EVALUATION OF NOVEL DUAL-HIT MODELS OF 'SCHIZOPHRENIA-LIKE' SYMPTOMS IN THE RAT

PHILIP LAURENCE ROY GASKIN, MPharmacol.

Thesis submitted to the University of Nottingham

for the degree of Doctor of Philosophy

JULY 2014

Abstract

Schizophrenia is a debilitating disorder comprising positive, negative and cognitive deficits with a poorly-defined neurobiological basis. Animal models with greater translational reliability and validity are essential to develop improved therapies and aid understanding of disease aetiology. This thesis utilised the well-established isolation rearing developmental disruption model of schizophrenia in the rat as the base for producing novel 'dual-hit' combination models of the disease, with the aim of improving disease validity and model robustness. Pharmacological insults were added to the isolation rearing model, first in the form of prenatal administration of the antimitotic agent methylazoxymethanol (MAM), and subsequently perinatal treatment with the N-methyl-D-aspartate receptor antagonist phencyclidine (PCP). The resulting 'dual-hit' models were assessed for behavioural and neurobiological validity to schizophrenia, and the incurred deficits challenged with the atypical antipsychotic risperidone and the putative adjunct therapy lamotrigine.

Combination of isolation rearing and prenatal MAM on gestational day 17 did not produce more robust behavioural deficits than isolation rearing alone, but did cause marked reductions in hippocampal volume, akin to those observed in the clinic. Addition of perinatal PCP treatment on post-natal days seven, nine and eleven to the isolation rearing protocol produced more robust behavioural deficits, with limitations. Baseline hyperlocomotion in a novel arena in three cohorts was accompanied by an elevated locomotor response to acute PCP treatment, highlighting sensitization. Visual and spatial learning deficits were

observed in the novel object discrimination task, whilst fear-motivated conditioning was impaired in a conditioned emotional response paradigm. Preattentional processing was also somewhat deficient in combination-treated animals in the prepulse inhibition of acoustic startle paradigm. Inconsistent deficits in visuo-spatial learning and cognitive flexibility were observed in a Morris water maze task.

Acute treatment with the atypical antipsychotic compound risperidone at 0.5mg/kg caused marked sedation. At lower doses, pretreatment 30 mins prior to behavioural testing elevated prepulse inhibition and reversed emotional conditioning deficits, and returned baseline locomotor activity to levels similar to control. There was no effect on visual reference memory deficits. Conversely, pretreatment with the sodium-channel blocker lamotrigine reversed a deficit in visual reference memory, but had no effect on sensorimotor gating or fear-motivated conditioning.

These data suggest that the combination of isolation rearing and perinatal PCP treatment to rats produces a model of schizophrenia-like symptoms that possesses some validity to the human condition, but lacks the desired robustness of a preclinical model. Further validation and improvement may allow this model to become a useful tool in on-going preclinical research.

Acknowledgements

Firstly, I would like to thank my supervisors Prof Kevin Fone and Dr Steve Alexander for their constant support and advice throughout my study at the University of Nottingham. Alongside my colleagues and friends Allison McIntosh, Sinead Shortall, Dave Watson, Caitlin Jones, Maddy King, Amy Warner, Stacey Knapp and Ian Topham, the community I have been part of in the School of Biomedical Sciences has been an on-going source of encouragement and assistance that I couldn't have done without. I would like to particularly acknowledge the contribution that Ian Topham and a plethora of undergraduate students have made to this thesis through assistance with *in vivo* data acquisition. Additional thanks go to Maria Toledo-Rodriguez for enthusiastically guiding me through the unfamiliar world of *ex vivo* biochemistry.

Outside of the lab, thanks go to all the teammates, coaches and opponents I have taken the field with as part of the Nottingham Outlaws and Nottingham Caesars American Football teams. There are few better ways to clear the mind after a hard day in the lab than banging heads in the trenches.

I would like to acknowledge my family. Huge thanks goes to my new wife Mims for putting up with me throughout my PhD study, and always putting a smile back on my face. I'd also like to thank my parents and sister for their unending support during my seemingly endless time at University. This thesis is for each of them, as without them it would not have existed.

Table of Contents

Chapter 1 – General Introduction……1

1.1 Schizophrenia……..2
1.1.1 Background…..…….2
1.1.2 Schizophrenia aetiology……..2
1.1.2.1 Genetics…….2
1.1.2.2 Environment……..4
1.1.2.3 Genetics x Environment……..7
1.1.3 Neurobiological Basis of Schizophrenia……...8
1.1.3.1 The Dopamine Hypothesis……..8
1.1.3.2 The Glutamate Hypothesis……9
1.1.4 Treatment of Schizophrenia……14
1.2 Preclinical Research in Schizophrenia……...……16
1.2.1 Locomotor Activity……17
1.2.2 Locomotor Response to Acute NMDA receptor antagonist……18
1.2.3 Novel Object Discrimination……...19
1.2.4 Prepulse Inhibition of the Acoustic Startle Response……20
1.2.5 Conditioned Emotional Response……...23
1.2.6 Morris Water Maze……25
1.3 Preclinical Models of Schizophrenia ……..……27
1.3.1 Isolation Rearing……29
1.3.2 Methylazoxymethanol (MAM)……...33
1.3.3 Phencyclidine (PCP)……37
1.3.4 Dual-Hit Models Models……43

Chapter 2 – Combined Gestational MAM Treatment and Post-Weaning Social Isolation as a Model of 'Schizophrenia-like' Symptoms in the Rat……..46

2.1 Introduction…….....47
2.1.1 Hypothesis……...49
2.2 Materials and Methods……50
2.2.1 Animals……50
2.2.2 Locomotor Activity……53
2.2.3 Novel Object Discrimination……...53
2.2.4 Prepulse Inhibition of Acoustic Startle……55
2.2.5 Conditioned Emotional Response……...56
2.2.6 Morris Water Maze……..57
2.2.7 Neuroanatomical Analysis……...60
2.2.8 HPLC Analysis……61
2.2.9 Statistical Analysis……..64
2.3 Results……65
2.3.1 Locomotor Activity……65
2.3.2 Novel Object Discrimination……..68
2.3.3 Prepulse Inhibition of Acoustic Startle……72
2.3.4 Conditioned Emotional Response……...75
2.3.5 Morris Water Maze……..78
2.3.6 Hippocampal Size……81
2.3.7 Hippocampus and Frontal Cortex Neurochemistry……...85
2.4 Discussion……89
2.4.1 Conclusion……104

Chapter 3 – Combined Rearing in Social Isolation and Perinatal Phencyclidine Treatment as a Model of 'Schizophrenia-like' Symptoms in the Rat……..105

3.1 Introduction……106
3.1.1 Hypothesis……107
3.2 Materials and Methods……...108
3.2.1 Animals……108
3.2.2 Morris Water Maze Modifications……110
3.2.3 Hyperactivity to Acute PCP Treatment in an Open Field……112
3.3 Results……...113
3.3.1 Locomotor Activity……...113
3.3.2 Novel Object Discrimination……117
3.3.3 Prepulse Inhibition of Acoustic Startle……..121
3.3.4 Conditioned Emotional Response……124
3.3.5 Morris Water Maze……126
3.3.6 Locomotor Response to Acute PCP……..141
3.4 Discussion……144
3.4.1 Conclusion……153

Chapter 4 – Evaluating the Effect of Risperidone in the Iso-PCP Dual-Hit Model of 'Schizophrenia-like' Symptoms in the Rat……....154

4.1 Introduction……155
4.1.1 Hypothesis……156
4.2 Materials and Methods……...157
4.2.1 Animals……..157
4.2.2 Drug Administration……..157
4.2.3 Behavioural Testing and Statistical Analysis……159
4.3 Results……...161
4.3.1 Locomotor Activity……...161
4.3.2 Novel Object Discrimination……165
4.3.3 Prepulse Inhibition of Acoustic Startle……..169
4.3.4 Conditioned Emotional Response……173
4.3.5 Morris Water Maze……180
4.4 Discussion……187
4.4.1 Conclusion……193

Chapter 5 – Evaluating the Effect of Lamotrigine in the Iso-PCP Dual-Hit Model of 'Schizophrenia-like' Symptoms in the Rat……....194

5.1 Introduction……195
5.1.1 Hypothesis……196
5.2 Materials and Methods……...197
5.2.1 Animals……..197
5.2.2 Drug Administration……..197
5.2.3 Behavioural Testing and Statistical Analysis……198
5.3 Results……...202
5.3.1 Pilot Study – Novel Object Discrimination……...202
5.3.2 Locomotor Activity……...204
5.3.3 Novel Object Discrimination……206
5.3.4 Prepulse Inhibition of Acoustic Startle……..208
5.3.5 Conditioned Emotional Response……210
5.4 Discussion……212
5.4.1 Conclusion……217

