dotted lines) or and increase (solid bold) in the neurotransmitter function identified. DA: dopamine; 5-HT: 5-hydroxytryptamine; Glu: glutamate; BDNF: brain derived neurotrophic factor; a2 ADR: a2 adrenoreceptor; HPA: hypothalamic pituitary adrenal axis and 5-HT receptors are named according to the IUPHAR nomenclature. *Impairment in recognition memory may involve deficits in the entorhinal and perirhinal cortex and HPC not represented on this figure (from Fone and Porkess, 2008**).

### *1.2.3 Pharmacological animal models*

Pharmacological models include acute challenge and chronic treatments with DA agonists (amphetamine, apomorphine), hallucinogens such as PCP or LSD, dizocilpine maleate (MK-801), ketamine and neurotoxins. The drugs may be given to adult animals or targeted to specific periods in development (Geyer and Moghaddam, 2002, Wong and Van Tol, 2003).

In humans, amphetamine produces a transient psychotic disorder which is defined as a thought disorder and induces positive symptoms. In contrast PCP induced positive, cognitive and negative symptoms of schizophrenia (Krystal et al., 2005). A retrospective study found that amphetamine abuse is associated with paranoia and PCP with bizarre delusions and alters sensory experiences (Rosse et al., 1994).

Compared to the amphetamine model, glutamatergic models show higher predictive and face validity (Rung et al., 2005). Indeed, if both models induce positive symptoms, glutamatergic antagonists also mimics negative and cognitive dysfunction (Javitt and Zukin, 1991) and furthermore shows better response to the atypical antipsychotic clozapine compared to dopaminergic models (Aultman and Moghaddam, 2001, Geyer and Moghaddam, 2002). Also, typical antipsychotics such as haloperidol which are antagonist D2 receptors, in general, do not treat the negative symptoms in schizophrenic patient (Blin, 1999, Abdul-Monim et al., 2006), in contrast partial dopamine

agonists (e.g. terguride) ameliorate these symptoms (Davis et al., 1991, Olbrich and Schanz, 1991) which taken together these results demonstrate that hyperdopaminergia is not directly linked with negative symptoms of schizophrenia (Davis et al., 1991). On the other hand glutamatergic model induces negative, positive and cognitive symptoms (Bakshi et al., 1994, Abdul-Monim et al., 2007, Grayson et al., 2007). Thus the glutamatergic model appears to be a more complete model to mimic schizophrenia in animals than the dopaminergic agonist model.

### 1.2.3.1 Glutamatergic Model

#### *1.2.3.1.1 Mk-801 model*

Compared to ketamine or PCP, MK-801 is the most potent NMDA receptor antagonist and has more selective effects than for example PCP (Seeman et al., 2005). Behavioural effects induced by acute injection MK-801 or PCP do not seem to differ for example both produce hyperactivity, PPI deficit, social withdrawal and impairment in various cognitive tasks (Zajaczkowski et al., 2003, Chartoff et al., 2005, Rung et al., 2005). However, when animals are tested after withdrawal from chronic administration the effects produced by PCP are different compared to those produced by MK-801. Withdrawal from subchronic administration induces working memory deficits, decrease social interaction and d-amphetamine-induced motor activity; while after

subchronic MK-801, there is only a slight decrease in social interaction (Seillier and Giuffrida, 2009).

*1.2.3.1.2 Ketamine model*

Ketamine is also often used as a model relevant to the symptoms of schizophrenia in rodent. As well as PCP ketamine has affinity at the D2 receptor (Seeman et al., 2005). Even if ketamine and PCP have structurally similarity and were both used as intravenous anaesthetics, ketamine but not PCP is still used as anaesthetics in animals. This is due to a less potent effect of ketamine than PCP on dopamine released in the amygdala, pyriform and prefrontal cortices (Bagchi, 1981, Rao et al., 1989). In contrast, ketamine decrease the DA release in striatum which is not affected by PCP (Rao et al., 1989). Furthermore, ketamine is acting faster than PCP but has a less persistent effect (Byrd et al., 1987).

*1.2.3.1.3 Subchronic PCP model*

PCP and PCP withdrawal -induced learning and memory deficits in rodents are consequently widely used to attempt to model the abnormalities in memory and other cognitive functions in schizophrenia (Jentsch and Roth, 1999, Wong and Van Tol, 2003, Mouri et al., 2007). This model is based on the hypoglutamatergic state observed in schizophrenia, which directly or indirectly affects the dopamine system.

In humans, there are multiple lines of evidence that NMDA receptor antagonists (e.g. ketamine and PCP) in healthy volunteers mimic schizophrenic-like symptoms such as positive and negative symptoms and cognitive deficits including episodic memory impairment (Luby et al., 1959, Javitt and Zukin, 1991, Krystal et al., 1994, Jentsch and Roth, 1999, Curran and Morgan, 2000, Hetem et al., 2000, Javitt, 2007). These drugs have also been shown to exacerbate psychosis in schizophrenic patients (Lahti et al., 1995). In addition to its antagonist effect on the NMDA receptors, PCP but not ketamine has affinity for the dopamine transporter (Tangui et al., 1991, Seeman et al., 2005) as well as sigma receptors which have been implicated in PCP-induced motor sensitization (Vignon et al., 1988, Xu and Domino, 1999).

Acute or chronic PCP (withdrawal) administration produces qualitatively different effects on neurochemical and behaviour changes, with a closer isomorphic symptomatology of schizophrenia after chronic PCP use. In humans and rodents symptoms are more relevant (social incompetence, poor attention and concentration) and recurrent with long term PCP treatment than acute (delusion and hallucination) (Jentsch and Roth, 1999). The behavioural effects of PCP in humans have been shown to persist for several weeks after drug discontinuation which is why withdrawal from repeated PCP administration is widely used as a pharmacological model relevant to schizophrenia (Jentsch and Roth, 1999, Enomoto et al., 2007, Mouri et al., 2007, Seillier and Giuffrida, 2009). In PCP withdrawal models

animals are free from the considerable sedative effects of acute PCP that may confound interpretation of impairments in memory tasks. In addition withdrawal models have an advantage in that they circumvent the “receptor tautology” confound in pharmacological translational studies, namely, that reversal of drug induced effects can simply reflect a pharmacological interaction and may not necessarily predict clinical efficacy (Young et al., 2009) (Table 1.2).

<table>
<tr><th>Positive symptoms</th><th>Negative symptoms</th><th>Sensorimotor gating deficit</th><th>Cognitive dysfunction</th></tr>
<tr><td colspan="4">Acute PCP treatment</td></tr>
<tr><td rowspan="4">Locomotor activity<br>↑<br><br>Sturgeon et al. (1979)<br>Nabeshima et al. (1983)<br>Nagai et al. (2003)</td><td>Social behavior in a social interaction test<br>↓<br><br>Sams-Dodd (1995)<br>Sams-Dodd (1996)</td><td rowspan="4">Prepulse inhibition<br>↓<br><br>Geyer et al. (2001)<br>Bakshi et al. (1994)<br>Bakshi and Geyer (1995)<br>Keith et al. (1991)</td><td>Memory impairment in a passive avoidance test<br>↓<br><br>Nabeshima et al. (1986)</td></tr>
<tr><td></td><td>Attention/latent learning in a water finding test<br>↓<br><br>Noda et al. (2001)</td></tr>
<tr><td>Immobility in a forced swimming test<br>→<br><br>Noda et al. (1995)</td><td>Attentional set shifting in a ID/ED test<br>↓<br><br>Egerton et al. (2005)</td></tr>
<tr><td></td><td>Reversal learning in an operant test<br>↓<br><br>Abdul-Monim et al. (2003)<br>Idris et al. (2005)</td></tr>
</table>

↑/↑↑ (enhancing): hyperactivity; ↓: impairment; →: non

**Table 1.2: Positive symptoms, negative symptoms and cognitive dysfunction in the PCP model animal (from Mouri et al., 2007).**

| Positive symptoms | Negative symptoms | Sensorimotor gating deficit | Cognitive dysfunction |
|---|---|---|---|
| **Repeated PCP treatment** | | | |
| Locomotor activity<br>↑↑ (enhancing)<br><br>Nabeshima<br>et al. (1987)<br>Kitaichi et al. (1995)<br>Nagai et al. (2003)<br>Jentsch et al. (1998) | Social behavior in a social interaction test<br>↓ (enduring)<br><br>Sams-Dodd (1995)<br>Sams-Dodd (1996)<br>Qiao et al. (2001) | Prepulse inhibition<br>↓<br><br>Martinez et al. (1999) | Working memory in a T-maze/radial arm maze test<br>↓ (enduring)<br><br>Jentsch et al. (1997b); Stefani and Moghaddam (2002)<br>Li et al. (2003); Marquis et al. (2003) |
| | | | Spatial or non-spatial learning in an object recognition test<br>↓ (enduring)<br><br>Mandillo et al. (2003)<br>Hashimoto et al. (2005) |
| | | | Attention/latent learning in a water finding test<br>↓ (enduring)<br><br>Mouri et al. (2007) |
| | Motivation in a forced swimming test<br>↓ (enduring)<br><br>Noda et al. (1995)<br>Noda et al. (1997)<br>Corbett et al. (1999)<br>Noda and Nabeshima (2000)<br>Guan et al. (2000)<br>Noda et al. (2001)<br>Nagai et al. (2003)<br>Murai et al. (2007) | | Attentional set shifting in a ID/ED test<br>↓ (enduring)<br>Rodefer et al. (2005)<br>→<br>Fletcher et al. (2005); Deschenes et al. (2006) |
| | | | Associative learning in a fear conditioning test<br>↓ (enduring)<br><br>Enomoto et al. (2005) |
| | | | Reversal learning in an operant test<br>↓ (enduring)<br><br>Abdul-Monim et al. (2006a); Abdul-Monim et al. (2006b) |

↑/↑↑ (enhancing): hyperactivity; ↓: impairment; →: non

**Table 1.2: Positive symptoms, negative symptoms and cognitive dysfunction in the PCP model animal (from Mouri et al., 2007) (continued).**

| Positive symptoms | Negative symptoms | Sensorimotor gating deficit | Cognitive dysfunction |
|---|---|---|---|
| **Perinatal PCP treatment** | | | |
| Locomotor activity<br>↑↑ (enhancing)<br>Wang et al. (2001)<br>Depoortere et al. (2005) | No data | Prepulse inhibition<br>↓ (enduring)<br>Wang et al. (2001)<br>Wang et al. (2003)<br>Takahashi et al. (2006) | Acquisition of delayed spatial alternation task<br>↓ (enduring)<br><br>Wiley et al. (2003) |
| | | | Reference memory in a Morris water maze test<br>↓ (enduring)<br><br>Sircar (2003)<br>Andersen and Pouzet (2004) |
| | | | Working memory in a Morris water maze test<br>↓ (enduring)<br><br>Andersen and Pouzet (2004) |
| | | | Recognition in social novelty discrimination<br>↓ (enduring)<br><br>Depoortere et al. (2005)<br>Harich et al. (2007) |

↑/↑↑ (enhancing): hyperactivity; ↓: impairment; →: non

**Table 1.2: Positive symptoms, negative symptoms and cognitive dysfunction in the PCP model animal (from Mouri et al., 2007) (continued).**

Repeated PCP administration induces functional impairments in the HPC, amygdala, PFCand nucleus accumbens (Shirayama et al., 2007). Chronic PCP administration in rodents induces behavioural deficits (e.g. radial maze, reversal learning, attentional set-shifting) (Li et al., 2003, Rodefer et al., 2005, McLean et al., 2009) that are selectively reversed by antipsychotic drugs, including memory tasks, (Bakshi et al., 1994, Verma and Moghaddam, 1996, Schwabe et al., 2005, Turgeon and Hulick, 2007) such as object recognition (Hashimoto et al., 2005, Grayson et al., 2007, McLean et al., 2009). However, chronic PCP treatment in rodent has not been tested in tasks that simultaneously evaluate “what” “where” “when” aspects of episodic memory.

Below a table recapitulates rodent studies which used different regimens of subchronic PCP model in object recognition task (Table 1.3). This table shows that the subchronic PCP treatment used in different studies do not change the result of the PCP-induced deficit in object recognition and that PCP induces a robust deficit in rodents (mice, female and male rats).

| Rodent | PCP Treatment | Other Treatments | Object recognition | Results | Conclusion | References |
|---|---|---|---|---|---|---|
| Mice<br>Male | **S.C.**<br>10 mg/kg -<br>10days<br><br>(Day:1-5, 8-12)<br>Other treatment:<br>Day 15 | **Donepezil cholinesterase inhibitor (i.p.):**<br>1 or 0.1 mg/kg -14 days<br><br>**Physostigmine, Cholinesterase Inhibitor (i.p.):**<br>0.25 mg/kg -14 days<br><br>**NE-100, Sigma-1 receptor antagonist (i.p.):**<br>1 mg/kg -14 days | 3 days of habituation<br><br>Object recognition<br>(5 min)<br><br>A-B 24 h A-A | - PCP ↓<br><br>- PCP +donepezil 0.1mg/kg: Ø<br>-PCP +Physostigmine: Ø<br><br>- PCP +donepezil 1mg/kg: ↑<br>- PCP +donepezil 1 mg/kg + NE-100: Ø<br><br>Only DI | Interaction between donepezil and sigma-1 receptors mechanism in cognitive deficit in schizophrenia | (Kunitachi et al., 2009) |
| Mice<br>Male | **S.C.**<br>10 mg/kg -<br>10days<br><br>(Day:1-5, 8-12)<br>Other treatment:<br>Day 15 | **Clozapine atypical antipsychotic (i.p.):**<br>5 mg/kg -1h before test -acute<br>5 mg/kg -14 days<br><br>**Haloperidol (i.p.):**<br>0.1 mg/kg -1h before test -acute<br>0.1 mg/kg -14 days | 3 days of habituation<br><br>Object recognition<br>(5 min)<br><br>A-B 24 h A-A | - PCP ↓<br><br>- PCP +clozapine (acute): Ø<br>-PCP +haloperidol (acute): Ø<br><br>- PCP +clozapine (chronic): ↑<br>- PCP +haloperidol (chronic): Ø<br><br>Only DI | Subchronic clozapine improved PCP-induced deficit but not haloperidol nor acute clozapine | (Hashimoto et al., 2005) |
| Rat<br>Female<br>hooded-lister | **I.P.**<br>2 mg/kg x2<br>-7 days/7days out | **SCH-23390, $D_1$-like receptor antagonist (i.p.)**<br>0.025 or 0.05 mg/kg<br><br>**SKF-38393, $D_1$-like receptor agonist (i.p.):**<br>6 mg/kg | 3 days of habituation<br>(1 h)<br><br>Object recognition<br>(3 min)<br><br>A-B 1 min A-A | - PCP ↓<br><br>-PCP +SKF: ↑<br>- PCP +SCH 0.025: Ø<br>-PCP +SCH 0.025 or 0.05 +SKF: Ø<br><br>DI and new vs old | Role of D1-like receptor in cognitive deficit in schizophrenia | (McLean et al., 2009) |

**Table 1.3: Different model of subchronic PCP treatment in rodent and their impact in object recognition paradigm.**
**DI: Discrimination Index, Ø: non effect; ↓: impaired; ↑: improved.**

| Rodent | PCP Treatment | Other Treatments | Object recognition | Results | Conclusion | References |
|---|---|---|---|---|---|---|
| Rat<br>Female<br>hooded-lister | **I.P.**<br>2 mg/kg x2<br>-7 days/7days out | **Haloperidol (i.p.):**<br>0.05 or 0.075 mg/kg -30 min before task<br><br>**Clozapine (i.p.) a week later:**<br>1 or 5 mg/kg -30 min before task<br><br>**Risperidone (i.p.):**<br>0.05 or 0.1 or 0.2 mg/kg -30 min before task | 3 days of habituation<br>(1 h)<br><br>Object recognition<br>(3 min)<br><br>A-B 1 min A-A | - PCP ↓<br><br>-PCP +haloperidol:Ø<br>- PCP +clozapine: ↑<br>-PCP +risperidone only 0.2 mg/kg: ↑<br><br>DI and new vs old | Clozapine and risperidone but not haloperidol reversed the deficit induced by subchronic PCP treatment | (Grayson et al., 2007) |
| Rat<br>Male<br>Wistar han | **I.P.**<br>10 mg/kg<br>-5 days/ 6days out | **PCP (acute) (i.p.):**<br>1 mg/kg -90 min before task (after sample)<br><br>**SSR103800 glycine transporter-1 inhibitor (p.o.):**<br>3 mg/kg -90 min before task (after sample) | 1 habituation<br><br>Object recognition<br>(Sample: 20sec on two objects or 5min total) Test: 3 min<br><br>A-B 90 min A-A | - PCP ↓<br><br>-PCP +SSR103800:↑<br><br>DI and new vs old | SSR103800 restored PCP deficit in object recognition | (Boulay et al., 2008) |
| Rat<br>Male<br>Wistar han | **I.P.**<br>10 mg/kg<br>-5 days/ 6days out | **PCP (acute) (i.p.):**<br>1 mg/kg -90 min before task (after sample)<br><br>**SSR180711 α7 nicotinic partial agonist (p.o.):**<br>3 mg/kg -90 min before task (after sample) | 1 habituation (same day than test)<br><br>Object recognition<br>(Sample: 20 sec on two objects or 5 min total) Test: 3 min<br><br>A-B 90 min A-A | - PCP ↓<br><br>-PCP +SSR180711:↑<br><br>DI and new vs old | SSR180711improved PCP deficit in object recognition | (Pichat et al., 2006) |
| Rat<br>Male<br>Sprague-dawley | **I.P.**<br>5 mg/kg x2<br>-7 days/7days out | **MKC-231 choline uptake enhancer (i.p.):**<br>3 mg/kg x2 -8 days (test day:9) | Object recognition<br>(15 min)<br><br>A-B 24 h A-A | - PCP ↓<br><br>- PCP +MKC-231: ↑<br><br>Only DI | PCP induced impairment which was antagonized by MKC-231 | (Shirayama et al., 2007) |

**Table 1.3: Different model of subchronic PCP treatment in rodent and their impact in object recognition paradigm (continued).**
**DI: Discrimination Index, Ø: non effect; ↓: impaired; ↑: improved.**

In rats, PCP challenge after repeated PCP administration increase locomotor activity (LMA) (Xu and Domino, 1994, Johnson et al., 1998). This specific phenomenon is called “reverse tolerance” or sensitization and it is correlated with apoptotic neuronal degeneration in different brain regions (Phillips et al., 2001). Increased locomotor activity induced by PCP is associated with dopamaniergic hyperactivity and is supposed to be related to clinical manifestations of schizophrenia (Steinpreis et al., 1994, Adams and Moghaddam, 1998). Dysfunctions in the dopaminergic and glutamatergic systems are the origin of behavioural sensitization due to neural adaptations in the mesocorticolimbic regions (Pierce and Kalivas, 1997, Cornish and Kalivas, 2001). Neural adaptations involve the nucleus accumbens which received dopaminergic projections from the VTA and glutamatergic excitatory inputs from the PFC (Cornish and Kalivas, 2001, Kalivas et al., 2005). Furthermore, clozapine, an atypical antipsychotic, prevents apoptotic degeneration of cortical neurons and reduces locomotor sensitization after chronic PCP treatment (Johnson et al., 1998, Phillips et al., 2001).

At the behavioural level, tasks currently used to assess episodic memory such as novel object recognition following PCP withdrawal may be limited for two reasons. First, PCP withdrawal induces object recognition deficits in rodents that are reversed by antipsychotic drugs (Hashimoto et al., 2005, Grayson et al., 2007). However, antipsychotic drugs appears to be ineffective at reversing memory impairments in

patients (Goldberg et al., 1993) representing what has been termed a ‘false positive’ (Young et al., 2009). Second, there is evidence that the brain circuitry that underpins performance in tasks that assess memory for “what” (which object is new?) may be different from that involved in the ability to remember “what- where-when” (Eacott and Norman, 2004, Langston and Wood, 2009). In this thesis both isolation rearing and subchronic PCP or PCP withdrawal models are tested in an episodic memory task the object-place-context recognition task (OPC recognition task) developed by Eacott and Norman (Eacott and Norman, 2004).

The table below briefly recapitulates the construct, face and predictive validity of the disease for both models (Table 1.4). Both models have been shown to be relevant model in schizophrenia and are widely used in this area of research. The table shows that on some aspects both models show strong construct validity and are similar on the face and predictive validities, both models are impaired in object recognition (Bianchi et al., 2006, Grayson et al., 2007), reversal learning (Abdul-Monim et al., 2007, Li et al., 2007), show hyperactivity (Gentsch et al., 1982) and respond positively with clozapine on PPI (Bakshi et al., 1994, Cilia et al., 2001).

| Animal Model | | Phencyclidine (PCP) | Isolation Rearing |
|---|---|---|---|
| **Protocol** | | Subchronic injection (5 mg/kg i.p.) twice a day during 7 days Followed by 7 days of wash out | Postnatal day 24 isolated in a cage experiments started 5 weeks after |
| **Construct validity** | Glutamatergic hypothesis | NMDA receptor antagonist | Glutamatergic hypofunction in the PFC (*Melendez et al., 2004*) |
| | Dopaminergic hypothesis | Direct and indirect effect on dopaminergic system (*Marsden, 2006*) | Elevated dopamine level in the nucleus accumbens (*Heidbreder et al., 2000*) |
| **Face validity** | Positive Symptoms | Locomotor sensitisation following acute administration of 3.2 mg/kg PCP (*Chapter 6*) | ↑ Locomotor activity (*Gentsch et al., 1982*) |
| | Negative symptoms | ↓ Reversal learning (*Abdul-Monim et al., 2007*) | ↓ Reversal learning (*Li et al., 2007*) |
| | Cognitive dysfunction | ↓ Object recognition (*Grayson et al., 2007*) | ↓ Object recognition (*Bianchi et al., 2006*) |
| **Predictive validity** | Clozapine | ↑ PPI (*Bakshi et al., 1994*) | ↑ PPI (Cilia et al., 2001) |

**Table 1.4: Brief comparative table of the subchronic PCP and isolation rearing model relevant to schizophrenia in rats.**

## 1.3 Treatment of schizophrenia

Antipsychotics have been classified into the typical (oldest group, such as haloperidol) and the atypicals (such as clozapine Figure 1.5) categories based on their affinity for dopaminergic receptors (Figure 1.6 and Table 1.5) (Meltzer et al., 1989). Both classes of drug are acting on the dopaminergic system with more or less affinity toward D2 receptors

(Table 1.5) compared to the typical antipsychotics, the atypical antipsychotics target a wider range of receptors. They are both efficient to treat the positive symptoms. However, they are far from impressive to treat the negative and cognitive symptoms even if some improvement have been noticed in attention for example after clozapine (Meltzer and McGurk, 1999, Gopal and Variend, 2005). Atypical antipsychotics appear to be more efficient to treat cognitive deficits than typicals (Meltzer and McGurk, 1999, Gopal and Variend, 2005).

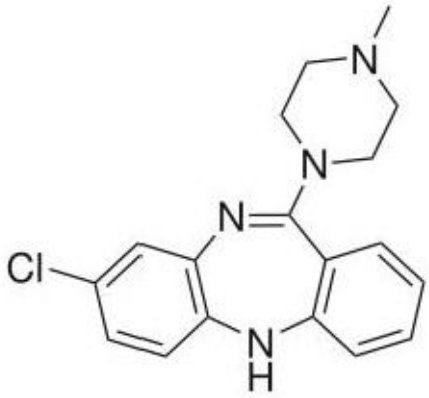



**Figure 1.5 Clozapine nomenclature 8-chloro-11-(4-methyl-1-piperazinyl)-5Hdibenzo(b,e)(1,4)diazepine.**

| | $D_1$-Like | | $D_2$-Like | | |
|---|---|---|---|---|---|
| | $D_1$ | $D_5$ | $D_2$ | $D_3$ | $D_4$ |
| *Antagonists* | | | | | |
| (+)-Butaclamol | +++ | ++ | +++ | ND | ++ |
| Chlorpromazine | + | + | +++ | ++ | ++ |
| Clozapine | + | + | + | + | ++ |
| Eticlopride | – | – | ++++ | ND | +++ |
| Haloperidol | + | + | ++++ | ++ | +++ |
| Nafadotride | ND | ND | +++ | ++++ | +/– |
| Nemonapride | ND | ND | ++++ | ++++ | ++++ |
| Raclopride | – | ND | +++ | +++ | +/– |
| SCH-23390 | ++++ | ++++ | +/– | +/– | +/– |
| (–)-Sulpiride | – | – | ++ | ++ | ++ |
| Spiperone | + | +/– | ++++ | +++ | ++++ |
| *Agonists* | | | | | |
| Apomorphine | +/– | + | +++ | ++ | +++ |
| Bromocriptine | + | + | +++ | +++ | + |
| Dopamine | +/– | + | + | ++ | ++ |
| Fenoldopam | +++ | +++ | ++ | ND | + |
| 7-OH-DPAT | +/– | ND | ++ | +++ | +/– |
| Quinpirole | – | ND | +/– | ++ | ++ |
| SKF-38393 | +++ | ++++ | + | +/– | +/– |

++++, Inhibition constant ($K_i$) <0.5 nM; +++, 0.5 nM < $K_i$ < 5 nM; ++, 5 nM < $K_i$ < 50 nM; +, 50 nM < $K_i$ < 500 nM; +/–, 500 nM < $K_i$ < 5 $\mu$M; –, $K_i$ >5 $\mu$M; ND, not determined; 7-OH-DPAT, 7-hydroxy-dipropylaminotetralin.

**Table 1.5: Pharmacological profile of dopamine receptors (From Missale et al., 1998).**

### *1.3.1 Antipsychotics*

Chlorpromazine, the first typical antipsychotic, was developed in 1950 (Brand et al., 1954). This drug was one of the biggest advances in psychiatric treatment. It truly changed the prognosis of patients with psychosis worldwide. Other antipsychotics such as haloperidol or chlorprothixene followed**.** These three different compounds are three different types of antipsychotics which derivate from different molecules: haloperidol is a butyrophenone, chlorpromazine a

phenothiazine and chlorprothixene a thioxanthenes. While typical antipsychotics are efficient against the positive symptoms of schizophrenia (e.g. hallucination and delusion), they appears to have a poor impact on the negative and the cognitive symptoms (Meltzer and McGurk, 1999, Gopal and Variend, 2005). The efficacy of the typical antipsychotics like chlorpromazine or haloperidol is correlated to their D2 receptor antagonist effect to reduce or abolish psychotic disorder. One of the major side effects of antipsychotics is that in the long-term antipsychotics induce at least some symptoms resembling those of Parkinson's disease called "extra pyramidal side effects": slowness in movement, lack of facial expression and general weakness due to their high affinity with D2 receptors (Bishnoi et al., 2007). The phenothiazine seems to have the best antipsychotic potency and antiemetic effect but also strong extrapyramidal side effects (Feinberg and Snyder, 1975).

### *1.3.2 Atypical antipsychotics*

On the other hand, atypical antipsychotics, like clozapine or risperidone, possess less dopamine D2 receptor affinity with high affinity for the dopamine D4 receptor compared to antipsychotics and target the nucleus accumbens rather the neostriatum locus responsible for "extra pyramidal effect" in Parkinson's disease (Marsden, 2006). Another particular effect of these medications is their multiple antagonist effect on different subtypes of adrenergic, cholinergic, histaminergic and serotonergic receptors (Figure 1.6) (Millan, 2000, Ma

et al., 2006). The rich pharmacological profile of the atypical antipsychotics allows a reduction of positive symptoms with less extra pyramidal side effects compare to typical antipsychotics (Tandon and Jibson, 2002). In clinical trials, they also improve negative symptoms and cognitive function more than typical antipsychotics (Lublin et al., 2005) but without successfully treating them (Marsden, 2006). As major side effects, Clozapine produces hypersalivation, weight gain, sedation, tachycardia and more dangerously can induce agranulocytosis and bone marrow toxicity (Buchanan, 1995, Pereira and Dean, 2006). Therefore, it is carefully used and is generally prescribed to patients who cannot tolerate typical antipsychotic drugs or non responders (Feldman et al., 1997).

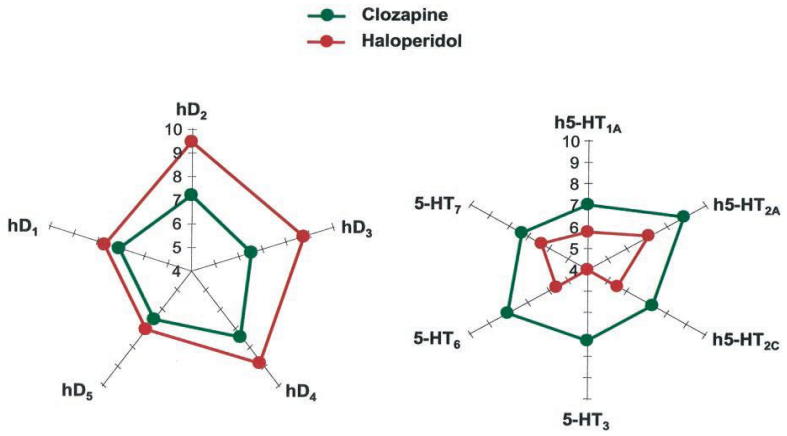



**Figure 1.6: Radar representations of the binding profile of clozapine compared with haloperidol at multiple dopaminergic and serotonergic receptors. All data were generated in the author's laboratory at cloned human (h) receptors, cloned rat (r) (5-HT6 and 5-HT7), and cloned mouse (m) (5-HT3) receptors. Note the broad-based and "equilibrated" interaction of clozapine at diverse dopaminergic and serotonergic sites, whereas haloperidol shows pronounced affinity for hD2 (hD3 and hD4) receptors versus modest or weak affinity at other sites (From Milan, 2000).**

In rodents, clozapine appears to be more efficient than typical antipsychotics in behavioural tasks which were correlated to the negative and cognitive symptom deficits of schizophrenia in animals. Indeed, clozapine reversed the chronic PCP deficit in conditional discrimination performance, reversal-learning, and object recognition whereas haloperidol did not (Abdul-Monim et al., 2006, Dunn and Killcross, 2006, Grayson et al., 2007). However, these results do not truly reflect the current clinical profile as both categories of antipsychotics have a minor effect on negative and cognitive symptoms such as episodic memory deficit (Lublin et al., 2005, Mortimer, 2005, Marsden, 2006, Young et al., 2009) even if clozapine appears to have some beneficial effects on some aspects (Meltzer, 1994, Meltzer and McGurk, 1999). One impediment to the identification and evaluation of potential new drug treatments is the lack of reliable animal models specifically designed to simulate cognitive impairment in schizophrenia. This has been identified by recent research consortia e.g. Measurement and Treatment Research to Improve Cognition in Schizophrenia" (MATRICS) (Sarter, 2006, Young et al., 2009). The MATRICS program brings together representatives of academia, industry and government in a consensus process for addressing the improvement of available antipsychotic medication to treat cognitive impairment in schizophrenia.

## 1.4. Cognitive enhancers

### *1.4.1 Cholinergic system*

The central cholinergic pathways which project to the cerebral cortex regulate various general functions which include motivation, attention, arousal, memory and consciousness (Woolf, 1991, 1996). Acetylcholine is one of the key chemical messengers in brain and is found in high concentrations in cortex and HPC (Jung et al., 1996). The majority of cholinergic neurons in the basal forebrain are distributed among three different nuclei: the medial septum (MS), the nucleus Basalis of Meynert (nBM) and the diagonal band of Broca (dBB) (Mesulam et al., 1983). Projections of these nuclei innervate the cerebral cortex, as well as the HPC and amygdala (Mesulam et al., 1983). The cholinergic and other excitatory inputs modulate the excitatory state of HPC and cortical neurons and are involved in cortical plasticity (Semba, 2000) (Figure 1.7).

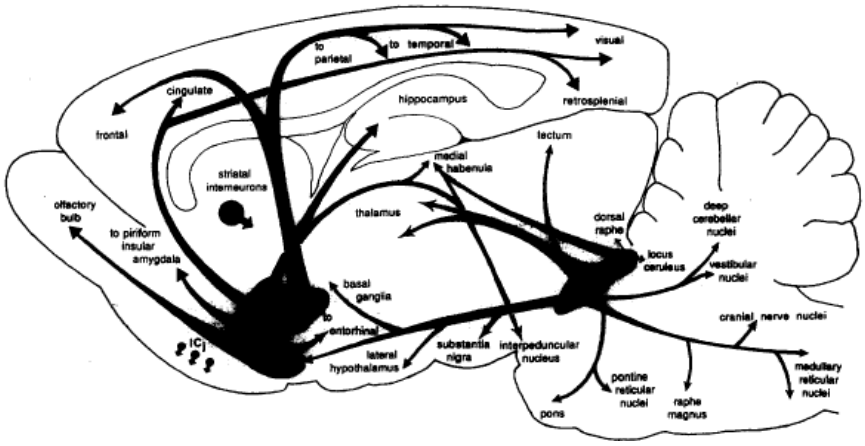



**Figure 1.7: The central projections of cholinergic cells are schematically represented on a parassagital section from rat brain. The entire cortical mantle is innervated by the basal forebrain subsystem and the subcortical mass is innervated by the pontomesencephale subsystem (From Woolf, 1991).**

Acetylcholine receptors are classified into two categories depending on their affinity for nicotine and muscarine. There are the nicotinic acetylcholine receptors (nACh receptors) and the muscarinic acetylcholine receptors (mACh receptors), the first type is ionotropic and the second type is metabotropic. The nACh receptors are classified according to their distribution in muscles or in the brain (neuronal types) (Deneris et al., 1991). The neuronal nACh receptors subtypes are a combination of 12 differents subunits: α2 to α 10 and β2 to β 4. In schizophrenic patient the mRNA level of α7 and α4 receptors are significantly lower than in healthy population (Perl et al., 2003). The mACh receptors belong to the superfamily of G-protein-coupled receptors. There are five identified mACh receptors: M1, M2, M3, M4 and M5 which are classified by their relation with the G protein types they are linked to (Van der Zee and Luiten, 1999).

### 1.4.1.1 Donepezil

The cholinergic system has been implicated in the regulation of attention, memory, processing speed and sensory gating processes (Cummings, 2000). In Alzheimer's disease acetylcholinesterase inhibitors (AChEi) such as galantamine or donepezil (Figure 1.8) improve cognitive deficit induced by the disease (Bullock and Dengiz, 2005, Tsuno, 2009).

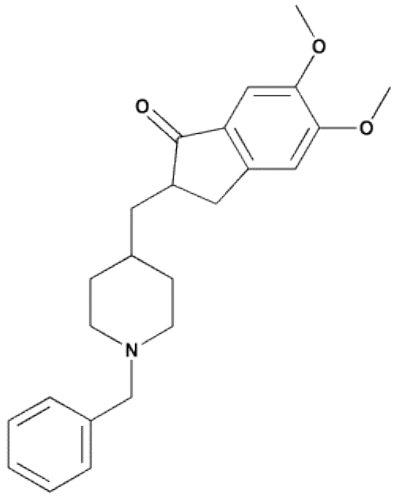



**Figure 1.8: Donepezil nomenclature, 2-[(1-Benzyl-4-piperidyl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one, C24 H29 N O3.**

In schizophrenia, anomalies in the cholinergic pathway have been reported such as lower numbers of muscarinic (Crook et al., 2001) and nicotinic receptors (Freedman et al., 1995) in the PFC and HPC (Raedler et al., 2006). Post mortem studies demonstrate the link between the alterations of Ach receptors subtypes such as muscarinic M1/M4 and nicotinic receptors and cognitive impairment observed in schizophrenia (Friedman et al., 2002). Indeed, there is a decrease in

low affinity of nAch receptors in HPC, cortex, striatum and thalamus (Friedman, 2004) but also a reduction of M1/M4 mACh receptors in the HPC, PFC and striatum (Cummings, 2000, Crook et al., 2001). Furthermore, a significant correlation has been found between impaired cognition in schizophrenia and the reduced level of choline acetyltransferase which is implicated in acetylcholine synthesis. (Powchik et al.,1998). In SPECT studies in living unmedicated schizophrenic patients, it has been shown that there are fewer mACh receptors in frontal, temporal and occipital cortex and in the striatum and thalamus compared to control group (Raedler et al., 2003).

Based on these results, donepezil and other acetylcholinesterase inhibitors AChEIs (Table 1.6) have been tested in initial clinical trials in schizophrenia. Controversial results has been published with some studies suggesting beneficial improvement in cognition (Howard et al., 2002, Buchanan et al., 2003, Risch et al., 2007, Chung et al., 2009) while others did not (Friedman et al., 2002, Tuğal O et al., 2004, Keefe et al., 2007).

| Target | Action | Drug |
|---|---|---|
| **Aceylcholinesterase** | Inhibition of acetylcholinesterase resulting in non-specific increase in synaptic acetylcholine at nicotinic and muscarinic recpetors | Tacrin, donepezil, rivastigmine, galantamine |
| **Muscarinic receptor** | M1 agonist | Xanomaline, CDD-0102, CI 1017, YM 796 |
| **Nicotinic receptor** | Nicotinic receptor agonist<br><br>Nicotinic receptor potentiator | GTS-21<br><br>Galantamine |

**Table 1.6: Potential cholinergic compounds for cognitive enhancement in schizophrenia (From Friedman, 2004).**

In rodent models donepezil reverses scopolamine-induced deficits in different memory tasks such as spontaneous alternation in the Y-maze, delayed non-match to place in radial maze, passive avoidance task and object recognition which assess working and spatial memories (Ogura et al., 2000 , Bontempi et al., 2003, Sambeth et al., 2007). Donepezil also improve aged rats performance in the water maze (Hernandez et al., 2006). However, few studies have investigated the cognitive effect of donepezil relevant to schizophrenia in rodents. Donepezil reverses the MK-801 and PCP-induced deficits in spatial reversal learning, contextual and cued memory, and one-trial object recognition (Csernansky et al., 2005, Kunitachi et al., 2009). In middle-aged mice, Béracochéa (2007) demonstrated that donepezil given alone improves contextual memory (Béracochéa et al., 2007). Furthermore human studies have also revealed episodic memory

improvement after donepezil administration in healthy young adult (Grön et al., 2005).

## 1.5 Cognitive impairment in schizophrenia

### *1.5.1 Generality*

As reported by a recent research consortium “MATRICS”, cognitive deficits in schizophrenia include wide impairments in areas such as attention, executive function and memory (Green et al., 2004). They are good predictors of long–term disability of the disease. Unfortunately, there is currently no appropriate medication to treat the cognitive deficits, but some areas of research such as acetylcholinesterase inhibitors,(Erickson et al., 2005, Grön et al., 2005, Chung et al., 2009). Attention and executive function have been shown to be impaired in schizophrenia (Brazo et al., 2005). There are three categories of attentional deficits: sustained, selective attention and shift attention (Hagh-Shenas et al., 2002). Diverse studies show attentional deficit in schizophrenia in selective attention in auditory task (Hagh-Shenas et al., 2002, Mulet et al., 2007), spatial selective attention, in sustained attention (Mulet et al., 2007). In rats different task are used to measure attentional deficit and respond to dopamine agonists such as latent inhibition (Bay-Richter et al., 2009)or overshadowing (O'Tuathaigh and Moran, 2004).

This thesis is focusing on the memory deficit observed in schizophrenia with particular attention on episodic memory deficits (Clare et al., 1993, Gold and Weinberger, 1995, Rushe et al., 1999, Danion et al., 2001, Achim and Lepage, 2003, Riutort et al., 2003, Toulopoulou et al., 2003, Al-Uzri et al., 2006).

### *1.5.2 Memory*

For a long time, psychologists and philosophers have thought that memory was not one single entity. For example, Maine de Biran in 1804, made the differentiation between mechanical memory, sensitive memory, and representative memory. In 1948, Tolman wrote at length on the proposition that there is more than one kind of memory (Tolman, 1948, Squire, 1992).

In the middle of the 20$^{th}$ century, the exploration of memory in patients with brain injury or disease that affects mental function showed that the multiple memory systems have their own localization in brain. Studies on brain lesions in monkeys and human patients have divided memory into two principal categories: the implicit or nondeclarative memory, characterised by knowledge of motor skills and habits, and explicit or declarative memory defins as knowledge of fact and events (Squire, 1992, Milner et al., 1998) (Figure 1.9).

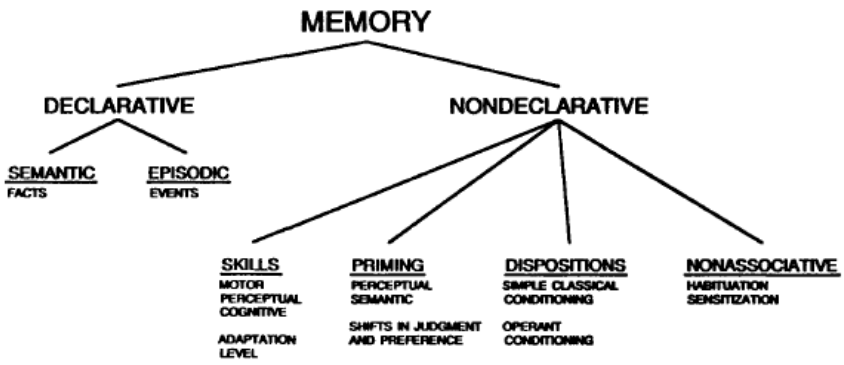



**Figure 1.9: A memory taxonomy. (Declarative memory includes memory for facts and events and depends on the integrity of the HPC and related structures. Nondeclarative memory refers to a heterogeneous collection of distinct learning and memory abilities where performance changes but without affording access to the experience or experiences that caused the change (From Squire, 1992).**

### 1.5.2.1 Distinction between memories: explicit memory

Explicit memory involves a specialized anatomical system in the medial temporal lobe, and particularly the HPC. This hypothesis comes mainly from three sources: human studies, brain lesions in primates and in studies amnesia and memory impairment in rats (Squire, 1992).