Chapter 6 – General Discussion……218

6.1 Aims…….....219
6.2 Findings & Implications……219
6.3 Future Studies……226
6.4 Conclusions……...228

Appendix ……..230

References……...233

List of Abbreviations

5CSRTT	Five-Choice Serial Reaction Time Task
5-HT	5-hydroxytryptamine, serotonin
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of Variance
BDNF	Brain-Derived Neurotrophic Factor
BLA	Basolateral Amygdala
CANTAB	Cambridge Neuropsychological Test Automated Battery
cDNA	Complimentary Deoxyribonucleic Acid
CE	Central Nucleus of the Amygdala
CER	Conditioned Emotional Response
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates
GABA	γ-Aminobutyric acid
GAD_{67}	Glutamate Decarboxylase 1 (brain, 67kDa isoform), aka GAD1
GAT-1	GABA Transporter-1
GD	Gestational Day
HPLC	High-Pressure Liquid Chromatography
IC	Inferior Colliculus
LDTg	Laterodorsal Tegmental Nucleus
LMA	Locomotor Activity
MAM	Methylazoxymethanol
MATRICS	Measurement and Treatment Research to Improve Cognition in Schizophrenia
mGluR	Metabotropic Glutamate Receptor
mPFC	Medial Prefrontal Cortex
mRNA	Messenger Ribonucleic Acid
MWM	Morris Water Maze
NAc	Nucleus Accumbens
NCBI	National Centre for Biotechnology Information
NICE	National Institute for Health and Care Excellence
NMDA	N-methyl-D-aspartate
NPS	Neuropeptide S
NOD	Novel Object Discrimination

PAG	Periacqueductal Grey
PCP	Phencyclidine
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PnC	Caudal Pontine Reticular Nucleus
PND	Post-natal Day
PPI	Prepulse Inhibition
PPTg	Pedunculopontine Tegmental Nucleus
PV	Parvalbumin
Q-PCR	Quantitative Polymerase Chain Reaction
RM ANOVA	Repeated-Measures Analysis of Variance
ROS	Reactive Oxygen Species
SC	Superior Colliculus
SEM	Standard Error of the Mean
SI	Social Interaction
SNc	Substantia Nigra Pars Compacta
SPECT	Single-Photon Emission Computed Tomography
US	Unconditioned Stimulus
vHipp	Ventral Hippocampus
VP	Ventral Pallidum
VTA	Ventral Tegmental Area

Chapter 1

General Introduction

1.1 Schizophrenia

1.1.1 Background

Schizophrenia is a major neuropsychiatric disorder with a lifetime incidence of 1% worldwide, rising to 10% of those with a family history of the condition. It most commonly presents to patients post-puberty, and can progress from a range of mild and non-specific symptoms at an early stage to a severe, persistent and unremitting disease in later progression (NICE 2009; Wood et al. 2011). Due to continuing debate over the exact aetiology of the disease, it is characterised by a core of psychological symptoms divided into three key domains: positive, negative, and cognitive, that may alter perception, thoughts and behavioural traits in a way neither specific, nor exclusive to the condition (NICE 2009). The positive domain encompasses "gain-of-function" symptoms such as visual/auditory hallucinations and delusions. Negative symptoms are associated with a loss of sociality, volition, or pleasure, and are hence categorised as "loss-of-function". Cognitive symptoms have been subdivided into seven key domains (attention and vigilance, working memory, reasoning and problem solving, processing speed, visual learning and memory, verbal learning and memory, and social cognition (Marder and Fenton 2004; Young et al. 2009)) and represent the poorest addressed symptoms in the clinic.

1.1.2 Schizophrenia aetiology

1.1.2.1 Genetics

It has long been concluded that schizophrenia development carries a genetic link, particularly considering the 10-fold increase in risk of developing the

condition if one parent suffers, and some have concluded that up to 80% of risk for schizophrenia is due to heritable/genetic factors (McGuffin and Gottesman 1999). However, no "Mendelian" forms of schizophrenia have been found, i.e. deterministically caused by rare mutations (Need et al. 2012), despite suggestions that *DISC-1* (disrupted in schizophrenia-1) may follow this profile (St Clair et al. 1990; Sullivan 2013). Up to eight rare copy number variations (CNVs) have been consistently associated with psychiatric disorders including schizophrenia (Levinson et al. 2011; Malhotra and Sebat 2012). These changes in the number of expected DNA copies can result in a change in "dosage" of genes in the affected region, and exert a large effect on disease development risk (Giusti-Rodriguez and Sullivan 2013). These CNVs often involve many genes, such as the 16p11.2 CNV which encompasses 29 genes, and are not specific to schizophrenia, so offer only limited understanding to the genetic basis of schizophrenia (McCarthy et al. 2009).

Genome-wide association studies (GWAS) have enabled better identification of specific loci that may be determinant of schizophrenia by massively increasing sample sizes through large collaborative projects. These large samples, with appropriate statistical power, can identify links between common genetic variations or single-nucleotide polymorphisms (SNPs) and disease not possible with smaller studies, highlighted by a recent GWAS which estimated that 8300 independent SNPs at 22 loci contribute to risk of schizophrenia (Ripke et al. 2013). This study noted that calcium signalling may be key in schizophrenia, with SNPs in the α_{1C} and β_2 subunits of voltage-gated L-type calcium channel reaching genome-wide significance. This is a

particularly intriguing finding due to calcium's long association with learning, memory and neuronal plasticity, and a disruption in calcium signalling may impact on multiple functions due to prevalence of calcium signalling cascades.

Further genetic involvement has been suggested in a neurodevelopmental hypothesis of schizophrenia (Murray et al. 1991). After identification that schizophrenia patients commonly exhibit enlarged cerebral ventricles and decreased hippocampal and temporal lobe volume, it was noted that patients commonly experienced obstetric complications and were born in late winter, suggesting a foetal origin for the disease (Murray et al. 1991). Genetic defects in processes of cell proliferation, migration, differentiation and death during neurodevelopment may lead to the structural abnormalities seen in patients, and may indeed be detectable earlier than the outwards symptoms of psychosis (Jones and Murray 1991). It is also plausible that obstetric complications could be important for these genetic susceptibilities to be unveiled. Furthermore, two key susceptibility genes for schizophrenia, *DISC1* and neuregulin-1, have emerged as having potentially prominent roles in neurodevelopment. Several studies have linked polymorphisms in these genes to schizophrenia, and rodent mutation models display abnormalities in neuroanatomy and behaviour akin to those seen in patients (Pletnikov et al. 2008; Wolpowitz et al. 2000).

1.1.2.2 Environment

Environmental aspects that may induce schizophrenia can be broadly subdivided into three categories: prenatal complications; social stress/adversity, particularly in early life; and pharmacological. Gestational problems and schizophrenia have long been associated, with two theories

producing a relatively consistent clinical linkage to schizophrenia: pre and perinatal foetal hypoxia (Clarke et al. 2006; Handford 1975), and maternal infection (Brown 2006; Brown and Derkits 2010). Hypoxia, starvation of oxygen to the brain, produces toxic effects on a cellular level mediated through changes in intracellular calcium leading to the creation of reactive oxygen species (ROS), mitochondrial dysfunction, and subsequent apoptosis (Thornton et al. 2012). The cellular damage caused has been shown through numerous clinical and preclinical studies (Golan and Huleihel 2006; Van Erp et al. 2002), and the epidemiological links to later development of schizophrenia in adulthood are strong (Joyce 2005; Schmidt-Kastner et al. 2006). Initial links between epidemic infections and later schizophrenia development in populations suggested a relationship between maternal infection and schizophrenia aetiology. Candidate infections including toxoplasmosis gondii (Brown et al. 2005) and herpes simplex virus type-2 (Buka et al. 2008), leading to elevated maternal serum immunoglobulin G (IgG) and pro-inflammatory cytokines such as interleukin-8 (IL-8) and tumour necrosis factor-alpha (TNFα), have been associated with significant increases in risk of schizophrenia. Evidence suggests that foetal exposure to elevated levels of pro-inflammatory cytokines may lead to disrupted maturation of oligodendrocytes through nitric oxide and excitatory amino acid production, and hence abnormalities in white matter, as seen in schizophrenia (Brown and Derkits 2010; Davis et al. 2003). Subsequently, activation of the immune system of gestating rodent dams has become a commonly studied preclinical model of schizophrenia. Offspring from dams treated with the synthetic viral

product polyinosinic-polycytidilic acid (Poly(I:C)) display a variety of behavioural deficits, including in cognitive and social domains (Meyer 2014; Pacheco-Lopez et al. 2013; Wolff and Bilkey 2010), associated with elevated maternal inflammatory cytokines, including TNFα (Cunningham et al. 2007).

A social aetiology for schizophrenia is also a popular evidence-based concept, with particular salience placed on early-life adversity such as maternal separation and childhood abuse (MacMillan et al. 2001; Schenkel et al. 2005; Wicks et al. 2005). Mechanistically, it has been suggested that these experiences may only lead to specific psychosis development in the presence of particular genetic predispositions, and that activation of the hypothalamic-pituitary-adrenal axis related to stress or altered dopaminergic transmission and sensitization may be viable links between environmental and genetic risk factors (Collip et al. 2008). Social stressors are a popular method for inducing schizophrenia-like deficits in animal models of the disease, with maternal separation, chronic social defeat, and rearing in social isolation all inducing behavioural and neurobiological abnormalities with salience to the disease (Fone and Porkess 2008; Millstein et al. 2006; Selten and Cantor-Graae 2007).

There have been continuing links between substance abuse and schizophrenia development, particularly based on the observation that N-methyl-D-aspartate (NMDA) receptor antagonists (such as phencyclidine (PCP)) taken for illicit recreational use can exacerbate psychotic symptoms in schizophrenia sufferers, and can induce auditory and visual hallucinations in healthy individuals (Javitt and Zukin 1991; Krystal et al. 1994; Luby et al. 1959). There is also some evidence that the symptoms induced by NMDA receptor antagonism endure

even after cessation of drug administration (Rainey and Crowder 1975), which has led to the development of an NMDA receptor hypofunction theory of schizophrenia (Olney et al. 1999). The co-morbidity between substance abuse disorders and schizophrenia is also notably high (Brown et al. 2012; Jones et al. 2011c; Koola et al. 2012), with particular focus paid to the potential causative links between adolescent cannabis use and schizophrenia (Andreasson et al. 1987). However until recently few studies highlighted whether cannabis use is causal or as a consequence of the disease (Arseneault et al. 2004). More recent work has indicated it may be associated with aberrant development of the prefrontal cortex (PFC) (Bossong and Niesink 2010), could cause significant alterations in dopamine transmission in the brain (Muller-Vahl and Emrich 2008), and may be associated with working-memory deficits and altered globus pallidus, striatum and thalamus structure by MRI (Smith et al. 2014). However, some inconsistencies still exist in the link between cannabis and schizophrenia, with recent evidence showing that cannabis use does not increase the risk of schizophrenia morbidity in people either with, or without, a familial history of the disease (Proal et al. 2014). Indeed, this study found that risk of schizophrenia was only significantly increased by familial history, lending yet more support to the genetic aetiology of the disease.

1.1.2.3 Gene x Environment Interactions

The most likely answer is that genes and environment interact to increase the risk of developing schizophrenia. A genetic predisposition to the disease may be subsequently precipitated by environmental factors. One example include the presence of polymorphisms which, when combined with prenatal immune

activation in rodents, may lead to aberrant neurodevelopment (Ibi et al. 2010; Lipina et al. 2013). The influence of post-natal environment on genetic predisposition is difficult to study clinically, but a number of rodent models examining the interplay between individual genes and early-life stress have emerged (Chesworth et al. 2012; Desbonnet et al. 2012; Haque et al. 2012; Ishihama et al. 2010; Jiang et al. 2013). Results of these studies have added to the suggestion that an underlying genetic susceptibility to neuropsychiatric disorders may be precipitated when environmental stressors are presented.

1.1.3 Neurobiological Basis of Schizophrenia

1.1.3.1 The Dopamine Hypothesis

The involvement of dopamine signalling in schizophrenia has long been established, since the discovery that primary drugs with efficacy against psychoses were dopaminergic in nature (Carlsson and Lindqvist 1963; Carlsson et al. 1957). Advanced imaging studies using PET and SPECT technology have demonstrated a modest increase in $D_{2/3}$ receptor density in the striatum of schizophrenia sufferers (Kestler et al. 2001), and striatal dopamine release significantly increases following amphetamine treatment to patients, coinciding with an exacerbation of positive symptoms (Abi-Dargham et al. 2000; Laruelle et al. 1996). Recent advances have also shown that elevated dopamine is limited to the precommissural dorsal caudate, and not the limbic or sensorimotor regions, of the associative striatum (Kegeles et al. 2010). This region is involved in associative information processing, and also accepts input

from the dorsolateral PFC, hence hyperdopaminergic activity may influence a range of disease symptoms (Kegeles et al. 2010; Lodge and Grace 2011).

Reduced dopaminergic activity in frontal regions (for example through lesion studies in rodents) seemingly mirrors negative symptoms of the disease (Davis et al. 1991; Howes and Kapur 2009). Prominent recent papers have shown increased frontal D_1 receptor density, reflecting an up-regulation in gene expression in response to hypodopaminergic activity that may be involved in the working memory deficits seen in schizophrenia (Abi-Dargham et al. 2002; Abi-Dargham et al. 2012). However, reproducible changes in frontal dopamine levels have not been observed (Akil et al. 1999).

A large meta-analysis covering all available neuroimaging data recently concluded that the largest abnormality in dopamine function is presynaptic, rather than in post-synaptic receptor density (Howes et al. 2012). The authors suggested that dopamine synthesis capacity, baseline dopamine levels and dopamine release may all be strongly implicated, and that future therapeutics should address this pathology.

1.1.3.2 The Glutamate Hypothesis

The NMDA receptor hypofunction model has been proposed to link the natural development of schizophrenia and the 'schizophrenia-like' effects of NMDA receptor antagonists (Cohen et al. 1962; Javitt and Zukin 1991; Krystal et al. 1994; Lahti et al. 1995; Luby et al. 1959; Olney et al. 1999). The model suggests that dysfunction of NMDA receptors may cause excess synaptic glutamate release to overcome the loss of signalling. This release of excitatory neurotransmitter may overstimulate post-synaptic cells by overcoming the

blockade or stimulating α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Excessive activation could lead to post-synaptic excitotoxicity, and subsequently cause neuronal cell death (Deutsch et al. 2001). This could explain the neuropathology identified in schizophrenia patients (Kreczmanski et al. 2007; Shenton et al. 2001).

The hippocampus' pivotal role in long-term memory implicates it strongly in the cognitive disease domain (Tamminga et al. 2010). Pathology of the hippocampus has long been observed in post-mortem tissue from patients (Bogerts et al. 1985), supported by imaging studies suggesting an inability to recruit the hippocampus when required for memory-based tasks (Heckers and Konradi 2002; Nelson et al. 1998). Basal activity of the hippocampus is increased in schizophrenia (Medoff et al. 2001) and atrophy of the hippocampus is seen in patients after psychosis onset (Schobel et al. 2013). Ketamine treatment to mice mirrors these effects, with pathology occurring in parvalbumin-expressing hippocampal interneurons, confirming the involvement of glutamate and NMDA receptors in hippocampal atrophy.

Hippocampal glutamate hyperfunction and excess striatal dopamine activity, both linked to the positive symptom domain, can be linked through a polysynaptic pathway from the ventral hippocampus (vHipp) to the ventral tegmental area (VTA) (Figure 1.1) (Blaha et al. 1997; Floresco et al. 1997; Floresco et al. 2001; Floresco et al. 2003). Top-down inputs are received from the prefrontal cortex (PFC) during cognitive tasks, further implicating frontal pathology in schizophrenia (Floresco et al. 1997; Jones and Wilson 2005).

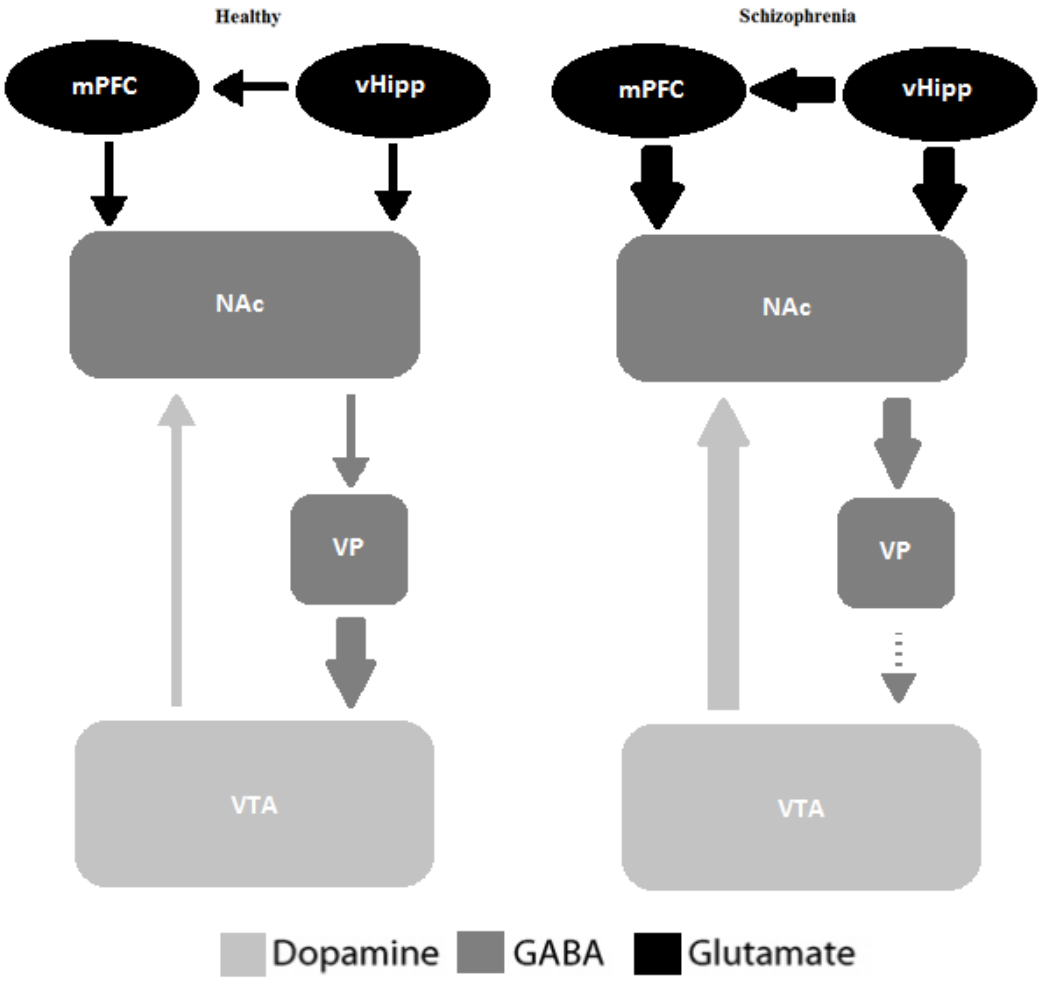

Figure 1.1 – Elevated activity in the ventral hippocampus (vHipp) and medial prefrontal cortex (mPFC) in schizophrenia leads to increased excitatory input to the nucleus accumbens (NAc). Hyperactive GABAergic projections from the NAc to ventral tegmental area (VTA) via the ventral pallidum (VP) lead to disinhibition of dopaminergic neurons, and hence excessive dopamine is released from the VTA into the limbic system. Adapted from (Lodge and Grace 2009).