Brenda Milner (1998) described patient H.M. who had sustained a bilateral resection of the medial structures of the temporal lobe including HPC. H.M. had a very profound impairment of recent memory without other intellectual loss; this defends the hypothesis of the essential role of the HPC in the learning of new facts and events (Milner et al., 1998).

Using a monkey model of human amnesia has confirmed this idea. A medial temporal lobe lesion in monkeys, including the HPC, produces deficits in tasks which require the utilisation of explicit memory like delayed nonmatching to sample (DNMS), a task that directly assessed responses related to object repetition (Squire, 1992).

In rodents, HPC lesion causes impairment of tasks which are used to explore explicit memory like water maze or radial maze (Morris et al., 1982, Kesner et al., 1993). Contextual information was shown to be impaired after HPC lesion in contextual fear conditioning (Kim et al., 1993, Young et al., 1994, Maren and Fanselow, 1997). Furthermore, other studies seem to confirm the role of HPC for episodic memory in tasks that involved object, places and context recognition paradigm (two samples phase) (Eacott and Norman, 2004, Kart-Teke et al., 2006, Good et al., 2007a). However, in the one trial object recognition test (one sample phase) results are contradictory: some did not find any impairment (Aggleton and Brown, 1999, Mumby et al., 2002) while other did (Clark et al., 2001) (Table 1.6).

| Task | Hippocampus | Amygdala | Reference |
|---|---|---|---|
| Water maze | + | − | Sutherland & McDonald, 1990 |
| Odor discrimination | + | − | Eichenbaum, Fagan, & Cohen, 1986[a] |
| Timing of events | + | − | Olton, Meck, & Church, 1987[a] |
| Learning cue relationships | + | − | Sutherland, McDonald, Hill, & Rudy 1989 |
| Spatial alternation | + | − | Aggleton, Hunt, & Rawlins, 1986; Aggleton, Blindt, & Rawlins, 1989 |
| Nonspatial alternation | + | − | Raffaele & Olton, 1988[a] |
| Radial maze | + | − | Becker, Walker, & Olton, 1980[a] |

*Note.* Plus sign indicates impairment; minus sign indicates no impairment.
[a] These lesions damaged the fornix rather than the hippocampus itself.

**Table 1.6: Effects of Lesions of the Hippocampal System or the Amygdala on Memory Tasks in the Rats (From Squire, 1992).**

## 1.6. Episodic memory

### *1.6.1 Definition*

In 1972, Endel Tulving defined explicit memory as having two different aspects, semantic memory and episodic memory (Tulving, 1972, Tulving, 1986).

According to Tulving's definition, episodic memory is the capacity to recollect an event in time and location. It refers to critical the triad "What-Where-When" but with the capacity to see oneself as an actor of the action (Tulving, 1972, Tulving, 1983, Tulving, 1985, 2002). For example, you can remember where you were, when you heard the news about the 11 September of 2001. Furthermore, just the evocation of this date (when) is a key memory of this particular event and helps you to recollect it: what (attack in New-York) and where (you heard the information). People see themselves in the situation: they are reliving it. Thus, episodic memory is the capacity to mentally travel in time which is called "mental time travel" (Tulving, 1985). Mental time travel requires a notion of the concept of self. The person who mentally time travels is fully conscious of himself as an actor in a past situation and therefore is defined as autonoetic awareness. Autonoetic awareness is required for remembering (Tulving, 1985, Suddendorf and Corballis, 1997). By contrast the semantic memory refers to the noetic awareness which characterised our ability to be aware about our environment

without any remembering but by knowing it based on familiarity. Semantic memory is the memory of facts and knowledge. These two states of awareness are interconnected and dependent from each other (Table 1.7) (Tulving, 1985). While, episodic memory is PFC and temporal lobe dependent (Vargha-Khadem et al., 1997, Tulving and Markowitsch, 1998), semantic memory belongs to a wider set of neocortical area which included frontal, temporal and occipital cortices. However, when people are asked to recognise previously encountered items based on “remember” or “know” judgments, results demonstrate that both judgments were not based on a degree of confidence and support the dissociation between two forms of recognition: one based on familiarity and one based on remembering (Rajaram, 1993). Therefore, people can have access to their personal past in terms of both autonoetic awareness (remembering) and in terms of noetic awareness too (knowing).

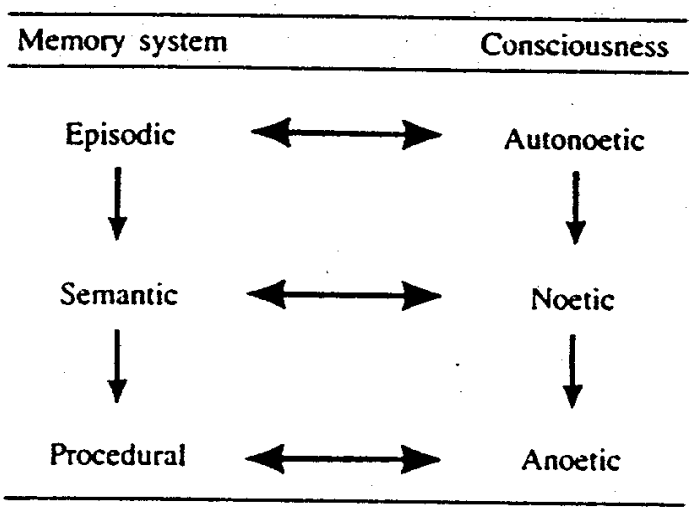



**Table 1.7: A schematic diagram of the relations between memory systems and varieties of consciousness (From Tulving, 1985).**

In order to understand better the origin of episodic memory, it is important to anatomically identify the brain region involved. Episodic memory reveals a large and complex interaction which involving the temporal lobe (perirhinal cortex, entorhinal cortex, postrhinal cortex and HPC) and the PFC.

### *1.6.2 Brain structures involved in episodic memory*

#### 1.6.2.1 Temporal lobe

The perirhinal cortex seems to have a role in object familiarity or "knowing" and is important in object recognition performance but not to discriminate between context such as in the object-in-context task (Norman and Eacott, 2005). Furthermore perirhinal neurons respond maximally in the first presentation of visual stimuli, but less to the second presentation ("what"). The integration of the object in context is postrhinal cortex (or parahipocampal cortex in mammal) dependent (Aggleton and Brown, 1999). Indeed, c-Fos studies have found postrhinal cortex activation in spatial task which is not the case for perirhinal cortex. Postrhinal lesion in rats impairs the encoding of egocentric spatial relations between objects, but not the object perception itself (Norman and Eacott, 2005).

Network information encoded in perirhinal/postrhinal cortex is directly linked to entorhinal cortex which is responsible for a mental

map in a spatial environment (“where”). The presence of “grid cell” in this cortex is responsible for spatial representation. It is shown that entorhinal cortex receives many inputs from every sensory modality such as the eye and ears while damage to this area lead to a sensory integration deficit and spatial learning impairment (Hafting et al., 2005). Furthermore entorhinal cortex possesses a main input in HPC, where the information is treated. HPC by the intermediate of “place cell” allows a perfect orientation in space. This activity combines with the “encoding map” and is responsible for spatial contextual recognition and allows free movement into a specific context toward a position (Davis et al., 2001, Hafting et al., 2005, Smith and Mizumori, 2006).

All the information of OPC is carried to other brain structures via the fornix from the HPC. The Fornix is involved in combination of object, place and context rather than any of these elements alone or in pairs. Indeed, fornix or HPC lesion induces memory impairment only in OPC recognition task but not in object or/and place or/and context recognition task (Eacott and Norman, 2004, Norman and Eacott, 2005, Dere et al., 2007, Langston and Wood, 2009, Ergorul and Eichenbaum, 2004).

#### 1.6.2.2 Prefrontal Cortex

The temporal relation of an event (“when”) is related to the PFC. Indeed, the PFC and more specifically the mPFC have been reported to play an important role in recency discriminations for objects or their

spatial locations in time (Wheeler et al., 1997, Barker et al., 2007). These findings were confirmed with lesion of mPFC in humans, non-human primates and rodents which were impaired in relative recency stimuli discriminations but not in recognition of novel and familiar stimuli (Dere et al., 2006).

Neural systems are interconnected, the rhinal cortex (perirhinal, postrhinal and entorhinal cortex) is connected to the HPC and via the fornix connect to the PFC, itself also connected to the rhinal cortex. This hippocampal cortical loop, is directly involved in episodic memory (Bachevalier et al., 1997, Aggleton and Brown, 1999, Gurden et al., 2000) (Figure 1.10).

Neurobiological studies confirm the link between temporal cortex and PFC. In vivo in rats, infusion of the dopamine D1 receptor agonist (SKF81297) or D1 receptor antagonist (SCH23390) in PFC, produces respectively an excitatory or inhibitory effect on hippocampal and PFC long term potentiation (LTP) (Gurden et al., 2000). These data combined with the cortical dopaminergic hypoactivity and glutamatergic hypofunction could highlight the episodic memory deficit in schizophrenia (Figure 1.10).

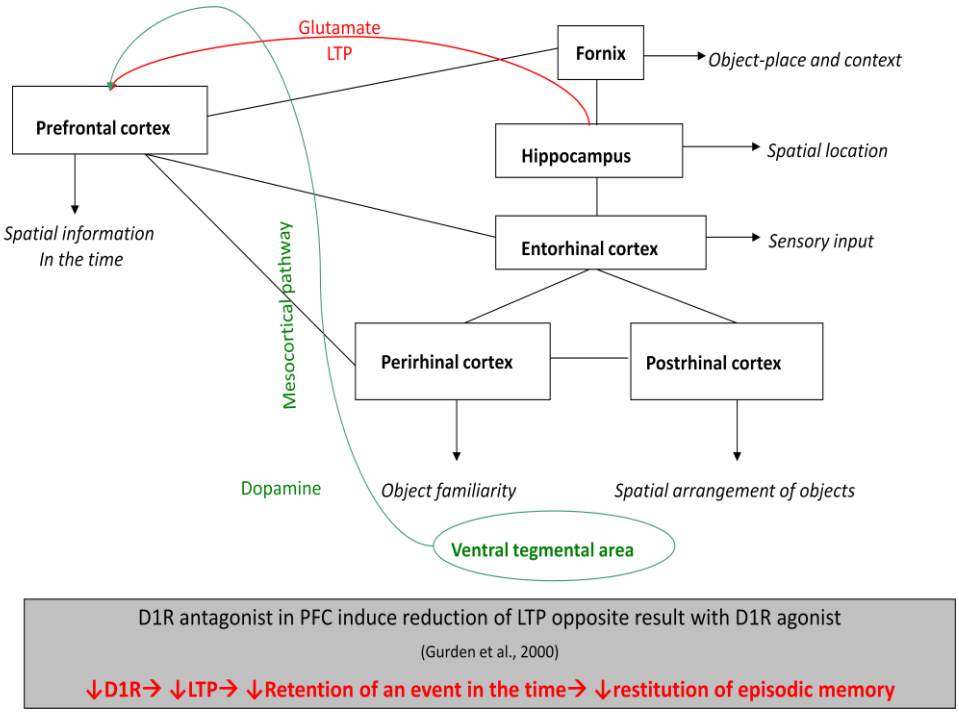



**Figure 1.10: Episodic memory process and relationship to glutamatergic and dopaminergic hypoactivity in prefrontal cortex**.

## 1.7 Episodic memory deficit in schizophrenia

People with schizophrenia have shown impairments in episodic memory in a variety of tasks (e.g. Rivermead Behavioural Memory Test (RBMT), The Autobiographical Memory Inventory (Kunitachi et al.) or Remember/Know paradigm) (Clare et al., 1993, Gold and Weinberger, 1995, Rushe et al., 1999, Danion et al., 2001, Achim and Lepage, 2003, Riutort et al., 2003, Toulopoulou et al., 2003, Al-Uzri et al., 2006). There is some evidence for dissociable impairments in autonoetic and noetic awareness. Indeed, schizophrenic people are impaired in autonoetic awareness (remembering) but not in noetic awareness (knowing) (Wheeler et al., 1997, Danion et al., 1999, Keefe et al., 2002,

Riutort et al., 2003, Sonntag et al., 2003, Neumann et al., 2006). Schizophrenic patients appear to not be able to link different elements of an event into a cohesive and memorable whole (Danion et al., 1999). Some studies using ketamine in humans (Hetem et al., 2000, Honey et al., 2005) have shown episodic memory deficit and they were able to distinguish between the two states of awarenesses. In the literature it is well established that NMDA receptor antagonist such as PCP or ketamine exacerbates and mimics schizophrenic-like symptoms in schizophrenic people and healthy volunteers (Luby et al., 1959, Javitt and Zukin, 1991, Krystal et al., 1994, Lahti et al., 1995, Jentsch and Roth, 1999, Curran and Morgan, 2000, Hetem et al., 2000, Javitt, 2007). NMDA antagonist receptor disrupts memory in human (Ghoneim et al., 1985, Krystal et al., 1994, Malhotra et al., 1996, Curran and Morgan, 2000, Hetem et al., 2000, Morgan and Curran, 2006, Javitt, 2007, Chrobak et al., 2008) without any difference between tasks: including recognition tasks (Ghoneim et al., 1985, Hetem et al., 2000, Honey et al., 2005); recall of passages of prose (Newcomer et al., 1999); recall of high and low-frequency word lists (Ghoneim et al., 1985, Malhotra et al., 1996, Hetem et al., 2000) and source memory tasks (Honey et al., 2003, Morgan et al., 2003).They also established that ketamine impairs encoding (i.e. capacity to encode the memory) but not the retrieval (i.e. capacity to recall the information into memory) part of memory (Hetem et al., 2000, Honey et al., 2005). Few studies investigated the effect of ketamine on conscious awareness in healthy volunteers (Hetem et al., 2000, Honey et al., 2005). Both studies

demonstrate an overall impairment of episodic memory but with some difference in how ketamine impairs the two awarenesses. Hetem (Hetem et al., 2000) established that ketamine does not selectively impair the two awarenesses as it is typically seen in patients with schizophrenia and it induces an overall deficit in episodic memory. Honey (Honey et al., 2005) suggests that ketamine only impaired the remembering but not the knowing part of the memory and that under ketamine participants had a tendency to guess source judgements. Both studies are contradictory and therefore, it is not clear what the effects of NMDAR antagonist are on both types of awarenesses.

Episodic memory impairment is of particular importance in schizophrenia as it is more closely associated with poor outcome than other symptoms in the disease such as hallucinations or delusions (Herlitz and Forsell, 1996, Green et al., 2004, Lepage et al., 2007, Berenbaum et al., 2008). Episodic memory impairment is also generally accompanied by other cognitive dysfunctions such as working memory, attention and executive functions (Mohamed et al., 1999, Nestor et al., 2007). Therefore, episodic memory is a key cognitive deficit in schizophrenia and as previously mentioned there is currently no treatment.

## 1.8 Episodic memory paradigms

### *1.8.1 Measuring episodic memory in humans*

Different tasks are used to assess episodic memory. It is possible to distinguish three types of test: one which assesses every day situations such as the Rivermead Behavioural Memory Test (RBMT), one which asses the general form of autonoetic awareness by asking the subject to remember specific events from their life such as The Autobiographical Memory Inventory (AMI), and one which discriminates the two forms of awareness based on remember and know paradigm such as the Remember/Know procedure.

The RBMT is used to asses every day memory by assessing every days situations that could be troublesome for people with different memory impairments. The task was firstly designed by Wilson (Wilson et al., 1985). Since now three different updates were made successively in 1999 with the RBMT-E, in 2003 with the RBMT-2 and now with the RBMT-3. Every update was created to restrict the memory deficit that could be assess but also to simplify and standardise the test. The task is based on 12 questions (14 for the RBMT-3) such as picture recognition, route: delayed or immediate recall, appointments: delayed recall and the score varies from 0 to 24 with 24 excellent memories and 0 no memory. People with schizophrenia have been shown to be impaired in this task in a numbered of studies (Cloud et al.,

1994, Doody et al., 1997, Al-Uzri et al., 2006). One criticism of the task it is not specific for episodic memory as it also addresses items that assess working or spatial memory (e.g. picture recognition).

The AMI is generally used to assess the ability or inability to remember information that preceds the onset of an illness or an accident. In schizophrenia, the task is an adaptation of Borrini (Borrini et al., 1989) version which was used to assess autobiographical memory in patients with Alzheimer's disease. The adaptation uses specific autobiographical questions which are more appropriate for the young schizophrenic population (e.g. question about retirement or work were changed). Typically people are asked to quote many personal events and name of friends of five different periods of their life in a short period of time (the preschool period; the period from ages 5 to 10; the period from age 11 to the onset of symptoms; the period from onset to 1 year before testing; and the current year). Respectively the score measures the episodic and semantic memory of the subject (Riutort et al., 2003). The AMI is generally modified through studies but generally assesses the same kinds of autobiographical questions. This type of task clearly reveals that Schizophrenic people are impaired in their capacity to remember events that occured in their past (Feinstein et al., 1998, Elvevag et al., 2003, Riutort et al., 2003, Piolino et al., 2009). Interestingly, schizophrenic people tend to report over general memories instead of specific, or events that last more than a day (e.g. during the week end) (Riutort et al., 2003, Harrison and Fowler, 2004).

Furthermore, they remember more pleasant than negative autobiographical memories or pictures, which is the opposite pattern of control group (Neumann et al., 2006).

The Remember/Know (R/K) paradigm is based on the capacity to remember or know if an item or word was paired with another item or object or belongs to a specific list in where it appears. A remember response is reflecting the capacity to be aware that an item or word has been encountered. A know answer is based on familiarity with the item or the object. In order to dissociate familiarity from guessing, a guess response is often introduced (Gardiner, 1988, Hetem et al., 2000, Sonntag et al., 2003, Honey et al., 2005). Previous studies showed that subjects used the know response when they were in fact guessing (Gardiner et al., 1997). The R/K paradigm is assumed to dissociate episodic memory (R) from semantic memory (K). This paradigm can also be analysed by Receiver Operating Curve (ROC) which shows dissociation between knowing and remembering (Yonelinas, 1994; Yonelinas, 1997; Heathcote et al., 2006) (Figure 1.11). A ROC is a function that associates the rate of correct answers (Y axis) and the rates of incorrect answers (X axis). It shows the trade-off between the two rates, the diagonal is the chance performance and how a curve is above it reflects more hit (correct answers) than false alarms (incorrect answers).

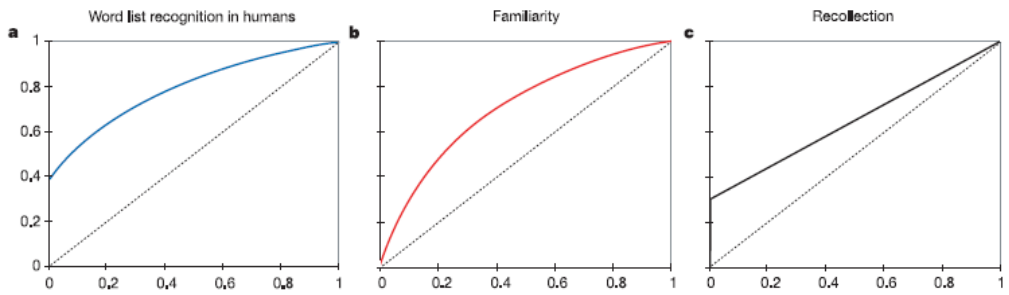



**Figure 1.11: ROCs for recognition performance of humans in verbal recognition (adapted from Yonelinas et al., 1997) (Fortin et al., 2004). On the X axis it is the number of hits (correct answers) and on the Y axis it is the number of false alarms. ROC curve distinguishes between familiarity and remembering (From Fortin et al., 2004).**

### *1.8.2 Measuring episodic memory in animals*

Until quite recently it was considered that animals did not exhibit episodic memory in a similar way to humans, as it was believed to be intrinsically associated with language and its development (Mitchell, 1993, Suddendorf and Corballis, 1997), in particular mental time travel aspects were thought to be particularly problematic in animals with several suggestions that animals do not have the notion of time (Roberts, 2002, Zentall, 2006). Nowadays, this idea has been challenged and with increasing behavioural sophistication of testing techniques it is clear that animals including rodents can encode events with specific "what", "when","where" components (Clayton and Dickinson, 1998, Aggleton and Pearce, 2001, Roberts, 2002, Eacott and Norman, 2004, Babb and Crystal, 2006, Dere et al., 2006, Kart-

Teke et al., 2006, Zentall, 2006, Eacott and Easton, 2007, Good et al., 2007b, Pillay et al., 2008).

Clayton (Clayton and Dickinson, 1998) was the first to report evidence of episodic-like memory in the food storing bird, the scrub jay. She demonstrates that scrub jays are able to remember where and when the storage of different types of food took place. This finding is described as “episodic-like” because the question of awareness or autonoetic consciousness cannot be addressed (Clayton and Dickinson, 1998). This discovery is the trigger of other experiments which have been revealed conclusive in animal capacity to integrate the “What”, “where” and “when” component of episodic memory as defined by Tulving (Tulving, 1983). Below there is a recapitulation of some studies which suggest episodic-like memory in animals (Table 1.8). For example, rats show the same capacity to remember what food they encountered (chocolate or pellets), in which arms (where) and when (delays between sample and test phase) (Babb and Crystal, 2006). Other studies demonstrate rodent’s capacity to discriminate a new object in time (or context) and place (Eacott and Norman, 2004, Dere et al., 2006, Kart-Teke et al., 2006) which is based on the object recognition paradigm which stipulates that rodents explore more a new object compared to a familiar one (Ennaceur and Delacour, 1988). These tasks are discussed in detail in the next section, and especially the OPC recognition task developed by Eacott and Norman (2004) which is used in the entire thesis.

| Species | What | Where | When | Who | Example Studies |
|---|---|---|---|---|---|
| Chimpanzees | Yes | Yes | ? | Yes | (Menzel 2005) |
| Crested Gibbons | Yes | Yes | ? | ? | (Scheumann & Call 2006) |
| Gorillas | Yes | Yes | ? | Yes | (Schwartz et al. 2002; Schwartz et al. 2005) |
| Hummingbirds | ? | Yes | Yes | ? | (Henderson et al. 2006) |
| Mice | Yes | Yes | Yes | ? | (Dere et al. 2005) |
| Orangutans | Yes | Yes | ? | ? | (Scheumann & Call 2006) |
| Rats | Yes | Yes | Yes | ? | (Babb & Crystal 2005; Eacott et al. 2005) |
| Rhesus monkeys | Yes | Yes | No | ? | (Hampton et al. 2005) |
| Scrub jays | Yes | Yes | Yes | Yes | (Clayton & Dickinson 1998; Clayton et al. 2001; Dally et al. 2006b) |

**Table 1.8: Recent studies suggesting knowledge of different "w" information in various species that form part of "episodic-like" memory (From Suddendorf and Corballis, 2007).**

## 1.9 The Object-Place-Context recognition task as a model of episodic memory in rodent?

Many studies demonstrate a rat's preference for items it sees which is dependent on what it saw, where it saw it and when it saw it in a time dependent manner (delay) (Dere et al., 2005a, Dere et al., 2005b, Kart-Teke et al., 2006, Good et al., 2007b). Eacott and Norman (2004) have shown that rats can remember what they saw and where depending on which past situation (context) they are being asked to remember. These studies exploit a rat's tendency to explore a novel object more than a familiar one. This tendency was first observed by Berlyne in 1950 (Berlyne, 1950) and then developed by Ennaceur (Ennaceur and Delacour, 1988) as the novel object recognition task (NOR). This task does not involve any food reinforcement which avoids confounding affects on palatability and does not involve any specific motivation for the rodent. Furthermore, it is relatively easy to set up (Ennaceur and

Delacour, 1988). This paradigm is widely used and has different variants such as the object in place recognition, the place recognition, context recognition or temporal recognition (Table 1.9). This task can be used to discriminate the effect of drugs in the acquisition (before sample phase) or retention phase (after sample phase) which can last up to 24 hours (Dere et al., 2005a). Lesion studies performed in the NOR task allowed the research to investigate the role of different subcortical (such as the entorhinal, postrhinal, HPC and perirhinal) or cortical (such as the PFC) unit into recognition (See Figure 1.10).

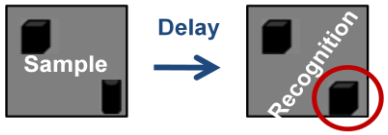
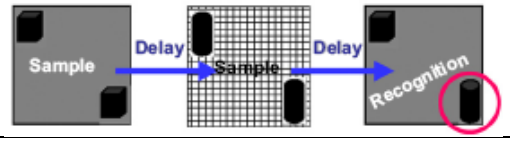
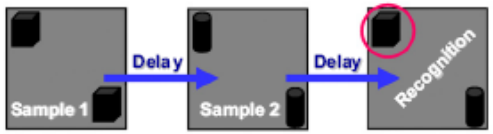
| Novelty preference paradigm | Protocol | What does it test? | Type of memory tested |
|---|---|---|---|
| Object recognition |  | Object (What?) | **Working memory.** Animals explore more the new object compared to the old one. |
| Object in place recognition |  | Object (What?)<br>Place (Where?) | **Working memory.** Animals explore more the object in a new place but based on the novelty effect of the object and place combination. |
| Place recognition |  | Place (Where?) | **Spatial memory.** Animals explore more the object in the new place compared to the old place. |
| Context recognition |  | Object (What?)<br>Context (When-Which?) | **Context discrimination.** Animals explore more the object not seen in the previously visited context. |
| Temporal recognition |  | Object (What?)<br>Recency (When?) | **Temporal memory recency versus primacy.** Animals explore more the object primarily seen compared to the object recently seen. |

**Table 1.9: The different object paradigms based on rat's tendency to explore a novelty compared to familiarity**

Compared to others episodic memory task previously used (Table 1.10), in the OPC recognition, the temporal (delay) information is secondary compared to the context in which rats first saw the objects. The context by itself acts as an “occasion specifier” and is used to discriminate between the two events (context 1 versus context 2). Similarly than in human, rats use contextual information, which is time related, to differentiate events that have occurred in the past (Smith et al., 2008). Indeed, based on the snapshot idea, episodic memory is an integration of cues (temporal or non-temporal cues) that will allow us to remember the past by reliving it: we are re-living a “specific occasion” as a snapshot (Easton et al., 2008). In order to distinguish similar events between each other, the context specificity of the event will be used to differentiate them, such as for example: the day you met this specific person for the first time at this conference, you have to remember at which conference you met them from amongst all of the conferences you have been to. Therefore, the contextual cues of that unique moment (such as conference location, where at the conference…) will be the key to remember the event and to relive it. Based on that Eacott and Norman (Eacott and Norman, 2004, Eacott and Easton, 2007, Easton et al., 2008) propose to redefine episodic memory as the recollection of an event in location depending on a specific occasion such as “what”-“where”-“which” as which the occasion or the scene memory define by the context. Therefore, the context (which) may be temporal (Clayton and Dickinson, 1998, Dix and Aggleton, 1999, Dere et al., 2005a, Dere et al., 2005b, Babb and

Crystal, 2006, Kart-Teke et al., 2006, Good et al., 2007a) or nontemporal (Gaffan, 1994, Eacott and Norman, 2004, Eacott and Easton, 2007). The OPC recognition developed by Eacott and Norman (2004) is investigating rats episodic memory using their capacity to discriminate object in place (what-where) between two contexts (or events, which) and therefore is a valid assessment of episodic memory in animal. Furthermore, after HPC or fornix lesion rats were not able to discriminate the new configuration of object, place and context (Eacott and Norman, 2004, Langston and Wood, 2009) which are consistent with previous studies which demonstrate an association episodic memory and fornix-HPC structure (Kennedy and Shapiro, 2004, Nestor et al., 2007). Eacott and Norman (2004) have also shown a task specificity of the OPC recognition compared to the object in place recognition task (what-where) in which none of the rats with any type of lesions (postrhinal, perirhinal and fornix lesion) were impaired after a similar delay (Eacott and Norman, 2004). Complementarily, it was shown that fornix lesion did not impair rats' capacity to recognize the new object in context task (what-when) which confirm the task specificity of OPC recognition (Norman and Eacott, 2005). In object or object in place recognition they only have to refer to one context (one event) whereas in the OPC recognition task rats have to discriminate objects in place between two contexts (events discrimination) which reflect the criteria of episodic memory definition in humans as the capacity to distinguish events depending of a specific context used as "scene memory" (Gaffan, 1994).

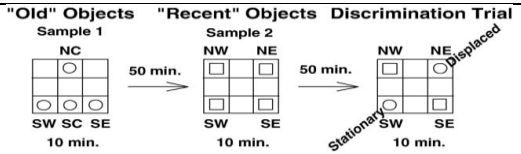
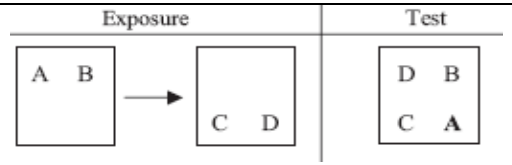
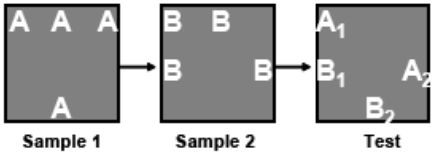
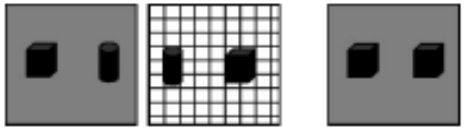
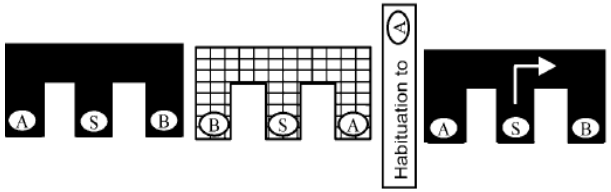
| Rodent | Episodic-like memory task | What-Where-When/which | Explanation | Reference |
|---|---|---|---|---|
| Male C57BL/6 mice |  | - Object<br>- Place<br>- Time | One object (NE) is in different place compared to when they previously saw them in sample 1 | Dere et al., 2005a |
| Male C57BL/6 mice |  | - Object<br>- Place<br>- Time | Objects A and D swap their place in the test. Object A is older than D Which should induce more exploration. | Good et al.,2007 |
| Male Wistar rats |  | - Object<br>- Place<br>- Time | Two objects (A2-B2) are in different place compared to when they previously saw them in sample 1. | Kart-Teke et al., 2006 |
| Male dark Agouti |  | - Object<br>- Place<br>- Context | One object (right one) is in a new position only depending of the context (event discrimination). | Eacott and Norman, 2004 |
| Male dark Agouti |  | - Object<br>- Place<br>- Context | R/K paradigm:<br>**Remember:** rats turn into the arm with the non habituated object (holding cage)<br>**Know:** ratio of exploration between habituated and non habituated object | Eacott and Easton, 2007 |

**Table 1.10: Episodic-like memory task in rodents based on object recognition paradigm. The OPC recognition task and the E-maze are based on contextual discrimination while he other task are based in temporal discrimination.**

## 1.10 The aims of the thesis

My studies attempt to establish an animal model of the episodic memory deficit in schizophrenia. NMDA antagonists have been shown to be psychotomimetic and induce episodic memory deficits in human studies. NMDA antagonists such as PCP induce behavioural deficits including memory that are selectively reversed by antipsychotic drugs (Grayson et al., 2007, McLean et al., 2009). Rearing rats in isolation produces behavioural (PPI, object recognition deficits) (Bianchi et al., 2006, McLean et al., 2008) and neurochemical alterations (elevated dopamine levels in the nucleus accumbens) similar to those observed in schizophrenia. Both models produce deficits in simple memory tasks in rats but have not been tested in tasks that simultaneously address the “what”, “when” and “where” aspect of episodic memory.

The following studies investigated whether PCP and/or social isolation can induce memory deficits in episodic memory tasks that capitulate the “what” “where” “when” triad. Results are compared to a task which only recapitulated the “what” and “where” aspect of working memory. The tasks used are the OPC and the object in place (OP) recognition task which have been demonstrated and replicated previously in rats (Eacott and Norman, 2004). Having established that PCP disrupted the task, it had been investigated whether the antipsychotic drug clozapine or the promnesic cholinesterase inhibitor donepezil affected PCP-withdrawal induced disruption of episodic memory in rats.

# - CHAPTER 2 -

# ESTABLISHEMENT OF THE OBJECT-PLACE-CONTEXT RECOGNITION TASK

## 2.1 Introduction

There is a considerable interest in animal models of episodic memory that might be relevant to schizophrenia, both as systems to test potential new treatments and as model systems to investigate its biological aetiology in the disease. This objective is clearly established by a consortium of researchers the “Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia: CNTRICS. One of their aims is to improve the behavioural cognitive animal models in schizophrenia. In an attempt to develop episodic memory models in animals, many studies develop different tasks which assess an animal’s capacity to encode events with specific “what”, “when”, and ”where” components (Clayton and Dickinson, 1998, Aggleton and Pearce, 2001, Roberts, 2002, Eacott and Norman, 2004, Dere et al., 2005a, Babb and Crystal, 2006, Kart-Teke et al., 2006, Eacott and Easton, 2007, Good et al., 2007a, Pillay et al., 2008).

As previously mentioned in the introduction, a study by Eacott and Norman (2004), using the OPC recognition task, demonstrated that rats

are able to remember what they saw and where, depending in which context they were in (Eacott and Norman, 2004). The OPC recognition task uses rats tendency to explore novelty (Ennaceur and Delacour, 1988) and their ability to differentiate events in term of context (context1 versus context 2) rather than time (recency versus primacy) (Eacott and Norman, 2004, Kart-Teke et al., 2006, Good et al., 2007a). Eacott and Norman (2004) also showed that the OPC recognition task is fornix dependent but that the one trial object in place recognition task (OP recognition task) is not. Recently another study using the OPC recognition task confirmed the HPC role in this task but not in one sample trial tasks that did not assess what-where-when simultaneously (Langston and Wood, 2009). These results clearly dissociated the two types of task and also corroborated previous findings showing the importance of HPC-fornix in episodic memory (Kennedy and Shapiro, 2004, Nestor et al., 2007).

In this chapter, the aim of the experiment was to replicate the OPC recognition task originally developed by Eacott and Norman (2004). However, based on previous studies on recency versus primacy (temporal) memory in rats, which showed that rats tend to explore more an object/place seen first than most recently (e.g. better memory for the last event compared to the first one) (Mumby et al., 2002, Dere et al., 2005a, Dere et al., 2005b, Barker et al., 2007), the OPC task was slightly adapted to eliminate this recency natural preference which could occur in the original task**.** In this thesis, rats were only tested in

sample phase 1 context (primacy), instead of the sample phase 1 and 2 context as tested in Eacott and Norman (2004) study.

## 2.2 Materials and Methods

### *2.2.1 Animals*

12 adult male Lister Hooded rats (Biomedical Services Unit, University of Nottingham Medical School, UK 150-200g, 300-350g at start of behavioural testing) were used. Animals received 1-2 minutes daily (9 am) handling the day after arrival at the unit and ending the day before the experiment. Animals were exposed to the test room one day before habituation. Animals for all experiments were kept in a temperature (21±2ºC) and humidity (40-60%) controlled environment on a 12 hour light/dark cycle (lights on 07:00-19:00). Food (standard animal chow, Harlan, US) and water were available *ad libitium*. Experiments were carried out in accordance with the Animals (Scientific Procedures) Act, 1986 and approved by a local ethical committee (PPL 40/2715).

### *2.2.2 Behavioural Testing*

#### 2.2.2.1 Apparatus

All testing was carried out in two different clear Perspex chambers (30 x 30 x 30 cm). A clear Perspex lid was placed on top of each chamber to prevent rats escaping but which allowed circulation of air. A video tracking was fixed to the lid to record each trial. One chamber (context 1) had a white plastic floor and the walls were covered in black and

white squares (3 $cm^2$). The second chamber (context 2) had a black plastic floor with wire mesh and four natural wood walls. Both chambers had crosses marked at the 2 locations where objects were presented. These locations were in the lower right and upper left corners 5cm from the walls of the chamber. The chambers stayed in the same position throughout the experiment. The room was lit by a single, centrally placed overhead fluorescent light (640 lux). Objects were chosen to fulfil the criteria of being easily cleaned and not easily gnawed by the rats. They were of a similar size and shape with different textures (e.g. glass, glass bottles surrounded by white tape), or colours (e.g. black and white), and heavy enough such that the rats could not push them over. Four copies of the objects were used during test trials to eliminate the use of odour cues. Objects and contexts were the same during all the experiment (Figure 2.1).

**Figure 2.1: Picture of the apparatus and the objects used in the OPC task.**

### 2.2.2.2 Habituation

Each rat received one habituation session per day for eight days. A habituation session consisted of placement of the rat in the behavioural chamber for 10 minutes with an object at the centre of the arena. Four of these habituation sessions were carried out in context 1 and four habituation sessions in context 2, the order of which was counterbalanced. The objects used during habituation were different from those used during the rest of the procedure. Objects had different shapes and sizes (Figure 2.2).

**Figure 2.2**: **Picture of the objects used during the habituation phase only and both contexts with an object in the middle**. **There was only one habituation session per day.**

### 2.2.2.3 Object-Place-Context recognition task

Trials consisted of two sample phases and a test phase separated by a delay. For all phases the rat started from the same point in the arena. Different copies of objects were used in each phase of the trial (sample1, sample 2 and test phase) to prevent the use of odour cues.

SAMPLE PHASE1: The first sample phase was carried out in context 1 with object A in the lower left corner and object B in the upper right corner (see Figures 2.3 and 2.4). Rats were allowed to explore freely for 4 minutes. Rats were then placed in their holding cage for one minute while the arena was cleaned using 70% alcohol.

SAMPLE PHASE 2: The second sample phase was carried out in context 2 with the locations of the objects switch (B now in lower left corner and A in upper right corner). Rats were allowed to explore freely for 4 minutes, after which they were placed in their holding cage for a delay period (2, 5, 10, 15, 30 or 120 minutes) before the test phase (see Figures 2.3 and 2.4).

TEST PHASE: The test phase was carried out in context 1 with two copies of the same object placed in lower left and upper right corners (either two copies of A or two copies of B). Rats were allowed to explore freely for 3 minutes. Each object, location and context was familiar to the rat, however one of the objects had never been seen before in this specific location in this specific context (see Figures 2.3 and 2.4).

Seven different delays were used: 2, 5, 10, 15, 30, 60, and 120 minutes. For each delay each rat underwent 4 different trials, one per day, comprising sample 1, sample 2 and test as described above according to the following schedule. In test phase 2 trials used context 1 and 2 trials use context 2. There were thus four possible combinations: context 1 or 2 with two objects A or two objects B (context 1 AA / BB and context 2 AA / BB). (See figure 2.4 for more

details on experimental design). The objects, places and contexts were counterbalanced and followed an irregularly sequence between animals and days. Time used between sample phase and test phase were also irregularly counterbalanced between animals and days.

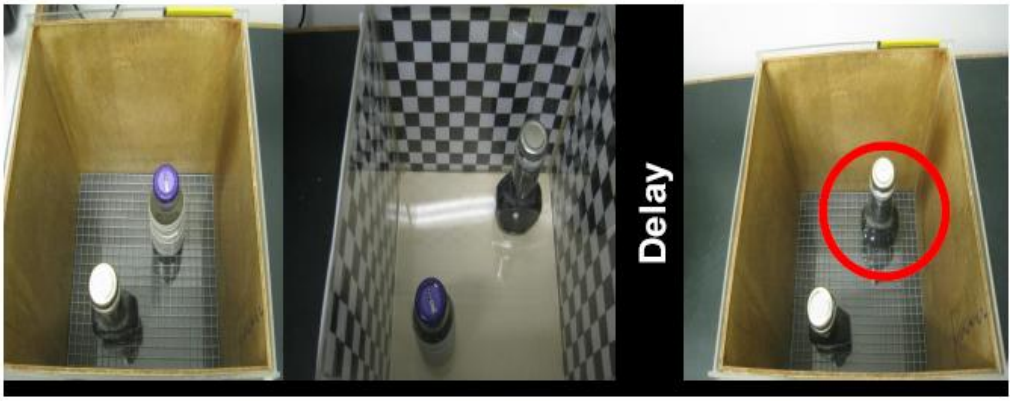



**Figure 2.3: Picture of the OPC recognition task. There was one test per day with only one delay. The new object in this condition was the one on the upper right corner (red circle), as this object (what?) had never been seen in this position (where?) in this context (when/which?).**

**1-Habituation**

- One habituation /day /animal
- 8 habituations /animals: 4 with context 1 and 4 with context 2
- One different junk object in the middle of the open field each day
- 8 days in total


**2- Test**

One test /day. Each animal is tested for all the delays
4 conditions /delay:
- 2 in context 1 (with two objects A or B)
- 2 in context 2 (with two objects A or B)
- 8 test days in total

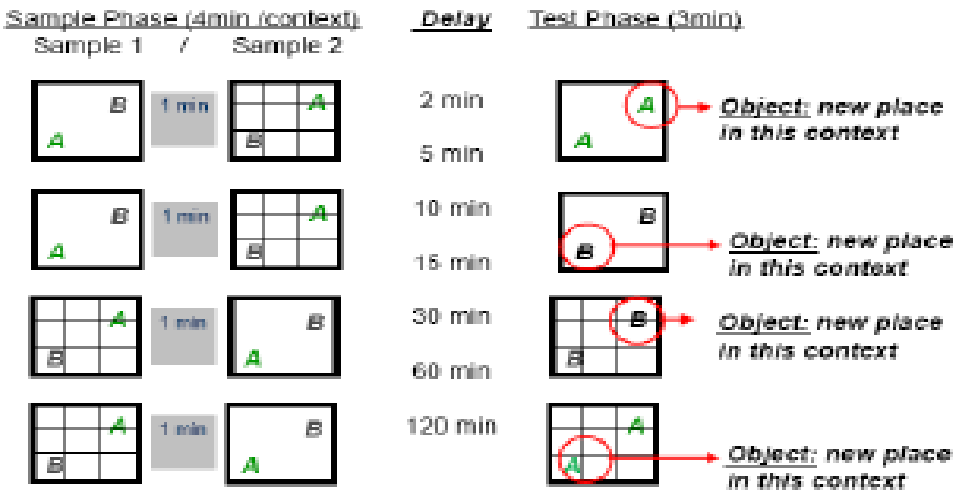



**Figure 2.4: Representation of the OPC recognition task experimental design. After 8 habituation days, rats were tested at several delays in their capacity to recollect an object in place depending on a context. Rats explored two contexts with two different objects in alternated places then, after 5 or 10 minutes delay, they were tested in one of the context (first one encountered) with two similar objects. Only one object ("what") will be on a new place ("where") depending of the context ("which-when").**

### *2.2.3 Data analysis*

Time spent (second: sec) exploring both objects during test was scored from a video recording of the test phase. A discrimination index (DI) was calculated as (time spent exploring the novel object - time exploring the old object) / (time spent exploring the novel object + time spent exploring the familiar object). A score of 0 indicated no discrimination between novel and familiar object. Note that the novel object in this procedure was the one that was in a new location for a specific context as the rat was already familiar with the object, location and context. The total amount of time spent exploring both objects was also added up and analysed separately. The video was independently scored twice and the Pearson correlation coefficient between two independent ratings of DI was r=0.8.