Particular attention has been focused on GABAergic interneuron pathology in the hippocampus and PFC in schizophrenia (Beasley and Reynolds 1997; Beasley et al. 2002; Benes et al. 2007; Hashimoto et al. 2003; Konradi et al. 2011; Lewis et al. 2005; Lodge et al. 2009; Zhang and Reynolds 2002). Post-mortem studies reveal a significant loss of interneurons in hippocampal sub-regions (Benes et al. 1998), and further analysis shows that the density of interneurons expressing the calcium-binding protein parvalbumin (PV) (Konradi et al. 2011; Zhang and Reynolds 2002) and the peptide hormone somatostatin (Konradi et al. 2011) is decreased. These findings have been replicated in a number of animal models of schizophrenia (Braun et al. 2007; Lodge et al. 2009; McKibben et al. 2010; Nakatani-Pawlak et al. 2009; Schobel et al. 2013), suggesting that loss of GABAergic inhibition could cause aberrant glutamatergic activity. By acting as a negative feedback loop to glutamatergic neurons, or as a route for glutamatergic regulation of inhibitory tone, hypofunction of NMDA receptors on these GABAergic interneurons may lead to excitotoxic cell death via excessive glutamate release as described above (Figure 1.2). The reported positive effects of benzodiazepines (positive GABAergic allosteric modulators) in alleviating psychotic symptoms in subset of schizophrenia patients further supports GABAergic involvement, however the varied side-effects including sedation and cognitive impairments limit benzodiazepines' utility (Wolkowitz and Pickar 1991). Targeting specific GABA receptor subtypes may provide a new therapeutic approach to the treatment of schizophrenia in the future (Lodge and Grace 2011).

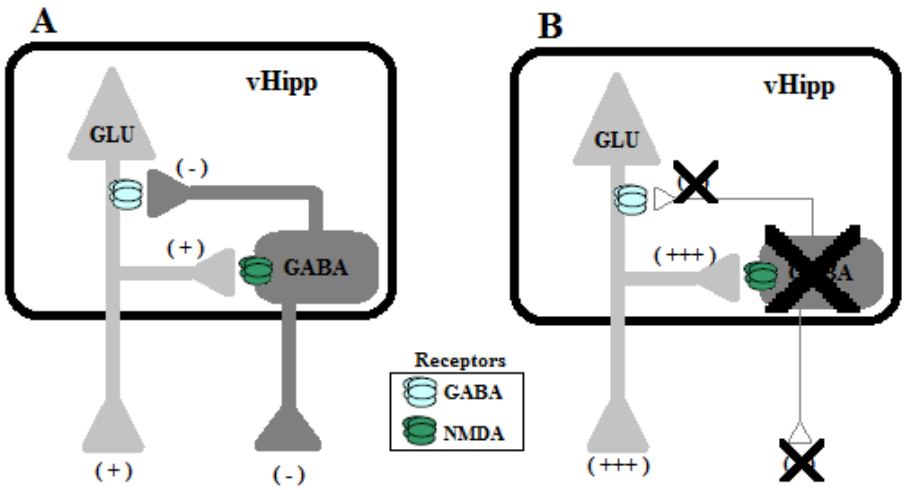

Figure 1.2 – Diagrammatic representation of a negative feedback loop within the ventral hippocampus through which glutamatergic output is regulated by GABAergic interneurons. (A) In a normal system, glutamatergic neurons (GLU) project onto GABAergic interneurons (GABA) within the ventral hippocampus (vHipp), and cause excitation through NMDA receptors on GABAergic cell bodies. Through a negative feedback loop, GABAergic projections onto GLU axons regulate GLU output. (B) Following loss of GABAergic interneuron function (via NMDA receptor dysfunction, apoptotic cell death etc.) this negative feedback loop is lost, and GLU output is disinhibited. Diagram adapted from (Del Arco et al. 2011).

1.1.4 Treatment of Schizophrenia

Broadly, available treatments for schizophrenia fall into two categories: typical and atypical antipsychotics. However, these groups have many characteristics in common, and are divided as much by their time of discovery as their differing pharmacology.

Typical antipsychotics, the first treatments to be discovered, are mostly defined as dopamine antagonists or inverse agonists. Drugs of this class, such as chlorpromazine and haloperidol, have relatively high affinities for the dopamine D_2 receptor, where binding levels correlate with successful treatment of positive symptoms (Nordstrom et al. 1993; Nyberg et al. 1995). However, dopamine antagonism produces significant side-effects along with benefits. Extrapyramidal side-effects due to dopamine antagonism in the basal ganglia produce ataxia, dystonia and Parkinsonism-like movement disorders. Further, typical antipsychotics produce a number of off-target side-effects, whilst having little benefit on the negative or cognitive disease symptoms. Chlorpromazine is a particularly "dirty" compound, with high affinity at dopamine receptors $D_{1\text{-}4}$, as well as at 5-$HT_{1/2}$ serotonin receptors, H_1 histamine receptors, α_1 adrenergic receptors and muscarinic acetylcholine receptors $M_{1/2}$ (Savelyeva et al. 1988). Antagonism of histamine and α-adrenergic receptors leads to strong sedation, which although may help to alleviate psychoses, does not aid a patients return to "normality". The anticholinergic effect of chlorpromazine leads to both common side-effects associated with this pharmacology (constipation, dry mouth etc.) and potentially to further cognitive impairment. Serotonin antagonism may aid the

activity of chlorpromazine, by reducing aggression and anxiety, and is also hypothesised to reduce extrapyramidal side-effects by reducing serotonin control of the basal ganglia (Kapur and Remington 1996; Kapur et al. 1999). But antagonism at serotonin receptors is also believed to lead to significant weight gain, a common side effect of antipsychotic treatment. Alternatively, haloperidol has a much cleaner binding profile, with highly selective dopamine receptor affinity. However, this specificity is accompanied by elevated extrapyramidal side-effects, overshadowing the positive symptom control.

The main pharmacological difference between the typical and atypical antipsychotics is 5-HT activity. The new class of compounds, whilst maintaining dopamine receptor affinity, also have greatly increased serotonin antagonism, particularly at 5-HT_{2A} receptors. Clozapine, the first atypical antipsychotic developed, in fact has a relatively low D_2 affinity compared to 5-HT_{2A}, and instead has greater D_4 affinity. Due to low D_4 expression in the basal ganglia, as well as the influence of serotonin antagonism, clozapine displays relatively few extrapyramidal side-effects. However, clozapine carries a black box warning for drug-induced agranulocytosis, which can cause death; hence use is limited to patients with symptoms resistant to other antipsychotic medication. Other compounds in this class, such as olanzapine and risperidone, are safer than clozapine but less efficacious. Both express high affinity at 5-HT_{2A} receptors compared to D_2, believed to aid with their antipsychotic action, but this is accompanied by the weight gain side-effects due to serotonin antagonism. Both are also potent H_1 antagonists, which like chlorpromazine leads to sedation. Again, drugs of this class are relatively successful in the

treatment of positive symptoms, but generally offer little in the way of negative or cognitive symptoms, although some potentially beneficial effects of particularly risperidone have been noted in clinical trials (Bilder et al. 2002; Houthoofd et al. 2008). There is also a subset of patients who do not respond to any antipsychotic medication, even the last line of treatment clozapine. These "clozapine-resistant patients" represent a further serious unmet medical need for novel antipsychotic drugs.

1.2 Preclinical Research in Schizophrenia

The lack of efficacy of current antipsychotic medication towards the cognitive symptoms of schizophrenia means the availability of preclinical animal models that accurately replicate this domain in the laboratory are vital. The utility of animal models is two-fold. Firstly, models can be used to determine the underlying mechanisms associated with the aetiology and risk-factors for disease development, including an assessment of potential interactions between different genetic and environmental factors. A greater disease understanding may reveal new avenues for drug development. Secondly, models are essential for evaluating novel treatments for their potential use in patients. This, however, relies on the validity of the model used, assessed in three domains: construct, predictive and face (Willner 1984). Face validity describes how similar an animal model looks to the human condition, in terms of behaviour in relevant paradigms and similar biological changes or markers. Construct validity examines similarities in the way deficits are created; if the

model and disease are induced in a similar manner. Predictive validity is gained by demonstrating similar responses to pharmacological challenge as seen in patients. This domain is therefore key when selecting a model to test putative new compounds.

With the development of the MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) and TURNS (Treatment Units for Research on Neurocognition and Schizophrenia) initiatives to develop reliable, valid and consensus-derived methods of assessing cognition, there is a clear framework established for preclinical models of schizophrenia-like symptoms to be judged against. With seven key cognitive domains identified as deficient in schizophrenia patients, a wide-ranging battery of behavioural techniques is available against which to assess the face and construct validity of animal models (Jones et al. 2011b; Young et al. 2009).