One sample t-test for individual delays was used to determine if the DI was significantly greater than zero according to Eacott and Norman (2004). During the test phase, DI and total time spent exploring objects was analysed using one way repeated measures ANOVA within delays. Total time spent on objects (total object exploration) was analysed by one way ANOVA with delay as within factor. ANOVA was followed by t-test between delays. Furthermore, in order to analysed the effect of a short term delay (<15 minutes) and a long term delay (>15 minutes), data were collapsed and analysed using a paired sample t-test. All data were statistically analysed using SPSS software, version 16.1 (SPSS Inc., USA).

## 2.3 Results

This experiment replicated that of Eacott and Norman (2004) establishing this model of episodic memory in rats as robust and reproducible (Figure 2.5 A). Rat's capacity to discriminate a new object in place depending of a context was delay dependent. There was no overall effect of delays on DI ($F_{(6,66)}$=1.97; p>0.05). Animals performed better than zero after 2, 5, 10 and 15 minute delays (respectively: $t_{(11)}$=3.06, p<0.05; $t_{(11)}$=3.28, p<0.05; $t_{(11)}$=4.76, p<0.005; $t_{(11)}$=3.09 p<0.005), but not after 30, 60 or 120 minutes of interval between the sample phase and the test (respectively: $t_{(11)}$=0.08, p>0.05; $t_{(11)}$=1.67, p>0.05; $t_{(11)}$=1.12, p>0.05). When data were collapsed (Figure 2.5 B) to analysed the difference between short term delay (<15 minutes) and long term delay (>15 minutes), statisticlally analysis revealed a significant difference between short and long term delay ($t_{(35)}$=3.63, p<0.05).

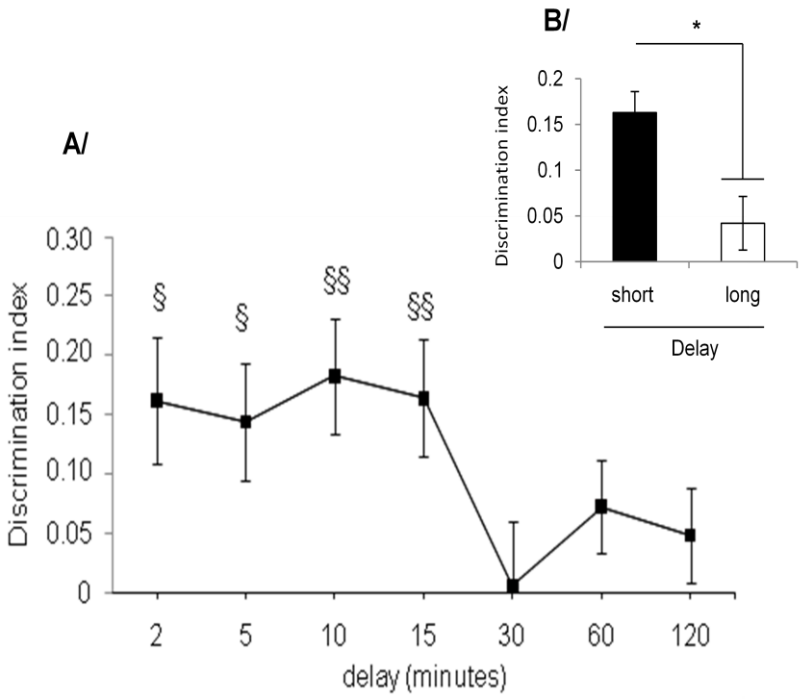



**Figure 2.5 (experiment 1): A/ The OPC task was delay dependent. Rats spent more time to explore the new object in place depending on the context after 2, 5, 10 and 15 minutes (§p<0.05 and §§p<0.005) but not after 30, 60 or 120 minutes. B/ When delays were collapsed between short term delays (<15 minutes) and long term delays (>15 minutes), there was a significant difference (*p<0.05). Data expressed as mean values (n=12) and errors bars represented ± SEM.**

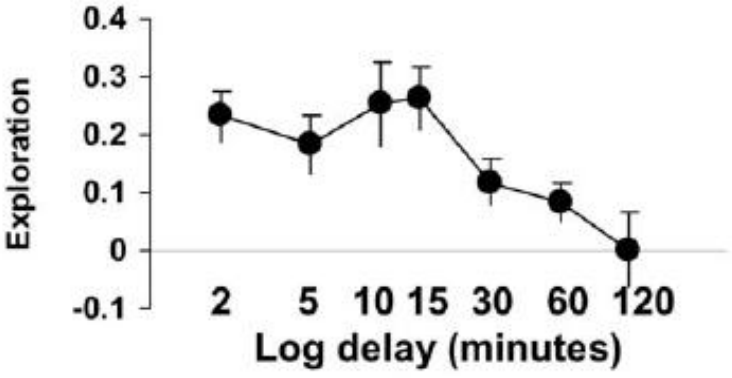



**Figure 2.6: Results of the OPC recognition task from Eacott and Norman (2004). Data (n=12) expressed as mean values and errors bars represented ± SEM.**

The total object exploration was found to be delay dependent, with a decrease in exploration between 2 minutes and15 minutes delay , after which exploration increased again (Figure 2.7). There was no significant effect of delays on total object exploration ($F_{(6,66)}$=1.64; p>0.05). However, as the delay increased, rats showed a tendency to reduce the exploration of the object with increasing delay. Indeed, rats spent more time exploring both objects at 2 minutes delay than after 15 minutes delay ($t_{(11)}$=2.54; p<0.05), with no other difference in total object exploration between delays.

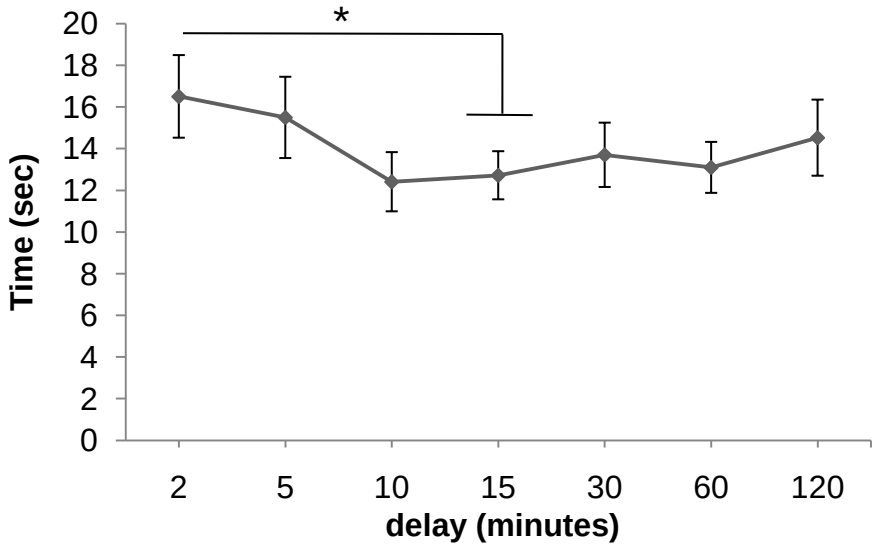



**Figure 2.7 (experiment 1): Total object exploration during the OPC recognition task. There was no general effect of delay but total object exploration reduced between 2 and 15 minutes (*p<0.05). Data expressed as mean values (n=12) and errors bars represented ± SEM.**

## 2.4 Discussion

These data show that rats performance in the modified OPC task was very similar to that in Eacott and Norman's study (2004) (Figure 2.6). Rats demonstrated a clear preference for the object in the new place depending on a specific context compared with an object in a more familiar configuration. This preference appeared to reduce when the delay increased and after 30 minutes delay rats were not able to perform the task. These data indicate that the OPC recognition task is a robust and a replicable phenomenon in rats.

A rat's memory for OPC task deteriorated after a short delay period (30 minutes) compared to rat's memory in one trial object recognition that can last over three weeks (Mumby et al., 2005, Nestor et al., 2007, Squire et al., 2007). Dix and Aggleton (Dix and Aggleton, 1999) showed that rats are sensitive to task difficulty, such that one trial object recognition is less complex than place recognition which is less complex than context recognition (Dix and Aggleton, 1999, Mumby et al., 2002) (Figure 2.8). Therefore, OPC task could be a more complex task for rats that lead to a faster decay of memory over time compared to other one trial object recognition tasks.

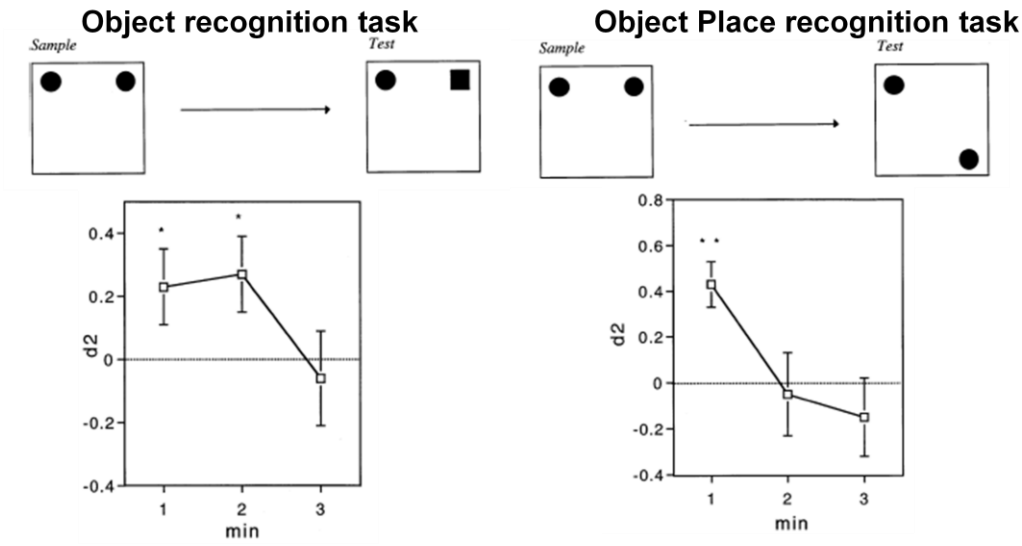



**Figure 2.8: During the test phase of the object recognition task rats spent more time to explore the new object compared to the old one during the first two minutes, but only one minute for the place recognition task. D2 ratio was defined as the difference of exploration between the newest components (object-place or object) and the oldest one divided by the total of exploration Data expressed as mean values and errors bars represented ± SEM (From Dix and Aggleton, 1999).**

In the OPC recognition task experiment, a rat's tendency to decrease exploration while the delay increased was observed. Rats tended to explore more the objects after 2 minute delay compared with 15 minute delay. However, rat's total object exploration seemed to slowly re-increase after 30 minutes delay. Rat's tendency to decrease exploration after a longer delay cannot be due to a habituation effect and therefore could be interesting to investigate further. Habituation is defined as the waning of a behavioural response due to the repetition of a stimulus. There are two forms of habituation, the long-term and the short-term form (Hinde, 1954, 1970, van der Staak, 1977). Long-term

habituation induces a decrease of exploration due to every days task repetition (rats explore more the objects on the first day than on the last day of the task) and short-term habituation is the decrease of exploration within the task (rats explore more during the first exposure into the context and less during the test phase). Therefore, the faster the rats are re-exposed to a context, the bigger the effect of short term habituation will be (less objects exploration). However, when the delay increases between the sample and test phase, there should be a dishabituation to the context and the objects and the object exploration should re-increase (Hinde, 1970, van der Staak, 1977). Unfortunately the sample phase was not recorded in the present experiment. However, in this experiment, when the delay increased, from 2 to 15 minutes, rats tended to reduce their exploration of the objects, which was the opposite pattern to that expected. Therefore, the decrease of object exploration was not due to short term habituation.

### *2.4.1 Conclusion*

In this experiment, rats performed the modified OPC task controlling for recency confound, similarly to those reported in Eacott and Norman (2004) task.

Thus, it was concluded that the task was suitable to use to assess episodic memory in rats. The next aim of these studies was to examine whether treatments that induce memory deficits in animals and humans or that mimic some of the symptoms of schizophrenia induce impairment in the task.

# - CHAPTER 3 -

# PCP WITHDRAWAL RELEVANT MODEL TO SCHIZOPHRENIA IN OPC AND OP RECOGNITION TASKS

## 3.1 Introduction

This chapter investigated the effect of withdrawal of subchronic PCP in episodic memory measured using the OPC task and a newly developed object-in-place (OP) task. As discussed previously (See chapter 1: page 31) withdrawal from subchronic PCP model is widely used as animal model relevant to schizophrenia in order to investigate the aetiology of the disease. This model demonstrates good construct, face and predictive validities of the disease (Jentsch and Roth, 1999, Enomoto et al., 2007, Mouri et al., 2007, Seillier and Giuffrida, 2009) (see Table 1.2 and 1.4).

Withdrawal from subchronic PCP induces impairment in simple object recognition task that assess the “what” (What is the new object?) dimension (Grayson et al., 2007, Karasawa et al., 2008, McLean et al., 2009) and had been defined as working memory (Ennaceur and

Delacour, 1988) but had never been tested in a task that capitulates the “what” “where” “when” triad as define in episodic memory in humans. In this chapter, withdrawal from subchronic PCP was tested in OPC recognition task. In order to further characterise the pattern of object exploration found in that experiment, an identical task addressing object in place only, without the contextual element was tested following the same withdrawal from subchronic PCP procedure. The OP recognition task was a similar task in term of number of days of habituation and conditions per delays to the OPC task; however, in this task there was only one sample phase instead of two. Therefore during the OP task rats had to remember the following event but did not have to discriminate between events.

## 3.2 Materials and Methods

### *3.2.1 Animals*

20 adult male per experiment (Experiment 2 and 3) Lister Hooded rats (Biomedical Services Unit, University of Nottingham Medical School, UK 150-200g, 300-350g at start of behavioural testing) were used. Animals received 1-2 minutes daily (9 am) handling the day after arrival at the unit and ending the day before the experiment. Animals were exposed to the test room one day before habituation. Animals for all experiments were kept in a temperature (21±2ºC) and humidity (40-60%) controlled environment on a 12 hour light/dark cycle (lights on 07:00-19:00). Food (standard animal chow, Harlan, US) and water were available *ad libitium*. Experiments were carried out in accordance with the Animals (Scientific Procedures) Act, 1986 and approved by a local ethical committee (PPL 40/2715).

### *3.2.2 Drug administration*

#### 3.2.2.1 Subchronic PCP treatment

Phencyclidine hydrochloride (PCP) was obtained from Sigma-Aldrich (Gillingham, UK) and dissolved in saline (0.9% w/v NaCl). PCP (5 mg/kg/ml in saline) was injected intraperitoneally (i.p.) to half the rats (n=10) twice daily (8 am and 6 pm) for 7 days followed by a 7 day drug

free period. The control group (n=10) received the same treatment regimen with sodium chloride 0.9% w/v (1ml/kg i.p.) The subchronic PCP regimen was established according to Jentsch's study in which they demonstrated cognitive impairment and reduction of the dopaminergic mPFC activity (Jentsch et al., 1997a).

### *3.2.3 Experiment 2: PCP withdrawal model relevant to schizophrenia in OPC recognition task*

#### 3.2.3.1 Behavioural testing

The OPC recognition followed the same procedure than used in chapter 2 (See page 82). Except that only delays of 5 and 10 minutes were used (Figures 3.1 and 3.2). Also, some modifications were made on context 2 (removed the wire mesh) and one of the object set had been changed (sand blasted glass replaced the glass surrounded by tape) (Figure 3.3). These modifications of context and objects were made because in previous experiments (not mentioned here) some rats were distracted by the wire mesh and the tape around the objects.

**1-Habituation**

- One habituation /day /animal
- 8 habituations /animals: 4 with context 1 and 4 with context 2
- One different junk object in the middle of the open field each day
- 8 days in total


**2- Test**

One test /day. Each animal is tested for all the delays
4 conditions /delay:
- 2 in context 1 (with two objects A or B)
- 2 in context 2 (with two objects A or B)
- 8 test days in total

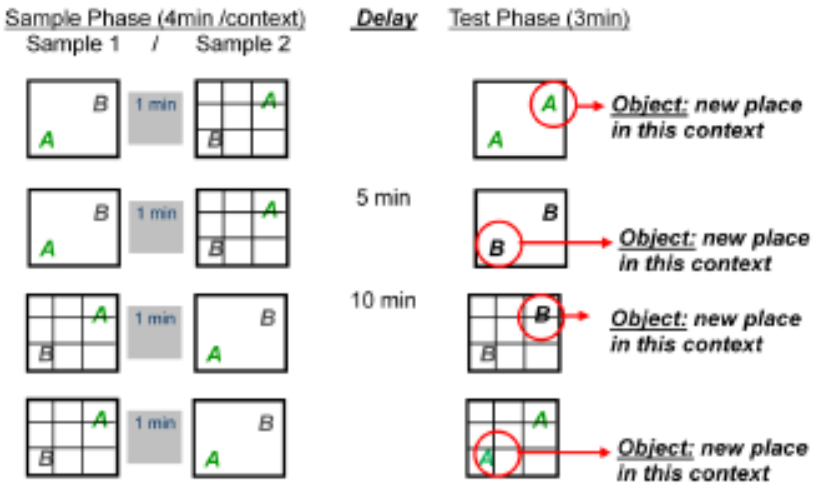



**Figure 3.1: Representation of the OPC recognition task experimental design. After 8 habituation days, rats were tested at 5 or 10 minutes delay in their capacity to recollect an object in place depending on a context. Rats explored two contexts with two different objects in alternated places then, after 5 or 10 minutes delay, they were tested in one of the context (first one encountered) with two similar objects. Only one object ("what") was on a new place ("where") depending of the context ("which-when")**

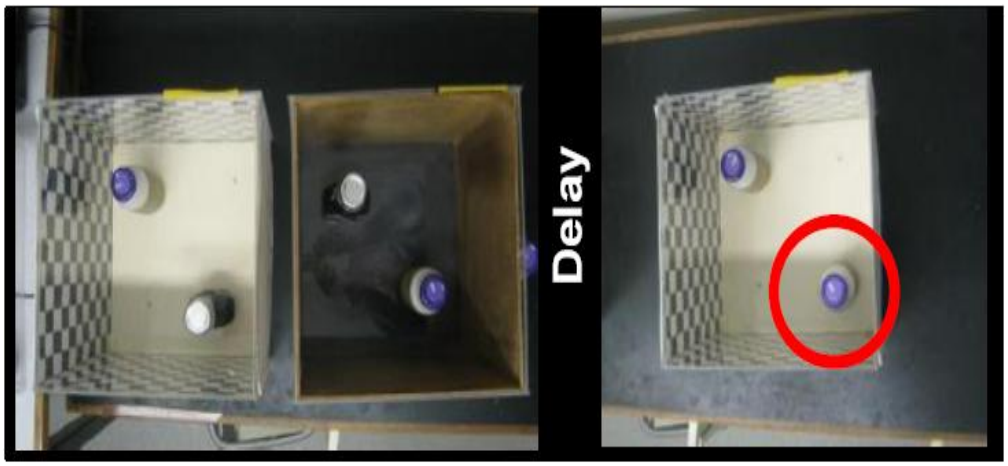



**Figure 3.2**: **Picture of the OPC recognition task. There was one test per day with only one delay. The new object in this condition was the one on the lower right corner (red circle), as this object (what?) was never seen in this position (where?) in this context (when/which?).**

**Figure 3.3: Picture of the apparatus and the objects used during the OPC and OP task.**

### *3.2.4 Experiment 3: PCP withdrawal model relevant to schizophrenia in OP recognition task*

#### 3.2.4.1 Behavioural testing

The OP recognition followed the same procedure than used in OPC recognition task, except that there was only one sample phase (no sample phase 2). The test phase was directly preceded by one sample phase and a delay of 5 or 10 minutes in between. The OP task took place in the same apparatus with similar objects than the previous OPC tasks.

*3.2.4.1.1 Habituation*

The habituation was similar to the OPC task

*3.2.4.1.2 OP recognition task*

Trials consisted of one sample phase and a test phase separated by a delay. For all phases the rats started from the same point in the arena (upper left corner, face to the wall). Different copies of objects were used in each trial (sample 1, sample 2 and test phase) to prevent the use of odour cues.

SAMPLE PHASE: The first sample phase was carried out in the context (context 1 or 2) with object A in the lower left corner and object B in the

upper right corner. Rats were allowed to explore freely for 4 minutes. Rats were then placed in their holding cage for 5 or 10 minutes delay while the arena was cleaned using 70% alcohol (see Figures 3.4 and 3.5).

TEST PHASE: The test phase was carried out in the same context than in sample phase (context 1 or 2) with two copies of the same object placed in lower left and upper right corners (two copies of A or two copies of B). Rats were allowed to explore freely for 3 minutes. Each objects and locations was familiar to the rat, however one of the objects had never been seen before in this specific location (see Figures 3.4 and 3.5).

Two delays were used (5 and 10 minutes), being selected on the basis the previous OPC task (not presented here). For each delay each rat underwent 4 different trials, one per day, comprising sample and test phase as described above according to the following schedule. 2 trials use context 1 and 2 trials used context 2 in test phase. There were thus four possible combinations: context 1 or 2 with two objects A or two objects B (context 1 AA / BB and context 2 AA / BB). (See figure 3.4 for more details on experimental design). The object's places during the sample phase were counterbalanced between delays and animals.

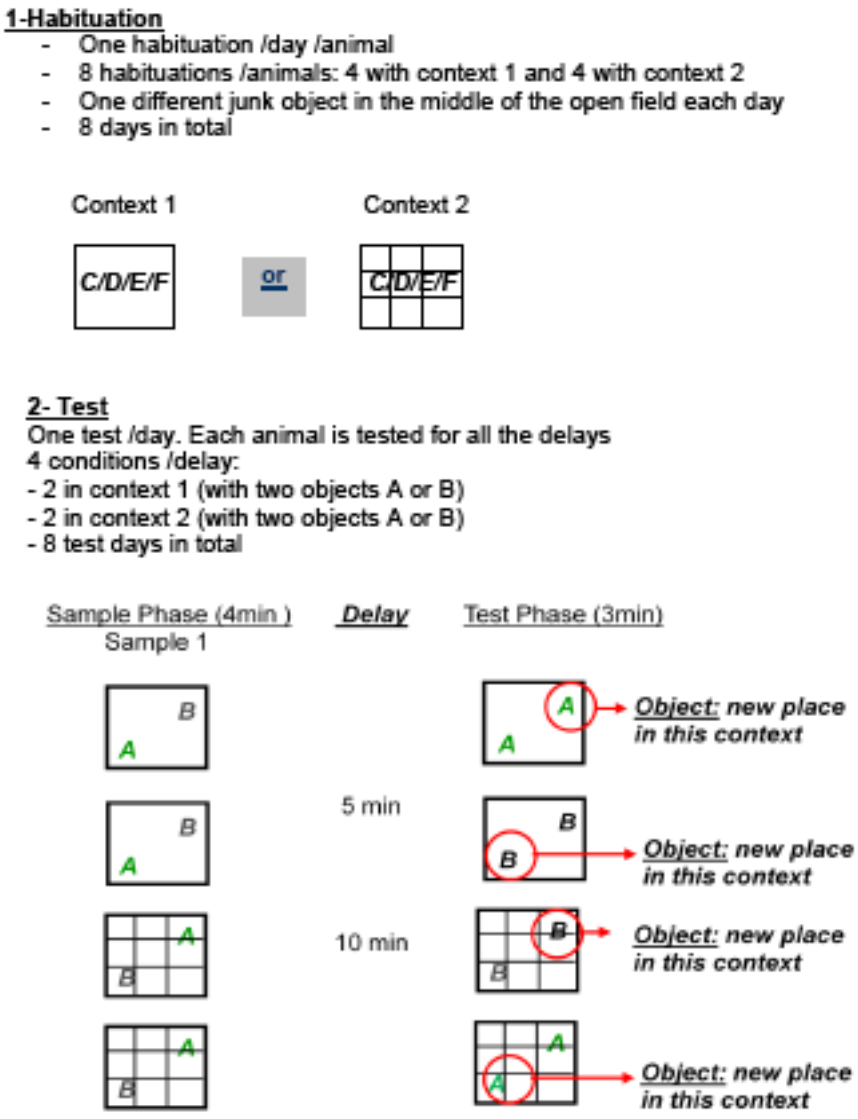



**Figure 3.4: Representation of the OP recognition task experimental design. After 8 habituation days, rats were tested at 5 or 10 minutes delay in their capacity to recollect an object in place. Rats explored only one of the two contexts with two different objects (A and B) then, after 5 or 10 minutes delay, they were tested in the same context with two similar objects (A-A or B-B). Only one object ("what") was be on a new place ("where") but in this task it did not depend on the context.**

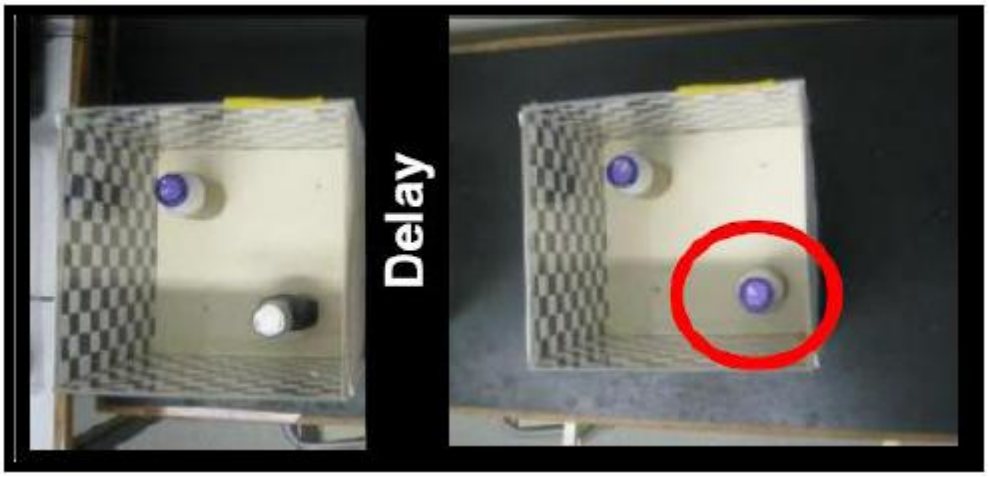



**Figure 3.5: Picture of the OP recognition task. There was one test per day with only one delay. The new object in this condition was the one on the lower right corner (red circle), as this object (what?) was never seen in this position (where).**

### *3.2.5 Data analysis*

Time spent (second: sec) exploring both objects during test was scored from a video recording of the test phase. A discrimination index (DI) was calculated as (time spent exploring the novel object - time exploring the old object)/ (time spent exploring the novel object + time spent exploring the familiar object). A score of 0 indicated no discrimination between novel and familiar object. Note that the novel object in this procedure was the one that was in a new location for a specific context as the rat was already familiar with the object, location and context. The total amount of time spent exploring both objects is also added up and analysed separately.

For the sample phase a split-plot ANOVA was used with exploration time (in seconds) as dependent variable and treatment and experimental day as factors.

For the test phase**,** one-way ANOVA for each delay was performed with DI as dependent variable with treatment as factor followed by planned *post-hoc* t-tests where appropriate. Two-way ANOVA was performed with exploration time (sec) as dependent variable between treatments and within delays followed by planned *post-hoc* t-tests where appropriate. One sample t-test for individual treatment groups was used to determine if the DI was significantly greater than zero as reported in Eacott and Norman (2004). All data were statistically analysed using SPSS software, version 16.1 (SPSS Inc., USA).

## 3.3 Results

### *3.3.1 Experiment 2: PCP withdrawal rats in OPC recognition task*

In the sample phase *(*Figure 3.6), there was a significant decrease in total object exploration over the 8 days of the procedure. There was a significant effect of days on total object exploration ($F_{(7,126)}$=46.16, $p<0.01$) but no effect of treatments ($F_{(1,18)}$=0.23, $p>0.05$) nor interaction between treatments and days ($F_{(7,126)}$=0.28, $p>0.05$).

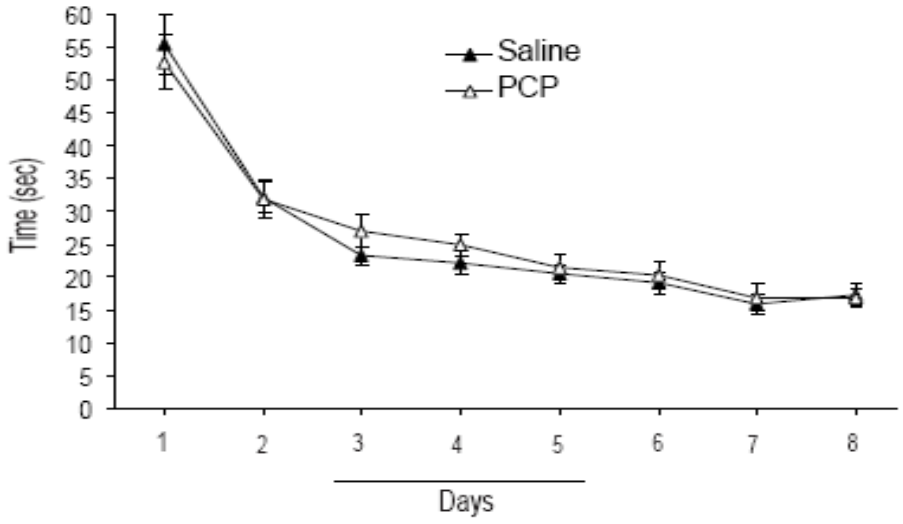



**Figure 3.6 (experiment 2): In the sample phase both saline and PCP withdrawal groups explored the two objects the same amounts of time (time in second: sec). Data expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

In the test phase *(*Figure 3.7*)*, at 5 minutes delay there was a significant effect of treatments on DI ($F_{(1,19)}$=8.01, p<0.05). Saline group DI was significantly greater than PCP group DI ($t_{(18)}$=2.83, p<0.05). Saline treated rats DI was significantly greater than zero at the 5 minutes delay ($t_{(9)}$=2.62, p<0.05) but not 10 minutes delay ($t_{(9)}$=1.45, p>0.05). PCP rats were impaired on the task at both delays; the DI was not significantly greater than 0 at either the 5 minutes ($t_{(9)}$=-1.32, p>0.05) or 10 minutes delay ($t_{(9)}$=0.63, p>0.05).

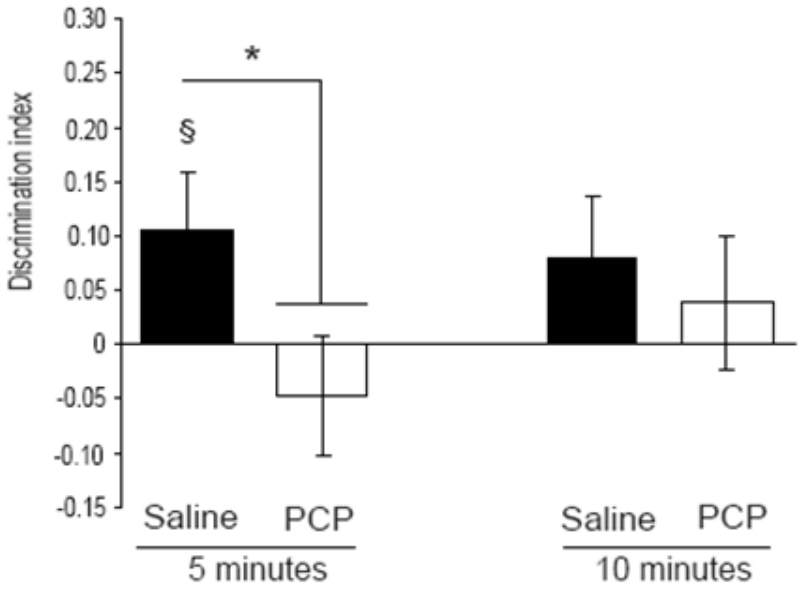



**Figure 3.7 (experiment 2): PCP withdrawal induced OPC recognition deficit in rats**. **After 5 minutes delay, only saline group performed above zero (§p>0.05) with a significant difference between scores compared to PCP group (*p>0.05). Data expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

Total object exploration during the test phase *(*Figure 3.8) was delay dependent for the saline group but not for the PCP withdrawal group. There was a significant effect of delays on total object exploration ($F_{(1,18)}$=10.51, p<0.05) but not of treatments ($F_{(1,18)}$=0.87, p>0.05) nor interaction between treatments and delays ($F_{(1,18)}$=2.40, p>0.05). Saline group explored the two objects more after 5 minutes delay than after 10 minutes ($t_{(9)}$=3.07, p<0.05) which was not the case for PCP withdrawal group for which total object exploration was not affected by delay ($t_{(9)}$=1.35, p>0.05).

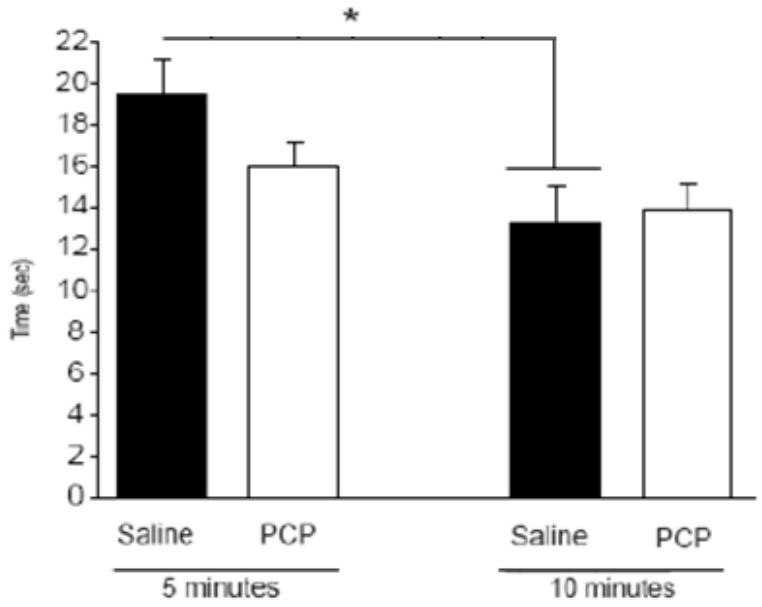



**Figure 3.8 (experiment 2): In the test phase, total object exploration reduced with increasing delay in the saline group but not for the PCP withdrawal group**. **Saline group after 5 minutes delay compared to 10 minutes delay (*p<0.05). Data expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

### *3.3.2 Experiment 3: PCP withdrawal rats in OP recognition task*

Total object exploration was not affected by treatment. There was a significant effect of days on total object exploration ($F_{(7,126)}$=63.84; p<0.001) but not of treatments ($F_{(1,18)}$=0.37; p>0.05) *(*Figure 3.9). This diminution of total object exploration by days can be explained by habituation to the task.

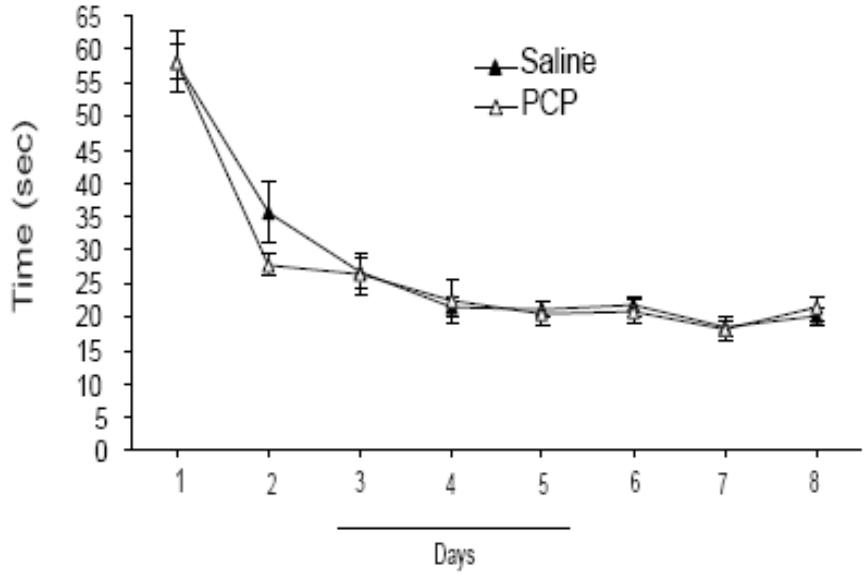
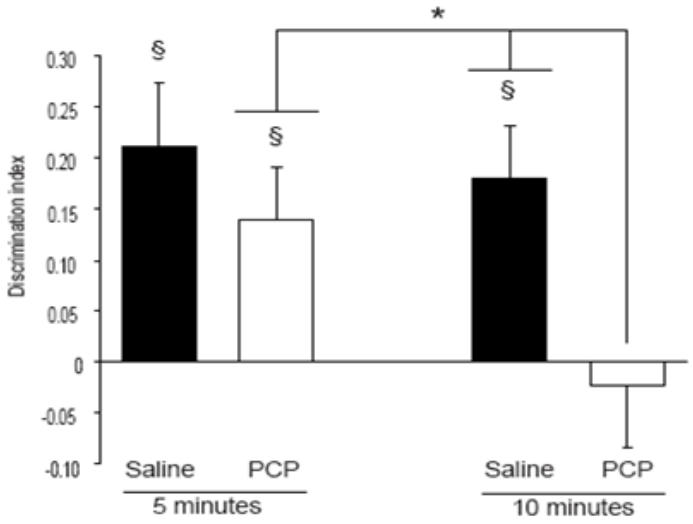



**Figure 3.9 (experiment 3): In the sample phase both saline and PCP withdrawal groups explored the two objects the same amounts of time (time in second: sec). Data expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

In the test phase (Figure 3.10), There was a significant effect of treatments ($F_{(1,18)}$=6.12, p<0.05) and delays ($F_{(1,18)}$=5.38, p<0.05) on DI with an interaction between treatments and delays ($F_{(1,18)}$=8.01, p<0.05). PCP group DI after 10 minutes delay was significantly different from saline group at the same delay ($t_{(18)}$=2.71, p<0.05) and also from PCP group after 5 minutes delay ($t_{(9)}$=2.23, p=0.05). Saline group DI was significantly greater than zero after 5 and 10 minutes delay ($t_{(9)}$=3.79, p<0.05 and $t_{(9)}$=5.23, p<0.05). Also PCP group DI was significantly greater than zero after 5 minutes delays ($t_{(9)}$=4.34, p<0.05) but not after 10 minutes delay ($t_{(9)}$=-0.35, p>0.05).




.

**Figure 3.10 (experiment 3): PCP withdrawal induced OP recognition deficit in rats**. **After 5 minutes delay, both groups were able to discriminate the new object in place ($^{§}$p<0.05). However, after 10 minutes delay, only the saline group spent more time exploring the object in a new place ($^{§}$p<0.05). There was a significant difference between PCP group at 10 minutes delay compared to PCP group after 5 minutes delay and saline group after 10 minutes delay (*p≤0.05). Data expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

Total object exploration in the test phase of the OP task (Figure 3.11) was neither treatment nor delay dependent. There was no significant effect of delays on total object exploration ($F_{(1,18)}$=0.30, p>0.05), nor treatments ($F_{(1,18)}$=0.43, p>0.05) nor interaction between treatments and delays ($F_{(1,18)}$=0.47, p>0.05).

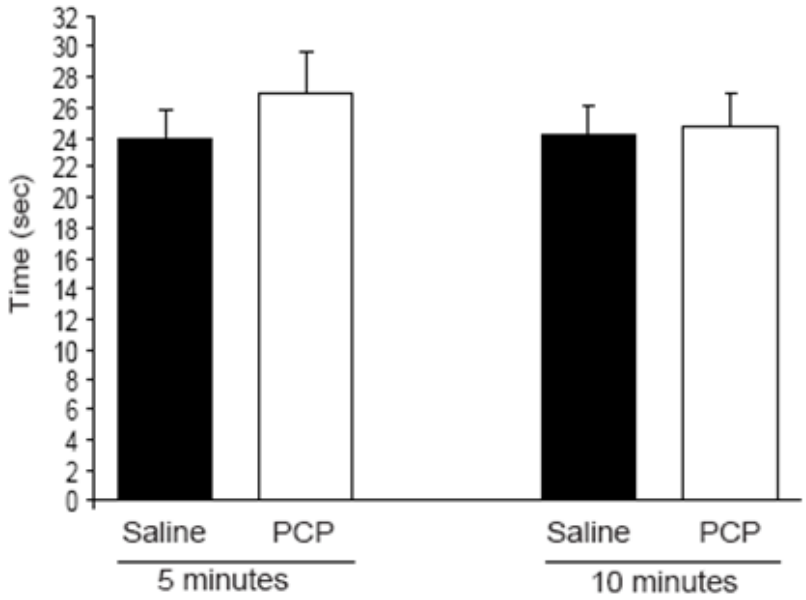



**Figure 3.11 (experiment 3): In the test phase, total object exploration was neither affected by PCP withdrawal nor delay. Data expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

## 3.4 Discussion

These data indicate that PCP withdrawal induces a deficit in recognition memory that requires simultaneous memory for the “what” “when” and “where” aspects. Reduced memory was seen at 10 minutes delay in saline controls confirming the sensitivity of the task to delay between acquisition and recall (Eacott and Norman, 2004), while PCP withdrawal rats showed a deficit at both 5 and 10 minute delays. Secondly, it was found unexpectedly that saline treated rats showed a delay dependent decrease in total object exploration which was not seen in PCP withdrawal rats. Thirdly, these results demonstrated that in the OP task that does not contain an explicitly contextual element, PCP withdrawal did induce a deficit in OP recognition. However, the delay induced total object exploration decrease identified in the previous experiment, in the OPC task, was not seen in controls. A direct comparison between the two tasks showed in control rats that after 10 minutes delay recognition was not affected in the OP recognition task but was in the OPC recognition task and also that after 5 minutes delay PCP-treated rats were able to recognise an object in a new place but not an object in a new place depending on the context. Finally, object exploration in the test phase was not affected by treatment and/or delay when the recognition task did not involve context discrimination. These results clearly demonstrated specific difference between the OPC recognition task and the OP recognition task which did not involve contextual discrimination. Therefore, the OPC recognition task may be

a useful model to assess episodic memory deficits consequent to treatments relevant to schizophrenia.

### *3.4.1 New component discrimination*

As shown in chapter 2, OPC recognition task assesses episodic memory in rats as the capacity to discriminate between two events. PCP withdrawal rats were unable to distinguish the newest object place and context combination. These results demonstrate a cognitive deficit in episodic memory after a subchronic PCP treatment in rats (5 mg/kg twice daily during 7 days). These data reinforce the cognitive list of impairment induced by subchronic PCP treatment in rodents (Castellani and Adams, 1981, Jentsch et al., 1997b, Hashimoto et al., 2005, Rodefer et al., 2005, Abdul-Monim et al., 2006, Grayson et al., 2007, Javitt, 2007, Hashimoto et al., 2008) and are consistent with human studies showing that acute non-competitive NMDA receptor antagonists disrupt episodic memory performance (Hetem et al., 2000) more specifically for the PCP-withdrawal model that these deficits are persistent after drug discontinuation (Malhotra et al., 1996). Subchronic PCP treatment in rats is reminiscent of the episodic memory deficit described in schizophrenic patients (Herlitz and Forsell, 1996, Rushe et al., 1999, Toulopoulou et al., 2003, Lepage et al., 2007, Nestor et al., 2007). Combined with the neuropathological changes induced by the PCP treatment (Olney et al., 1989), this study confirms the utility of the subchronic PCP model as a model relevant to schizophrenia and

confirms the importance of the NMDA glutamatergic systems in memory and more specifically in episodic memory.

As this model involves withdrawal from PCP rats are not tested under the acute influence of the drug and thus it is unlikely that results are mediated by extraneous effects of PCP on general performance in the rats. There were no significant differences in object exploration between PCP and control rats in the sample phases confirming that withdrawal from PCP had no effect on general exploration pattern. Furthermore, PCP withdrawal did not affect rats' memory in the OPC task after 5 minutes delay, but did in the OPC task. The control group, after 5 minutes but not after 10, recognised the object in a new place and context in a task that involved "what", "where", "when". However when the task did not involve a context discrimination rats were able to perform the task after 10 minutes delay. These results clearly established a task difference between the OPC recognition and the OP recognition task. To some extent, it is possible to postulate that the OPC recognition task assesses episodic memory which recapitulates the what-where-when (Eacott and Norman, 2004, Langston and Wood, 2009) and the OP recognition task assesses working memory as it had been originally postulated by Ennaceur and Delacour, (Ennaceur and Delacour, 1988). Therefore, the OP recognition task is a clearly distinct task which involves different types of memory as it has been previously demonstrated in the literature (Eacott and Norman, 2004, Langston and Wood, 2009). Indeed, the OPC recognition task is HPC/fornix

dependent which is not the case of the OP recognition task (Eacott and Norman, 2004, Langston and Wood, 2009).

There are a number of possible underlying mechanisms that could mediate PCP-induced deficit in episodic memory. Subchronic PCP reduces parvalbumin-immunoreactive (parvalbumin-IR) neurons in the CA2/3 sub-region of the HPC and reduces prefrontal cortical dopaminergic activity (Jentsch et al., 1997b, Abdul-Monim et al., 2007). Given that prior studies have demonstrated a role of both the HPC (Eichenbaum, 2003) and frontal cortical networks in the temporal discrimination of events and episodic memory (Lee and Kesner, 2003, Barker et al., 2007) these are possible mechanisms that could be explored in future studies.

### *3.4.2 Total object exploration*

In the sample phase, total object exploration for saline/PCP rats showed a long term habituation effect of days (reduction of the exploration by days). This effect was constant for all the groups in both experiments.

In the OPC recognition task, in the test phase, total object exploration was delay dependent for saline-treated rats. The Control group explored the two objects after a 5 minutes delay more than after a 10 minutes delay. This effect was abolished either by PCP withdrawal or

when the task is not context dependent. In the OP recognition task saline group's object exploration was not affected by delay. This effect on total object exploration, as previously explained in chapter 2, cannot be due to a short term habituation to the context-object. Also, this effect was task dependent and seemed to be related to context discrimination. These results plus those in subsequent experiments have suggested that this measure may be representative of remembering (autonoetic awareness) in rats which could be distinct from rats capacity to discriminate the newest combination of object-place-context based on familiarity (noetic awareness) (Tulving, 1985, Fortin et al., 2004, Eacott and Easton, 2007) (see chapter 8: page: 189).

### *3.4.3 Conclusion*

These experiments established that PCP withdrawal abolished rat's memory in a task that simultaneously assessed the what-where-when. This deficit was not due to rat's incapacity to discriminate novelty as, after 5 minutes delay, PCP-treated rats were able to recognise an object in a new place in the OP recognition task. Therefore, results showed that both tasks did not assess the same type of memory (Eacott and Norman, 2004, Dere et al., 2007, Easton et al., 2008, Langston and Wood, 2009) and that one task was more complex than the other one, as control rats performed better into the OP task than in the OPC task. Furthermore, a delay dependent effect on total object

exploration in control group was noticed in the test phase of the OPC task but not in the OP task. This total object exploration seemed to be induced by the context and cannot be due to habituation effect as in the sample phase both groups explored the objects at the same extent.

The next step was to test another animal model relevant of schizophrenia, the isolated rearing model, in the OPC and OP recognition task, in order to correlate the findings with the subchronic PCP model.

# - CHAPTER 4 -

# ISOLATION REARING MODEL RELEVANT TO SCHIZOPHRENIA IN OPC AND OP RECOGNITION TASKS

## 4.1 Introduction

This chapter investigated the effect rearing rats pups from weaning in isolation on episodic memory in adulthood. Isolation rearing model is used as a model relevant to neurodevelopmental aspects of schizophrenia (see Figure 1.4 and Table 1.6). As with the subchronic PCP model, the isolation rearing model induces a robust increase in natural forgetting in the simple non-spatial object recognition task (Bianchi et al., 2006, McLean et al., 2008) (See chapter 1: page 26 for fuller treatment effects of isolation rearing**).** However, the effect of isolation rearing has not been previously examined in an episodic memory task. The following chapter describes the effects of isolation rearing on both OPC recognition (Eacott and Norman, 2004, Langston and Wood, 2009) and OP recognition using the same tasks established in chapter 3.

## 4.2 Materials and Methods

### *4.2.1 Animals*

For each experiment (experiment 4 and 5), 20 experimentally naïve male Lister Hooded rats (Biomedical Services Unit, university of Nottingham Medical School, UK) weighing 50-100g were obtained immediately after weaning on postnatal day (PND) 24. Animals were pseudo-randomly assigned (counter-balanced by weight and litter) into two rearing groups, housed either singly (socially isolated) or in groups of 4 (group housed) per cage. For the duration of the experiment, all rats were reared for at least 5 weeks, with minimal handling, in plastic cages with sawdust lined, solid bases and no additional environmental enrichment prior to experimental testing. Animals were housed in the same temperature (21±2ºC) and humidity (40-60%) controlled holding room on a reversed 12 hour light/dark cycle (lights on 07:00-19:00) and received the same auditory, olfactory and visual cues but only group-housed rats were able to make physical contact with littermates. All experiments were carried out during the light phase. Food (standard animal chow, Harlam, US) and water were available *ad libitium*. Experiments were carried out in accordance with the Animals (Scientific Procedures) Act, 1986 and approved by a local ethical committee.

### *4.2.2 Behavioural tasks before the OPC or the OP recognition task*

Rats were tested in the OPC or the OP recognition task eight weeks after their isolation (post natal day (PND) 79). On PND 58, rats were tested in a locomotor activity task in a novel arena which was followed twenty four hours later by a two trial non-spatial object recognition with an inter trial interval of 2 hours (PND 59). On PND 65 rats performed a PPI task and then a conditioned emotional response task (CER) on PND 72 (Figure 4.1). This part of the protocol was not performed by me but has been utilised previously to assess behavioural alterations that are thought to have translational relevance to some of the core positive, negative and cognitive defects seen in schizophrenia (Fone and Porkess, 2008) and the resultant data from the these test is therefore only given in the appendix to validate the successful development of the neurodevelopment alterations in this experiment.

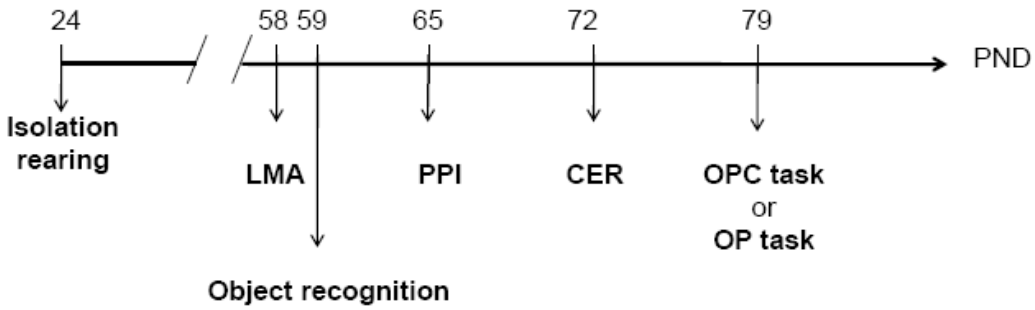



**Figure 4.1: Summary of experimental procedures undergone by the isolated rats before the OPC or the OP task.**

### *4.2.3 Experiment 4 and 5: Isolation rearing model relevant to schizophrenia in OPC recognition task*

#### 4.2.3.1 Behavioural testing

The OPC and OP recognition followed the same procedure used in chapter 3 (See page 97 and 100). Results were analysed following the same procedure than in chapter 3 (Seee page: 103).

## 4.3 Results

### *4.3.1 Experiment 4: Isolated rats in OPC recognition task*

In the sample phase, overall, isolated rats spent more time exploring the two different objects (Figure 4.2). There was a significant effect of treatments ($F_{(1,18)}$=4.69; p<0.05) and days ($F_{(7,126)}$=22.16; p<0.001) on total object exploration but no interaction between treatments and days ($F_{(7,126)}$=1.70; p>0.05).

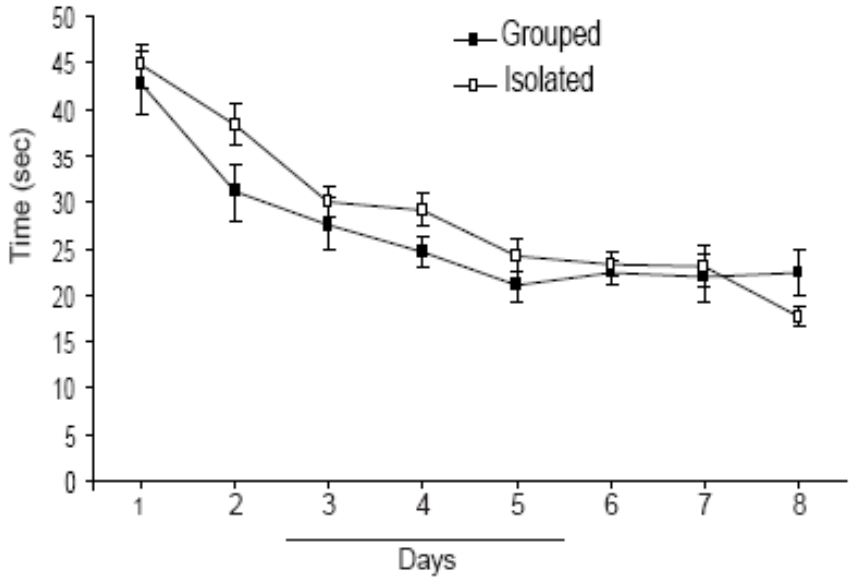



**Figure 4.2 (experiment 2): In the sample phase isolated group explored more the two objects compared to grouped group (time in second: sec). Data are expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

In the test phase (Figure 4.3), there was a significant effect of delays on DI ($F_{(1,18)}$=6.24; p<0.05) but no effect of treatments ($F_{(1,18)}$=0.08; p>0.05) nor interaction between treatments and delays ($F_{(1,18)}$=0.12; p>0.05). At the 5 minutes delay both isolated and group housed rats spent more time exploring the object in new location depending on context (group-housed rats: $t_{(9)}$=2.40; p<0.05 and isolated rats: $t_{(9)}$=2.74; p<0.05 compared to zero). At 10 minutes delay neither group did (group-housed rats: $t_{(9)}$=0.22; p>0.05 and isolated rats: $t_{(9)}$=0.71; p>0.05 compared to zero).

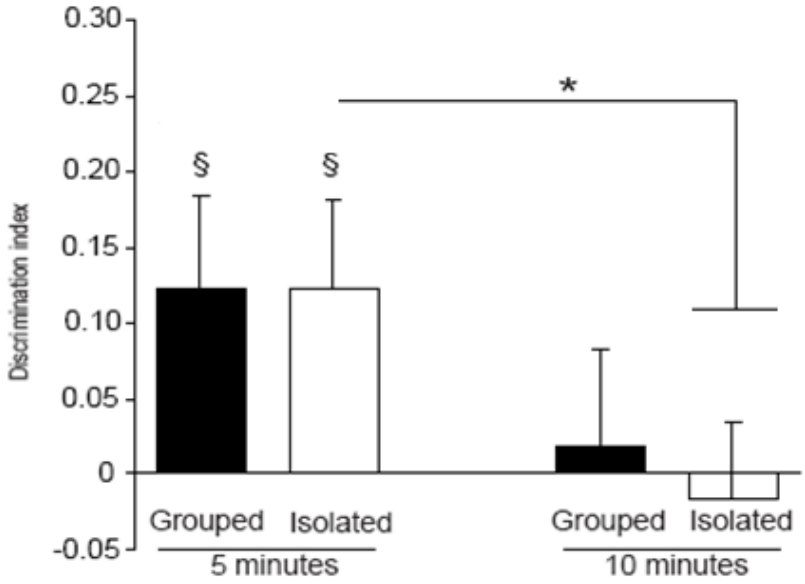



**Figure 4.3 (experiment 4): Isolation reared and group housed rats were not impaired in the OPC task. Both groups of rats recognised the object in the new place depending on the context after 5 minutes delay ($^{§}$p<0.05) but not after 10 minutes delay (*p<0.05 isolated rats within delays). Data are expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

In the test phase, the total object exploration was affected by delay for the group-housed rats but not the isolated littermates (Figure 4.4). There was no significant effect of treatments ($F_{(1,18)}$=0.73; p>0.05) nor delays ($F_{(1,18)}$=3.69; p>0.05) on total object exploration but there was an interaction between delays and treatments ($F_{(1,18)}$=5.11; p<0.05). Group-housed rats spent significantly more time exploring the two objects after 5 minutes delay than after 10 minutes delay ($t_{(9)}$=2.95; p<0.05).

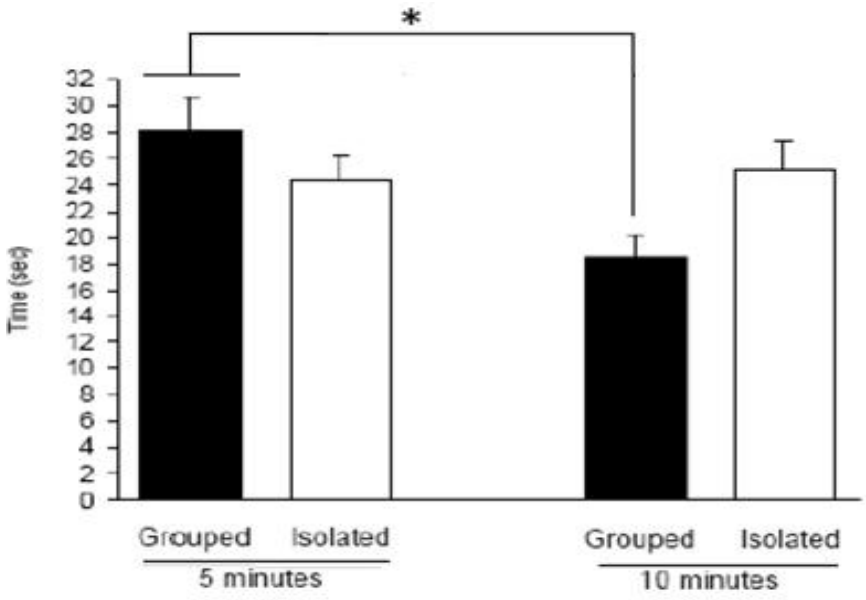



**Figure 4.4 (experiment 4): In the test phase, total object exploration was affected by isolation rearing and delay. Grouped rats after 5 minutes delay compared to 10 minutes delay (*p<0.05). .Data are expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

### *4.4.2 Experiment 5: Isolated rats in OP recognition task*

In the sample phase, isolated rats explored more the two objects than the grouped one (Figure 4.5). There was a significant effect of treatments ($F_{(1,18)}$=4.91; p<0.05) and days ($F_{(7,126)}$=30.99; p<0.001) on total object exploration but no interaction between treatments and days ($F_{(7,126)}$=0.63; p>0.05).

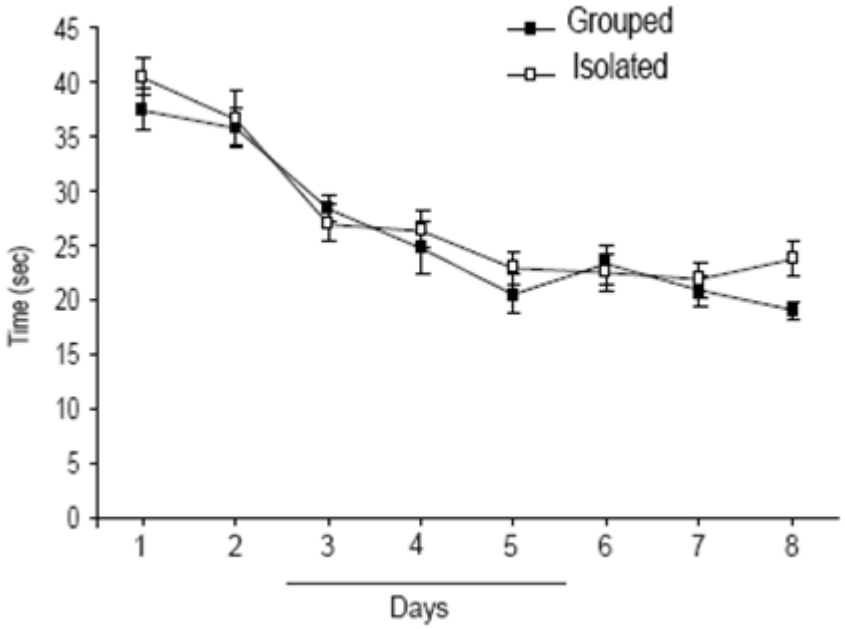



**Figure 4.5 (experiment 5): In the sample phase isolated group explored more the two objects compared to grouped group (time in second: sec). Data are expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

In the test phase, there was no effect of treatment on rat's capacity to distinguish the familiar from the novel object *(*Figure 4.6). There was no effect of treatments ($F_{(1,18)}$=0.25; p>0.05) nor delays ($F_{(1,18)}$=0.12; p>0.05) nor interaction between treatments and delays ($F_{(1,18)}$=0.37; p>0.05) on DI. DI compared to zero highlighted that both groups performed the task at both delays (group-housed rats: at 5 minutes $t_{(9)}$=3.52; p<0.05; at 10 minutes $t_{(9)}$=3.36; p<0.05 and isolated rats: at 5 minutes $t_{(9)}$=4.04; p<0.05; at 10 minutes $t_{(9)}$=2.89; p<0.05).

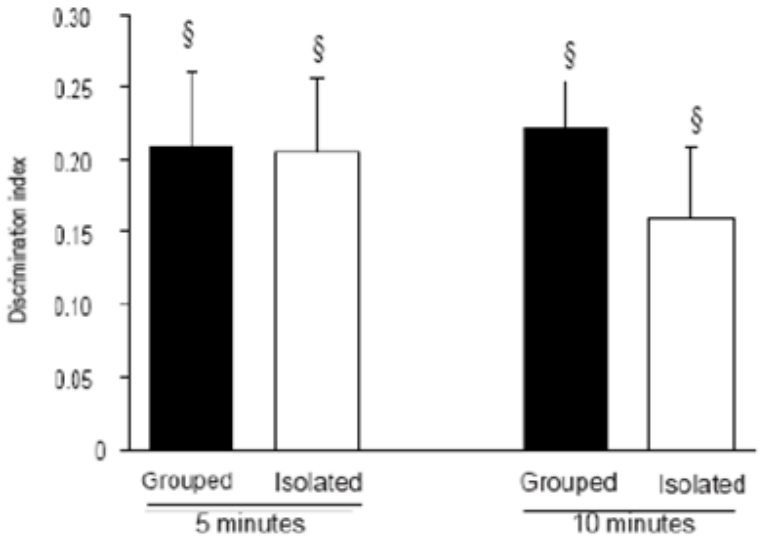



**Figure 4.6 (experiment 5): Isolation reared and group housed rats were not impaired in the OP task. Both groups of rats recognised the object in the new place after 5 and 10 minutes delay (§p<0.05 compared to 0). Data are expressed as mean values and errors bars represented ± SEM.**

Total object exploration in the test phase of the OP task (Figure 4.7) was neither treatment nor delay dependent. There was no significant effect of treatments on total object exploration ($F_{(1,18)}$=0.61; p>0.05) nor delays ($F_{(1,18)}$=0.94; p>0.05) nor interaction between treatments and delays ($F_{(1,18)}$=1.50; p>0.05).

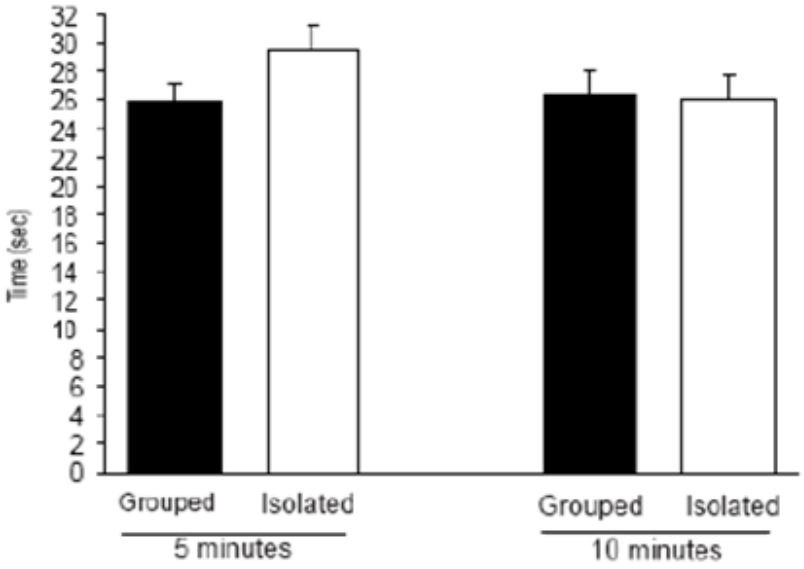



**Figure 4.7 (experiment 5): In the test phase, total object exploration was neither affected by isolation rearing nor delay. Data are expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

## 4.4 Discussion

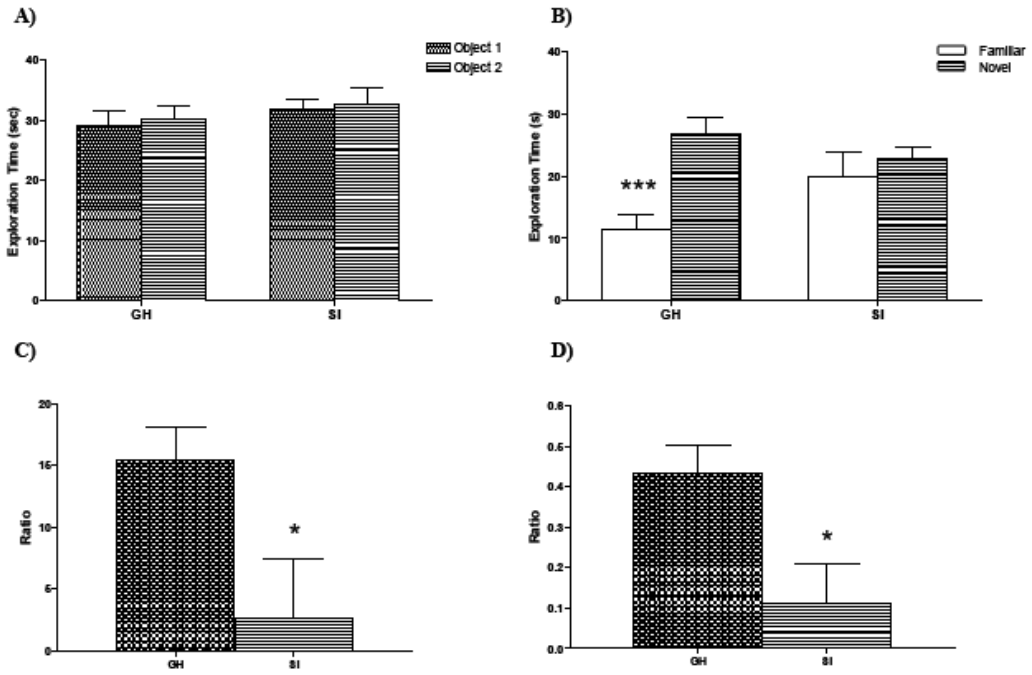
These experiments showed that rats which were housed in social isolation from weaning were able to discriminate the newest combination of what-where-when compared to a most familiar combination. This suggests they do not have a deficit in episodic memory-like memory. There was no difference in DI between group-housed and isolated group. After 10 minutes delay, both groups showed a delay dependent decrease and did not perform the OPC task .Furthermore in the OP task, there was also no difference between the two groups at either delay tested, however in this task both groups were able to distinguish an object in a new place after 10 minutes delay. On the other hand, in the test phase, the total object exploration in the grouped housed but not isolated group decreased with delay.

### *4.4.1 New component discrimination*

These results in the OP and OPC task combined with the results in object recognition demonstrated that isolated rats were able to discriminate a new object (for at least 1 hour) (Bianchi et al., 2006, McLean et al., 2008), an object in a new place and also an object in a new place depending on context. However, memory for one trial recognition task (OP task, >10 minutes) seems to last much longer than for two trials object recognition (OPC task, <10 minutes).

Isolation reared rats were not impaired in either of the OPC and OP recognition tasks. However, delays used in these experiments were extremely short compared with the 1 hour used by other studies in object recognition task (Bianchi et al., 2006, McLean et al., 2008) (Appendix 1.2) and may account for the inability to show any deficit.

Four weeks before the OPC or the OP recognition task, isolated rats showed a deficit in one trial object recognition task after two hours delay (Appendix 1.2) which confirmed that isolated rats were impaired in the cognitive task which assessed object recognition. Therefore, in the OPC task, one possibility was that the short delay between the sample and the test phase (5 and 10 minutes) may have been insufficient to expose the relatively mild impairment in memory which might be produced by isolation compared with that associated with chronic PCP. Indeed, it may have narrowed the possibility of detection of a deficit between the control and the experimental group. In the OPC task, only animals which cannot discriminat the newest combination after 5 minutes delay are identified as being impaired in episodic memory.




**Appendix 1.2. Animals reared in social isolation (SI) demonstrated NOR deficits. The time spent exploring two identical objects in the familiarisation trial (A) and a novel and familiar object in the choice trial (B) are presented as mean sec ± s.e.m. (n=10 per group). Each trial was separated by an ITI of 2 hours. The ability to discriminate between the novel and familiar object was calculated as discrimination ratios D1 and D2 (mean ± s.e.m.; C and D). * = p≤0.05, *** = p≤0.0001 by paired Student's t-test from Group housed (GH) control.**

These results demonstrate a task specificity of the OPC recognition task compared to a task which does not involve context discrimination. Indeed, both groups performed the OP task after 10 minutes delay but not the OPC task at similar delay.

### *4.4.2 Total object exploration*

Total object exploration in the test phase, in isolated groups remained constant and was not delay dependent in both OPC and OP tasks. However in controls, total object exploration decreased with increasing delay in controls in the OPC task but not in the OP task. In the OP version of the task in which contextual information was kept constant and therefore not necessary for object recognition the delay-induced decrease in total object exploration was not seen. These results corroborate the results found with PCP-withdrawal model, which suggested that total object exploration differences seen in isolated rats and PCP-treated rats may reflect an abnormality in processing contextual information that was common to both models was and as also reported in schizophrenia (Cohen and Servan-Schreiber, 1992, Bazin et al., 2000, Waters et al., 2004). However impairment in the explicit recall of contextual information was not seen as socially isolated rats were able to perform the OPC task (which requires contextual information too) just as well as controls (at least at the time intervals used herein). It may be possible that rats can therefore use two potential strategies to solve the task, one based on familiarity toward one or more of the components in the test phase and the other based on remembering the general recognition of the context and the object previously seen in the sample phase (context-object association) (Tulving, 1985, Fortin et al., 2004, Eacott and Easton, 2007). Therefore, The OPC and OP recognition task demonstrate that the capacity to

discriminate an object in a new place in a context using familiarity was generally not impaired in isolated rats but remembering the context-object association previously seen in the sample phase was disrupted, but only when the task involved contextual discrimination (comparing events).

### *4.4.3 Conclusion*

In view of the robust effect of PCP withdrawal but not isolation rearing to impair OPC recognition, it was decided to use PCP withdrawal model to evaluate pharmacological treatment in the model. In the next chapter, this OPC task with the subchronic PCP model relevant to schizophrenia was used to investigate the effect of the atypical antipsychotic clozapine.

# - CHAPTER 5 –

# THE EFFECT OF ANTIPSYCHOTIC CLOZAPINE ON PCP WITHDRAWAL-INDUCED EPISODIC MEMORY DEFICIT IN RATS

## 5.1 Introduction

As discussed, in Chapter 1 (See page 37) , PCP-induced learning and memory deficit is widely used as an animal model of abnormalities in memory and other cognitive functions in schizophrenia (Jentsch and Roth, 1999, Wong and Van Tol, 2003). However tasks currently used to assess episodic memory such as novel object recognition may be limited for two reasons; first, PCP induces object recognition deficits in rodents that are reversed by antipsychotic drugs (Hashimoto et al., 2005, Grayson et al., 2007). However, antipsychotic drugs do not generally (Meltzer, 1994) reverse memory impairments in patients (Goldberg et al., 1993) (See chapter 1 page: 45); second, there is evidence that the brain circuitries that underpin performance in tasks that assess memory for “what” differ from those that underpin the ability to remember “what”, “when” and “where” (Eacott and Norman, 2004, Dere et al., 2007, Langston and Wood, 2009) (chapter 3 and 4) which suggests a memory dissociation between the two tasks

In chapter 3, it has been shown that PCP withdrawal disrupted memory in rats in a task that simultaneously includes the “what” “where” “when” aspects that characterise human episodic memory. In the present study, it was examined whether the antipsychotic drug clozapine known to reverse PCP withdrawal impairment in one trial object recognition (Hashimoto et al., 2005, Grayson et al., 2007) affects impairment in OPC recognition.

## 5.2 Materials and Methods

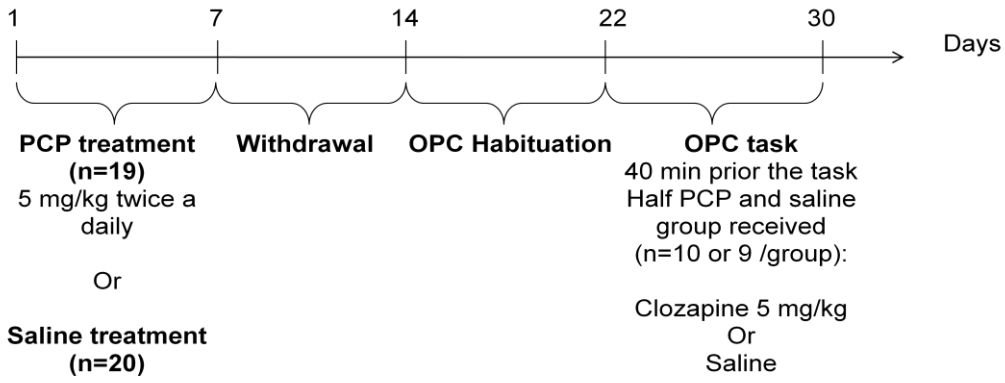



**Figure 5.1: Time line of the experimental procedure of the experiment 6. 19 rats received subchronic PCP treatment and 20 received saline injections. Half of each group were then injected, 40 minutes prior the OPC task, with clozapine 5 mg/kg or saline.**

### *5.2.1 Animals*

39 adult male Lister Hooded rats (Biomedical Services Unit, University of Nottingham Medical School, UK 150-200g, 300-350g at start of behavioural testing) were used. Animals received 1-2 minutes daily (9 am) handling the day after arrival at the unit and ending the day before the experiment. Animals were exposed to the test room one day before habituation. Animals for all experiments were kept in a temperature (21±2ºC) and humidity (40-60%) controlled environment on a 12 hour light/dark cycle (lights on 07:00-19:00). Food (standard animal chow, Harlan, US) and water were available *ad libitium*. Experiments were

carried out in accordance with the Animals (Scientific Procedures) Act, 1986 and approved by a local ethical committee (PPL 40/2715).

### *5.2.2 Subchronic PCP treatment*

PCP treatment schedule was as described previously (See chapter 3 page 96)

### *5.2.3 Experiment 6: Effect of clozapine on PCP withdrawal rats in OPC recognition task*

#### 5.2.3.1 Clozapine

Clozapine was obtained from Sigma-Aldrich (Gillingham, UK). Clozapine was dissolved in a minimum volume of acetic acid, pH was adjusted to 5.5 with 1 M sodium hydroxide (NaOH) and saline (0.9% w/v NaCl) was added to adjust the volume. Rats received 5 mg/kg i.p. clozapine or saline (0.9% w/v NaCl) (1ml/kg, i.p., n=20) 40 minutes prior the task for 8 consecutive days. This regimen has previously been shown to reverse object recognition deficits in rats treated with NMDAR antagonists PCP and MK801 (Grayson et al., 2007, Karasawa et al., 2008). One PCP-treated rat died after two days of treatment for unknown reasons (leaving n=9 for PCP-saline group).

### *5.2.4 Behavioural testing: OPC recognition*

The OPC recognition followed the same procedure used in chapter 3 and 4 (See chapter 3 page 97)

### *5.2.5 Data analysis*

Statistical analysis were similar than in previous chapters (See chapter 3 page 103 for more detail). However in this case, treatment levels were: saline-saline, PCP-saline; saline-clozapine, PCP-clozapine.

## 5.3 Results

Clozapine reduced total object exploration during the sample phase *(*Figure 5.2*)*. There was a significant effect of days ($F_{(7,245)}$=57.53, p<0.01) and treatments ($F_{(3,35)}$=307.28, p<0.01) on total object exploration and interaction between treatments and days ($F_{(21,245)}$=8.66, p<0.01).

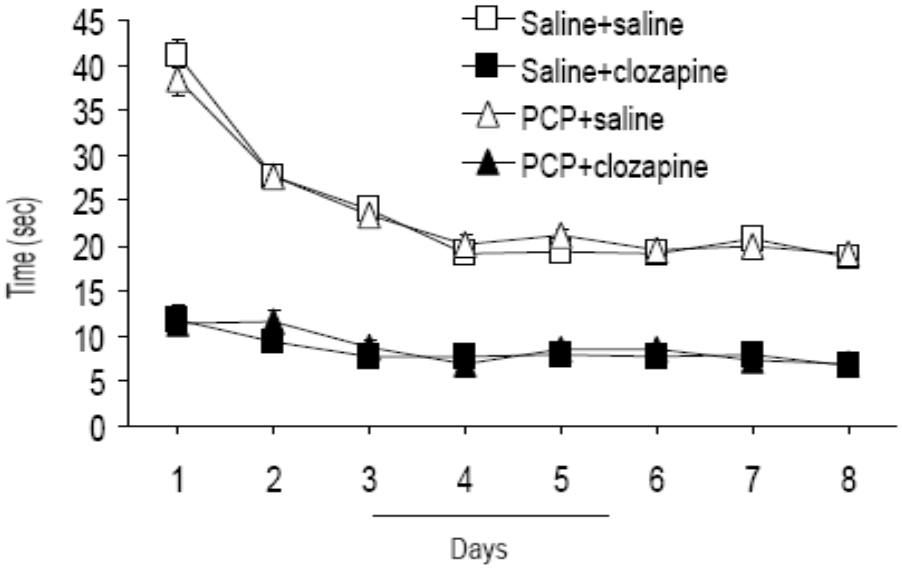



**Figure 5.2 (experiment 6): In the sample phase all groups explored the two objects the same amounts of time (time in second: sec). Data are expressed as mean values (n=10 for saline-saline/saline-clozapine and PCP-clozapine groups and n=9 for PCP-saline group) and errors bars represented ± SEM.**

In the test phase (Figure 5.3), at 5 minutes delay there was a significant effect of treatment on the DI ($F_{(3,35)}$=3.08, p<0.05). Clozapine did not impair saline treated rat's capacity to perform the task (DI was significantly better than chance, $t_{(9)}$=2.33, p<0.05), also clozapine did not restore the PCP-induced deficit after the same delay ($t_{(9)}$=-0.81, p>0.05). Therefore, clozapine affected rat's general exploration but not their capacity to discriminate object in place and context. A table showing time spent exploring each objects (new combination versus familiar one) is presented in appendix 2.1. Saline-saline treated rats were able to perform the task after 5 minutes delay (DI significantly better than zero, $t_{(9)}$=3.82, p<0.01). None of the PCP or saline treated rats were able to recognise the correct new triad of object, place and context at any delay (p>0.05). Similarly to in experiment 2 there was a difference between saline-saline DI and PCP-saline DI ($t_{(17)}$=2.25, p<0.05) and also between PCP-clozapine DI ($t_{(18)}$=2.51, p<0.05).