1.2.1 Locomotor Activity

Locomotor activity (LMA) in an open field is a long established behavioural paradigm that has a wide variety of uses, from assessing motor co-ordination to sedation. The "positive" symptoms of schizophrenia are difficult to model in rodents, as symptoms of hallucinations are highly subjective; however neophobia is seen as a suitable correlate for positive human symptoms based on its reasonable predictive and construct validity (Fone and Porkess 2008; Levine et al. 2007; McIntosh et al. 2013). Rats placed in a novel environment show elevated exploratory activity decreasing with time as they habituate to the novelty. However, rodents of a schizophrenia-like phenotype exhibit

elevated horizontal exploration and vertical rearing and habituate at a slower rate than control counterparts (Fone and Porkess 2008; Gentsch et al. 1988; Le Pen et al. 2011; Zhang et al. 2012b). This hyperlocomotion has been interpreted as an elevated propensity to escape from the novel arena, and hence reflects fear of novelty (Fone et al. 1996). This hyperlocomotion is robust across groups, cohorts and methods of interference (Fone and Porkess 2008; Jones et al. 2011b), and also displays predictive validity as it is consistently reversed by standard antipsychotic compounds notably strong at reversing positive symptoms in the clinic (Fabricius et al. 2011; Le Pen et al. 2011; McIntosh et al. 2013). Elevations in locomotor activity have been correlated with increased striatal dopamine release in rodents (Flagstad et al. 2004; Paulson and Robinson 1995), similar to the correlation between striatal dopamine and the onset of psychosis in schizophrenia patients (Abi-Dargham et al. 1998; Abi-Dargham et al. 2000; Laruelle et al. 1996). The PFC is also implicated, with a strong correlation between hyperlocomotion in rodent models and differential expression of genes involved in cell differentiation and apoptosis (Kaiser et al. 2004; Levine et al. 2007; Liu et al. 2010; Turnock-Jones et al. 2009).

1.2.2 Locomotor Response to Acute NMDA receptor antagonist

An additional arm of locomotor activity analysis involves an altered response to acute NMDA receptor antagonist administration. As discussed previously, NMDA receptor antagonists produce symptoms similar to the positive domain of schizophrenia in healthy individuals, and exacerbate positive symptoms in

patients (Javitt and Zukin 1991; Krystal et al. 1994; Lahti et al. 1995). Preclinically, acute NMDA receptor antagonists cause hyperlocomotion in many animal models of schizophrenia, particularly those using NMDA receptor antagonist pre-treatment to induce the 'schizophrenia-like' syndrome, providing good face validity (Boctor and Ferguson 2010; Le Pen et al. 2011; Nakatani-Pawlak et al. 2009; Wang et al. 2001).

1.2.3 Novel Object Discrimination

Novel object discrimination (NOD) is a common task often utilized for cognition research, particularly relative to schizophrenia (Akkerman et al. 2012; Dere et al. 2007; Ennaceur and Delacour 1988; Grayson et al. 2007; McIntosh et al. 2013; McLean et al. 2010a). NOD relies on an innate tendency to explore novelty, tending to previously unseen objects placed into an arena with vibrissae, nose and forepaws (Dere et al. 2007), and has been identified as a key correlate for visual learning and memory impairment by MATRICS (Young et al. 2009). After exposure to two identical objects, rodents will preferentially explore a novel object over one of those they have been pre-exposed to following a delay away from the context. However, animals that have impairments in visual learning and memory may be unable to encode the necessary information during the first trial, or not able to retain/retrieve the information when required in the second trial, and hence explore novel and familiar objects to a similar extent, showing a measurable behavioural deficit (Ennaceur and Delacour 1988).

Evidence suggests that NOD performance relies heavily on the entorhinal and perirhinal cortex over the more traditional "memory centre" of the hippocampus, with perirhinal lesions able to impair NOD in a study where hippocampal lesions did not (Winters et al. 2004). However, some hippocampal involvement is likely, particularly with longer intervals between trials (Clark et al. 2000). Alterations in the perirhinal cortex of schizophrenia patients have been noted through MRI studies (Turetsky et al. 2003), and deficits in visual memory tasks in patients with schizophrenia are directly comparable to those with temporal lobe epilepsy that have a clear hippocampal lesion impairing performance (Yoo et al. 2006). Hence, NOD displays a level of face validity and etiological relevance. However, the predictive validity of this model is highly questionable. The effects of current antipsychotic medication on NOD performance varies greatly between studies, but both acute (Grayson et al. 2007) and subchronic clozapine (Hashimoto et al. 2005), acute risperidone (Grayson et al. 2007; McIntosh et al. 2013) and repeated aripiprazole (Nagai et al. 2009) have all been shown to reverse NOD deficits. Considering the very limited efficacy of current antipsychotic compounds on cognition in schizophrenia, these reversals of NOD deficits represent a weakness of this paradigm (Keefe 2007; Young et al. 2009)

1.2.4 Prepulse Inhibition of the Acoustic Startle Response

Considered as a paradigm with high translational relevance to schizophrenia, despite a lack of consistency in preclinical studies (Fone and Porkess 2008), prepulse inhibition (PPI) of the acoustic startle response is thought to evaluate

preattentional processing, identified as a key domain in the MATRICS battery (Young et al. 2009). In PPI, a low amplitude, non-startle eliciting sound presented immediately before a startle eliciting tone causes a decreased startle response than elicited with the tone alone. Thus, PPI is considered to require sensorimotor gating, the ability of the brain to filter out unnecessary sensory inputs. Deficits in this behaviour have been observed schizophrenia patients (Braff et al. 2001; Swerdlow et al. 2006a), although the link between PPI and cognitive deficits is unclear as they do not necessarily co-exist in the same patients (Swerdlow et al. 2006a). Regardless, this test has face validity in a preclinical model as it is highly conserved across species and can be directly measured in humans (Swerdlow et al. 2006a).

The networks involved in PPI are highly complicated, and their modulation of the primary auditory startle network from the cochlear to the caudal pontine reticular nucleus (PnC) and spinal motor neurons has been extensively reviewed (Campbell et al. 2007; Fendt et al. 2001; Swerdlow et al. 2001) (Figure 1.3). Key to the link between PPI and schizophrenia are the inputs of the NAc, hippocampus and frontal regions feeding into the primary networks controlling auditory startle response. With previously described pathology in these regions, the impairment of PPI in schizophrenia is unsurprising.

Atypical antipsychotic treatments can normalise PPI responses in patients or "low-PPI" phenotype individuals (Swerdlow et al. 2006b; Vollenweider et al. 2006; Wynn et al. 2007), and similar results have been reported in rodent models of schizophrenia (Bakshi et al. 1994; Geyer et al. 2001). Hence, the predictive validity and relevance of this paradigm is strong.

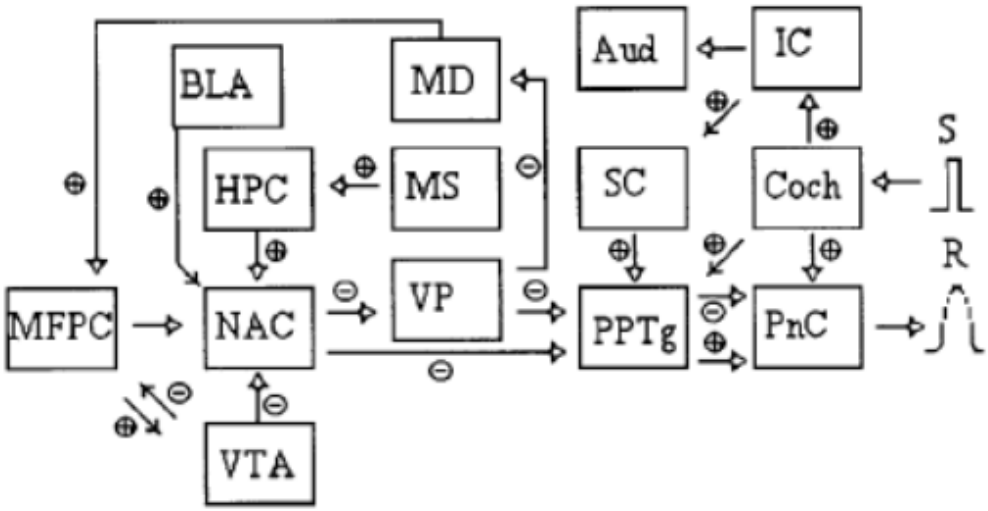

Figure 1.3 – Neural circuitry involved in acoustic startle and prepulse inhibition. The acoustic stimulus ("S") stimulates a simple circuit through the cochlea (Coch) and the caudal pontine reticular nucleus (PnC) to elicit a startle response ("R"). A secondary circuit, via the inferior (IC) and superior colliculus (SC) to the pendunculopontine tegmentum (PPTg) can also influence startle response, and is regulated by a network of descending projections from the forebrain, importantly via the ventral pallidum (VP) and nucleus accumbens (NAC), and implicating the hippocampal formation (HPC), medial prefrontal cortex (MPFC), and ventral tegmental area (VTA). Also included in the diagram are the mediodorsal thalamus (MD), the medial septal nucleus (MS), the basolateral amygdala (BLA) and the auditory cortex (Aud). Diagram adapted from Swerdlow, 2001.

1.2.5 Conditioned Emotional Response

Although not considered a core part of the MATRICS test battery, there is significant evidence that associative learning is impaired in schizophrenia (Herbener 2009; Rushe et al. 1999). The conditioned emotional response (CER) paradigm involves associating a non-aversive conditioned stimulus (CS), e.g. a light and sound tone, with an aversive unconditioned stimulus (US), like a mildly-aversive foot-shock. Successful association of the stimuli results in a reaction to the conditioned stimulus that would normally be observed upon presentation of just the US.