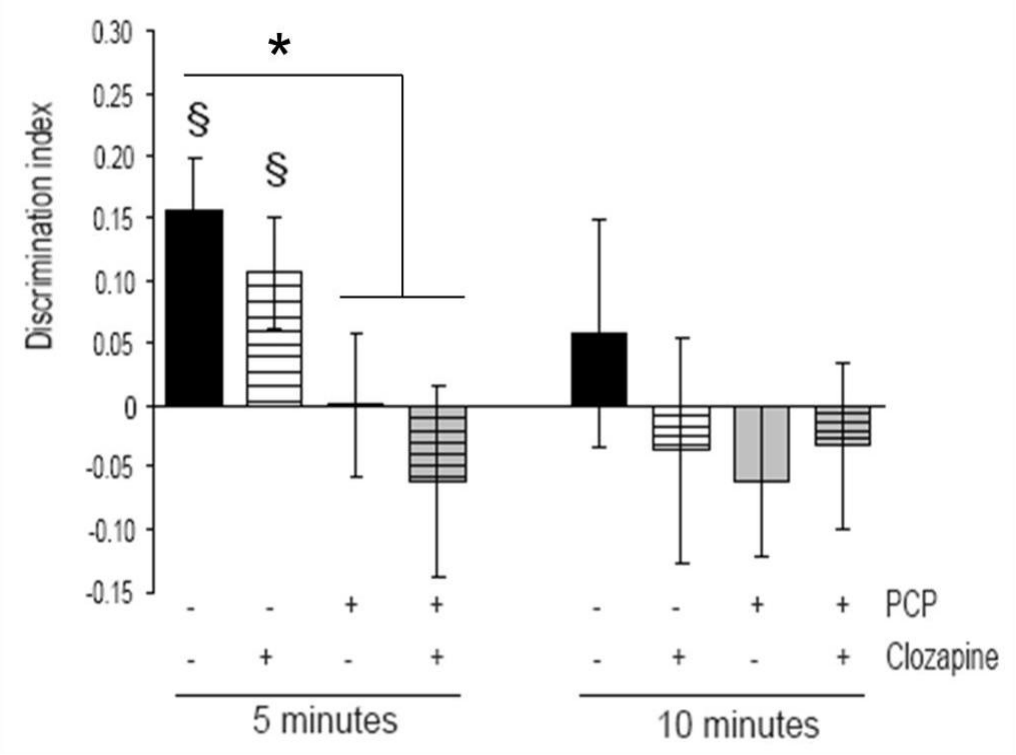



**Figure 5.3 (experiment 6): Clozapine did not reverse the PCP withdrawal-induced deficit in OPC task in rats. After 5 minutes delay, both saline-saline and saline-clozapine group performed above zero (§p<0.05). Clozapine affected rat's exploration but not their capacity to discriminate new object in place and context. Data are expressed as mean values (n=10 for saline-saline/saline-clozapine and PCP-clozapine groups and n=9 for PCP-saline group) and errors bars represented ± SEM.**

As noticed in the sample phase, clozapine reduced rats object exploration (Figure 5.4).There was a significant effect of treatments on total object exploration ($F_{(3,35)}$=20.32, p<0.001) and an interaction between treatments and delays ($F_{(3,35)}$=4.40, p<0.01). Clozapine-treated rats independently of their first treatment (saline or PCP) were impaired in exploration at both delays (saline-saline compared to saline-clozapine group after 5 minutes delay: $t_{(18)}$=4.32, p<0.001 and after 10 minutes $t_{(18)}$=2.58, p<0.05; PCP-saline compared to PCP-clozapine group after 5 minutes delay: $t_{(17)}$=4.93, p<0.001 and after 10 minutes delays compared to saline-saline group after 10 minutes delay: $t_{(17)}$=5.31, p<0.001.). Also, total object exploration in the control group was higher at 5 minutes delay compared to 10 minutes delay ($t_{(9)}$=4.04, p<0.05) but that was not the case for the other groups (Saline-clozapine group: $t_{(9)}$=0.08, p>0.05; PCP-saline group: $t_{(8)}$=-0.25, p>0.05 and PCP-clozapine group: $t_{(9)}$=-1.06, p>0.05). Furthermore total object exploration, after 5 minutes delay, in the control group was higher compared to the PCP-saline group ($t_{(17)}$=2.44, p<0.05) but not after 10 minutes delay.

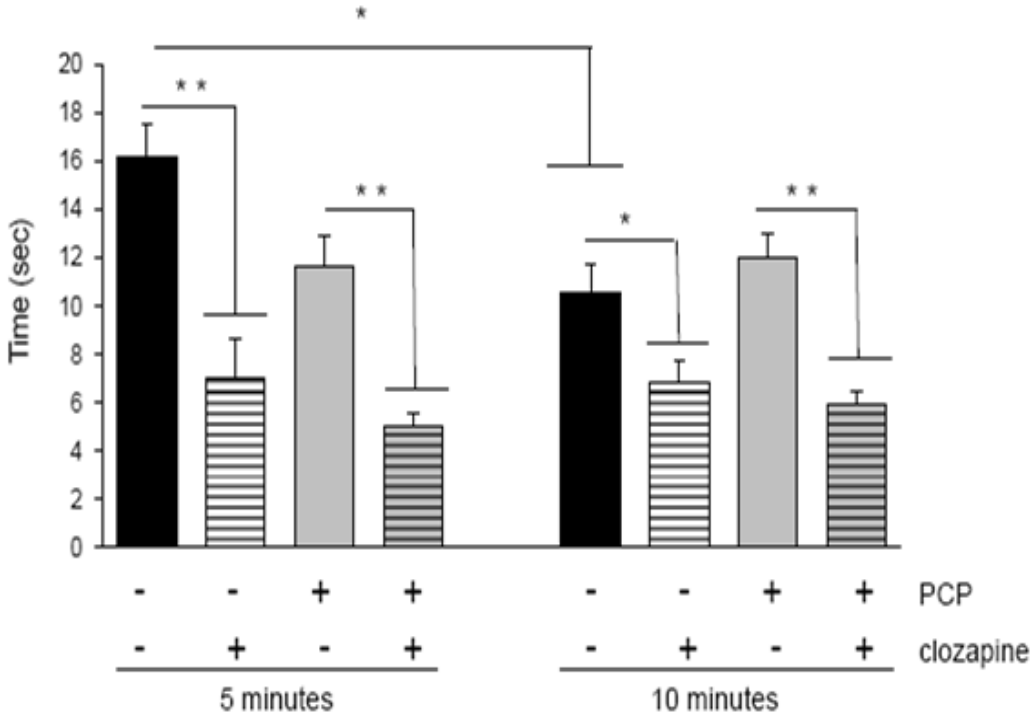



**Figure 5.4 (experiment 6): In the test phase, total object exploration was affected by delays in control group (*p<0.05) but not in PCP-saline group and by clozapine (*p<0.05 and **P<0.001). Data are expressed as mean values (n=10 for saline-saline/saline-clozapine and PCP-clozapine groups and n=9 for PCP-saline group) and errors bars represented ± SEM.**

## 5.4 Discussion

Clozapine (5 mg/kg) did not affect PCP-induced episodic memory deficit in OPC recognition nor did it affect reduced delay induced reduction in total object exploration. The dose used produced significant sedation which was reflected in reduced exploration times during the sample phase of the task. Clozapine has known sedative effects in both humans and in experimental animals (Kumra et al., 2008, Wiley, 2008). However, there was no disruption in DI in clozapine treated rats suggesting that in this model a degree of sedation was not confound to memory performance. It is possible that clozapine may have an influence on memory performance at higher doses than the dose used in this study test here. However, same dose has been shown to reverse PCP withdrawal effects in one-trial object recognition in rats (Grayson et al., 2007, Karasawa et al., 2008) which addresses “what” (which object is new?) which only refer to a previous event, and refers to working memory task (Ennaceur and Delacour, 1988). This is in contrast to the OPC recognition task in the present study which requires a comparison between events (Eacott and Norman, 2004, Dere et al., 2007, Langston and Wood, 2009) (see chapter 3 and 4). In the present study clozapine did not improve the PCP withdrawal induced-deficit in OPC recognition which suggest that this reversal does not extend to episodic memory defined as memory for what-where-when.

The sedative effect of clozapine (Kumra et al., 2008, Wiley, 2008), reduced the reliability of investigating the effects on total object exploration in the test phase.

### *5.4.4 Conclusion*

These data indicate that in contrast to prior studies using tasks that do not require recognition of both context and component clozapine does not reverse PCP withdrawal disruption of memory (Hashimoto et al., 2005, Grayson et al., 2007, Karasawa et al., 2008). These data suggest that it is possible to pharmacologically distinguish between memory for “what-where-when” (OPC recognition) which do not respond to atpical antipsychotic and “what” (one-trial object recognition) which is improved by typical antipsychotic.

# - CHAPTER 6 -

# THE EFFECT OF DONEPEZIL ON PCP WTHDRAWAL-INDUCED EPISODIC MEMORY DEFICIT IN RATS

## 6.1 Introduction

In chapter 5, it was shown that clozapine did not reverse the PCP withdrawal-induced episodic memory deficit in the OPC task. It was important to demonstrate that PCP withdrawal induced deficit in OPC recognition could be reversed if this model is to have utility for detecting novel memory enhancing drugs. Thus, it was postulated that consistent with data suggesting a cholinergic substrate to episodic memory and with prior reports of enhancement of episodic memory in humans (Grön et al., 2005) and rodents (Béracochéa et al., 2007) that this impairment would be reversible by the acetylcholinesterase drug donepezil (See chapter 1 page 50).

## 6.2 Materials and Methods

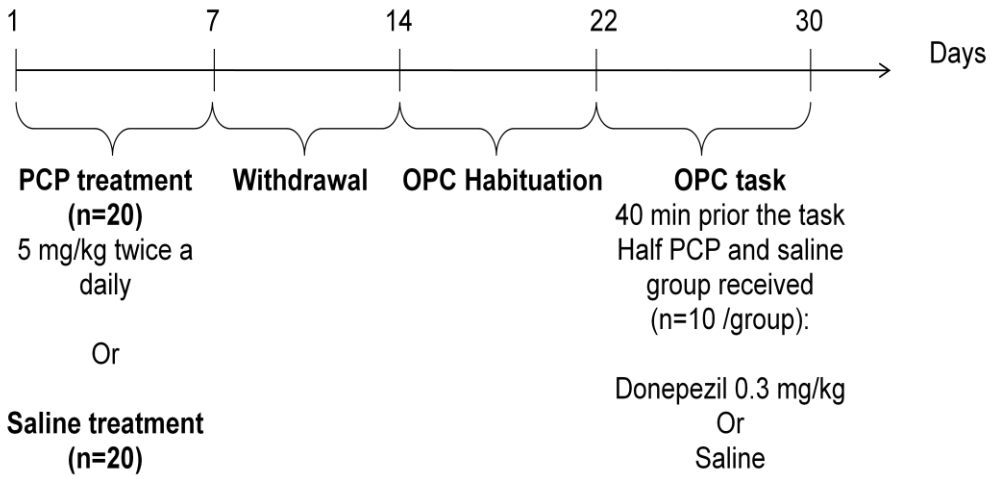



**Figure 6.1: Time line of the experimental procedure of the experiment 6. 20 rats received subchronic PCP or saline treatment. Half of each group were then injected, 40 minutes prior the OPC task, with donepezil 0.3 mg/kg or saline.**

### *6.2.1 Animals*

40 adult male Lister Hooded rats (Biomedical Services Unit, University of Nottingham Medical School, UK 150-200g, 300-350g at start of behavioural testing) were used. Animals received 1-2 minutes daily (9 am) handling the day after arrival at the unit and ending the day before the experiment. Animals were exposed to the test room one day before habituation. Animals for all experiments were kept in a temperature (21±2ºC) and humidity (40-60%) controlled environment on a 12 hour light/dark cycle (lights on 07:00-19:00). Food (standard animal chow, Harlan, US) and water were available *ad libitium*. Experiments were

carried out in accordance with the Animals (Scientific Procedures) Act, 1986 and approved by a local ethical committee (PPL 40/2715).

### *6.2.2 Subchronic PCP treatment*

PCP treatment schedule was as described previously (See chapter 3 page 96)

### *6.2.3 Experiment 7: Effect of donepezil on PCP withdrawal rats in OPC recognition task*

#### 6.2.3.1 Donepezil

Donepezil was obtained from The National Institute of Mental Health: NIMH (Bethesda, USA). Donepezil was dissolved in saline (0.9% w/v NaCl). Rats received 0.3 mg/kg i.p. donepezil or saline (0.9% w/v NaCl) (1ml/kg, i.p., n=20) 40 minutes prior the task during 8 days. This regimen has been shown to improve memory in other behavioural tasks in rodents such as object recognition (Prickaerts et al., 2005).

### *6.2.4 Behavioural testing: OPC recognition*

The OPC recognition followed the same procedure used in chapter 3, 4 and 5 (See chapter 3 page 97)

### *6.2.5 Data analysis*

Statistical analysis were similar than in previous chapters (see chapter 3 page 104 for more detail). However in this case, treatment levels were: saline-saline, PCP-saline; saline-donepezil, PCP-donepezil.

## 6.3 Results

As previously observed, in the sample phase (Figure 6.2) there was a significant decrease on total object exploration over the 8 days of procedure, but neither donepezil nor PCP affected rat's exploration. There was a significant effect of days on total object exploration ($F_{(7,252)}$=83.24, p<0.01), but no effect of treatment ($F_{(3,36)}$ =0.56, p>0.05) and nor interaction between treatment and days ($F_{(21,252)}$=0.75, p<0.01).

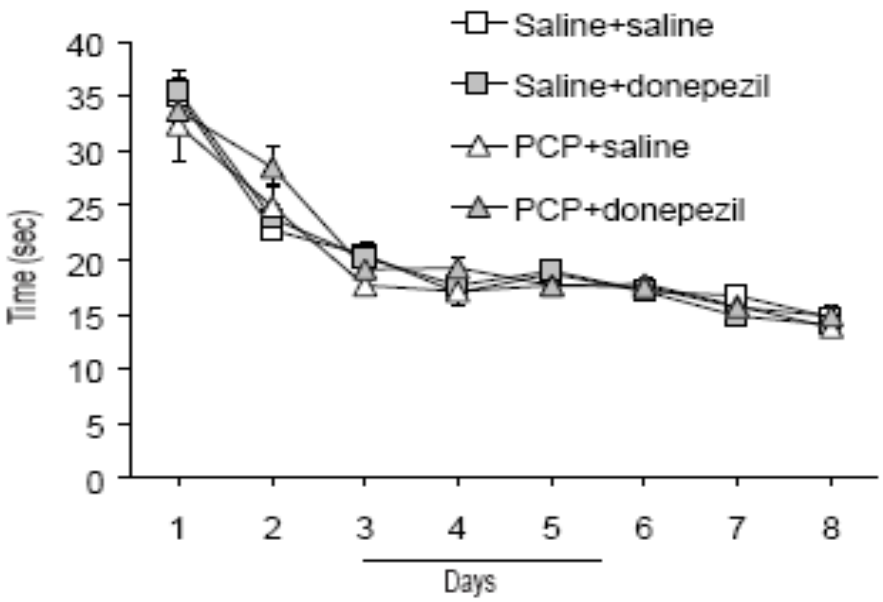



**Figure 6.2 (experiment 7): In the sample phase all groups explored the two objects the same amounts of time (time in second: sec). Data are expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

In the test phase (Figure 6.3), At 5 minutes delay there was a significant effect of treatments on DI ($F_{(3,36)}$=2.81, p=0.05). After 5 minutes delay, PCP-saline group DI was significantly lower than saline-saline, saline-donepezil and PCP-donepezil group DI (respectively: $t_{(18)}$=2.43, p<0.01; $t_{(18)}$=2.50, p<0.05 and $t_{(18)}$=2.21, p<0.05 ). Donepezil reversed PCP-induced episodic memory deficit in OPC task after 5 minutes delay (DI significantly better than zero: $t_{(9)}$=2.74, p<0.05) but not after 10 minutes (DI not significantly better than zero $t_{(9)}$=1.21, p>0.05). Donepezil improved saline treated rats memory after 10 minutes delay (DI after 10 minutes delay is significantly better than zero (saline-donepezil group DI $t_{(9)}$=2.45, p<0.05 and saline-saline group DI $t_{(9)}$=-0.083, p>0.05). Both saline-saline and saline-donepezil treated rats performed the task after 5 minutes delay (DI significantly better than zero after 5 minutes delay: saline-saline group $t_{(9)}$=3.71, p<0.05 and saline-donepezil group $t_{(9)}$=3.58, p<0.05).

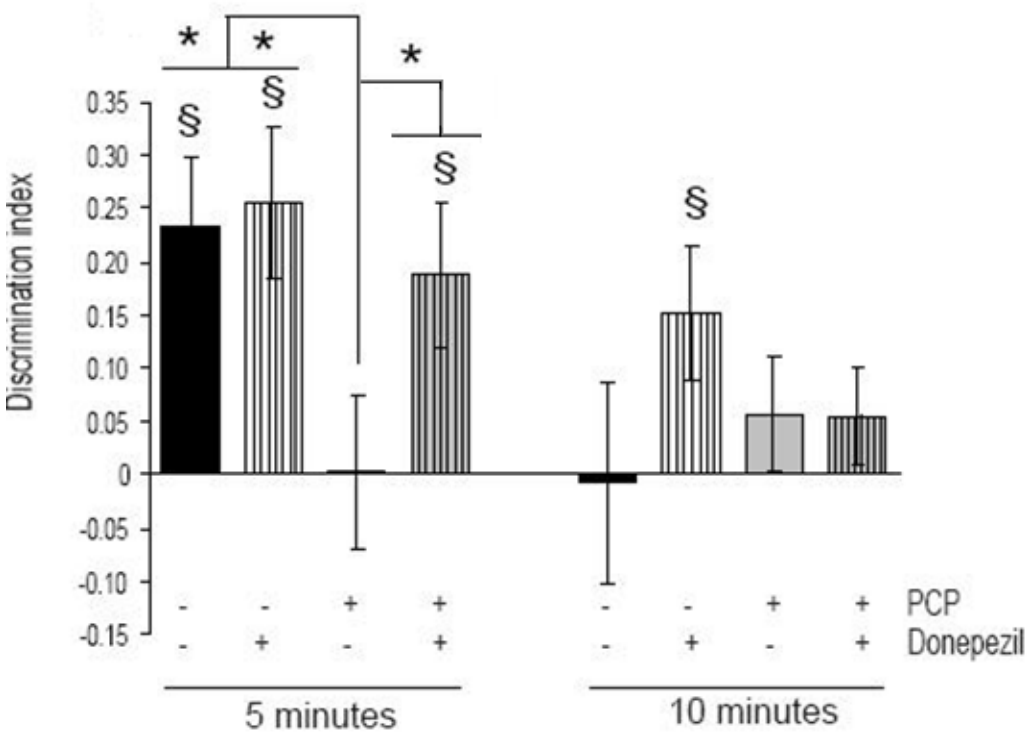



**Figure 6.3 (experiment 7): Donepezil reversed the PCP withdrawal-induced deficit in OPC task in rats. After 5 minutes delay, only PCP-saline group did not perform above chance (§p<0.05) and there were a significant difference between DI from PCP-saline group with every other groups was revealed (*p<0.05). After 10 minutes delay, saline-donepezil treated rats still performed the task (§p<0.05) but not the other groups. Data are expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

Total object exploration in the test phase was delay and treatment dependent (Figure 6.4). There was a significant effect of delays on total object exploration ($F_{(3,36)}$=28.05, p<0.001) and an interaction between treatments and delays ($F_{(3,36)}$=6.62, p<0.001). Both saline-saline and saline-donepezil groups showed more total object exploration after the short delay compared to the longer one (respectively: $t_{(9)}$=5.44, p<0.001 and $t_{(9)}$=6.54, p<0.001), which was not the case for PCP-saline and PC-donepezil ($t_{(9)}$=-0.69, p>0.05 and $t_{(9)}$=1.71, p>0.05). After 5 minutes delay, there was no significant effect of treatment on total object exploration ($F_{(3,36)}$=0.92, p>0.05), however after 10 minutes delay, there was a significant effect of treatment on total object exploration ($F_{(3,36)}$=7.30, p<0.001). Indeed, after 10 minutes delay, PCP-saline group explored more the object compared to all the other groups (saline-saline group: $t_{(18)}$=-3.81, p<0.05; saline-donepezil group: $t_{(18)}$=-3.73, p<0.05 and PCP-donepezil group: $t_{(18)}$=-3.18, p<0.05).

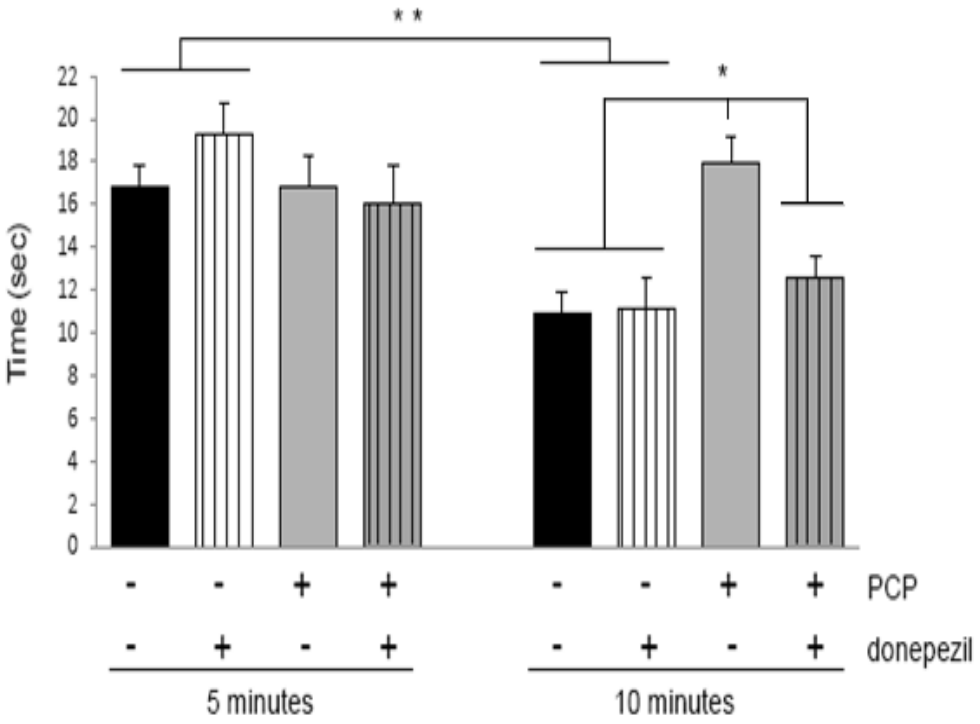



**Figure 6.4 (experiment 7): In the test phase, total object exploration was affected by PCP withdrawal nor delay, donepezil did not reverse these PCP withdrawal/delay-induced deficit. Total object exploration was delay dependent for the saline-saline (**p<0.001) and saline-donepezil (**p<0.001) group but not for PCP-saline (p>0.05) and PCP-donepezil (p>0.05) group. Data are expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

## 6.4 Discussion

Donepezil (0.3 mg/kg) increased performance in saline-treated rats and reversed the PCP-induced deficit in the episodic memory task of OPC recognition but did not affect PCP withdrawal disruption of delay dependent reduction of total object exploration. Donepezil is known to have a beneficial effect on learning and memory in Alzheimer's disease (Bullock and Dengiz, 2005, Tsuno, 2009). In schizophrenia, anomalies in the cholinergic pathway have been reported such as lower numbers of both muscarinic and nicotinic receptors in the PFC and HPC (Raedler et al., 2006, Crook et al., 2001, Freedman et al., 1995). Few studies have investigated the cognitive effect of donepezil in rat models relevant to schizophrenia. Studies in mice have shown donepezil reversal of MK-801 and PCP-induced deficits in spatial reversal learning, contextual and cued memory, and one-trial object recognition (Csernansky et al., 2005, Kunitachi et al., 2009). In middle-aged mice, Béracochéa (Béracochéa et al., 2007) demonstrates that donepezil given alone improves contextual memory. Furthermore human studies have also revealed episodic memory improvement after donepezil administration (Grön et al., 2005). In human studies, some studies show a beneficial effect of AChEIs in general cognitive impairment in schizophrenia in task such as Rey Auditory Verbal Learning Test (RAVLT), Wisconsin Card Sorting Test (WCST) and selective attention Digit Span Distraction Test (DSPT), howver others studies do not found

any improvement or a minimal benefits (Friedman et al., 2002, Howard et al., 2002, Liddle, 2002, Buchanan et al., 2003, Tuğal O et al., 2004, Keefe et al., 2007, Risch et al., 2007, Chung et al., 2009). The studies performed to date have however used general cognitive assessment batteries typically measuring executive function and attention and do not include specific tests of episodic memory.

Donepezil alone did not improve delay-induced total object exploration deficit after 10 minutes delay and nor improved the PCP withdrawal-induced deficit in total object exploration after 5 minutes. In the light of my suggestion that total object exploration reduction after delay may reflect autonoetic awareness while OPC performance (DI) reflects noetic awareness, these results suggest that donepezil might improve rats' capacity to discriminate OPC based on familiarity (noetic awareness) but not rats' capacity to remember the previous visited context and objects (autonoetic awareness) (see chapter 8 page 189 for more details).

### *5.4.4 Conclusion*

This suggests that PCP withdrawal disruption of OPC is potentially reversible and it may be a new sensitive preclinical model for to study the episodic memory impairments in schizophrenia. Furthermore, these data suggest that the AChEI's may be useful as treatment for memory impairment in schizophrenia.

# - CHAPTER 7 -

# LOCOMOTOR ACTIVITY TASKS IN PCP-WITHDRAWAL RATS

## 7.1 Introduction

This chapter investigated locomotor activity in subchronic PCP treated rats given either acute PCP (3.2 mg/kg) challenge, clozapine (5 mg/kg), and with donepezil (0.3 mg/kg). The PCP challenge was performed to validate and to analyse the long term effect of the subchronic PCP treatment; but also to investigate if the potential chronic effect of clozapine and donepezil treatment received during the OPC task could reverse PCP sensitization on LMA task. Clozapine and donepezil injections were tested in order to analyse their effects on locomotor activity.

In rats, PCP challenge after repeated PCP administration increases locomotor activity (Xu and Domino, 1994, Johnson et al., 1998). This specific phenomenon is called “reverse tolerance” or sensitization and it is correlated with apoptotic neuronal degeneration in different parts of the brain including the anteterior cingulate, parietalcingulate, temporal, piriform, entorhinal cortices, HPC and amygdala (Olney et al., 1989,

Phillips et al., 2001). Increased locomotor activity induced by PCP sensitization is associated with dopamaniergic hyperactivity and is supposed to be related to clinical manifestations of schizophrenia (Steinpreis et al., 1994, Adams and Moghaddam, 1998). Dysfunctions in the dopaminergic and glutamatergic systems are the origin of behavioural sensitization due to neural adaptations in the mesocorticolimbic regions (Pierce and Kalivas, 1997, Cornish and Kalivas, 2001). Clozapine prevents apoptotic degeneration of cortical neurons and reduced locomotor sensitization after chronic PCP treatment (Johnson et al., 1998, Phillips et al., 2001). On the other hand, the effect of donepezil on behavioural locomotor sensitization after PCP challenge had never been investigated.

While donepezil does not affect general locomotor activity (Sonkusare et al., 2005), clozapine is known to induce sedative effects in patients and in experimental animals (Kumra et al., 2008, Wiley, 2008). The sedative effects were observed during the OPC recognition task and affected object exploration during the sample and the test phases.

## 7.2 Materials and Methods

### *7.2.1 Animals*

Animals were the same as those used in chapter 6 and 7 (See chapter 5 and 6: page 134 and 145)

### *7.2.2 Apparatus*

All locomotor experiments take place in the same open fields. Each activity monitor consist of a clear Perspex box (40 x 20 x 25 cm), surrounded by SDI photobeam Activity System frame with 4x8 photobeams (spaced between them: 1 $^{15/16}$”) crossing the arena to track the animal’s specific path. All photocells are interfaced to a Windows XP computer and the number of beam breaks relayed as counts in 5 minute time bins using SDI Photobeam Activity software.

### *7.2.3 Drug administration*

#### 7.2.3.1 PCP

PCP preparation was previously described (See chapter 3 page 97). Rats received 3.2 mg/kg i.p. PCP or saline (0.9% w/v NaCl) (1ml/kg, i.p., n=20) after 40 minutes of habituation to the LMA boxes. This

regimen has been shown to induce PCP sensitization in LMA (Philip et al. 2001).

#### 7.2.3.2 Clozapine

Clozapine preparation was previously described (See chapter 5 page 135)

#### 7.2.3.3 Donepezil

Donepezil preparation was previously described (See chapter 6 page 146)

### *7.2.4 Behavioural testing*

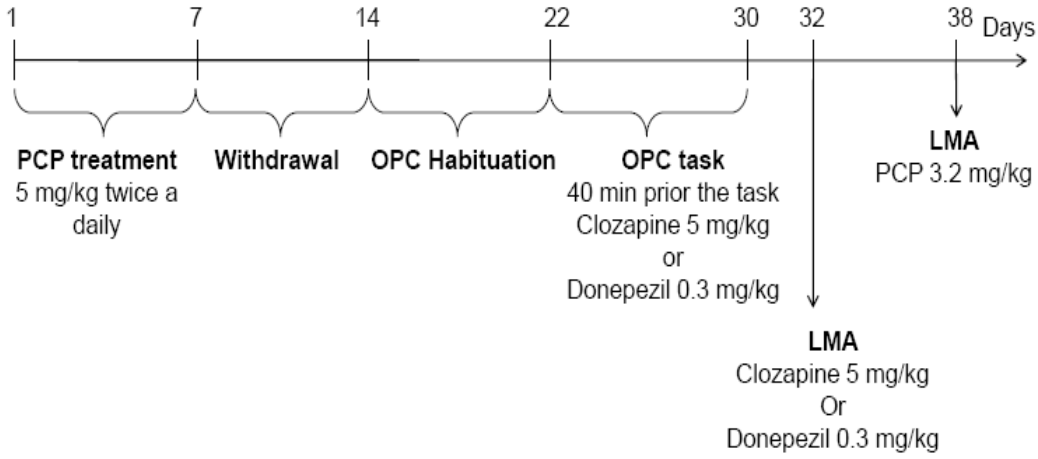



**Figure 7.1: Summary of experimental procedures. Chronic PCP treatment was followed by a 7 day withdrawal period and then animals were tested in the OPC task. LMA with clozapine (5 mg/kg, experiment 8) or donepezil (0.3 mg/kg experiment 9) was carried out two days after the OPC task and seven days after rats were tested on LMA with PCP challenge (3.2 mg/kg experiment 10 and 11).**

### 7.2.4.1 Experiment 8 and 9: PCP (3.2 mg/kg) challenge after OPC task with clozapine and with donepezil

The LMA task was carried out 9 days after the last OPC t ask or 31 days after the last PCP injections. Each animal (n=40) received 40 minutes of habituation in the activity monitor box, before an acute injection of PCP (3.2 mg/kg i.p.) and then were allowed to move freely in the box for 40 minutes. Their behaviour was monitored throughout the experiment by SDI Photobeam Activity soft (see Figure 7.2).

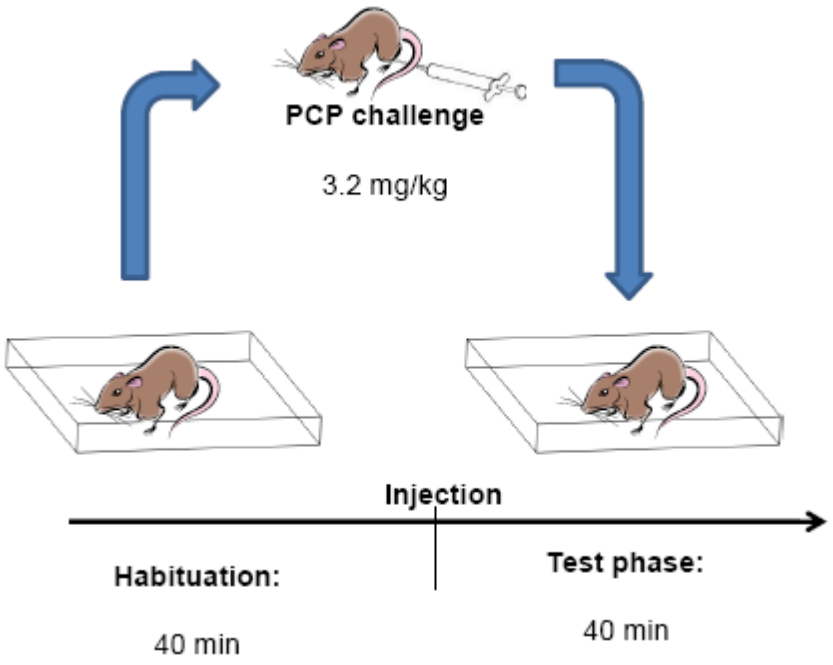



**Figure 7.2: Experimental design of the LMA task. Rats had 40 minutes of habituation into the open field, followed by PCP (3.2 mg/kg) injection (i.e. experiment 8 and 9) and then they came back in the open field for 40 minutes.**

### 7.2.4.2 Experiment 10-11: LMA after clozapine (5 mg/kg) and donepezil 0.3 mg/kg

LMA task was carried out two days after the last day of the OPC task. Each group of rats previously treated in experiment 6 (n=39) such as: saline-saline, saline-clozapine, PCP-saline and PCP-clozapine and in experiment 7 (n=40) such as: saline-saline, saline-donepezil, PCP-saline and PCP-donepezil were divided in two subgroups. Half of them received the clozapine (5 mg/kg i.p.) injection and half of them the saline injection (See Appendix 3.1 for diagram). Prior to injection, animals received 20 minutes of habituation in the activity monitor box (see Figure 7.2). Then, they were kept in their holding cage (post injection) for 40 minutes before replacement in the activity monitor box for 70 minutes. They were allowed to move freely in the box and their behaviour was monitored throughout the experiment by SDI Photobeam Activity soft (see Figure 7.3).

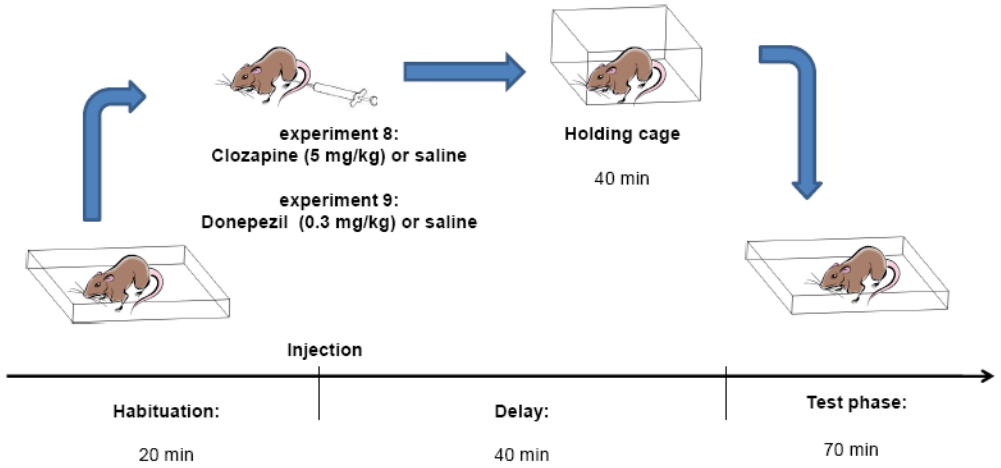



**Figure 7.3: Experimental design of the LMA task after clozapine (5 mg/kg) or donepezil (0.3 mg/kg) which was carried out two days after the OPC task (See Figure 7.1). Rats had 20 minutes of habituation into the open field, followed by clozapine (5 mg/kg) (experiment 10) or donepezil (0.3 mg/kg) (experiment 11) or saline injection. After 40 minutes delay in their holding cage, they came back into the open field for 70 minutes.**

### *7.2.5 Data analysis*

LMA was calculated as mean ± SEM activity per 5 minutes for each group. Two-way ANOVA was performed with beam break (counts) as dependent variable between treatments and within delays followed by planned post-hoc LSD where appropriate. All data were statistically analysed using SPSS software, version 16.1 (SPSS Inc., USA).

## 7.3 Results

### *7.3.1 Experiment 8: PCP (3.2 mg/kg) challenge after OPC task under clozapine*

Results showed that there was a PCP sensitisation of the rats which received subchronic PCP treatment (Figure 7.6). Previous clozapine treatment during the OPC recognition task did not reverse PCP sensitisation. There was a significant effect of treatments ($F_{(3,35)}$=5.03; p<0.001) and time ($F_{(6,210)}$=25.85; p<0.001) on LMA but no interaction between treatments and time ($F_{(18,210)}$=1.10; p>0.05). There was a significant difference between saline-saline group and PCP-saline group (p<0.05), saline-clozapine group and PCP-clozapine (p<0.05) but not between saline-saline group and saline-clozapine group (p>0.05) or PCP-saline group and PCP-clozapine (p>0.05). In the habituation phase, there was no difference of LMA between groups. There was a significant effect of time on LMA ($F_{(7,245)}$=86.94; p<0.001) but not of treatment ($F_{(3,35)}$=2.05; p>0.05) and no interaction between treatment and time ($F_{(21,245)}$=1.54; p>0.05).

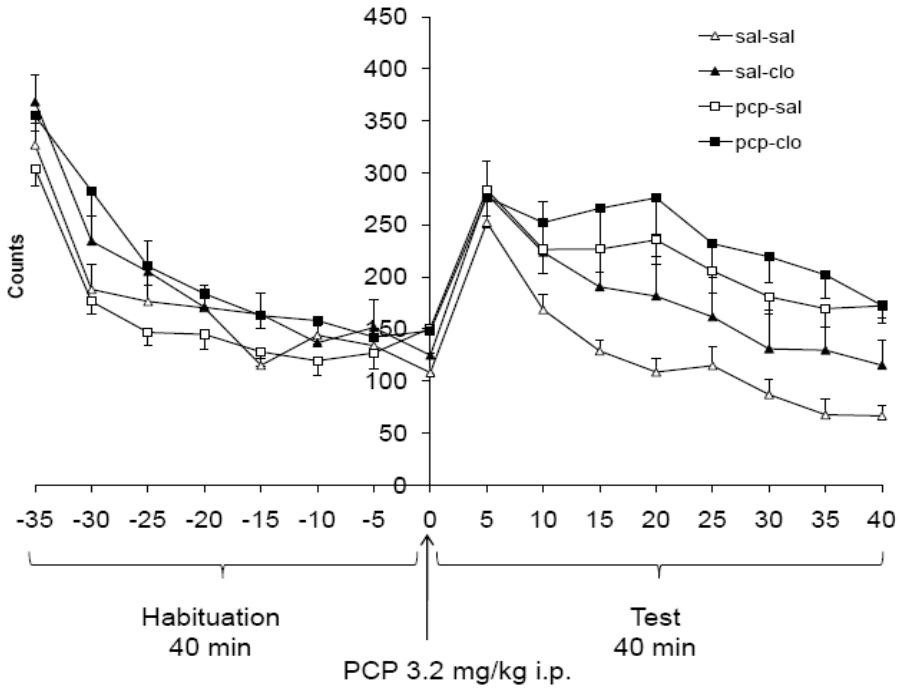



**Figure 7.4 (experiment 8): There was PCP sensitization after a PCP challenge (3.2 mg/kg) even 31 days after the last PCP injection. Clozapine (5 mg/kg) received during the OPC recognition task did not reverse the PCP sensitization. There was a significant difference of LMA between saline-saline (sal-sal) group and PCP-saline (pcp-sal) group ($p<0.05$) and between saline-clozapine (sal-clo) group and PCP-clozapine (pcp-clo) group ($p<0.05$). Data are expressed as mean values (n=10 for saline-saline/saline-clozapine and PCP-clozapine groups and n=9 for PCP-saline group) and errors bars represented ± SEM.**

### *7.3.2 Experiment 9: PCP (3.2 mg/kg) challenge after OPC task under donepezil*

Rats PCP sensitisation was observed in PCP withdrawal rats (Figure 7.7). Donepezil did not restore the hyperlocomotor activity induced by the PCP treatment. There was a significant effect of treatments ($F_{(3,36)}$=3.71; $p<0.05$) and times ($F_{(6,216)}$=27.98; $p<0.001$) on LMA without any interaction between treatments and times ($F_{(18,216)}$=1.91; $p>0.05$). There was a significant difference between saline-saline group and saline-donepezil group ($p<0.05$), saline-donepezil group and PCP-donepezil ($p<0.05$) but not between saline-saline group and saline-donepezil group ($p>0.05$) or PCP-saline group and PCP-donepzil ($p>0.05$). In the habituation phase, there was no difference on LMA between groups. There was a significant effect of times on LMA ($F_{(7,252)}$=104.02; $p<0.001$) but not of treatments ($F_{(3,36)}$=0.90; $p>0.05$) and nor interaction between treatments and times ($F_{(21,252)}$=0.70; $p>0.05$).

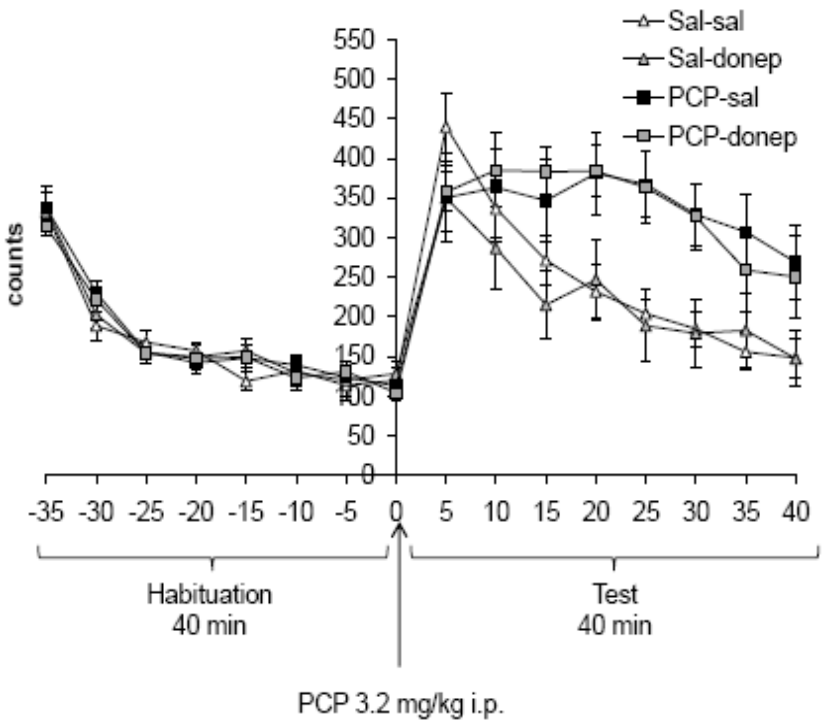



**Figure 7.5 (experiment 9): 31 days after the last injection of the chronic PCP treatment, there was PCP sensitization after a PCP challenge (3.2 mg/kg). Donepezil (0.3 mg/kg) received during the OPC recognition task did not reverse the PCP sensitization. There was a significant difference on LMA between saline-saline (sal-sal) group and PCP-saline (pcp-sal) group ($p<0.05$) and between saline-donepezil (sal-donep) group and PCP-donepezil (pcp-donep) group ($p<0.05$). Data are expressed as mean values (n=10 per group) and errors bars represented ± SEM.**

### *7.3.3 Experiment 10: LMA after Clozapine (5 mg/kg)*

40 minutes after the 5 mg/kg clozapine injection, rats locomotor activity was reduced compared to rats which received saline injection (Figure 7.4). There was a significant effect of treatments ($F_{(3,35)}$=34.53; $p<0.05$) and times ($F_{(13,455)}$=36.01; $p<0.05$) on LMA but no interaction between treatments and times ($F_{(39,455)}$=1.46; $p>0.05$). There was a significant difference between groups which had been injected with clozapine and those which had been injected with saline ($p<0.05$), but there was no difference between groups previously treated with saline and PCP ($p>0.05$). In habituation, there was a significant effect of times on LMA ($F_{(3,105)}$=87.82; $p<0.05$) but not of treatments ($F_{(3,35)}$=0.60; $p>0.05$) and no interaction between treatments and times ($F_{(9,105)}$=0.66; $p>0.05$).

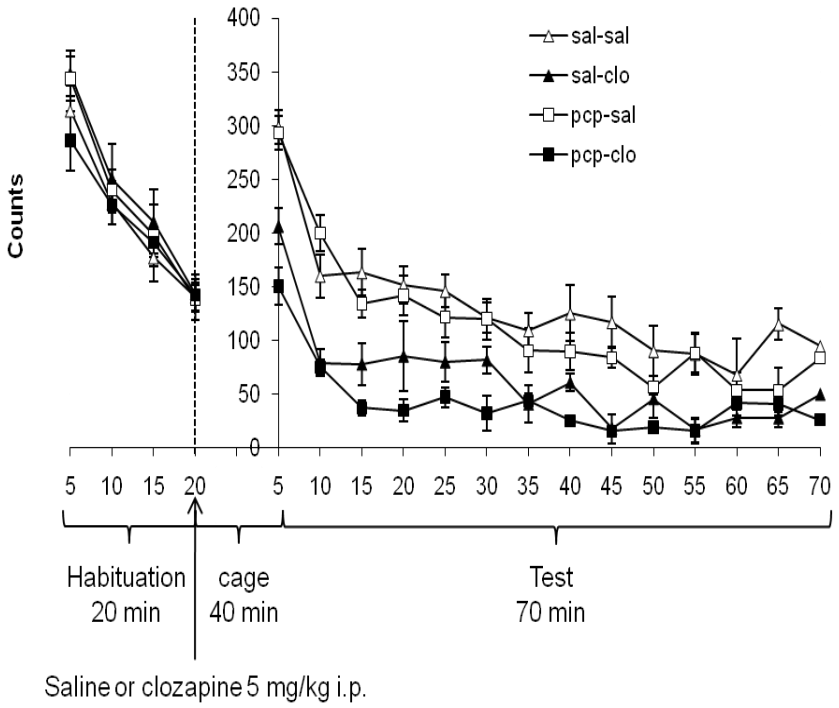



**Figure 7.6 (experiment 10): Clozapine (5 mg/kg) 40 minutes after injection impaired rat's locomotor activity. There was a significant difference on LMA between saline-saline (sal-sal) group and saline-clozapine (sal-clo) group ($p<0.05$) and between PCP-saline (pcp-sal) group and PCP-clozapine (pcp-clo) group ($p<0.05$). Data are expressed as mean values (n=10 for saline-saline/saline-clozapine and PCP-clozapine groups and n=9 for PCP-saline group) and errors bars represented ± SEM.**

### *7.3.4 Experiment 11: LMA after donepezil (0.3 mg/kg)*

Donepezil (0.3 mg/kg) did not affect rat's locomotor activity (Figure 7.5). There was a significant effect of times on LMA ($F_{(13,468)}$=48.94; p<0.05) but not of treatments ($F_{(3,36)}$=0.03; p>0.05) no interaction ($F_{(39,468)}$=0.92; p>0.05). In the habituation phase there was also no difference in locomotor activity between groups. There was a significant effect of time ($F_{(3,108)}$=206.03; p<0.05) but not of treatment ($F_{(3,36)}$=0.03; p>0.05) nor interaction between treatments and times ($F_{(9,108)}$=0.58; p>0.05).

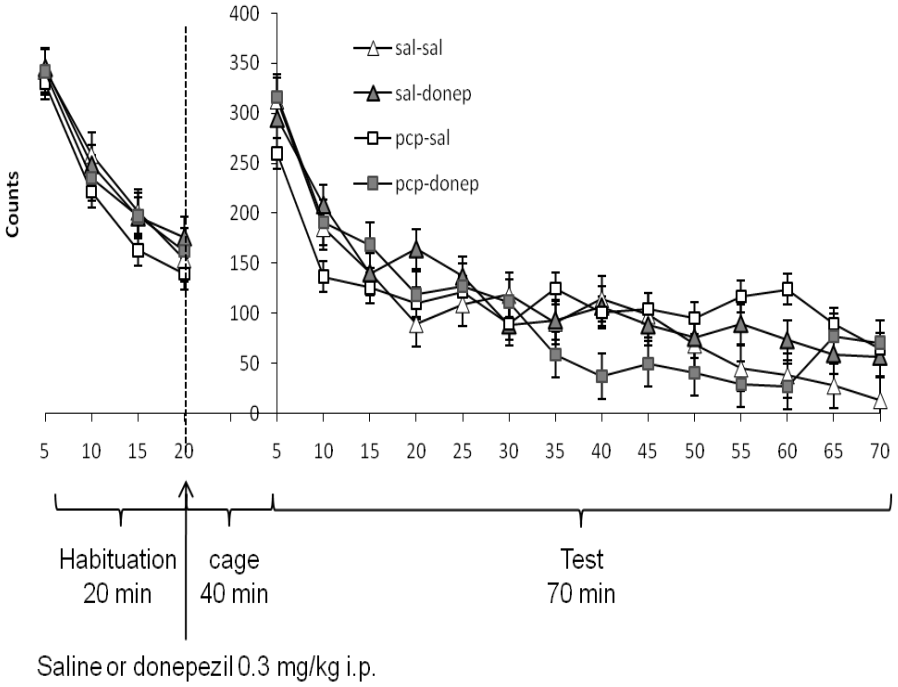



**Figure 7.7 (experiment 11): Donepezil (0.3 mg/kg) 40 minutes after injection did not affect rat's locomotor activity. There was no significant difference between groups on LMA (saline-saline (sal-sal); saline-clozapine (sal-donep); PCP-saline (pcp-sal); PCP-clozapine (pcp-donep)). Data expressed as mean (n=10 per group) values and errors bars represented ± SEM.**

## 7.4 Discussion

PCP (3.2 mg/kg) challenge showed PCP withdrawal rats' sensitisation. This result demonstrated that the subchronic PCP treatment has persisted for at least over 31 days after the last PCP injection the PCP withdrawal effect during the OPC and OP tasks recognition. Also, chronic clozapine and chronic donepezil treatment received during the OPC task and LMA task did not reverse the PCP-induced hyperactivity after acute PCP challenge 7 days later.

Clozapine (5 mg/kg) but not donepezil (0.3 mg/kg) had a sedative effect that reduced locomotor activity (experiment 8 and 9: Figure 7.5 Figure 7.6) and objects exploration (experiment 6: Figure 5.2). This sedative effect is well established in the literature (Kumra et al., 2008, Wiley, 2008). However, in saline treated rats, clozapine did not impair rat's capacity to discriminate between the newest combination of object, place and context (See chapter 5 for more detail).

Overall these data confirmed the sedative effect induced by clozapine but not by donepezil. Also, they showed a long lasting effect of subchronic PCP treatment certainly induced by apoptotic neuronal degeneration in different brain regions (Phillips et al., 2001). Chronic clozapine treatment (8 days) did not last 7 days after the last injection and was not able to reverse the PCP-induced sensitization, this effect has been interpreted as clozapine withdrawal effect which induced

dopaminergic hypersensitivity and psychosis relapse in human (Borison et al., 1988, Shore et al., 1995) (See chapte 8: page 192).

### *7.4.1 Conclusion*

The LMA tasks establish a long term effect of PCP withdrawal in the subchronic PCP model of rats and also that clozapine but not donepezil induce sedation in rats that could interfere with the total object exploration in the OPC task. However clozapine sedative effect did not affect rats' capacity to discriminate the object in place depending on the context (See chapter 5 Figure 5.3 and 5.4).

# - CHAPTER 8 –

# GENERAL DISCUSSION

The results, presented in this thesis 1) established a modified OPC recognition task in our laboratory 2) demonstrated that withdrawal from subchronic PCP induced a deficit in OPC recognition 3) found that donepezil but not clozapine restored this PCP-induced deficit, 4) established that isolation reared rats did not show a deficit in OPC recognition after a short delay but did after 1h in standard object recognition paradigm, 5) found that both PCP and isolation rearing abolished delay sensitive decrease in total object exploration at test 6) confirmed that reduction of exploration is associated with context as OP recognition does not show total object exploration decrease at delay and 7) confirmed that OP recognition is disrupted by PCP withdrawal 8) PCP sensitisation of the locomotor response was shown after acute PCP (3.2 mg/kg) injection 31 days after the last PCP injection confirming persistent changes induced by withdrawal from subchronic PCP 9) showed that neither PCP hyperactivity nor abolition of delay sensitive in reduction in total object exploration was clozapine or donepezil treatment.

## 8.1 Differentiation between the effects of PCP withdrawal and isolation rearing in the OPC and OP recognition task

The OPC recognition task recapitulates the human episodic memory definition defined by Tulving as the capacity to recollect an event in time and place (what-where-when) (Tulving, 1983). On the other hand, the OP recognition task is a one trial object recognition task and only assesses what-where, therefore this task is more associated with working memory and the capacity to remember the previous situation (Ennaceur and Delacour, 1988, Dere et al., 2007). Both tasks were matched for the number of days of the procedure and the number of conditions by delays, only the number of sample phases was different (i.e. two sample phases for the OPC task and one sample phase for the OP task).The choice of this protocol has been decided in order to investigate further the effect of the second trial (context dependent task) on total object exploration deficit seen in the OPC task after a longer delay.

PCP withdrawal and isolation rearing models demonstrated different patterns of results in OPC and OP tasks. Indeed, in the OPC task but not in the OP task after 5 minutes, PCP withdrawal rats were impaired in their capacity to discriminate the new object (experiment 2 and 3: Figure 3.7 and Figure 3.10). On the other hand, in both tasks, isolated rats did not show any impairment compared to the control group and

were impaired after 10 minutes delay in the OPC task (experiment 4: Figure 4.3) and not impaired in the OP task after 10 minutes delay (experiment 5: Figure 4.6). Interestingly, it is important to note that before the OPC and the OP recognition task the isolated rats have been previously tested and have been shown to be impaired on a one trial object recognition task with a delay of 1 hour between sample and test, replicating results from previous studies (Appendix 1.2) (Bianchi et al., 2006, McLean et al., 2008). Therefore, it cannot be argued that isolated rats were not impaired due to incomplete isolation during rearing or an other procedural problem. One possible explanation for a lack of effect of isolation rearing is the short delay interval between the two samples and the test phase (5 and 10 minutes). The likelihood of identifying a deficit between the control and the experimental group is necessarily short and only animals which cannot discriminate the newest combination after 5 minutes delay can be identified as impaired in episodic memory. Also, isolated rats show a much more robust memory in object recognition task compared to PCP withdrawal rats. In prior studies in female rats PCP withdrawal rats were impaired after a 1 minute delay (Grayson et al., 2007, McLean et al., 2009) and male after 10 minutes delay in object in place (i.e. chapter 4, OP recognition task), while isolated rats were impaired in the object recognition task after 1 hour (Appendix 1.2) (Bianchi et al., 2006, McLean et al., 2008). Thus, isolation rearing induces a general milder impairment in memory compared with that associated with withdrawal from subchronic PCP administration.

On the other hand during the OP task, PCP treated rats showed a deficit at 10 minutes but not 5 minutes delay (experiment 3: Figure 3.10). Previous studies found that PCP-treated rats were not able to discriminate the familiar object from the new one even after 1 minute delay (Grayson et al., 2007, McLean et al., 2009). The OP recognition task developed in this chapter assessed spatial-working memory as only the combination of object and place created the newest environment and not the object or the place on their own, therefore there was no purely spatial orientation involved in this task. Thus, the task specificity of the OP recognition may have influenced PCP-treated rats' capacity to remember the combination of the object at a never encountered place after 5 minute delay. Also, as PCP-treated rats were not impaired after 5 minutes delay in the OP task, it suggests that PCP-treated rats have a general tendency to explore a new environment and that their impairment after 10 minutes delay or in the OPC task was only due to a longer retention delay or task difficulty and not to their lack of interest in novelty.

These results clearly established a robust impairment in both OPC and OP tasks induced by PCP withdrawal which was not the case for isolation rearing. Therefore, withdrawal from subchronic PCP model appears to be a better model to investigate the episodic memory deficit in this OPC task. It also support previous suggestions that the OPC task and the OP task investigate different types of memory (different delays of retention between the OPC and the OP tasks) as previously

demonstrated in other studies (Eacott and Norman, 2004, Langston and Wood, 2009).

## 8.2 Differentiation between OPC recognition task and OP recognition task

A direct comparison of results, between the OPC and the OP tasks clearly suggests that both tasks investigated different types of memory and lower DI, after 10 minutes delay, in the OPC task suggests that it is a more complex task than the OP task. Control and isolated/group housed rats performed better in the OP task (memory not impaired after 10 minutes delay) than in the OPC task (memory impaired after 10 minutes delay). This was also the case for PCP withdrawalrats which showed memory impairment after 5 minutes delay in the OPC task but not in the OP task. Previous studies using both tasks also clearly established a differentiation between the two tasks (Eacott and Norman, 2004, Langston and Wood, 2009). Indeed, after fornix (Eacott and Norman, 2004) or HPC (Langston and Wood, 2009) lesion both studies showed that animals were able to perform the OP task but not the OPC task after 2 minutes delay. In both studies, they concluded that both tasks assess different types of memory such that OPC is investigating episodic memory and OP investigating working memory (Eacott and Norman, 2004, Langston and Wood, 2009).

PCP-treated rats were relatively more impaired into their capacity to integrate the context as a part of the information of a coherent memory (OPC task) compared to when the memory did not involve context specificity (OP task). In the OPC task, PCP withdrawal rats did not integrate the context as part of the information and, in the test phase, cannot differentiate the two objects as both of the objects had been previously seen in those places but in different contexts (sample phase 1 and 2). On the other hand, when the task does not involve context discrimination (OP task), PCP-treated rats were able to distinguish the newest combination of place and object, at least after 5 minutes. These results are reminiscent of human studies in schizophrenia which highlighted a context memory deficit which demonstrated difficulties for schizophrenic patients to bind different memory components such as the context of the information in episodic memory (Cohen and Servan-Schreiber, 1992, Bazin et al., 2000, Waters et al., 2004). Therefore, in the OPC task, the context appeared to be crucial and characteristic of episodic memory definition which reflects rats' capacity to integrate different features (place and object) within a specific context in which event has co-occurred.

Furthermore, it has been found that in the OPC task but not in OP task, there was a delay dependent reduction in total object exploration in controls that was not seen in PCP withdrawal or isolated rats.

## 8.3 Total object exploration time is delay dependent in the OPC task but not in the OP task

In all experiments (experiment 1, 2, 4, 6 and 7), in control groups, delay induced a decrease of rat's exploration during the test phase. Rats spent less time exploring both objects at 5 min delay compared to 10 minutes delay.

Multiple exposures to a context will lead to a diminution of exploration because rats will learn that there is no novelty present and therefore they will explore the open field less during the second exposure (Broadbent et al., 2009). Rats will be habituated to the context. Habituation is defined as the waning of a behavioural response due to the repetition of a stimulus. Two forms of habituation can be distinguished, the long-term and the short-term forms (Hinde, 1954, 1970, van der Staak, 1977). In the OPC recognition task the long term habituation is the repetition of the task, more a task is repeated more the rats will be habituated to the contexts and the objects, therefore, the less they will explore them the next time they will encounter them (Broadbent et al., 2009). Thus, exploration should decrease day by day, which was the case as shown in sample phase (experiments 2, 3, 4, 5, 6 and 7: Figure 3.6, 3.9, 4.2, 4.5, 5.2 and 6.2). The short term habituation is within the task itself, rats should explore more during the test phase compared to the sample phase as the objects and context will appear more familiar. Also, when the delay is increased between the sample phase and the test phase rats should explore more as there

is a dishabituation of the context and the objects (they become less familiar). The faster the rats are re-exposed to a context the greater the habituation will be (Hinde, 1970, van der Staak, 1977). In the OPC task in experiments 2, 4, 6 and 7, when the delay increased, from 5 to 10 minutes, there was a reduction rather than an increase in time spent exploring the objects, therefore short term habituation is unlikely to explain the effect obtained.

If the postulate is that rats explore the familiar less (Powel et al., 2004; Broadbent et al., 2009), it is possible that after 10 minutes delay the environment, which is the association of the context and the objects (context-object association) seems more familiar than after 5 minutes delay. One of the possible reasons for this diminution of objects exploration could therefore be that rats after 10 minutes delay were more confused than after 5 minutes delay by the similarity of the context-object association during the test phase compared to the context-object association during the sample phases 1 and 2. Total object exploration time is reduced after a longer delay because rats are more confused, they do not fully remember the previous context-object association seen during the sample phase 1 (context-object association look similar). Rats did not remember that the context-object association was different between the test and the sample phase 1. In other words, after a short delay rats remembered the sample context-objects association but not after a longer delay which was leading to less exploration.

In the OP task in which contextual information had been kept constant and therefore not necessary for object recognition, the delay-induced decrease in total object exploration was not seen (experiments 3 and 5: Figure 3.11 and 4.7). This might suggest that total object exploration differences seen in PCP-treated rats and isolated rats may reflect an abnormality in processing contextual information that was common to both models. Contextual processing deficits have been widely reported in schizophrenia (Cohen and Servan-Schreiber, 1992, Bazin et al., 2000, Waters et al., 2004). However, it cannot be concluded that isolated rats have impairment in the explicit recall of contextual information as they successfully performed the OPC task (which requires contextual information) as well as controls. One potential explanation might be that rats can use two potential strategies to solve the task, one based on familiarity with one or more of the components (object, place, context) encountered during the test phase and one based on remembering the context-object association previously seen. It has been recently shown that rats can use familiarity/recall strategies in task that involved odour discrimination associated with reward (Fortin et al., 2004) and also in the E-shape recognition task based on rats spontaneous tendency to explore novelty (Table 1.10) (Eacott and Easton, 2007). Scrub jays are also able to remember a past situation in order to act at a present time which suggests that animals can mentally travel in time and can remember previous events (Clayton and Emery, 2009). This interpretation of the results might suggest that knowing (DI) and

remembering (delay-induced total object exploration reduction) are dissociable within the OPC task but not within the OP task. PCP but not isolation rearing disrupts knowing (experiments 2 and 4) while both PCP and isolation disrupt remembering (experiments 2 and 4).

This capacity to remember the context-object association as indexed by reduced delay-induced total object exploration may be analogous to autonoetic awareness or remembering the past situation, which is distinguishable from noetic awareness or knowing which will be in this case the capacity to discriminate the most familiar object at the present time (test phase). To remember the context-object association of both sample phases and test phase is the capacity to compare events in time, and to be able to refer to a past situation in order to behave in a present situation (e.g. increase or decrease exploration). This interpretation of total object exploration corresponds to Tulving's what-where-where definition of episodic memory (Tulving, 1983). In this case, the "what" will be the context-object association, the "when" will be during the first exposure of the sample phase (first event: sample phase 1 compared to the second event: sample phase 2) and the "where" will be the context of the first exposure.

However, in experiment 1, which looked at a number of different delays at 15 minutes delay, rats were still able to discriminate the new object in place and context (experiment 1: Figure 2.5), even if rats did not fully remember the difference between context-object associations from the sample compared to the test phase. That was not the case in

other experiments (experiment 2, 4, 6 and 7) in which, for control group, lower exploration was always associated with rats' incapacity to discriminate the new object in place and context. This result does not contradict the previous statement, as the capacity to discriminate the newest object configuration is based on familiarity and refers to knowing or to the noetic awareness and this is distinct from autonoetic awareness which refers to remembering the context-object association (Tulving, 1985, 1986, 2002, Tulving, 2005). Therefore, rats could potentially recognise the new object in place and context only based on which object shares the most familiar aspects in term of place and context. This discrimination of the newest object configuration refers to Tulving's definition of episodic memory (Tulving, 1983) as the recollection of an object in place and context (what, where and which/when), however, it does not necessarily refer to rats capacity to remember the previous events as the task could be potentially solved with familiarity toward one or more of the three dimensions (objects, places and contexts).

Mumby et al., (2002) studied the effect of HPC lesion in rats in object recognition, place recognition and context recognition task (Figure 8.1). They rank ordered object recognition tasks according to complexity with object recognition being easier than place or context recognition. While these authors were explicitly studying lesion effects and do not discuss this effect, examination of figure 2 (see figure 8.1) below shows that increasing complexity is associated with decreasing total object

exploration at test, in the absence of differences in exploration times in the sample phase. It is possible therefore that a new object could be more salient than a new place or context.

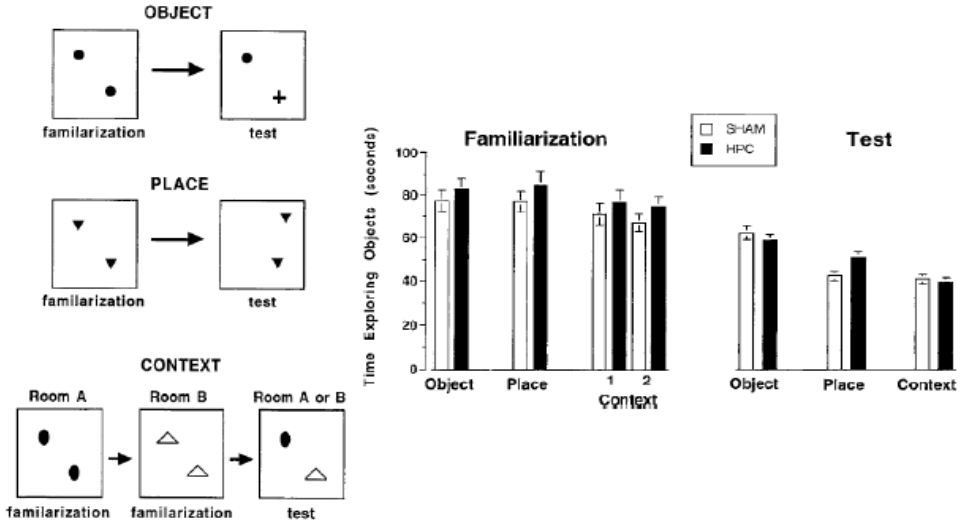



**Figure 8.1: Time engaged in object exploration during the familiarisation and test phases for each trial type (Object recognition, place recognition and context recognition). For context trials data are shown separately for the first (1) and second (2) familiarization phases. Error bars represent SEM. (From Mumby et al., 2002).**

Good et al., (2007a) also studied the effect of HPC lesion in rats in different object recognition paradigms (e.g. object recognition, temporal and spatial context recognition, and spatio-temporal context recognition). Examination of total time spent exploring object (approximate time spent in second on the new object + time spent on the familiar object) according to the results presented in figure 2 (see figure 8.2) highlights similar results to those in Mumby's study (Mumby et al., 2002) with a decrease of total object exploration depending of the

task complexity, with higher exploration when the object-context association in the test phase shared less similarity than in the sample phase (e.g. spatio-temporal context recognition as less similarity than spatial recognition). However, in this study, they did not report exploration time of the sample phase.

| approximate Total object exploration | Sham | HPC |
|---|---|---|
| Novelty | 63 sec | 73 sec |
| Temporal Context | 32 sec | 49 sec |
| Spatial Context | 25 sec | 34 sec |
| Spatio-temporal Context | 75 sec | 89 sec |

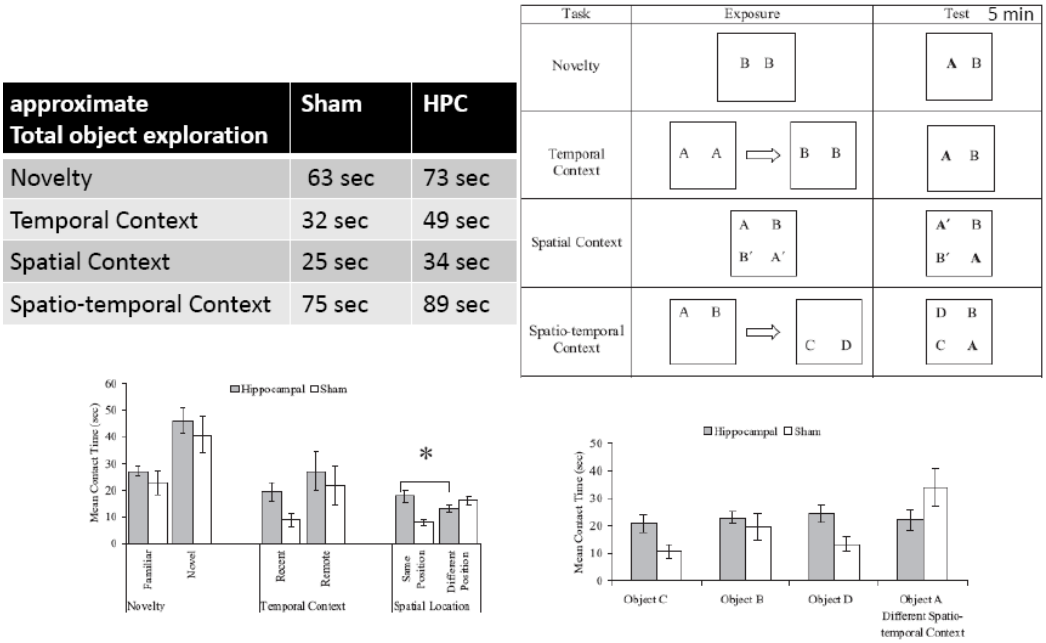



**Figure 8.2: Approximate summation of the time spent exploring objects in the test phase during the novelty, temporal, spatial context and spatio-temporal context tests in sham rats and rats with hippocampal lesions (From Good et al., 2007a).**

These results are reminiscent of the findings in the present thesis. This could suggest that the more a task is complex the less the rats explored the objects. In the OPC task, total object exploration time is reduced after a longer delay, but not after a short delay, because context-object association in the test appeared more complicated to

distinguish from the context-object association seen in the sample phase 1. Sample phase 2 is interfering between sample phase 1 and test phase which makes the whole context-object association similar during the test phase and the sample phase 1. In both studies (Mumby et al., 2002, Good et al., 2007a), sham rats were able to discriminate the newest combination of objects compared to a more familiar one in every paradigms, which suggests that decrease in total object exploration is independent from rats ability to recall which object is new at test time. Thus, it could be possible to postulate that, in the OPC task, rats can use two potential strategies to identify a new object in place and context, one based on simple recognition (does this look familiar?) and one based on contextual recognition of the objects (does it the same context and objects?). These two strategies may correspond to the current distinction between knowing/remembering in episodic memory (Tulving, 2002)

The delay-induced deficit was abolished by both PCP withdrawal and social isolation (experiment 2, 4, 6 and 7: Figure 3.8, 4.4, 5.3 and 6.4). The delay effect on total object exploration could be interpreted as remembering or autonoetic awareness deficit and rat's capacity to discriminate the familiar object in place depending of a context (DI) as knowing or noetic awareness.

Isolation rearing abolished delay induced total object exploration (remembering) but not their capacity to distinguish the newest object combination (knowing) is consistent with studies showing that episodic

memory in schizophrenia was impaired in remembering but not in knowing (Wheeler et al., 1997, Danion et al., 1999, Keefe et al., 2002, Riutort et al., 2003, Sonntag et al., 2003, Neumann et al., 2006). As, the behavioural effects of PCP in humans have been shown to persist for several weeks a (Jentsch and Roth, 1999, Enomoto et al., 2007, Seillier and Giuffrida, 2009), PCP withdrawal results in rats are consistent with human study using ketamine administration which showed impairment in both remembering and knowing (Hetem et al., 2000), in this case is impairment in both total object exploration (remembering) and object recognition (knowing) (Table 8.1).

| ***Human Studies*** | Autonoetic awareness (Remembering) | noetic awareness (Knowing) |
|---|---|---|
| Control group | *OK* | *OK* |
| Schizophrenic patient* | ↓ | *OK* |
| Ketamine/PCP human** | ↓ | ↓ |
| ***OPC task (5 minutes delay)*** | Total Object exploration (Remembering) | Object-Place-Context (Knowing) |
| Control group | *OK* | *OK* |
| Isolation rearing | ↓ | *OK* |
| PCP withdrawal | ↓ | ↓ |

**Table 8.1: Comparative study between human and animal showing a potential interpretation of the total object exploration and object-place-context recognition (DI) effect in PCP withdrawal and isolation rearing model of schizophrenia (*(Sonntag et al., 2003);**(Hetem et al., 2000)).**

It has been shown that people with schizophrenia have damage to the HPC which could be responsible for cognitive impairments including

episodic memory (Chambers et al., 1996, Shenton et al., 2001, Bergeron et al., 2005). Subchronic PCP administration, as well as isolation, induces some permanent disruption into the HPC such as dopaminergic hyperfunction (Jones et al., 1992, Pierce and Kalivas, 1997, Hall, 1998, Cornish and Kalivas, 2001). On the other hand, HPC lesion in rats impairs performance in the OPC task. Similar deficit had been seen after PCP withdrawal but not isolation rearing (Eacott and Norman, 2004, Langston and Wood, 2009). Therefore, it can be suggested that subchronic PCP induces more damages into the HPC which provoke major memory impairment, such as in the OPC, OP and object recognition task, compared with that associated with isolation (only impaired in object recognition after 1h delay). Furthermore, in human, HPC damage or lesion impaired both recall and recognition (Wais et al., 2006). Thus, it will have been interesting to know if Eacott and Norman (2004) or Langston and Wood (2009) would have obtained similar delay-induced deficit as well as the impairment in recognition in their OPC task if they will have tested different delays with the HPC lesion in rats.

Overall, these data suggest that the OPC task is sensitive to glutamate antagonists withdrawal and that it can identify highly specific memory impairments common to both PCP and social isolation rearing (total object exploration deficit). This suggests that this may prove to be a sensitive preclinical model for episodic memory impairments in schizophrenia. This model integrated contextual information which

provided dissociation between the noetic awareness (knowing/familiarity) and the autonoetic awareness (remembering/recall). Thus, PCP withdrawal model compared to isolation may be a relevant model to study a possible dissociative improvement induced by atypical antipsychotics such as clozapine or by cognitive enhancers such as donepezil in both the autonoetic and/or the noetic awareness aspect of the OPC task.

## 8.4 Donepezil but not clozapine restore the PCP-induced deficit in episodic memory but not the delay-induced deficit in total object exploration

Donepezil (0.3 mg/kg) has been shown to increase performance in saline-treated rats and to restore the PCP-induced deficit in the OPC recognition task (experiment 7: Figure 6.3). In rodents donepezil has been shown to restore the NMDA antagonist-induced deficit in spatial reversal learning, contextual and cued memory, and one-trial object recognition (Csernansky et al., 2005, Kunitachi et al., 2009) and improve episodic contextual memory in middle aged mice (Béracochéa et al., 2007). This finding corroborates previous human studies which have also revealed episodic memory improvement after donepezil administration (Grön et al., 2005). Results are consistent with these findings as in the present study at the 10 minutes delay donepezil rats showed clear object discrimination where controls did not, suggesting that it enhances basal memory performance. AChEIs have been tested

in initial clinical trials in schizophrenia some studies suggesting a beneficial effect in general cognitive impairment (e.g., Rey Auditory Verbal Learning Test (RAVLT); Wisconsin Card Sorting Test (WCST); selective attention Digit Span Distraction Test (DSPT)) in schizophrenia but others demonstrating minimal benefits (Friedman et al., 2002, Howard et al., 2002, Liddle, 2002, Buchanan et al., 2003, Tuğal O et al., 2004, Keefe et al., 2007, Risch et al., 2007, Chung et al., 2009). The studies performed to date have however used general cognitive assessment batteries typically measuring executive function and attention and do not include specific tests of episodic memory. While large scale double blind studies are required to fully assess the therapeutic potential of AChEIs, our study suggests that episodic memory impairment in particular might benefit from the effects of AChEIs in schizophrenia.

Delay-induced reduction in total object exploration time was abolished in PCP-saline group as in other experiments but donepezil failed to restore it (experiment 7: Figure 6.4). According to our interpretation of what total object exploration time reduction is measuring it could be that donepezil restored the noetic awareness (knowing, which object in place in this context is new?), but did not improve the PCP-induced deficit in autonoetic awareness (remembering, is it the same context-objects association?).

Therefore, it is possible to conclude a role of the cholinergic system in memory (Bontempi et al., 2003, Bullock and Dengiz, 2005, Seltzer, 2005) including episodic memory (Grön et al., 2005). In

animals, interactions between the cholinergic and glutamatergic systems have been established in some forms of memory such as spatial reversal learning, contextual and cued memory, and one-trial object recognition (Csernansky et al., 2005, Kunitachi et al., 2009). During episodic memory, acetylcholine may facilitate glutamate activity by coordinating states of acquisition and recall in the cortex and the HPC (Cox et al., 1994). Cholinergic projections modulates the glutamatergic pathway into the HPC and is responsible for encoding new information into different hippocampal subregions (Yun et al., 2000). In order to corroborate our findings, further analysis with donepezil in humans using specific tasks which investigate the knowing/remembering part in schizophrenia would be of future interest.

Clozapine did not reverse the PCP-induced deficit in OPC task (experiment 6: Figure 5.3), and total object exploration could not have been investigated further as clozapine treated rats explored less than non clozapine treated rats due to a sedative effect (experiment 6: Figure 5.4) (Kumra et al., 2008, Wiley, 2008). However no disruption in DI in clozapine treated rats suggests that in this task this degree of sedation did not confound memory performance. The present findings confirm that this reversal does not extend to episodic memory defined as memory for what-where-when while the present findings suggest a reproduction of the lack of clinical effectiveness of antipsychotics such as clozapine to treat episodic memory deficits in schizophrenia seen in schizophrenia in RBMT or AMI test (Riutort et al., 2003, Al-Uzri et al.,

2006). It is possible that clozapine may have an influence at a higher dose than that tested here. This is unlikely, as at this dose clozapine reverses PCP- and MK801 (a non-competitive NMDA receptor antagonist) induced deficits in one-trial object recognition (Hashimoto et al., 2005, Grayson et al., 2007, Karasawa et al., 2008). These studies used one-trial object recognition tasks addressing “what” (which object is new?), that only refer to a previous event, and are considered as working memory tasks (Ennaceur and Delacour, 1988). This is in contrast to OPC recognition in the present study which requires a comparison between events. The possibility that these tasks may be dissociable is suggested by the finding that fornix/HPC lesion does not impair one-trial object recognition but does impair OPC recognition (Eacott and Norman, 2004, Langston and Wood, 2009). However, further studies using a variety of doses and antipsychotics including perhaps the putatively antipsychotic mGluR2/3 agonists, are warranted before such a conclusion could be definitively drawn (Gold and Weinberger, 1995, Harvey and Keefe, 2001, Riutort et al., 2003).

## 8.5 Long lasting impairment in locomotor activity induced by subchronic PCP treatment is not restored by chronic clozapine or donepezil injections

These data demonstrated a robust hyperactivity in PCP withdrawal rats after acute PCP challenge. Sensitisation was observed 31 days after the last PCP injection when challenged with 3.2 mg/kg PCP. Neither donepezil nor clozapine administred chronically during the OPC task 7 days before LMA reversed PCP sensitization. Also, data confirmed that clozapine (5 mg/kg) but not donepezil (0.3 mg/kg) induced sedation in rats which last for more than 110 minutes after injection.

Subchronic PCP treatment produced a long lasting effect which was lasting for at least 31 days. Indeed, PCP-treated rats are hyperactive after an acute PCP (3.2 mg/kg) compared to saline-treated rats (experiment 8 and 9: Figure 7.4 and 7.5). This locomotor activity has been used to model psychosis in animals (Goldman-Rakic, 1996, Verma and Moghaddam, 1996). Also, subchronic PCP exposure attenuates the PFC dopaminergic release induced by a single PCP injection (Jentsch et al., 1997b) and after an acute PCP challenge, this attenuation of dopamine efflux is interpreted as a reduction of dopamine neurons in the PFC (Jentsch et al., 1997a, Jentsch et al., 1997b, Easton et al., 2008). Chronic clozapine treatment (9 days treatment) received during the OPC task and LMA task 7 days earlier

did not reverse the PCP hyperlocomotion induced by PCP challenge in subchronic PCP rats (Figure 7.4). Previous studies in rats have shown that acute clozapine (10 mg/kg i.p.) reverses the PCP (3.2 mg/kg i.p.) challenge-induced effect on locomotor activity 48 hours after injection in chronic PCP-treated rats (20 mg/kg s.c. once a day for 5 days) (Phillips et al., 2001)and that chronic clozapine administration (10 mg/kg i.p. once a day for 14 days) reversed, 11 days after treatment, the acute PCP (7.5 mg/kg i.p.) -induced hyperlocomotor activity (Abekawa et al., 2007). Thus, in LMA task, the long term effect of the chronic PCP treatment had never been investigated after such a long period of time and appeared to be robust. Also, chronic clozapine treatment did not restore the deficit which could be due to the delay (7 days) between the last clozapine injection and the PCP challenge task. Also, chronic donepezil (8 days treatment) did not reverse hyperlocomotor activity induced by NMDA antagonist (experiment 9: Figure 7.5) as it has been previously shown in other study (Csernansky et al., 2005). However, in humans, clozapine withdrawal induces abnormal behaviour, psychosis symptoms and dopaminergic supersensitivity (Borison et al., 1988, Shore et al., 1995, Stanilla et al., 1997), which could explain why PCP-clozapine and saline-clozapine group had a tendency to be more hyperactive than PCP-saline and saline-saline group respectively (experiment 10: Figure 7.5) after the acute PCP injection.

The clozapine (5 mg/kg) dose used during the LMA task produced significant sedation which was clearly established by a reduction of

locomotor activity 40 minutes after injection. This result confirmed previous finding about the sedative effect of clozapine in patients and rodents (Kumra et al., 2008, Wiley, 2008) and can explain the low object exploration observed during the OPC task. However, clozapine sedative effect did not impair rat's capacity to discriminate new environment. On the other hand, donepezil did not induce any difference in locomotor activity task compared to control group which is consistent with a previous study (Sonkusare et al., 2005).

## 8.6 Conclusion

- The OPC recognition task has been refined and previous results found in the original study by Eacott and Norman (2004) replicated.
- It has been demonstrated that PCP withdrawal rats but not isolated rats are impaired in the OPC task, however they both demonstrate impairment into total object exploration during the test phase compared to control groups. These results could suggest that the task can differentiate between autonoetic awareness (total object exploration, is the context-object association familiar?) and the noetic awareness (capacity to discriminate the newest combination of object, place and context). If confirmed this would corroborate findings in human studies on episodic memory with NMDA antagonist and with schizophrenic persons.

- A task specificity has been shown between a two sample recognition task (the OPC recognition task) compared to a one-sample recognition task (OP recognition task).
- It has been found that donepezil but not clozapine restore the PCP-induced deficit in OPC recognition task as a model of episodic-like memory which suggests a role of AChEIs in cognitive deficit in schizophrenia. However neither clozapine nor donepezil restored the total object exploration noticed during the test phase which could reflect the limitation of AChEIs into autonoetic aspects of cognitive improvement in schizophrenia.
- In summary these studies have shown that OPC recognition in rats may have some potential as a behavioural measure for animal models of episodic memory in schizophrenia.

# - REFERENCES -


Abdul-Monim Z, Neill JC, Reynolds GP (2007) Sub-chronic psychotomimetic phencyclidine induces deficits in reversal learning and alterations in parvalbumin-immunoreactive expression in the rat. J Psychopharmacol 21:198-205.

Abdul-Monim Z, Reynolds GP, Neill JC (2006) The effect of atypical and classical antipsychotics on sub-chronic PCP-induced cognitive deficits in a reversal-learning paradigm. Behavioural Brain Research 169:263-273.

Abekawa T, Ito K, Koyama T (2007) Different effects of a single and repeated administration of clozapine on phencyclidine-induced hyperlocomotion and glutamate releases in the rat medial prefrontal cortex at short- and long-term withdrawal from this antipsychotic. Naunyn-Schmiedeberg's Archives of Pharmacology 375:261-271.

Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Seibyl JP, Bowers M, van Dyck CH, Charney DS, Innis RB, Laruelle M (1998) Increased Striatal Dopamine Transmission in Schizophrenia: Confirmation in a Second Cohort. American Journal of Psychiatry 155:761-767.

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M (2000) Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. ProcNatlAcadSciUSA 97:8104-8109.

Abi Dargham A (2002) Recent evidence for dopamine abnormalities in schizophrenia. European Psychiatry 17:341-347.

Achim AM, Lepage M (2003) Is associative recognition more impaired than item recognition memory in Schizophrenia? A meta-analysis. Brain and Cognition 53:121-124.