The CER protocol has been well validated in rodents (Woods et al. 2012), and the neurobiological basis of conditioning has been mapped (Figure 1.4) (Maren 2001). The amygdala is the centre for integration of physiological responses to sensory inputs and is a likely centre for association between aversive and non-aversive inputs (Maren 2001). Hippocampal involvement in contextual conditioning circuitry is also evident from indirect projections to the CE via the mPFC, where pre-limbic projections to the BLA enhance fear renewal while infra-limbic projections to intercalated cells have an inhibitory effect on the CE to reduce fear-responses (Maren et al. 2013). Post-mortem studies have highlighted a reduced volume and neuron number in the lateral nucleus of the amygdala of patients (Kreczmanski et al. 2007), suggesting pathology may be responsible for observed associative learning deficits (Herbener 2009; Rushe et al. 1999).

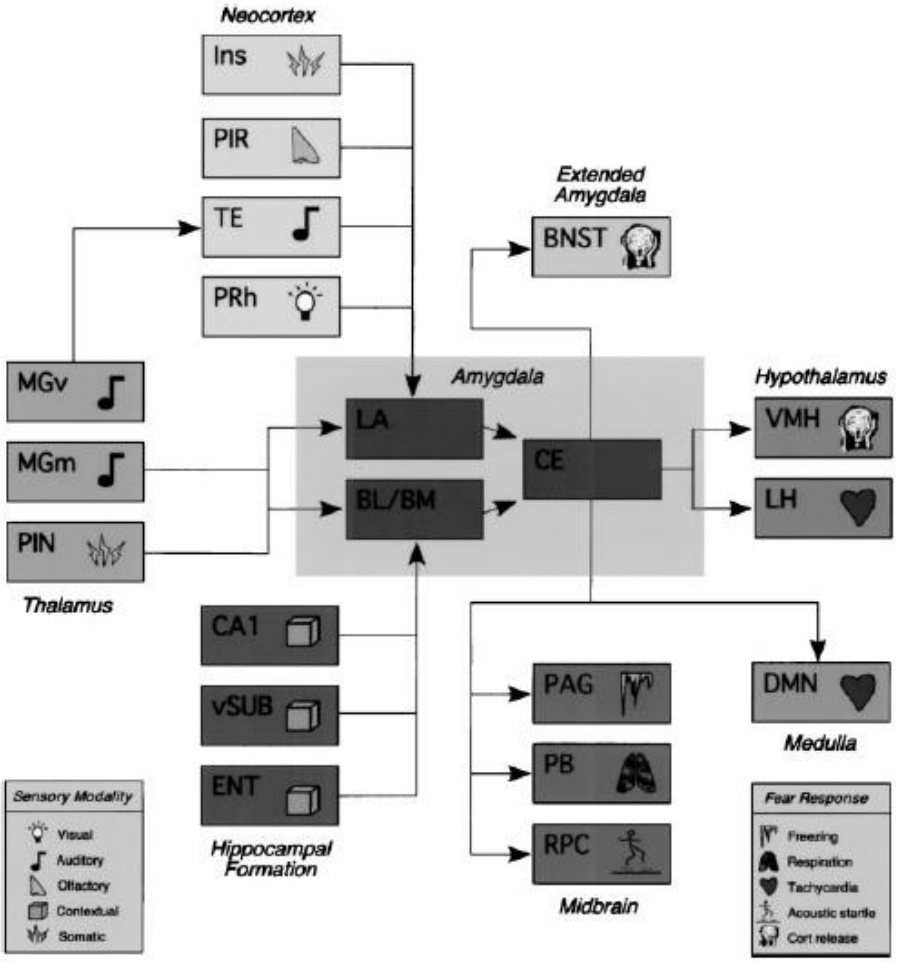

Figure 1.4 – Neural circuits governing fear conditioning. The basolateral amygdala complex (the lateral (LA), basolateral (BL) and basomedial (BM) muclei) and the central nucleus (CE) combine to form the amygdaloid nuceli. Sensory information from the thalamus (medial and ventral thalamic medial geniculate, MGm and MGv; posterior intralaminar nucleus, PIN), neocortex (insular cortex, INS; piriform cortex, PIR; primary auditory cortex, TE; perirhinal cortex, PRh), and hippocampus (CA1 area; ventral subiculum, vSUB; entorhinal cortex, ENT) is received by the basolateral complex, and is conveyed to the CE by intra-amygdaloid circuitry, suggested as a location for conditioned-unconditioned stimuli to be integrated. From the CE, divergent projections mediate physiological responses such as freezing, potentiated startle, and altered cardiac rhythm via the midbrain (periacqueductal grey, PAG; parabrachial nucleus, PB; caudal pontine reticular nucleus, RPC), medulla (dorsal motor nucleus of the vagus, DMN), hypothalamus (ventromedial hypothalamus, VPN; lateral hypothalamus), and bed nucleus of the stria terminalis (BNST) in the extended amygdala. Diagram adapted from Maren, 2001.

Numerous groups have shown that deficits in contextual and cue-based conditioning can be readily reproduced in animal models of cognitive dysfunction, and that these can be ameliorated by pharmacological treatments including antipsychotics (Csernansky et al. 2005; Martin et al. 2005; Woods et al. 2012), providing some predictive validity to the paradigm. However, the exclusion of any kind of conditioning-based paradigm from the MATRICS initiative does indicate that the translational relevance of CER is somewhat limited compared to others described here (Young et al. 2009).

1.2.6 Morris Water Maze

In contrast to CER, the Morris Water Maze (MWM) (Morris 1984) is highly translatable to the MATRICS battery, and has a protocol that can include working memory, visual learning and memory, and reasoning and problem solving (Young et al. 2009). The MWM task involves a repetitive training regime that requires rodents to locate a hidden, fixed-location platform below the surface of a water-filled pool using visual extra-maze clues. Over repeated trials, rodents can successfully locate the platform with decreasing latency, and can be tested for their level of task acquisition by removing the platform and assessing the search profile. This simple protocol requires inputs from the dorsal hippocampus particularly, based on the strong spatial aspect of the task (Moser et al. 1993), but the habenula has also been implicated. Lesions of this nucleus in rat impairs water maze performance (Lecourtier et al. 2004; Lecourtier et al. 2005; Wang et al. 2013) and calcification of the habenula and neighbouring pineal gland has been seen in schizophrenia patients (Sandyk

1992), although the correlation between calcification and cognitive performance has not been examined.

In addition, a more complicated reversal task can be performed. The fixed location of the platform can be changed, requiring extinction of a previously learnt rule and re-acquisition of a new location. Alternatively, a new search strategy can be implemented by altering the position of the platform between each trial, or each day, following a consistent rule, requiring working memory and problem solving to successfully complete (Young et al. 2009). Schizophrenia patients display cognitive deficits in multi-modal 3D virtual maze tasks that require continual working memory to complete, adding face validity (Sorkin et al. 2005; Sorkin et al. 2006). Although not analogous to the MWM task, the use of altered visual and spatial clues to update a maze-escape procedure does provide considerable cross comparison between the human and rodent tasks. Some difficulty in construct validity occurs when considering the neural aspects of working memory that may be involved in a MWM reversal task, as the hippocampus, key in rodent studies (Dong et al. 2013; Duffy et al. 2008), contrasts with findings that the frontal cortex is integral to working memory performance in humans (Bor et al. 2006; Bor et al. 2001).

Many antipsychotics cause impairments in the MWM, including clozapine (Didriksen et al. 2006), olanzapine (Didriksen et al. 2006; Terry et al. 2002), and haloperidol (Terry et al. 2002; Terry et al. 2003). Short term risperidone causes a slight improvement in performance (Terry et al. 2003), but longer-term treatment causes impairment (Terry et al. 2007). This data is partially comparable to the clinic, where most antipsychotics have little beneficial effect

on cognition (Keefe 2007), whilst some potentially positive effects of risperidone have been noted (Bilder et al. 2002; Houthoofd et al. 2008).

The MATRICS battery includes further tests to assess social cognition through interactions, and attentional processing through tasks like the five choice serial reaction time task (5CSRTT), however as these have not been utilised in this thesis they have not been further discussed.

1.3 Preclinical Models of Schizophrenia

With continuing uncertainty over the aetiology of schizophrenia, it is unsurprising that a number of methods for producing 'schizophrenia-like' symptoms in rodents also exist. Broadly, these fit into four categories: developmental, pharmacological, lesion, and genetic, each with strengths in terms of validity to schizophrenia (Jones et al. 2011b).

Developmental models of schizophrenia, including those based on prenatal immune challenge or neurodevelopmental disruption, social stress and early-life adversity, hold a high level of construct validity to the disease. Each is closely linked to a specific risk factor for schizophrenia development, identified through clinical risk studies: maternal immune activation to the co-incidence of maternal viral infection and subsequent psychoses in offspring; isolation rearing to the social stress theory of schizophrenia aetiology; and prenatal MAM treatment to the neurodevelopmental disruption hypothesis (Jones et al. 2011b). Each also has considerable face and some predictive

validity, with behaviours relating to positive, negative and cognitive symptoms known to be deficient and reversible by some current antipsychotic agents. The weakness of these models, however, is that they do not address the clear genetic linkage of schizophrenia.

Specific genetic models of schizophrenia hold a high level of construct validity by addressing this issue. DISC-1, neuregulin-1, and other genetic mutation/ knockout lines in rodents hold deficits in potentially key susceptibility genes that may underlie the development of schizophrenia. Dual-hit models where these genetic predispositions are exacerbated by subsequent developmental challenges have been developed to further increase the validity (discussed below). However, as no Mendelian forms of schizophrenia have been discovered, the relevance of these single knockout/point-mutation models is difficult to conclude.

Pharmacological models, particularly those based around NMDA receptor antagonists also hold some construct validity, inducing NMDA receptor hypofunction and impeding glutamate signalling. Dopamine-elevating compounds such as amphetamine can also be used to model particularly the positive symptoms of the disease, but these are less successful in inducing either negative or cognitive deficits, so have limited face validity. One considerable issue with NMDA receptor antagonist models of schizophrenia are the false-positive effects of current and putative antipsychotics on the behavioural deficits of these models. This is considered in greater depth below.

Lesion models, such as the ventral hippocampal lesioning approach, hold limited construct validity. However, their face validity in terms of behavioural

responses and changes in neurotransmitter systems are equal to those produced by pharmacological challenge, and are potentially much longer-lasting (Jones et al. 2011b).

In this investigation, research focused on models with a strong developmental/ perinatal insult, following the early-life adversity theory of aetiology.

1.3.1 Isolation Rearing

Rearing rats in social isolation following weaning (postnatal day (PND) 20-25), maintaining auditory, olfactory and visual, but not physical contact with conspecifics, prevents establishment of a hierarchy in a litter which is key in neurodevelopment. Alterations in behaviour and neurobiology are produced that persist into adulthood (Fone and Porkess 2008; Lapiz et al. 2003; Schubert et al. 2009). This developmental disruption protocol mimics the early-life adversity hypothesis of schizophrenia aetiology, and the model phenotype is termed 'isolation syndrome' (Fone and Porkess 2008; Jones et al. 2011b).

Neophobia, fear of novelty, can be examined in LMA tasks as described previously (see Chapter 1.2.1), and this is one of the most robust changes induced in isolation-reared rats. Multiple groups have shown elevated horizontal locomotor activity and vertical rears following two to six weeks of single housing (Del Arco et al. 2004; Fone and Porkess 2008; Powell et al. 2002; Robbins et al. 1996). The early onset of this behaviour and the simple testing procedure make LMA a useful marker for assessing the establishment of isolation syndrome. As discussed previously (see Chapter 1.2.1), one theory of the underlying basis of this hyperlocomotion is alteration in PFC function.

Structural deficits in the PFC of isolation reared animals have been noted (Day-Wilson et al. 2006; Schubert et al. 2009), which replicates results seen in schizophrenia patients (Hirayasu et al. 2001; Wible et al. 2001). Predictive validity is also provided with evidence that clinically relevant antipsychotics such as risperidone (McIntosh et al. 2013), haloperidol and olanzapine (Fabricius et al. 2011) are able to reverse the hyperactivity seen.

The impairment of PPI of acoustic startle in isolation syndrome (Cilia et al. 2001; Geyer et al. 1993; Powell et al. 2002; Schubert et al. 2009; Varty and Higgins 1995; Wilkinson et al. 1994) is analogous to commonly observed deficits in schizophrenia patients (Braff and Geyer 1990; Braff et al. 2001; Swerdlow et al. 2006a), but an increasing number of negative studies have called the robustness of the deficit into question (Cilia et al. 2005; Fone and Porkess 2008; Jones et al. 2011a; McIntosh et al. 2013). The isolation rearing-induced deficit in PPI can be reversed by infusion of 6-hydroxydopamine into the NAc to deplete dopamine levels, which does not occur in socially housed animals (Powell et al. 2003). This suggests that excess dopamine in the NAc may be a key neuropathology in isolation-reared animals, providing construct validity linking the model to dopaminergic theories of schizophrenia. Isolation rearing deficits in PPI are also susceptible to reversal with antipsychotics including quetiapine, olanzapine, clozapine, haloperidol and risperidone (Cilia et al. 2001; Varty and Higgins 1995).

In paradigms with strong cognitive substrates such as NOD, isolation causes impairment (Bianchi et al. 2006; King et al. 2004; McIntosh et al. 2013; McLean et al. 2010a; Watson et al. 2012a). There is also evidence that this can

be reversed by current and putative antipsychotics, including risperidone (McIntosh et al. 2013) and 5-HT_6 receptor antagonists (King et al. 2004), providing some predictive validity. Whilst there is some face validity to the model as it mirrors the visual recognition deficits seen in schizophrenia (McClure et al. 2007), there is no published evidence on the effect of isolation rearing on the perirhinal cortex in rats thought crucial for NOD performance (see Chapter 1.2.3). In the Morris Water Maze and T-Maze, involving spatial cognition, isolation rearing fails to induce impairment in acquisition of a fixed rule (Quan et al. 2010; Schrijver et al. 2002), but reduces retention of a learned task (Quan et al. 2010), and causes deficits in reversal protocols that require cognitive flexibility (Schrijver et al. 2004). Adding validity to the model, these deficits are accompanied by reduced long-term potentiation in the hippocampus (Ibi et al. 2008) and PFC (Quan et al. 2010). Although not considered a core cognitive symptom of schizophrenia, rats reared in isolation are impaired in their retention of both context and cue in the rodent CER paradigm, deficits that were not reversed by risperidone (McIntosh et al. 2013). In correlation with schizophrenia and cognitive symptoms (Goldman-Rakic et al. 2004), isolates have a reduced D_1 dopamine receptor density in the PFC (Toua et al. 2010) and increased density in the striatum (Gill et al. 2013). There is some evidence that isolation rearing does not affect basal dopamine levels in the NAc (Fulford and Marsden 1998; Howes et al. 2000; Jones et al. 1992), but may cause higher levels and altered asymmetry in the mPFC (Jones et al. 1992) and decreased overall turnover in this region (Heidbreder et al. 2000). Treatment with clozapine and olanzapine, but not haloperidol, causes elevated

dopamine in the mPFC of isolates, which may underlie some of their clinical efficacy against positive symptoms (Heidbreder et al. 2001).

Much less focus has been on the glutamatergic system in isolates, perhaps due to clinically relevant dopamine receptor antagonists being readily available for study. A variety of NMDA receptor subunits and metabotropic glutamate receptors have been shown to be differentially regulated in the PFC (Melendez et al. 2004; Turnock-Jones et al. 2009) and hippocampus (Zhao et al. 2009) of isolation reared animals, suggesting an important role for glutamate function in the isolation phenotype.

Following 11 weeks of isolation rearing, female Sprague-Dawley rats have decreased levels of parvalbumin and calbindin in the hippocampus *ex vivo*, correlated with deficits in PPI performance (Harte et al. 2007). A reduced expression of the GABA-transporter 1 (GAT-1) in the ventral prelimbic cortex, a marker of alterations in prefrontal GABAergic chandelier cartridges, has also been noted in (Bloomfield et al. 2008), with GAT-1 reductions at a level comparable to those seen in schizophrenia patients (Woo et al. 1998). These results suggest a decrease in GABAergic control throughout the brain of isolation reared animals may be a common origin of a number of the behavioural deficits observed.

The isolation rearing model does have some limitations. As discussed previously, the model is highly susceptible to alterations in protocol, with even minimal repeated handling post-weaning preventing the induction of some behavioural changes, including hyperlocomotion to novelty (Holson et al. 1991) and PPI deficits (Krebs-Thomson et al. 2001; Rosa et al. 2005).

Furthermore, some behaviours such as PPI lack robustness (Cilia et al. 2005; Cilia et al. 2001; Fone and Porkess 2008; Jones et al. 2011a; McIntosh et al. 2013). Additional understanding of the molecular basis underlying the behavioural deficits observed would also increase the strength of this model. Work presented in this thesis attempts to address some of these issues by comparing and combining the isolation rearing model with pharmacological protocols to produce a dual-hit treatment with increased robustness and validity to schizophrenia.

1.3.2 Methylazoxymethanol (MAM)

Administration of the anti-mitotic and anti-proliferative agent methylazoxymethanol (MAM) (Matsumoto and Higa 1966) to pregnant rat dams on gestational day 17 (GD17) can also be considered a developmental disruption model of schizophrenia (Lodge and Grace 2009). By methylating DNA, selectively targeting developing neuronal cells without causing teratogenic effects elsewhere (Cattabeni and Di Luca 1997), gestational MAM impairs the development of brain structures in the foetus undergoing rapid mitosis, with construct validity to the neurodevelopmental theory of schizophrenia aetiology (Jones and Murray 1991; Murray et al. 1991). When administered on GD15, a time of peak neurogenesis in developing rat pups (Bayer 1995), MAM treatment induces gross neuronal histopathology and microencephaly (Banfi et al. 1984), reducing total cortical mass and brain weight (Moore et al. 2006). This pathology does not mimic the subtle changes in tissue morphology throughout the prefrontal cortical and temporal lobe in

schizophrenia (Shenton et al. 2001). By contrast, MAM treatment on GD17, when neuronal proliferation has peaked in cortical and subcortical regions (Bayer 1995), produces subtle reductions in cortical thickness throughout the mPFC, parahippocampal cortices, mediodorsal thalamus and the hippocampus (Chin et al. 2011; Flagstad et al. 2004; Le Pen et al. 2006; Matricon et al. 2010; Moore et al. 2006), increased neuronal packing in the mPFC (Moore et al. 2006), disorganisation and sporadic density of hippocampal pyramidal neurons (Le Pen et al. 2006; Moore et al. 2006), and enlargements of the lateral and third cerebral ventricles (Chin et al. 2011), all noted clinically in schizophrenia (Harrison 1999; Kovelman and Scheibel 1986; Shenton et al. 2001). This similarity in pathology lends considerable validity to the model.