Adams B, Moghaddam B (1998) Corticolimbic Dopamine Neurotransmission Is Temporally Dissociated from the Cognitive and Locomotor Effects of Phencyclidine. Journal of Neuroscience 18:5545-5554.

Aggleton JP, Brown MW (1999) Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. The Behavioral and brain sciences 22:425-444.

Aggleton JP, Pearce JM (2001) Neural systems underlying episodic memory: insights from animal research. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 356:1467-1482.

Al-Uzri MM, Reveley MA, Owen L, Bruce J, Frost S, Mackintosh D, Moran P (2006) Measuring memory impairment in community-based patients with schizophrenia. Case-control study. British Journal of Psychiatry 189:132-136.

Ananth J, Djenderdjian A, Shamasunder P, Costa J, Herrera J, Sramek J (1991) Negative symptoms: psychopathological models. JPsychiatry Neurosci 16:12-18.

Andreasen N (1995) Symptoms, signs, and diagnosis of schizophrenia. The Lancet 346:477-481.

Andreasen NC (1982 ) Negative symptoms in schizophrenia. Definition and reliability. Archives of General Psychiatry 39:784-788.

Asarnow JR, Goldstein MJ (1986) Schizophrenia during adolescence and early adulthood: A developmental perspective on risk research. Clinical Psychology Review 6:211-235.

Aultman J, Moghaddam B (2001) Distinct contributions of glutamate and dopamine receptors to temporal aspects of rodent working memory using a clinically relevant task. Psychopharmacology Volume 153:353-364.

Babb SJ, Crystal JD (2006) Episodic-like memory in the rat. Current biology 16:1317-1321.

Bachevalier J, Meunier M, Lu MX, Ungerleider LG (1997) Thalamic and temporal cortex input to medial prefrontal cortex in rhesus monkeys. ExpBrain Res 115:430-444.

Bagchi SP (1981) Effects of phencyclidine on synaptosomal dopamine continuously appearing from phenylalanine: Sensitivity to reserpine. Neuropharmacology 20:845-851.

Bakshi VP, Swerdlow NR, Geyer MA (1994) Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. The Journal of pharmacology and experimental therapeutics 271:787-794.

Barker GR, Bird F, Alexander V, Warburton EC (2007) Recognition memory for objects, place, and temporal order: a disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. Journal of Neuroscience 27:2948-2957.

Bay-Richter C, O'Tuathaigh C, O'Sullivan G, Heery DM, Waddington JL, Moran PM (2009) Enhanced latent inhibition in dopamine receptor-deficient mice is sex-specific for the D1 but not D2 receptor subtype: implications for antipsychotic drug action. International Journal of Neuropsychopharmacology 12:403-414.

Bazin N, Perruchet P, Hardy-Bayle MC, Feline A (2000) Context-dependent information processing in patients with schizophrenia. Schizophrenia Research 45:93-101.

Bell DS (1973) The experimental reproduction of amphetamine psychosis. Archives of General Psychiatry 29:35-40.

Béracochéa D, Philippin J, Meunier S, Morain P, Bernard K (2007) Improvement of episodic contextual memory by S 18986 in middle-aged mice: comparison with donepezil. Psychopharmacology 193:63-73.

Berenbaum H, Kerns JG, Vernon LL, Gomez JJ (2008) Cognitive correlates of schizophrenia signs and symptoms: III. Hallucinations and delusions. Psychiatry Research 159:163-166.

Bergeron R, Coyle JT, Tsai G, Greene RW (2005) NAAG reduces NMDA receptor current in CA1 hippocampal pyramidal neurons of acute slices and dissociated neurons. Neuropsychopharmacology 30:7-16.

Berlyne DE (1950) novelty and curiosity as determinants of exploratory behavior. British Journal of Psychology 41:68-80.

Bianchi M, Fone KF, Azmi N, Heidbreder CA, Hagan JJ, Marsden CA (2006) Isolation rearing induces recognition memory deficits accompanied by cytoskeletal alterations in rat hippocampus. European Journal of Neuroscience 24:2894-2902.

Bishnoi M, Chopra K, Kulkarni SK (2007) Neurochemical changes associated with chronic administration of typical antipsychotics and its relationship with tardive dyskinesia. Methods and findings in experimental and clinical pharmacology 29:211-216.

Blin O (1999) A comparative review of new antipsychotics. Canadian Journal of Psychiatry 44:235-244.

Bontempi B, Whelan KT, Risbrough VB, Lloyd GK, Menzaghi F (2003) Cognitive Enhancing Properties and Tolerability of Cholinergic Agents in Mice: A Comparative Study of Nicotine, Donepezil, and SIB-1553A, a Subtype-Selective Ligand for Nicotinic Acetylcholine Receptors. Neuropsychopharmacology 28:1235-1246.

Borison RL, Diamond BI, Sinha D, Gupta RP, Ajiboye A (1988) Clozapine withdrawal rebound psychosis. Psychopharmacology Bulletin 24:260-263.

Borrini G, Dall'Ora P, Della Sala S, Marinelli L, Spinnler H (1989) Autobiographical memory. Sensitivity to age and education of a standardized enquiry. Psychological Medicine 19:215-224.

Brazo P, Delamillieure P, Morello R, Halbecq I, Marié R-M, Dollfus S (2005) Impairments of executive/attentional functions in schizophrenia with primary and secondary negative symptoms. Psychiatry Research 133:45-55.

Breier A, Schreiber JL, Dyer J, Pickar D (1991) National Institute of Mental Health longitudinal study of chronic schizophrenia. Prognosis and predictors of outcome. Archives of General Psychiatry 48:239-246.

Broadbent NJ, Gaskin S, Squire LR, Clark RE (2009) Object recognition memory and the rodent hippocampus. Learning & memory 17:794-800.

Brozoski TJ, Brown RM, Rosvold HE, Goldman PS (1979) Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. Science 205:929-932.

Buchanan RW (1995) Clozapine: Efficacy and Safety. Schizophrenia Bulletin21:579-591.

Buchanan RW, Summerfelt A, Tek C, Gold J (2003) An open-labeled trial of adjunctive donepezil for cognitive impairments in patients with schizophrenia. Schizophrenia Research 59:29-33.

Bullock R, Dengiz A (2005) Cognitive performance in patients with Alzheimer's disease receiving cholinesterase inhibitors for up to 5 years. International Journal of Clinical Practice 59:817-822.

Byrd LD, Standish LJ, Howell LL (1987) Behavioral effects of phencyclidine and ketamine alone and in combination with other drugs. European Journal of Pharmacology 144:331-341.

Carllson A, Lindqvist M (1963) Effect of chorpromazine or haloperidol on formation of 3metoxytyramine and normetanephrine in mousse brain. Acta pharmacologica et toxicologica 20:140-144.

Carlsson A (1978) Antipsychotic drugs, neurotransmitters, and schizophrenia. American Journal of Psychiatry 135:165-173.

Castellani S, Adams PM (1981) Acute and chronic phencyclidine effects on locomotor activity, stereotypy and ataxia in rats. European Journal of Pharmacology 73:143-154.

Chambers RA, Moore J, McEvoy J, Levin ED (1996) Cognitive effects of neonatal hippocampal lesions in a rat model of schizophrenia. Neuropsychopharmacology 15:587-594.

Chartoff EH, Heusner CL, Palmiter RD (2005) Dopamine is not required for the hyperlocomotor response to NMDA receptor antagonists. Neuropsychopharmacology 30:1324-1333.

Chrobak JJ, Hinman JR, Sabolek HR (2008) Revealing Past Memories: Proactive Interference and Ketamine-Induced Memory Deficits. Journal of Neuroscience 28:4512-4520.

Chung YC, Lee CR, Park TW, Yang KH, Kim KW (2009) Effect of donepezil added to atypical antipsychotics on cognition in patients with schizophrenia: An open-label trial. World Journal of Biological Psychiatry 10:156 - 162.

Cilia, Cilia J, Reavill, Reavill C, Hagan, Hagan J, Jones, Jones D (2001) Long-term evaluation of isolation-reared prepulse inhibition deficits in rats. Psychopharmacology 156:327-337.

Clare L, McKenna P, j., Mortimer AM, Baddeley AD (1993) Memory in schizophrenia: what is impaired and what is preserved? Neuropsychologia 31 1225-1241.

Clark RE, West AN, Zola SM, Squire LR (2001) Rats with lesions of the hippocampus are impaired on the delayed nonmatching-to-sample task. Hippocampus 11:176-186.

Clayton NS, Dickinson A (1998) Episodic-like memory during cache recovery by scrub jays. Nature 395:272-274.

Clayton NS, Emery NJ (2009) What do jays know about other minds and other times? In: Neurobiology of “Umwelt”, pp 109-123.

Cloud BS, White AO, Tracy J, Oesterling R, de Leon J, Josiassen R, Simpson G (1994) The effects of chronic psychosis on rivermead behavioral memory test performance. Biological Psychiatry 35:637-637.

Cohen JD, Servan-Schreiber D (1992) A neural network model of disturbances in the processing of context in schizophrenia. Psychiatric Annals 22 131–136.

Contreras F, Fouillioux C, Bolivar A, Simonovis N, Hernandez-Hernandez R, rmas-Hernandez MJ, Velasco M (2002) Dopamine, hypertension and obesity. Journal of human hypertension 16 Suppl 1:S13-7.:S13-S17.

Cornish JL, Kalivas PW (2001) Cocaine Sensitization and Craving: Differing Roles for Dopamine and Glutamate in the Nucleus Accumbens. Journal of Addictive Diseases 20:43-54.

Cox CL, Metherate R, Ashe JH (1994) Modulation of cellular excitability in neocortex: Muscarinic receptor and second messenger-mediated actions of acetylcholine. Synapse 16:123-136.

Coyle JT (2006) Glutamate and schizophrenia: beyond the dopamine hypothesis. Cellular and molecular neurobiology 26:365-384.

Coyle JT, Tsai G (2004) The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia. Psychopharmacology (Berlin) 174:32-38.

Coyle JT, Tsai G, Goff DC (2002) Ionotropic glutamate receptors as therapeutic targets in schizophrenia. Current drug targets. CNS and neurological disorders 1:183-189.

Crook JM, Tomaskovic-Crook E, Copolov DL, Dean B (2001) Low Muscarinic Receptor Binding in Prefrontal Cortex From Subjects With Schizophrenia: A Study of Brodmann's Areas 8, 9, 10, and 46 and the Effects of Neuroleptic Drug Treatment. American Journal of Psychiatry 158:918-925.

Crow TJ (1980) Molecular pathology of schizophrenia: more than one disease process? British medical journal 280:66-68.

Csernansky JG, Martin M, Shah R, Bertchume A, Colvin J, Dong H (2005) Cholinesterase Inhibitors Ameliorate Behavioral Deficits Induced by MK-801 in Mice. Neuropsychopharmacology 30:2135-2143.

Cummings JL (2000) Cholinesterase Inhibitors: A New Class of Psychotropic Compounds. American Journal of Psychiatry 157:4-15.

Curran HV, Morgan C (2000) Cognitive, dissociative and psychotogenic effects of ketamine in recreational users on the night of drug use and 3 days later. Addiction 95:575-590.

Danion JM, Meulemans T, Kauffmann-Muller F, Vermaat H (2001) Intact Implicit Learning in Schizophrenia. American Journal of Psychiatry 158:944-948.

Danion JM, Rizzo L, Bruant A (1999) Functional mechanisms underlying impaired recognition memory and conscious awareness in patients with schizophrenia. Archives of General Psychiatry 56:639-644.

Davis AE, Gimenez AM, Therrien B (2001) Effects of entorhinal cortex lesions on sensory integration and spatial learning. Nursing research 50:77-85.

Davis KL, Kahn RS, Ko G, Davidson M (1991) Dopamine in schizophrenia: a review and reconceptualization. American Journal of Psychiatry 148:1474-1486.

Day-Wilson KM, Jones DNC, Southam E, Cilia J, Totterdell S (2006) Medial prefrontal cortex volume loss in rats with isolation rearing-induced deficits in prepulse inhibition of acoustic startle. Neuroscience 141:1113-1121.

Deneris ES, Connolly J, Rogers SW, Duvoisin R (1991) Pharmacological and functional diversity of neuronal nicotinic acetylcholine receptors. Trends in Pharmacological Sciences 12:34-40.

Deng X, Shibata H, Ninomiya H, Tashiro N, Iwata N, Ozaki N, Fukumaki Y (2004) Association study of polymorphisms in the excitatory amino acid transporter 2 gene (SLC1A2) with schizophrenia. BMC Psychiatry 4.

Deng X, Shibata H, Takeuchi N, Rachi S, Sakai M, Ninomiya H, Iwata N, Ozaki N, Fukumaki Y (2007) Association study of polymorphisms in the glutamate transporter genes SLC1A1, SLC1A3, and SLC1A6 with schizophrenia. American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics 144:271-278.

Dere E, Huston JP, De Souza Silva MA (2005a) Episodic-like memory in mice: simultaneous assessment of object, place and temporal order memory. Brain research protocols 16:10-19.

Dere E, Huston JP, De Souza Silva MA (2005b) Integrated memory for objects, places, and temporal order: Evidence for episodic-like memory in mice. Neurobiology of Learning and Memory 84:214-221.

Dere E, Huston JP, De Souza Silva MA (2007) The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neuroscience & Biobehavioral Reviews 31:673-704.

Dere E, Kart-Teke E, Huston JP, De Souza Silva MA (2006) The case for episodic memory in animals. Neuroscience and biobehavioral reviews 30:1206-1224.

Dix SL, Aggleton JP (1999) Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. Behavioural brain research 99:191-200.

Doody GA, Sanderson TL, Cunningham-Owens DG, Johnstone EC (1997) Why poor performance on the rivermead behavioural memory test in schizophrenia is indicative of a specific disease process. Schizophrenia Research 24:101-101.

Dunn MJ, Killcross S (2006) Clozapine but not haloperidol treatment reverses sub-chronic phencyclidine-induced disruption of conditional discrimination performance. Behavioural Brain Research 175:271-277.

Eacott MJ, Easton A (2007) On familiarity and recall of events by rats. Hippocampus 17:890-897.

Eacott MJ, Norman G (Integrated memory for object, place, and context in rats: a possible model of episodic-like memory? Journal of Neuroscience 24:1948-1953.2004).

Easton A, Eacott MJ, Ekrem D, Nadel L, Huston J, P. (2008) Chapter 2.3 A new working definition of episodic memory: replacing "when" with "which". In: Handbook of Behavioral Neuroscience, vol. Volume 18, pp 185-196: Elsevier.

Eichenbaum H (2003) The hippocampus, episodic memory, declarative memory, spatial memory...where does it all come together? International Congress Series 1250:235-244.

Elvevag K, Kerbs M, Malley JD, Seeley E, Goldberg T (2003) Autobiographical memory in schizophrenia: an examination of the distribution of memories. Neuropsychology 17:402–409.

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behavioural Brain Research 31:47-59.

Enomoto T, Noda Y, Nabeshima T (2007) Phencyclidine and genetic animal models of schizophrenia developed in relation to the glutamate hypothesis. Methods Find Clinical and experimental pharmacology & physiology 29:291-301.

Ergorul C, Eichenbaum H (2004) The Hippocampus and Memory for "What," "Where," and "When. Learning & Memory 11(4):397–405.

Erickson SK, Schwarzkopf SB, Palumbo D, Badgley-Fleeman J, Smirnow AM, Light GA (2005) Efficacy and tolerability of low-dose donepezil in schizophrenia. Clinical Neuropharmacology 28:179-184.

Feinberg AP, Snyder SH (1975) Phenothiazine drugs: structure-activity relationships explained by a conformation that mimics dopamine. 72:1899–1903.

Feinstein A, Goldberg TE, Nowlin B, Weinberger DR (1998) Types and characteristics of remote memory impairment in schizophrenia. Schizophrenia Research 30:155-163.

Feldman RS, Meyers JS, Quenzer LF (1997) Principles of Neuropsychopharmacology. Sinauer Associates: Sunderland MA.

Fone KCF, Porkess MV (2008) Behavioural and neurochemical effects of post-weaning social isolation in rodents-Relevance to developmental neuropsychiatric disorders. Neuroscience & Biobehavioral Reviews 32:1087-1102.

Fortin NJ, Wright SP, Eichenbaum H (2004) Recollection-like memory retrieval in rats is dependent on the hippocampus. Nature 431:188-191.

Freedman R, Hall M, Adler LE, Leonard S (1995) Evidence in postmortem brain tissue for decreased numbers of hippocampal nicotinic receptors in schizophrenia. Biological Psychiatry 38:22-33.

Friedman J, Adler DN, Howanitz E, Harvey PD, Brenner G, Temporini H, White L, Parrella M (2002) A double blind placebo controlled trial of donepezil adjunctive treatment to risperidone for the cognitive impairment of schizophrenia. Biological Psychiatry 51:349-357.

Friedman JI (2004) Cholinergic targets for cognitive enhancement in schizophrenia: focus on cholinesterase inhibitors and muscarinic agonists. Psychopharmacology 174:45-53.

Fujii Y, Shibata H, Kikuta R, Makino C, Tani A, Hirata N, Shibata A, Ninomiya H, Tashiro N, Fukumaki Y (2003) Positive associations of polymorphisms in the metabotropic glutamate receptor type 3 gene (GRM3) with schizophrenia. Psychiatric Genetics 13:71-76.

Ganda C, Schwab SG, Amir N, Heriani H, Irmansyah I, Kusumawardhani A, Nasrun M, Widyawati I, Maier W, Wildenauer DB (2009) A family-based association study of DNA sequence variants in GRM7 with schizophrenia in an Indonesian population. Int J Neuropsychopharmacol 12:1283-1289.

Gaffan D (1994) Scene-specific memory for objects: a model of episodic memory impairment in monkeys with fornix transection. Journal of Cognitive Neuroscience 6:305-320.

Gardiner JM (1988) Functional aspects of recollective experience. Memory & cognition 16:309-313.

Gentsch C, Lichtsteiner M, Kraeuchi K, Feer H (1982) Different reaction patterns in individually and socially reared rats during exposures to novel environments. Behavioural Brain Research 4:45-54.

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 156:117-154.

Geyer MA, Moghaddam B (2002) Neurosphychopharmacology: the fifth generation of progress. In: Animal models relevant to schizophrenia disorders, pp 689-701: American College of Neuropsychopharmacology.

Geyer MA, Wilkinson LS, Humby T, Robbins TW (1993) Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. Biological Psychiatry 34:361-372.

Ghoneim MM, Hinrichs JV, Mewaldt SP, Petersen RC (1985) Ketamine: behavioral effects of subanesthetic doses. Journal of clinical psychopharmacology 5:70-77.

Gold JM, Randolph C, Carpenter CJ, Goldberg TE, Weinberger DR (1992) Forms of memory failure in schizophrenia. Journal of Abnormal Psychology 101:487-494.

Gold JM, Weinberger DR (1995) Cognitive deficits and the neurobiology of schizophrenia. Current opinion in neurobiology 5:225-230.

Goldberg TE, Greenberg RD, Griffin SJ, Gold JM, Kleinman JE, Pickar D, Schulz SC, Weinberger DR (1993) The effect of clozapine on cognition and psychiatric symptoms in patients with schizophrenia. The British Journal of Psychiatry 162:43-48.

Goldman-Rakic PS (1996) Memory: recording experience in cells and circuits: diversity in memory research. Proceedings of the National Academy of Sciences of the United States of America 93:13435–13447.

Gonzalez MC, Abreu P, Barroso-Chinea P, Cruz-Muros I, Gonzalez-Hernandez T (2004). Effect of intracerebroventricular injection of lipopolysaccharide on the tuberoinfundibular dopaminergic system of the rat. Neuroscience 127: 251-259.

Good MA, Barnes P, Staal V, McGregor A, Honey RC (2007a) Context- but not familiarity-dependent forms of object recognition are impaired following excitotoxic hippocampal lesions in rats. Behavioural Neuroscience 121:218-223.

Good MA, Hale G, Staal V (2007b) Impaired "episodic-like" object memory in adult APPswe transgenic mice. Behavioural Neuroscience 121:443-448.

Gopal YV, Variend H (2005) First-episode schizophrenia: review of cognitive deficits and cognitive remediation. Advances in Psychiatric Treatment 11:38-44.

Grayson B, Idris NF, Neill JC (2007) Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. Behavioural Brain Research 184:31-38.

Green MF, Kern RS, Heaton RK (2004) Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. Schizophrenia Research 72:41-51.

Grön G, Kirstein M, Thielscher A, Riepe M, Spitzer M (2005) Cholinergic enhancement of episodic memory in healthy young adults. Psychopharmacology 182:170-179.

Guidry J, Kent TA (1999) New genetic hypothesis of schizophrenia. Medical hypotheses 52:69-75.

Gur RE, Cowell PE, Latshaw A, Turetsky BI, Grossman RI, Arnold SE, Bilker WB, Gur RC (2000) Reduced dorsal and orbital prefrontal gray matter volumes in schizophrenia. Archives of General Psychiatry 57:761-768.

Gurden H, Takita M, Jay TM (2000) Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentiation at hippocampal-prefrontal cortex synapses in vivo. Journal of Neuroscience 20:RC106:1-5.

Hafting T, Fyhn M, Molden S, Moser MB, Moser EI (2005) Microstructure of a spatial map in the entorhinal cortex. Nature 436:801-806.

Hagh-Shenas H, Toobai S, Makaremi A (2002) Selective, sustained, and shift in attention in patients with diagnoses of schizophrenia. Perceptual and motor skills 95:1087-1095.

Hall FS (1998) Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences. Critical Reviews in Neurobiology 12:129-162.

Harris T, Brown GW, Bifulco A (1986) Loss of parent in childhood and adult psychiatric disorder: The role of lack of adequate parental care. Psychological Medicine 16:641-659.

Harrison CL, Fowler D (2004) Negative symptoms, trauma, and autobiographical memory: An investigation of individuals recovering from psychosis. Journal of Nervous and Mental Disease 192:745-753.

Harvey PD, Keefe RSE (2001) Studies of Cognitive Change in Patients With Schizophrenia Following Novel Antipsychotic Treatment. American Journal of Psychiatry 158:176-184.

Hashimoto K, Fujita Y, Shimizu E, Iyo M (2005) Phencyclidine-induced cognitive deficits in mice are improved by subsequent subchronic administration of clozapine, but not haloperidol. European Journal of Pharmacology 519:114-117.

Hashimoto K, Ishima T, Fujita Y, Matsuo M, Kobashi T, Takahagi M, Tsukada H, Iyo M (2008) Phencyclidine-Induced Cognitive Deficits in Mice Are Improved by Subsequent Subchronic Administration of the Novel Selective [alpha]7 Nicotinic Receptor Agonist SSR180711. Biological Psychiatry 63:92-97.

Hatzipetros T, Yamamoto BK (2006) Dopaminergic and GABAergic modulation of glutamate release from rat subthalamic nucleus efferents to the substantia nigra. Brain Research 1076:60-67.

Heidbreder CA, Weiss IC, Domeney AM, Pryce C, Homberg J, Hedou G, Feldon J, Moran MC, Nelson P (2000) Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. Neuroscience 100:749-768.

Herlitz A, Forsell Y (1996) Episodic memory deficit in elderly adults with suspected delusional disorder. Acta psychiatrica Scandinavica 93:355-361.

Hernandez CM, Gearhart DA, Parikh V, Hohnadel EJ, Davis LW, Middlemore ML, Warsi SP, Waller JL, Terry AV (2006) Comparison of galantamine and donepezil for effects on nerve growth factor, cholinergic markers, and memory performance in aged rats. The Journal of pharmacology and experimental therapeutics 316:679-694.

Hetem LA, Danion JM, Diemunsch P, Brandt C (2000) Effect of a subanesthetic dose of ketamine on memory and conscious awareness in healthy volunteers. Psychopharmacology (Berl) 152:283-288.

Hietala J, Syvalahti E, Vuorio K, Nagren K, Lehikoinen P, Ruotsalainen U, RakkOlainen V, Lehtinen V, Wegelius U (1994) Striatal D2 Dopamine Receptor Characteristics in Neuroleptic-Naive Schizophrenic Patients Studied With Positron Emission Tomography. Archives of General Psychiatry 51:116-123.

Hinde RA (1954) Changes in responsiveness to a constant stimulus. The British Journal of Animal Behaviour 2:41-55.

Hinde RA (1970) Behavioral habituation: Short-term Changes in Neural Activity and Behaviour. University Press, Cambridge 3-40.

Honey G, Honey R, Sharar S, Turner D, Pomarol-Clotet E, Kumaran D, Simons J, Hu X, Rugg M, Bullmore E, Fletcher P (2005) Impairment of specific episodic memory processes by sub-psychotic doses of ketamine: the effects of levels of processing at encoding and of the subsequent retrieval task. Psychopharmacology 181:445-457.

Honey RAE, Turner DC, Honey GD, Sharar SR, Kumaran D, Pomarol-Clotet E, McKenna P, Sahakian BJ, Robbins TW, Fletcher PC (2003) Subdissociative Dose Ketamine Produces a Deficit in Manipulation but not Maintenance of the Contents of Working Memory. Neuropsychopharmacology 28:2037-2044.

Howard AK, Thornton AE, Altman S, Honer WG (2002) Donepezil for memory dysfunction in schizophrenia. Journal of Psychopharmacology 16:267-270.

Howes OD, McDonald C, Cannon M, Arseneault L, Boydell J, Murray RM (2004) Pathways to schizophrenia: the impact of environmental factors. International Journal of Neuropsychopharmacology Suppl 1: S7-S13

Javitt DC (2007) Glutamate and Schizophrenia: Phencyclidine, N-Methyl-d-Aspartate Receptors, and Dopamine-Glutamate Interactions. International review of neurobiology 78:69-108.

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. American Journal of Psychiatry 148:1301-1308.

Jay TM, Thierry AM, Wiklung L, Glowinski J (1992) Excitatory amino acid pathway from the hippocampus to the prefrontal cortex. Contribution of AMPA receptors in hippocampo-prefrontal cortex transmission. European Journal of Neuroscience 4:1285-1295.

Jentsch JD, Redmond Jr DE, Elsworth JD, Taylor JR, Youngren KD, Roth RH (1997a) Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. Science 277:953-955.

Jentsch JD, Roth RH (1999) The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20:201-225.

Jentsch JD, Tran A, Le D, Youngren KD, Roth RH (1997b) Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. Neuropsychopharmacology 17:92-99.

Johnson KM, Phillips M, Wang C, Kevetter GA (1998) Chronic phencyclidine induces behavioral sensitization and apoptotic cell death in the olfactory and piriform cortex. Journal of Neuroscience Research 52:709-722.

Jones GH, Hernandez TD, Kendall DA, Marsden CA, Robbins TW (1992) Dopaminergic and serotonergic function following isolation rearing in rats: Study of behavioural responses and postmortem and in vivo neurochemistry. Pharmacology Biochemistry and Behavior 43:17-35.

Jung Y-W, Frey KA, Mulholland GK, del Rosario R, Sherman PS, Raffel DM, Van Dort ME, Kuhl DE, Gildersleeve DL, Wieland DM (1996) Vesamicol Receptor Mapping of Brain Cholinergic Neurons with Radioiodine- Labeled Positional Isomers of Benzovesamicol1. Journal of Medicinal Chemistry 39:3331-3342.

.

Kalivas PW, Volkow N, Seamans J (2005) Unmanageable Motivation in Addiction: A Pathology in Prefrontal-Accumbens Glutamate Transmission. Neuron 45:647-650.

Karasawa J, Hashimoto K, Chaki S (2008) d-Serine and a glycine transporter inhibitor improve MK-801-induced cognitive deficits in a novel object recognition test in rats. Behavioural Brain Research 186:78-83.

Kart-Teke E, De Souza Silva MA, Huston JP, Dere E (2006) Wistar rats show episodic-like memory for unique experiences. Neurobiology of Learning and Memory 85:173-182.

Kay SR (1990) Significance of the Positive-negative Distinction in Schizophrenia. Schizophrenia Bulletin 16:635-652.

Keefe RSE, Arnold MC, Bayen UJ, McEvoy JP, Wilson WH (2002) Source-monitoring deficits for self-generated stimuli in schizophrenia: multinomial modeling of data from three sources. Schizophrenia Research 57:51-67.

Keefe RSE, Malhotra AK, Meltzer HY, Kane JM, Buchanan RW, Murthy A, Sovel M, Li C, Goldman R (2007) Efficacy and Safety of Donepezil in Patients with Schizophrenia or Schizoaffective Disorder: Significant Placebo//Practice Effects in a 12-Week, Randomized, Double-Blind, Placebo-Controlled Trial. Neuropsychopharmacology 33:1217-1228.

Kegeles LS, bi-Dargham A, Zea-Ponce Y, Rodenhiser-Hill J, Mann JJ, Van Heertum RL, Cooper TB, Carlsson A, Laruelle M (2000) Modulation of amphetamine-induced striatal dopamine release by ketamine in humans: implications for schizophrenia. Biological psychiatry 48:627-640.

Kennedy PJ, Shapiro ML (2004) Retrieving Memories via Internal Context Requires the Hippocampus. Journal of Neuroscience 24:6979-6985.

Kesner RP, Bolland BL, Dakis M (1993) Memory for spatial locations, motor responses, and objects: Triple dissociation among the hippocampus, caudate nucleus and extrastriate visual cortex. Experimental Brain Research 93:462-470.

Kim JJ, Rison RA, Fanselow MS (1993) Effects of amygdala, hippocampus, and periaqueductal gray lesions on short- and long-term contextual fear. Behavioral Neuroscience 107:1093-1098.

Kim SW, Jang YJ, Chang JW, Hwang O (2003) Degeneration of the nigrostriatal pathway and induction of motor deficit by tetrahydrobiopterin: an in vivo model relevant to Parkinson's disease. Neurobiology of Disease 13:167-176.

Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB, Jr., Charney DS (1994) Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Archives of General Psychiatry 51:199-214.

Krystal JH, Perry EB, Jr., Gueorguieva R, Belger A, Madonick SH, Abi-Dargham A, Cooper TB, MacDougall L, Abi-Saab W, D'Souza DC (2005) Comparative and Interactive Human Psychopharmacologic Effects of Ketamine and Amphetamine: Implications for Glutamatergic and Dopaminergic Model Psychoses and Cognitive Function. Archives of General Psychiatry 62:985-994.

Kumra S, Oberstar J, Sikich L, Findling R, McClellan J, Vinogradov S, Charles Schulz S (2008) Efficacy and tolerability of second-generation antipsychotics in children and adolescents with schizophrenia. Schizophrenia Bulletin 34:60-71.

Kunitachi S, Fujita Y, Ishima T, Kohno M, Horio M, Tanibuchi Y, Shirayama Y, Iyo M, Hashimoto K (2009) Phencyclidine-induced cognitive deficits in mice are ameliorated by subsequent subchronic administration of donepezil: Role of sigma-1 receptors. Brain Research 1279:189-196.

Lahti A, Koffel B, LaPorte D, Tamminga CA (1995) Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 13:9-19.

Langston RF, Wood ER (2009) Associative recognition and the hippocampus: Differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. Hippocampus 9999:NA.

Lapiz MDS, Fulford A, Muchimapura S, Mason R, Parker T, Marsden CA (2003) Influence of Postweaning Social Isolation in the Rat on Brain Development, Conditioned Behavior, and Neurotransmission. Neuroscience and Behavioral Physiology 33:13-29.

Laruelle M, bi-Dargham A, van DC, Gil R, D'Souza DC, Krystal J, Seibyl J, Baldwin R, Innis R (2000) Dopamine and serotonin transporters in patients with schizophrenia: an imaging study with [(123)I]beta-CIT. Biological psychiatry 47:371-379.

Lee I, Kesner RP (2003) Time-Dependent Relationship between the Dorsal Hippocampus and the Prefrontal Cortex in Spatial Memory. Journal of Neuroscience 23:1517-1523.

Leng A, Feldon J, Ferger B (2004) Long-term social isolation and medial prefrontal cortex: Dopaminergic and cholinergic neurotransmission. Pharmacology Biochemistry and Behavior 77:371-379.

Lepage M, Sergerie K, Pelletier M, Harvey P O (2007) Episodic memory bias and the symptoms of schizophrenia. Canadian Journal of Psychiatry 52:702-709.

Levine JB, Youngs RM, MacDonald ML, Chu M, Leeder AD, Berthiaume F, Konradi C (2007) Isolation rearing and hyperlocomotion are associated with reduced immediate early gene expression levels in the medial prefrontal cortex. Neuroscience 145:42-55.

Lewis DA, Moghaddam B (2006) Cognitive dysfunction in schizophrenia: convergence of gamma-aminobutyric acid and glutamate alterations. Archives of Neurology 63:1372-1376.

Li N, Wu X, Li L (2007) Chronic administration of clozapine alleviates reversal-learning impairment in isolation-reared rats. Behavioural Pharmacology 18:135-145.

Li Z, Kim CH, Ichikawa J, Meltzer HY (2003) Effect of repeated administration of phencyclidine on spatial performance in an eight-arm radial maze with delay in rats and mice. Pharmacology, biochemistry, and behavior 75:335-340.

Liddle PE (2002) Negative symptom and cognitive deficit treatment response in schizophrenia. The British Journal of Psychiatry 180:284.

Lipska BK (2004) Using animal models to test a neurodevelopmental hypothesis of schizophrenia. Journal of Psychiatry Neuroscience 29:282-286.

Lipska BK, Weinberger DR (2000) To model a psychiatric disorder in animals: schizophrenia as a reality test. Neuropsychopharmacology 23:223-239.

Lublin H, Eberhard J, Levander S (2005) Current therapy issues and unmet clinical needs in the treatment of schizophrenia: a review of the new generation antipsychotics. International clinical psychopharmacology 20:183-198.

Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R (1959) Study of a new schizophrenomimetic drug; sernyl. A. M. A. archives of neurology and psychiatry 81:363-369.

Lynch DR, Guttmann RP (2001) NMDA receptor pharmacology: perspectives from molecular biology. Current Drug Targets 2:215-231.

Lynch MR (1992) Schizophrenia and the D1 receptor: Focus on negative symptoms. Progress in Neuro-Psychopharmacology and Biological Psychiatry 16:797-832.

Ma J, Ye N, Cohen BM (2006) Expression of noradrenergic alpha1, serotoninergic 5HT2a and dopaminergic D2 receptors on neurons activated by typical and atypical antipsychotic drugs. Progress in neuro-psychopharmacology & biological psychiatry 30:647-657.

Malhotra AK, Pinals DA, Weingartner H, Sirocco K, David Missar C, Pickar D, Breier A (1996) NMDA receptor function and human cognition: The effects of ketamine in healthy volunteers. Neuropsychopharmacology 14:301-307.

Maren S, Fanselow MS (1997) Electrolytic Lesions of the Fimbria/Fornix, Dorsal Hippocampus, or Entorhinal Cortex Produce Anterograde Deficits in Contextual Fear Conditioning in Rats. Neurobiology of Learning and Memory 67:142-149.

Marsden CA (2006) Dopamine: the rewarding years. British Journal of Pharmacology 147 Suppl 1:S136-S144.

Martinot JL, Peron-Magnan P, Huret JD, Mazoyer B, Baron JC, Boulenger JP, Loc'h C, Maziere B, Caillard V, Loo H (1990) Striatal D2 dopaminergic receptors assessed with positron emission tomography and [76Br]bromospiperone in untreated schizophrenic patients. American Journal of Psychiatry 147:44-50.

McLean SL, Grayson B, Harris M, Protheroe C, Bate S, Woolley ML, Neill JC (2008) Isolation rearing impairs novel object recognition and attentional set shifting performance in female rats. Journal of Psychopharmacology 24(1):57-63.

McLean SL, Idris NF, Woolley ML, Neill JC (2009) D1-like receptor activation improves PCP-induced cognitive deficits in animal models: Implications for mechanisms of improved cognitive function in schizophrenia. European Neuropsychopharmacology 19:440-450.

Meador-Woodruff JH, Clinton SM, Beneyto M, McCullumsmith RE (2003) Molecular abnormalities of the glutamate synapse in the thalamus in schizophrenia. Annals of the New York Academy of Sciences 1003:75-93.

Melendez RI, Lee Gregory M, Bardo MT, Kalivas PW (2004) Impoverished Rearing Environment Alters Metabotropic Glutamate Receptor Expression and Function in the Prefrontal Cortex. Neuropsychopharmacology 29:1980-1987.

Meltzer HY (1994) An overview of the mechanism of action of clozapine. Clinical Psychiatry 55:47-52.

Meltzer HY, Matsubara S, Lee JC (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pKi values. The Journal of pharmacology and experimental therapeutics 251:238-246.

Meltzer HY, McGurk SR (1999) The effects of clozapine, risperidone, and olanzapine on cognitive function in schizophrenia. Schizophrenia Bulletin 25:233-256.

Mesulam MM, Mufson EJ, Wainer BH, Levey AI (1983) Central cholinergic pathways in the rat: An overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience 10:1185-1201.

Millan MJ (2000) Improving the treatment of schizophrenia: focus on serotonin (5-HT) (1A) receptors. The Journal of pharmacology and experimental therapeutics 295:853–861.

Milner B, Squire LR, Kandel ER (1998) Cognitive neuroscience and the study of memory. Neuron 20:445-468.

Mitchell RLC, Elliott R, Woodruff PW (2001) fMRI and cognitive dysfunction in schizophrenia. Trends in cognitive sciences 5:71-81.

Mitchell RW (1993) Mental models of mirror-self-recognition: Two theories. New Ideas in Psychology 11:295-325.

Mohamed S, Paulsen JS, O'Leary D, Arndt S, Andreasen N (1999) Generalized cognitive deficits in schizophrenia: a study of first-episode patients. Archives of General Psychiatry 56:749-754.

Morgan C, Curran H (2006) Acute and chronic effects of ketamine upon human memory: a review. Psychopharmacology 188:408-424.

Morgan CJA, Mofeez A, Brandner B, Bromley L, Curran HV (2003) Acute Effects of Ketamine on Memory Systems and Psychotic Symptoms in Healthy Volunteers. Neuropsychopharmacology 29:208-218.

Morris RGM, Garrud P, Rawlins JNP, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297:681-683.

Mortimer AM (2005) The neuropsychology of schizophrenia. Psychiatry 4:26-29.

Mouri A, Noda Y, Enomoto T, Nabeshima T (2007) Phencyclidine animal models of schizophrenia: Approaches from abnormality of glutamatergic neurotransmission and neurodevelopment. Neurochemistry International 51:173-184.

Mulet B, Valero J, Gutiérrez-Zotes A, Montserrat C, Cortés MJ, Jariod M, Martorell L, Vilella E, Labad A (2007) Sustained and selective attention deficits as vulnerability markers to psychosis. European Psychiatry 22:171-176.

Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H (2002) Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. Learning & memory (Cold Spring Harbor, N.Y.) 9:49-57.

Mumby DG, Tremblay A, Lecluse V, Lehmann H (2005) Hippocampal damage and anterograde object-recognition in rats after long retention intervals. Hippocampus 15:1050-1056.

Nestor PG, Kubicki M, Kuroki N, Gurrera RJ, Niznikiewicz M, Shenton ME, McCarley RW (2007) Episodic memory and neuroimaging of hippocampus and fornix in chronic schizophrenia. Psychiatry Res 155:21-28.

Neumann A, Blairy S, Lecompte D, Philippot P (2007) Specificity deficit in the recollection of emotional memories in schizophrenia. Consciousness and cognition 16:469-485.

Newcomer JW, Farber NB, Jevtovic-Todorovic V, Selke G, Melson AK, Hershey T, Craft S, Olney JW (1999) Ketamine-Induced NMDA Receptor Hypofunction as a Model of Memory Impairment and Psychosis. Neuropsychopharmacology 20:106-118.

Nieoullon A (2002) Dopamine and the regulation of cognition and attention. Progress in neurobiology 67:53-83.

Nordstrom A-L, Farde L, Eriksson L, Halldin C (1995) No elevated D2 dopamine receptors in neuroleptic-naive schizophrenic patients revealed by positron emission tomography and [11C]N-methylspiperone. Psychiatry Research: Neuroimaging 61:67-83.

Norman G, Eacott MJ (2005) Dissociable effects of lesions to the perirhinal cortex and the postrhinal cortex on memory for context and objects in rats. Behavioural Neuroscience 119:557-566.

O'Tuathaigh CP, Moran PM (2004) The effect of sulpiride on amphetamine-induced disruption of overshadowing in the rat. Progress in neuro-psychopharmacology & biological psychiatry 28:1249-1253.

Ogura H, Kosasa T, Kuriya Y, Yamanishi Y (2000) Donepezil, a centrally acting acetylcholinesterase inhibitor, alleviates learning deficits in hypocholinergic models in rats. Methods and Findings Clinical and experimental pharmacology & physiology 22:89-95.

Okubo Y, Suhara T, Suzuki K, Kobayashi K, Inoue O, Terasaki O, Someya Y, Sassa T, Sudo Y, Matsushima E, Iyo M, Tateno Y, Toru M (1997) Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. Nature 385:634-636.

Olbrich R, Schanz H (1991) An evaluation of the partial dopamine agonist terguride regarding positive symptoms reduction in schizophrenics. Journal of Neural Transmission 84:233-236.

Olney J, Labruyere J, Price MT (1989) Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. Science 244:1360-1362.

Pearce BD (2001) Schizophrenia and viral infection during neurodevelopment: a focus on mechanisms. MolPsychiatry 6:634-646.

Pereira A, Dean B (2006) Clozapine bioactivation induces dose-dependent, drug-specific toxicity of human bone marrow stromal cells: A potential in vitro system for the study of agranulocytosis. Biochemical Pharmacology 72:783-793.

Perl O, Ilani TAL, Strous RD, Lapidus R, Fuchs S (2003) The {alpha}7 nicotinic acetylcholine receptor in schizophrenia: decreased mRNA levels in peripheral blood lymphocytes. FASEB J 17:1948-1950.

Peters YM, O'Donnell P (2005) Social Isolation Rearing Affects Prefrontal Cortical Response To Ventral Tegmental Area Stimulation. Biological Psychiatry 57:1205-1208.

Phillips M, Wang C, Johnson KM (2001) Pharmacological characterization of locomotor sensitization induced by chronic phencyclidine administration. The Journal of pharmacology and experimental therapeutics 296:905-913.

Pierce RC, Kalivas PW (1997) A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants. Brain Research Reviews 25:192-216.

Pillay NS, Kellaway LA, Kotwal GJ (2008) Early detection of memory deficits and memory improvement with vaccinia virus complement control protein in an Alzheimer's disease model. Behavioural Brain Research 192:173-177.

Pin JP, Duvoisin R (1995) The metabotropic glutamate receptors: structure and functions. Neuropharmacology 34:1-26.

Piolino P, Desgranges B, Eustache F (2009) Episodic autobiographical memories over the course of time: Cognitive, neuropsychological and neuroimaging findings. Neuropsychologia 47:2314-2329.

Powchik P, Davidson M, Haroutunian V, Gabriel SM, Purohit DP, Perl DP, Harvey PD, Davis KL (1998) Postmortem Studies in Schizophrenia. Schizophrenia Bulletin24:325-341.

Prickaerts J, Şık A, van der Staay FJ, de Vente J, Blokland A (2005) Dissociable effects of acetylcholinesterase inhibitors and phosphodiesterase type 5 inhibitors on object recognition memory: acquisition versus consolidation. Psychopharmacology 177:381-390.

Raedler TJ, Bymaster FP, Tandon R, Copolov D, Dean B (2006) Towards a muscarinic hypothesis of schizophrenia. Molecular psychiatry 12:232-246.

Raedler TJ, Knable MB, Jones DW, Urbina RA, Egan MF, Weinberger DR (2003) Central Muscarinic Acetylcholine Receptor Availability in Patients Treated with Clozapine. Neuropsychopharmacology 28:1531-1537.

Rajaram S (1993) Remembering and knowing: two means of access to the personal past. Memory & cognition 21:89-102.

Rao TS, Kim HS, Lehmann J, Martin LL, Wood PL (1989) Differential effects of phencyclidine (PCP) and ketamine on mesocortical and mesostriatal dopamine release. Life Sciences 45:1065-1072.

Risch SC, Horner MD, McGurk SR, Palecko S, Markowitz JS, Nahas Z, DeVane CL (2007) Double-blind donepezil-placebo crossover augmentation study of atypical antipsychotics in chronic, stable schizophrenia: A pilot study. Schizophrenia Research 93:131-135.

Riutort M, Cuervo C, Danion JM, Peretti CS, Salame P (2003) Reduced levels of specific autobiographical memories in schizophrenia. Psychiatry Research 117:35-45.

Roberts WA (2002) Are animals stuck in time? Psychology Bulletin 128:473-489.

Rodefer JS, Murphy ER, Baxter MG (2005) PDE10A inhibition reverses subchronic PCP-induced deficits in attentional set-shifting in rats. European Journal of Neuroscience 21:1070-1076.

Rosse RB, Collins JP, Jr., Fay-McCarthy M, Alim TN, Wyatt RJ, Deutsch SI (1994) Phenomenologic comparison of the idiopathic psychosis of schizophrenia and drug-induced cocaine and phencyclidine psychoses: a retrospective study. Clinical Neuropharmacology 17:359-369.

Rung JP, Carlsson A, Rydén Markinhuhta K, Carlsson ML (2005) (+)-MK-801 induced social withdrawal in rats; a model for negative symptoms of schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry 29:827-832.

Rushe TM, Woodruff PWR, Murray RM, Morris RG (1999) Episodic memory and learning in patients with chronic schizophrenia. Schizophrenia Research 35:85-96.

Sambeth A, Riedel WJ, Smits LT, Blokland A (2007) Cholinergic drugs affect novel object recognition in rats: Relation with hippocampal EEG? European Journal of Pharmacology 572:151-159.

Sarter M (2006) Preclinical research into cognition enhancers. Trends in Pharmacological Sciences 27:602-608.

Schmitz Y, Lee CJ, Schmauss C, Gonon F, Sulzer D (2001) Amphetamine distorts stimulation-dependent dopamine overflow: effects on D2 autoreceptors, transporters, and synaptic vesicle stores. Journal of Neuroscience 21:5916-5924.

Schwabe K, Brosda J, Wegener N, Koch M (2005) Clozapine enhances disruption of prepulse inhibition after sub-chronic dizocilpine- or phencyclidine-treatment in Wistar rats. Pharmacology Biochemistry and Behavior 80:213-219.

Seeman P, Bzowej NH, Guan HC, Bergeron C, Reynolds GP, Bird ED, Riederer P, Jellinger K, Tourtellotte WW (1987) Human brain D1 and D2 dopamine receptors in schizophrenia, Alzheimer's, Parkinson's, and Huntington's diseases. Neuropsychopharmacology 1:5-15.

Seeman P, Ko F, Tallerico T (2005) Dopamine receptor contribution to the action of PCP, LSD and ketamine psychotomimetics. Molecular psychiatry 10:877-883.

Seillier A, Giuffrida A (2009) Evaluation of NMDA receptor models of schizophrenia: Divergences in the behavioral effects of sub-chronic PCP and MK-801. Behavioural Brain Research 204:410-415.

Seltzer B (2005) Donepezil: a review. Expert Opinion on Drug Metabolism & Toxicology 1:527-536.

Semba K (2000) Multiple output pathways of the basal forebrain: organization, chemical heterogeneity, and roles in vigilance. Behavioural Brain Research 115:117-141.

Sham PC, MacLean CJ, Kendler KS (1994) A typological model of schizophrenia based on age at onset, sex and familial morbidity. Acta psychiatrica Scandinavica 89:135-141.

Shenton ME, Dickey CC, Frumin M, McCarley RW (2001) A review of MRI findings in schizophrenia. Schizophrenia Research 49:1-52.

Shirayama Y, Yamamoto A, Nishimura T, Katayama S, Kawahara R (2007) Subsequent exposure to the choline uptake enhancer MKC-231 antagonizes phencyclidine-induced behavioral deficits

and reduction in septal cholinergic neurons in rats. European Neuropsychopharmacology. 17:616-626.

Shore D, Matthews S, Cott J, Lieberman JA (1995) Clinical implications of clozapine discontinuation: Report of an NIMH workshop. Schizophrenia Bulletin 21:333-338.

Smith DM, Mizumori SJ (2006) Hippocampal place cells, context, and episodic memory. Hippocampus 16:716-729.

Smith DM, Ekrem Dere AELN, Joseph PH (2008) Chapter 4.4 The hippocampus, context processing and episodic memory. In: Handbook of Behavioral Neuroscience, vol. Volume 18, pp 465-481, 629-630: Elsevier.

Sonkusare S, Srinivasan K, Kaul C, Ramarao P (2005) Effect of donepezil and lercanidipine on memory impairment induced by intracerebroventricular streptozotocin in rats. Life Sciences 77:1-14.

Sonntag P, Gokalsing E, Olivier C, Robert P, Burglen F, Kauffmann-Muller F, Huron C, Salame P, Danion JM (2003) Impaired strategic regulation of contents of conscious awareness in schizophrenia. Consciousness and cognition 12:190-200.

Squire LR (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychological review 99:195-231.

Squire LR, Wixted JT, Clark RE (2007) Recognition memory and the medial temporal lobe: a new perspective. Nature reviews. Neuroscience 8:872-883.

Stanilla JK, De Leon J, Simpson GM (1997) Clozapine withdrawal resulting in delirium with psychosis: A report of three cases. Journal of Clinical Psychiatry 58:252-255.

Steinpreis RE, Sokolowski JD, Papanikolaou A, Salamone JD (1994) The effects of haloperidol and clozapine on PCP- and amphetamine-induced suppression of social behavior in the rat. Pharmacology Biochemistry and Behavior 47:579-585.

Stevens ER, Esguerra M, Kim PM, Newman EA, Snyder SH, Zahs KR, Miller RF (2003) D-serine and serine racemase are present in the vertebrate retina and contribute to the physiological activation of NMDA receptors. Proceedings of the National Academy of Sciences of the United States of America 100:6789-6794.

Suddendorf T, Corballis MC (1997) Mental time travel and the evolution of the human mind. vol. 123, pp 133-167.

Talbot PS, Laruelle M (2002) The role of in vivo molecular imaging with PET and SPECT in the elucidation of psychiatric drug action and new drug development. European neuropsychopharmacology 12:503-511.

Tandon R, Jibson MD (2002) Extrapyramidal Side Effects of Antipsychotic Treatment: Scope of Problem and Impact on Outcome. Annals of Clinical Psychiatry 14:123-129.

Tangui M, Jacques V, Jean-Marc K, Robert C (1991) Differential Interaction of Phencyclidine-Like Drugs with the Dopamine Uptake Complex In Vivo. Journal of Neurochemistry 56:553-559.

Thompson PM, Vidal C, Giedd JN, Gochman P, Blumenthal J, Nicolson R, W. TA, Rapoport JL (2001) Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. Proceedings of the National Academy of Sciences of the United States of America 98:11650-11655.

Tolman EC (1948) Cognitive maps in rats and men. Psychological Review 55:189-208.

Toulopoulou T, Rabe-Hesketh S, King H, Murray RM, Morris RG (2003) Episodic memory in schizophrenic patients and their relatives. Schizophrenia Research 63:261-271.

Tsuno N (2009) Donepezil in the treatment of patients with Alzheimer's disease. Expert Review of Neurotherapeutics 9:591-598.

Tuğal O, Yazici KM, Anil Yağcioğlu AE, Göğüş A (2004) A double-blind, placebo controlled, cross-over trial of adjunctive donepezil for cognitive impairment in schizophrenia. The International Journal of Neuropsychopharmacology 7:117-123.

Tulving E (1972) Episodic and semantic memory. In E. Tulving and W. Donaldson (Eds.). Organization of Memory pp. 381-403.

Tulving E (1983) Elements of Episodic Memory. Oxford Clarendon Press.

Tulving E (1985) Memory and consciousness. Canadian Psychology/Psychologie canadienne 26:1-12.

Tulving E (1986) What kind of a hypothesis is the distinction between episodic and semantic memory? Journal of Experimental Psychology: Learning, Memory, and Cognition 12:307-311.

Tulving E (2002) Episodic memory: From Mind to Brain. Annual Review of Psychology 53:1-25.

Tulving E (2005) Episodic memory and autonoesi: Uniquely human? In: H.S. Terrace and J. Metcalfe, Editors, The missing link in cognition: Evolution of self-knowing consciousness, . Oxford University Press.

Tulving E, Markowitsch HJ (1998) Episodic and declarative memory: Role of the hippocampus. Hippocampus 8:198-204.

Turgeon SM, Hulick VC (2007) Differential effects of acute and subchronic clozapine and haloperidol on phencyclidine-induced decreases in voluntary sucrose consumption in rats. Pharmacology, biochemistry, and behavior 86:524-530.

Van der Staak C (1977) Habituation of open field activity during cortical spreading depression in rats. Physiology & Behavior 18:369-374.

Van der Zee EA, Luiten PGM (1999) Muscarinic acetylcholine receptors in the hippocampus, neocortex and amygdala: a review of immunocytochemical localization in relation to learning and memory. Progress in Neurobiology 58:409-471.

Vargha-Khadem F, Gadian DG, Watkins KE, Connelly A, Van Paesschen W, Mishkin M (1997) Differential Effects of Early Hippocampal Pathology on Episodic and Semantic Memory. Science 277:376-380.

Velakoulis D, Wood SJ, McGorry PD, Pantelis C (2000) Evidence for progression of brain structural abnormalities in schizophrenia: beyond the neurodevelopmental model. The Australian and New Zealand journal of psychiatry 34 Suppl:S113-26.

Verma A, Moghaddam B (1996) NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: modulation by dopamine. Journal of Neuroscience 16:373-379.

Vignon J, Pinet V, Cerruti C, Kamenka JM, Chicheportiche R (1988) [3H]N-[1-(2-benzo(b)thiophenyl)cyclohexyl]piperidine ([3H]BTCP): A new phencyclidine analog selective for the dopamine uptake complex. European Journal of Pharmacology 148:427-436.

Wais PE, Wixted JT, Hopkins RO, Squire LR (2006) The Hippocampus Supports both the Recollection and the Familiarity Components of Recognition Memory. Neuron 49:459-466.

Ward KE, Friedman L, Wise A, Schulz SC (1996) Meta-analysis of brain and cranial size in schizophrenia. Schizophrenia Research 22:197-213.

Waters FAV, Maybery MT, Badcock JC, Michie PT (2004) Context memory and binding in schizophrenia. Schizophrenia Research 68:119-125.

Weinberger DR (1988) Schizophrenia and the frontal lobe. Trends in Neurosciences 11:367-370.

Weinberger DR, Aloia MS, Goldberg TE, Berman KF (1994) The frontal lobes and schizophrenia. The Journal of neuropsychiatry and clinical neurosciences 6:419-427.

Weinberger DR, Bigelow LB, Kleinman JE, Klein ST, Rosenblatt JE, Wyatt RJ (1980) Cerebral ventricular enlargement in chronic schizophrenia. An association with poor response to treatment. Archives of General Psychiatry 37:11-13.

Weinberger DR, Torrey EF, Neophytides AN, Wyatt RJ (1979) Lateral cerebral ventricular enlargement in chronic schizophrenia. Archives of General Psychiatry 36:735-739.

Weissman MM, Pilowsky DJ, Wickramaratne PJ, Talati A, Wisniewski SR, Fava M, Hughes CW, Garber J, Malloy E, King CA, Cerda G, Sood AB, Alpert JE, Trivedi MH, Rush AJ (2006) Remissions in maternal depression and child psychopathology: a STAR*D-child report. The Journal of the American Medical Association 295:1389-1398.

Wheeler MA, Stuss DT, Tulving E (1997) Toward a theory of episodic memory: the frontal lobes and autonoetic consciousness. Psychological bulletin 121:331-354.

Wiley JL (2008) Antipsychotic-induced suppression of locomotion in juvenile, adolescent and adult rats. European Journal of Pharmacology 578:216-221.

Willner P (1991) Behavioural models in psychopharmacology. In: Willner P (ed) Behavioural models in psychopharmacology: theoretical, industrial and clinical perspectives Cambridge University Press: Cambridge 3-18.

Wilson B, Cockburn J, Baddeley A (1985) The Rivermead Behavioural Memory Test. Bury St Edmunds: Thames Valley Test Co.

Wong AH, Van Tol HH (2003) Schizophrenia: from phenomenology to neurobiology. Neuroscience and biobehavioral reviews 27:269-306.

Woolf NJ (1991) Cholinergic systems in mammalian brain and spinal cord. Progress in Neurobiology 37:475-524.

Woolf NJ (1996) The Critical Role of Cholinergic Basal Forebrain Neurons in Morphological Change and Memory Encoding: A Hypothesis. Neurobiology of Learning and Memory 66:258-266.

Xu X, Domino EF (1994) Phencyclidine-induced behavioral sensitization. Pharmacology Biochemistry and Behavior 47:603-608.

Xu X, Domino EF (1999) A Further Study on Asymmetric Cross-Sensitization Between MK-801 and Phencyclidine-Induced Ambulatory Activity. Pharmacology Biochemistry and Behavior 63:413-416.

Young JW, Powell SB, Risbrough V, Marston HM, Geyer MA (2009) Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. Pharmacology & Therapeutics 122:150-202.

Young SL, Bohenek DL, Fanselow MS (1994) NMDA processes mediate anterograde amnesia of contextual fear conditioning induced by hippocampal damage: Immunization against amnesia by context preexposure. Behavioral Neuroscience 108:19-29.

Yun SH, Cheong MY, Mook-Jung I, Huh K, Lee CJ, Jung MW (2000) Cholinergic modulation of synaptic transmission and plasticity in entorhinal cortex and hippocampus of the rat. Neuroscience 97:671-676.

Zajaczkowski W, Czyrak A, Wedzony K (2003) A competitive antagonist of NMDA receptors CGP 40116 attenuates experimental symptoms of schizophrenia evoked by MK-801. Polish Journal of Pharmacology 55:703-711.

Zentall TR (2006) Mental time travel in animals: a challenging question. Behavioural processes 72:173-183.

# - APPENDIX –

## 1.1 Material, method and results of the behavioural task that isolated rats performed before the OPC task and OP task *(From Caitlin Jones: 1st Year Treansfert Report, July 2008).*

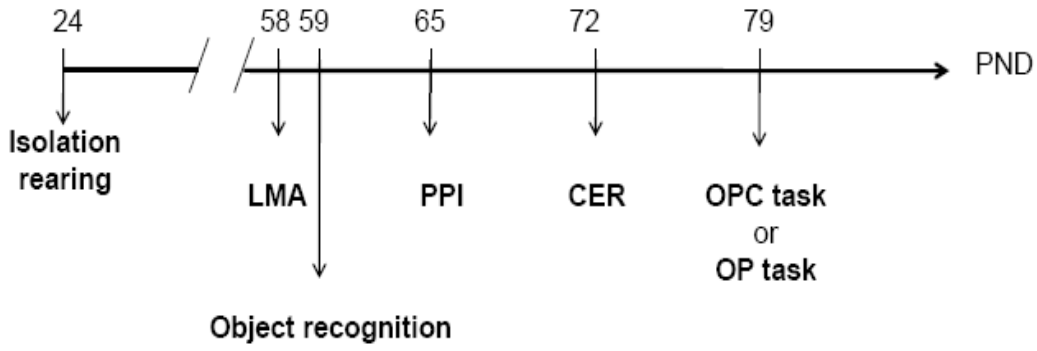



**Figure 4.1: Summary of experimental procedures underwent by the isolated rats before the OPC or the OP task.**

### *1.1.1 Appendix 1: Locomotion and Rearing Activity in a Novel Environment on PND 58*

Locomotion (LMA) and rearing activity was measured using 12 automated infra-red activity monitors consisting of plexiglas cages (39cm x 23.5cm x 24.5cm) with wire mesh lids and exchangeable floors, fitted with 2 layers of 5 parallel horizontal photobeams (designed

by Dept. Medical Physics, University of Nottingham, Nottingham, UK). The consecutive breaking of 2 adjacent photobeams in the lowest set of beams, situated 9 cm from the bottom of the cage produce counts of ambulatory locomotor activity. The breaking of any individual beam located in the upper set (20 cm from the bottom of the cage) produced counts indicative of rearing activity. Cumulative beam break counts were automatically recorded by computer software (Activity Monitor Alias, Dept. Medical Physics, University of Nottingham,
UK) in 5 min time epochs for a period of 60 min and store for subsequent off-line analysis.

#### 1.1.1.1 Data Analysis

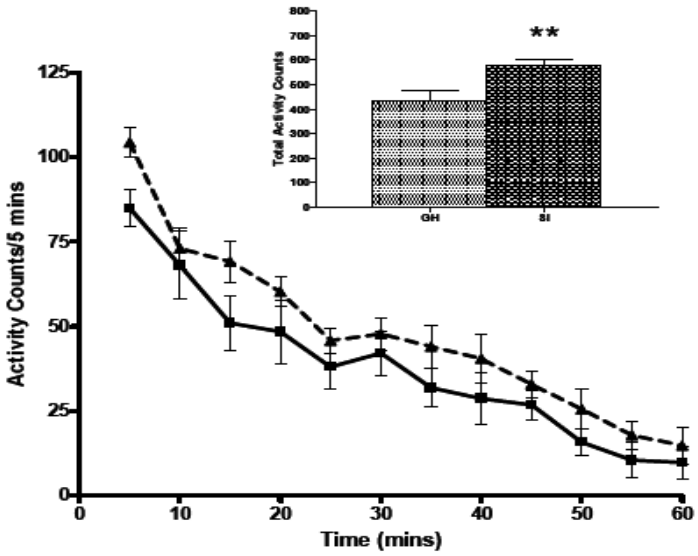
LMA and rearing activity were calculated as mean ± SEM activity per 5 min for each group. Mean ± s.e.m. total LMA and rears over 60 min were also calculated.

A repeated measures analysis of variance (RM ANOVA) was used to analyse LMA data. Housing condition was set as the “between” variable in all studies, whilst time, was identified as “within group” variables. Post – hoc analyses were performed by Bonferroni’s where appropriate. Significance levels were set at $p \leq 0.05$ and all analyses were performed using GraphPad Prism® v.4.0.3 (GraphPad Software Inc, San Diego, USA). Any raw data values which were ± 2 X standard

deviations (SD) away from the mean were deemed outliers and not included in subsequent data analyses.

#### 1.1.1.2 Result

Housing animals in social isolation increased novelty-induced LMA (Appendix 1.1). A repeated measures analysis of variance (RM ANOVA) revealed a significant difference between the LMA of GH and SI animals ($F_{(11,198)}$= 4.70, p≤0.04) with a post-hoc Bonferroni test indicating hyperactivity in SI compared to GH controls. LMA decreased over time in both groups of animals but this was significantly faster in GH controls (main effect ANOVA $F_{(11,198)}$ = 40.85, p≤0.0001). There was no interaction between Housing Condition X Time. Furthermore, an unpaired Student's t-test identified a significant greater total LMA in SI animals (p≤0.007; Figure 1; inset).




**Appendix 1.1: Rearing Lister Hooded rats in social isolation from weaning increases their spontaneous locomotor activity in a novel area. Group housed (squares and GH in the inset) or socially isolated (triangles and SI in the inset) animals (n =10 per group) are placed in automated photobeam activity cages for 60 min. LMA is represented as activity counts (beam breaks) per 5 min (main) or the total LMA over 60 min (inset). Data are presented as mean activity ± s.e.m. for each rearing condition. ** = p≤0.01 by unpaired Student's t-test vs. GH controls.**

### *1.1.2 Appendix 2: Recognition of a Novel Object in a Familiar Environment on PND 59*

The novel object recognition task was adapted from studies initially described by and based on those previously described by Marsden and colleagues (Bianchi et al., 2006; Lapiz et al., 2003). 24 h following the measurement of locomotion and rearing activity, animals were re-habituated to the same infra-red activity monitor cages for a period of 3

min. Animals were then returned to their homecages for 1 min whilst 2 identical objects (upturned 8 cm x 5 cm water-filled, plastic bottles covered with white masking tape) were placed securely in each activity cage. During this familiarisation trial (T1), animals were allowed to explore both objects for a total period of 3 min and the time (sec) spent exploring each object is recorded manually. Following an inter-trial interval (ITI) of 2 hours (based on previously published findings by (King et al., 2004) which was spent in their homecages, animals were reintroduced to the activity cage whereupon one of the familiar objects was replaced with a novel object (an upturned 8 cm x 5 cm waterfilled plastic bottle covered in white masking tape, with 3 additional 1.2 cm black stripes). During this choice trial (T2), exploration of either object was recorded as previously described for T1. Objects were located in the front left and back right of the cage, 5 cm from the side and 10 cm from the back of the cage. The location of the novel object varied in a pseudorandom order within groups and both objects and cages were cleaned with 20% w/v ethanol (EtOH) between trials to remove any olfactory cues. Exploratory behaviour was defined as sniffing, touching and direct attention to the object - indicated by moving vibrissae whilst the nose was within 1 cm of the object. Climbing on or chewing the object were not considered as exploratory behaviours and therefore not counted.

### 1.1.2.1 Data Analysis

Data were calculated according to methods established by Ennaceur and Delacour (Ennaceur and Delacour, 1988) and previously described by (Bianchi et al., 2006). In order to discern mean total exploratory behaviour (± s.e.m.), the total time spent exploring both objects in the familiarisation (Object 1 (s) + Object 2 (s)) and choice
(Novel (s) + Familiar (s)) trials were calculated for each animal. Data were presented as the mean exploration time (s) for each object ± s.e.m. for each group. In order to established the ability of either group to discriminate between the familiar and novel object in the choice trial, two discrimination ratios were calculated according to the following equations;

$$\mathbf{D1 = Novel\ (sec) - Familiar\ (sec)}$$

$$\mathbf{D2 = \frac{Novel\ (sec) - Familiar\ (sec)}{Novel\ (sec) + Familiar\ (sec)}}$$

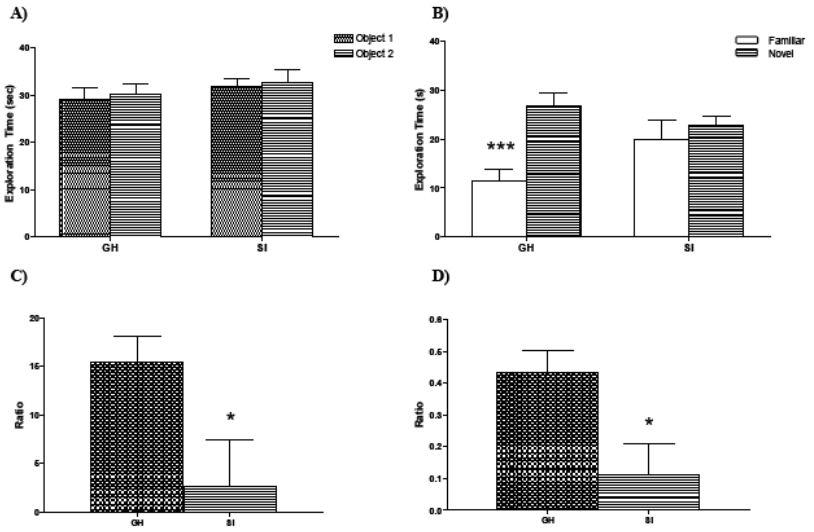
Data are presented as mean D1 and D2 ± s.e.m. for each treatment group.

Statistical differences between groups both in the familiarisation and choice trials in the NOR task were identified using paired Student's t-tests, whilst D1 and D2 ratios were analysed one-way ANOVA and post hoc Bonferroni tests. Significance levels were set at $p \leq 0.05$ and all analyses were performed using GraphPad Prism® v.4.0.3 (GraphPad Software Inc, San Diego, USA). Any raw data values which were ± 2 X

standard deviations (SD) away from the mean were deemed outliers and not included in subsequent data analyses.

#### 1.1.2.2 Result

Social isolation induced recognition memory deficits in rats. During the familiarisation trial of the NOR task, both GH and SI rats spent an equal amount of time exploring both identical objects (Appendix 1.2; A) with no significant place preference for either object. During the choice trial (Appendix 1.2; B) GH animals spent a significantly longer ($p \leq 0.0002$ by Student's paired t-test) time exploring the novel rather than the familiar object whist SI animals spent equal time exploring both objects. Failure to recognise the novel object was confirmed by statistical analyses of the D1 and D2 discrimination ratios (Appendix 1.2; C and D). In both cases, the indices of SI animals were significantly lower than those of the GH animals ($p \leq 0.02$ in all cases). Total object-directed exploration time significantly decreased between the familiarisation and choice trials in both groups of animals ($p \leq 0.01$ and $p \leq 0.001$ for GH and SI animals respectively by paired Student's t-test).




**Appendix 1.2: Animals reared in social isolation demonstrated NOR deficits. The time spent exploring two identical objects in the familiarisation trial (A) and a novel and familiar object in the choice trial (B) are presented as mean sec ± s.e.m. (n=10 per group). Each trial is separated by an ITI of 2 hours. The ability to discriminate between the novel and familiar object is calculated as discrimination ratios D1 and D2 (mean ± s.e.m.; C and D). * = p≤0.05, *** = p≤0.0001 by paired Student's t-test from GH control.**

### *1.1.3 Appendix 1.3: Prepulse Inhibition of the Acoustic Startle Response on PND 65*

All studies were carried out based on those previously reported by Geyer and colleagues (Geyer et al., 1993; Varty et al., 2000). Studies were conducted using four SR-Lab (San Diego Instruments, San Diego, USA) startle response systems. Each system consisted of a clear Plexiglas non-restrictive cylinder (8.8cm x 19.5cm) mounted on a solid perspex base situated inside a well lit (15W) and ventilated sound attenuating chamber (39cm x 38cm x 58cm). Background noise (65dB)

and acoustic stimuli – 120dB startle response and 76dB, 80dB and 84dB prepulse stimuli produced by a noise generator controlled by SR-Lab and administered through a microphone located 24cm above the restraint chamber. Individual whole body startle responses were transduced by a piezoelectric accelerometer attached to the base of the system and recorded as 100 x 1-msec readings by Startle Reflex Testing software (San Diego Instruments, San Diego, USA) starting from the initiation of the startle pulse. Each chamber was calibrated prior to experimental use to ensure consistent readings. animals were placed in the startle chambers receiving 65dB background noise for an acclimatisation period of 5 min. Animals were then exposed to a series of ten 120dB pulse alone (120PULSE) trials in order to familiarise each animal with the 120dB tone. Once completed, animals underwent 46 pulse alone or prepulse + pulse trials consisting of a 120dB, 20 msec startle tone or a 40 msec 76dB (76PP120), 80dB (80PP120) or 84dB (84PP120) tone followed by a startle tone. The interval between the prepulse and pulse tones was set as 100 msec. All trials were administered in a pseudorandom, unpredicitive order with a variable inter-trial interval. Each animal received 15 x 120dB trials, and 7 trials of each prepulse intensity in total.

### 1.1.3.1 Data Analysis

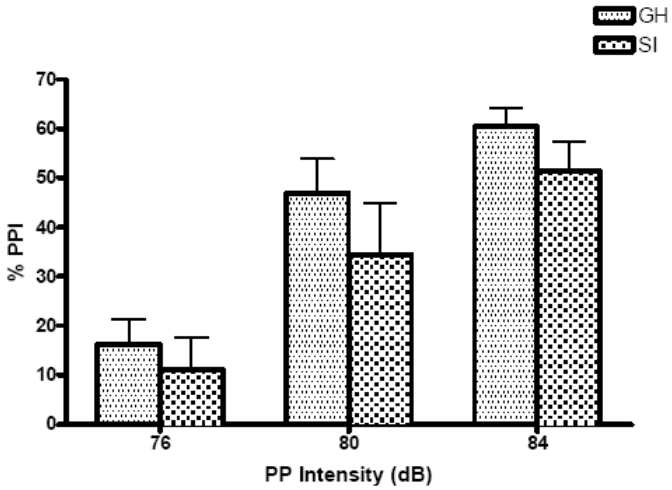
Initial and final startle responses for each group were calculated as a mean ± s.e.m. of the five 120dB tone trials preceding and following the series of 46 prepulse + pulse and pulse alone trials respectively. Prepulse inhibition for each prepulse intensity (xxPP) was calculated using a Microsoft Excel spreadsheet according to the following equation and expressed as a percentage.

$$\%PPI = \frac{(\text{mean 120PULSE}) - (\text{mean xxPP120})}{(\text{mean 120PULSE})}$$

A repeated measures analysis of variance (RM ANOVA) was used to analyse PPI data. Housing condition was set as the “between” variable whilst prepulse intensity was identified as “within group” variables. Post – hoc analyses were performed by Bonferroni’s test where appropriate. Significance levels were set at $p \leq 0.05$ and all analyses were performed using GraphPad Prism® v.4.0.3 (GraphPad Software Inc, San Diego, USA). Any raw data values which were ± 2 X standard deviations (SD) away from the mean were deemed outliers and not included in subsequent data analyses.

### 1.1.3.2 Result

Post-weaning social isolation had no effect on prepulse inhibition (Béracochéa et al.) of acoustic startle responses (Appendix 1.3). A repeated measure ANOVA identified a significant effect of prepulse intensity on %PPI ($F_{(2,36)}$= 30.79, p≤0.0001) however no significant differences between rearing conditions were seen. ANOVA showed that %PPI increased with increasing prepulse intensity for all treatment groups. No significant Housing Condition X Prepulse Intensity interaction was present. Social isolation had no effect on the magnitude of the initial startle response when compared to group housed littermates (mean startle amplitude GH = 348±5; SI = 389±5).




**Appendix 1.3: Raising animals in post-weaning social isolation did not disrupt prepulse inhibition (Béracochéa et al.) of the acoustic startle response. Startle responses were induced by a 120dB pulse, inhibition of the startle response was induced by 76dB, 80db and 84dB prepulse tones. Values represent mean %PPI ± s.e.m. (n=10 per group). A significant effect of increasing prepulse intensity was identified (p≤0.0001 by repeated measures ANOVA).**

### *1.1.4 Appendix 4: Conditioned Emotional Response Paradigm on PND 72*

All studies were based on those previously described by Marsden and colleagues (Fulford and Marsden, 1997; Saulskaya and Marsden, 1995). using a two-way rat shuttle box consisting of a light side and a dark side (25 x 25 x 27 cm) separated by an automated trap door (8 x 8 cm) linked to a shuttle box control and automated shocker (Panlab SLab, Barcelona, Spain). Animals were placed in the light side of the

box for 30 sec, where upon a door into the dark side was automatically opened. The latency time for all four paws of the animal to transfer to the dark side was automatically recorded using a floor sensor by the computer software (ShutAvoid software v.1.8.2., Panlab S.L, USA) and caused closure of the inter-chamber door. Animals were allowed to explore the dark side for 30 sec before a conditioned stimulus (Csernansky et al.) consisting of a concurrent light and tone (3kHz, 89dB) were administered continuously for 5 sec. The unconditioned stimulus (US) consisted of a mild inescapable 0.4mA footshock administered through a grid floor and was given in the final 1 sec of the CS. Each animal underwent a total of three CS/US trials separated by a fixed ITI of 55 sec. Upon completion of the conditioning trial, animals returned immediately to their home cages and the shuttle box was cleaned thoroughly with 20% EtOH between animals. 24 h and 48 h post-conditioning animals returned to the dark side of the shuttle box however no light/tone or mild footshocks were applied. The time spent in a frozen position was recorded manually for a maximum of 300 sec in both the retention (24h post conditioning) and extinction trials (48 h post conditioning). Freezing behaviour was defined as the complete absence of movement except that essential for respiration (including moving vibrissae) and adoption of a hunched posture. During the extinction trial, the CS was re-administered to the animals after initial freezing time has been measured for 300 sec, for a period of 5 sec and (re)freezing behaviour was monitored as described previously.

### 1.1.4.1 Data Analysis

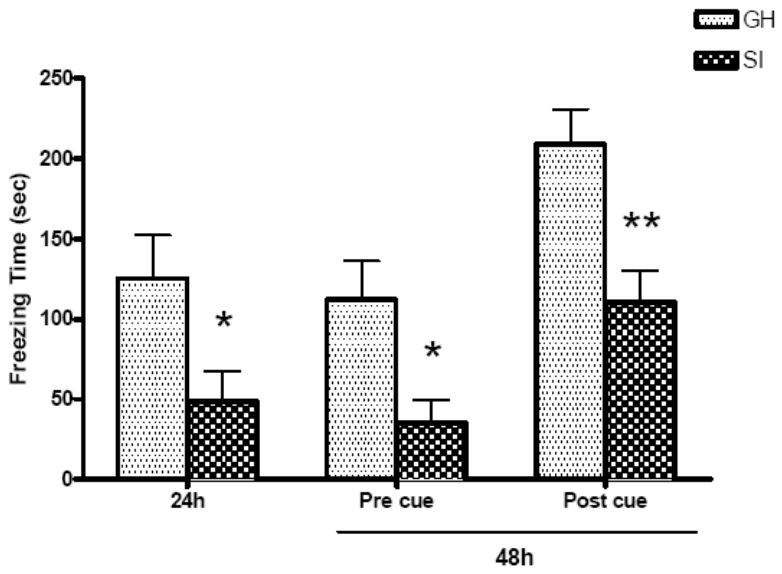
Data are presented as the mean total freezing time (sec) ± s.e.m. for each trial/condition in both experiments.

A repeated measures analysis of variance (RM ANOVA) was used to analyse CER data. Housing condition was set as the “between” variable, whilst trial type was identified as “within group” variables. Post – hoc analyses were performed by Bonferroni’s test where appropriate. Significance levels were set at $p \leq 0.05$ and all analyses were performed using GraphPad Prism® v.4.0.3 (GraphPad Software Inc, San Diego, USA). Any raw data values which were ± 2 X standard deviations (SD) away from the mean were deemed outliers and not included in subsequent data analyses.

### 1.1.4.2 Result

The freezing behaviour of SI animals was significantly reduced compared to that of GH control animals (Appendix 1.4). Following a significant RM ANOVA ($F_{(1,36)} = 10.24$, $p \leq 0.005$), a post-hoc Bonferroni test highlighted reduced freezing behaviour in SI animals following all trial types compared with GH controls. A significant effect of trial type was also highlighted ($F_{(2,36)} = 26.25$, $p \leq 0.0001$), indicating that animals froze for a longer during the CS alone trial (48h post-cue) when compared to the retention (24h) and extinction trials (48h pre-cue). The

Trial Type X Housing interaction did not reach statistical significance ($F_{(2,36)}$ = 0.48, p=0.62).



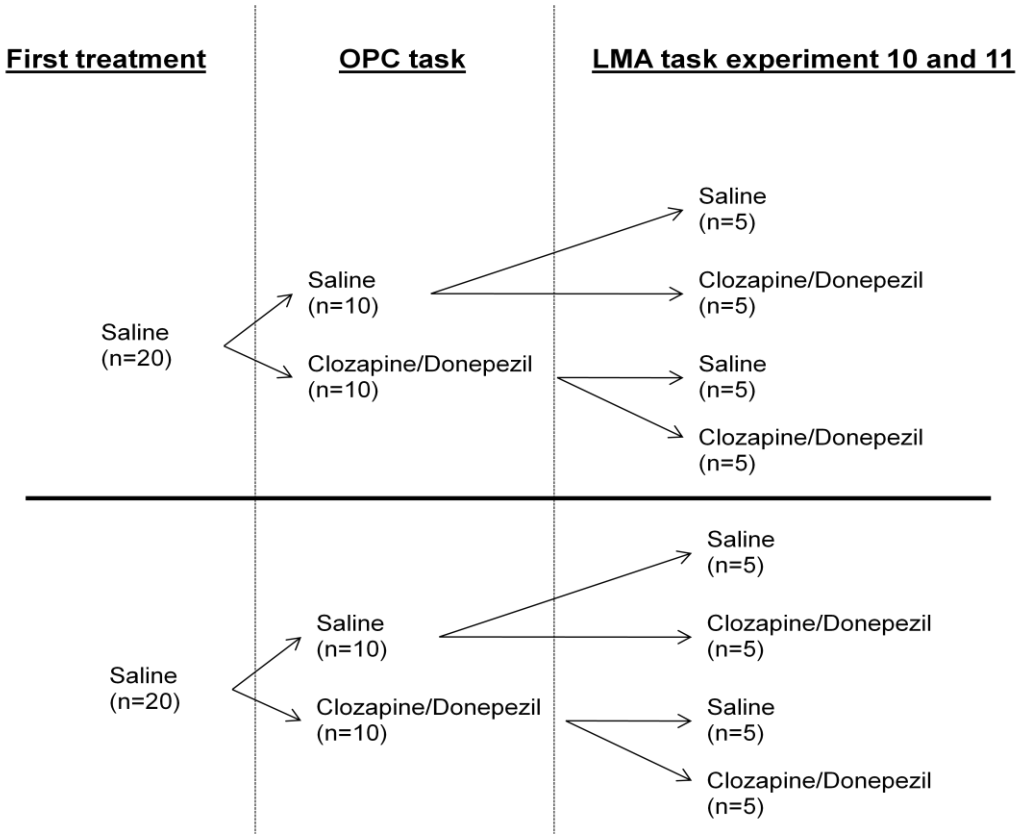

**Appendix 1.4. Socially isolated rats display impaired conditioned emotional responses following non-aversive inescapable foot shocks. GH or SI rats (*n*=10 per group) were administered 3 light/tone cues paired with a 0.4mA foot shocks. Freezing behaviour was scored 24h and 48h later in the same context, in the absence and presence (48h post cue) of light/tone cues. Data presented as mean freezing (sec) ± s.e.m., * = p≤0.05 *vs.* GH Veh, ** = p≤0.01 *vs.* GH Veh calculated by repeated measures ANOVA and post-hoc Bonferroni test.**

**2.1 Table (appendix 2.1) showing the time spent exploring each objects during the 3 minutes test phase in experiment 6 (chapter 5): "The effect of antipsychotic clozapine on PCP withdrawal-induced episodic memory deficit in rats."**

| Delay | Treatment | Total exploration | Novel OPC | Familiar OPC | p value |
|---|---|---|---|---|---|
| 5 minute | saline-saline | *16.1655* | 9.594 | 6.5715 | *****<0.05 |
| | saline-clozapine | *6.97175* | 4.1705 | 2.80125 | *****<0.05 |
| | PCP-saline | *11.66972* | 5.898333 | 5.771389 | >0.05 |
| | PCP-clozapine | *5.00475* | 2.335 | 2.66975 | >0.05 |
| 10 minute | saline-saline | *10.58825* | 5.8645 | 4.72375 | >0.05 |
| | saline-clozapine | *6.80725* | 3.4195 | 3.38775 | >0.05 |
| | PCP-saline | *11.97972* | 5.637778 | 6.341944 | >0.05 |
| | PCP-clozapine | *5.9485* | 2.90675 | 3.04175 | >0.05 |

***P<0.05** significant difference in time exploring the novel OPC compared with the familiar OPC.

## 3.1 Drug treatments by rats received from the subchronic PCP treatment, the OPC task and LMA task after clozapine and donepezil




**Appendix 3.1: Diagram of treatment by rats adminstrated from the subchronic treatment, followed by the OPC task after clozapine (5 mg/kg) and donepezil (0.3 mg/kg), and LMA after clozapine (5 mg/kg) and donepezil (0.3 mg/kg) injection.**