GD17 MAM produces a number of neurochemical and activity-related changes with further similarities with schizophrenia, particularly in dopaminergic function. Elevated dopamine release in the NAc of MAM-treated animals in response to amphetamine treatment, akin to hyperdopaminergia in schizophrenia (Breier et al. 1997; Howes and Kapur 2009), correlates with spontaneous hyperactivity of dopaminergic neurons in the VTA (Flagstad et al. 2004; Lodge and Grace 2007). This is proposed to be through hippocampal pathology (Matricon et al. 2010; Moore et al. 2006), with modulatory activity on the VTA and NAc dopaminergic neurons via glutamatergic projections elevated in MAM rats (Floresco et al. 2001; Floresco et al. 2003; Lodge and Grace 2007), replicating pathology in schizophrenia patients (Heckers and Konradi 2002; Shenton et al. 2001). A decrease in hippocampal PV-positive interneuron number has also been identified (Lodge et al. 2009; Penschuck et

al. 2006), providing the GD17 MAM model with further construct validity (Beasley et al. 2002; Benes et al. 2007; Konradi et al. 2011; Lewis et al. 2005). The behavioural phenotypes created by MAM treatment are dependent on the time of drug administration. Deficits in PPI are evident following GD17, but not GD15, treatment to rats (Moore et al. 2006) and display a post-pubertal onset profile (Hazane et al. 2009; Le Pen et al. 2006), akin to the emergence of schizophrenia. Basal locomotion has been shown to be elevated post-puberty in some studies of GD17 MAM treatment (Hazane et al. 2009; Le Pen et al. 2006), and is reproducible following stimulant treatment (Flagstad et al. 2004; Le Pen et al. 2006; Lodge and Grace 2007; Moore et al. 2006; Penschuck et al. 2006); correlated with increased NAc dopamine release (Flagstad et al. 2004). There is some limited data examining the effect of prenatal MAM on cognition, but it is spread across multiple time points, making direct comparisons difficult. Of the GD17-based studies, a reproducible impairment in hippocampus-dependent spatial learning and memory emerges, with deficits in the radial arm maze (Featherstone et al. 2009), Y-maze (Hazane et al. 2009; Le Pen et al. 2006), and Morris Water Maze (Hazane et al. 2009; Snyder et al. 2013). Few studies have examined the effect of MAM treatment in the novel object discrimination task. GD17 treatment caused a deficit in NOD (Flagstad et al. 2005), whilst GD11/12 treatment produced no impairment (Fiore et al. 1999). Deficits in reversal learning and cognitive flexibility have also been identified with GD17 MAM (Gastambide et al. 2012; Moore et al. 2006), as have impairments in social interaction paradigms (Hazane et al. 2009; Le Pen et al. 2006); the latter representing an important correlate to the negative

symptoms of human schizophrenia. Earlier MAM administration does not necessarily translate to a more severe behavioural phenotype, despite severe neuropathology, with GD9-15 MAM producing severe reductions in total brain mass and entorhinal, prefrontal and striatal volumes, but no accompanying deficit in PPI, latent inhibition, or a conditioned freezing paradigm (Jongen-Relo et al. 2004), and only minor reversal learning deficits (Leng et al. 2005).

Comparatively few studies examining the predictive validity of the MAM model with standard or putative antipsychotic agents have been published. Whilst a single treatment with risperidone is able to selectively reverse basal hyperlocomotion induced by GD17 MAM treatment in rats, haloperidol and clozapine are non-selective in causing reductions in locomotor activity across MAM- and saline-treated animals (Le Pen et al. 2011).

The lack of available drug-reversal data limits the predictive validity of the MAM model. Combined with the apparent disparity between the degree of neuropathology and behavioural deficits induced by treatment on different gestational days, many questions still remain over this model. However, with very clear and reproducible neuroanatomical deficits that closely mirror those seen in schizophrenia, the GD17 MAM model has some value. Thus, work in this thesis attempts to combine the MAM and isolation rearing models to establish whether the two might have synergistic effects on behaviours in the adult rat, with greater translational relevance to schizophrenia.

1.3.3 Phencyclidine (PCP)

By reversibly binding within the channel of ionotropic NMDA receptors, antagonists such as PCP block calcium ion influx following voltage- and ligand-activated channel pore opening, preventing the activation of calcium-triggered signalling cascades. The ability of NMDA receptor antagonists to artificially induce NMDA receptor hypofunction makes these compounds an attractive option for use in a variety of preclinical models, and provides construct validity (Anastasio and Johnson 2008b; Csernansky et al. 2005; Keilhoff et al. 2004; Li et al. 2011a; Neill et al. 2010; Sabbagh et al. 2012; Wang et al. 2001).

Whilst acute administration of PCP in adulthood has been used to cause alterations in behaviours with salience to all symptom domains of schizophrenia including hyperlocomotion (Kalinichev et al. 2008), impaired social interaction (Sams-Dodd 1995), decreased PPI response (Bakshi et al. 1994; Li et al. 2011a; Takahashi et al. 2006) and cognitive impairment (Idris et al. 2009; Thomson et al. 2010), a number of these deficits are short-lived. A stable, longer-lasting profile of changes more valid to schizophrenia is achieved with repeated PCP administration. This protocol was hypothesised to recapitulate effects observed in the clinic where schizophrenia-like symptoms persist in recreational PCP users following cessation of their abuse (Rainey and Crowder 1975), and structural deficits in the frontal and temporal lobes can be observed using PET scanning (Hertzmann et al. 1990).

One of the most reproducible effects of repeated PCP administration in rodents is sensitization to subsequent PCP challenge. Acute treatment causes an

elevation in locomotor activity in controls, but the degree of hyperlocomotion is markedly increased by previous drug exposure (Abekawa et al. 2002; Fletcher et al. 2005; Xu and Domino 1994), mirroring the clinical exacerbation of positive symptoms (Lahti et al. 1995; Malhotra et al. 1997), providing face validity. Both acute (Bakshi et al. 1994; Li et al. 2011a) and repeated/subchronic PCP treatment (Li et al. 2011a; Martinez et al. 1999; Takahashi et al. 2006; Tunstall et al. 2009) induce PPI deficits in rodents, however this deficit is transient, even after repeated exposure, and the ability to recover from PCP-induced deficits may be partially responsible for the variability in reported outcomes.

There is also face validity to the negative and cognitive symptoms of schizophrenia, with deficits in social interaction (Sams-Dodd 1998a; b; Snigdha and Neill 2008a; b), visual and spatial cognition (Beninger et al. 2010; McKibben et al. 2010; Nagai et al. 2009; Roseman et al. 2012), and reasoning and problem solving (Idris et al. 2009; Large et al. 2011) observed in PCP-based models. Some of these deficits are also susceptible to challenge with antipsychotic compounds (Grayson et al. 2007; Hashimoto et al. 2005; McLean et al. 2010b), potentially providing predictive validity. However, as the evidence for efficacy of these compounds against cognitive deficits in the clinic is limited at best, these results are suggestive of false-positive effects in PCP-based models rather than true predictive validity.

A number of relevant neuropathological changes are readily produced by PCP treatment. As well as amphetamine-induced hyperlocomotion (Beninger et al. 2010) and elevated mesolimbic dopamine activity (Jentsch et al. 1998), basal

levels of dopamine in the PFC are reduced by chronic PCP treatment to rats (Jentsch et al. 1997). Changes in receptor expression levels are also common, with striatal D_1 receptor expression decreased (Choi et al. 2009), and GABA subunit levels altered throughout the frontal cortex, hippocampus and striatum (Beninger et al. 2010). Furthermore, alterations in NMDA receptor binding in the hippocampus, NAc, striatum and cortex occur following chronic PCP treatment (Newell et al. 2007). A reduction in PV-positive GABAergic interneuron immunoreactivity in the hippocampus and frontal cortex is noted after subchronic PCP treatment (Abdul-Monim et al. 2007; Jenkins et al. 2010b; McKibben et al. 2010), along with reductions in GAT-1 and the GABA synthesising enzyme glutamate decarboxylase 1, 67kDa-isoform (GAD_{67}) mRNA levels in the cerebellum (Bullock et al. 2009). Both protein and mRNA levels of GAD_{67} and GAT-1 are decreased throughout the brain of schizophrenia patients (Akbarian et al. 1995; Bullock et al. 2008; Guidotti et al. 2000; Hashimoto et al. 2003; Lewis et al. 2005) and may be linked to disease development (Curley et al. 2013; Zhao et al. 2007).

In order to add a neurodevelopmental aspect to the PCP model, repeated administration of high dose PCP in early life was examined (Wang et al. 2001). Administration of PCP at 10mg/kg on PND 7, 9 and 11 produced PPI impairments, a delay in acquisition of the spatial learning task, and hyper responsiveness to an acute PCP dose (2mg/kg) in a locomotor task post-adolescence (Wang et al. 2001). Subsequently, perinatal PCP administration to rats has been shown to induce lasting deficits in spatial working memory and reversal learning in the Morris Water Maze (Andersen and Pouzet 2004; Sircar

2003), impaired delayed and continuous spatial alternation performance (Boctor and Ferguson 2010; Wiley et al. 2003), impaired novel object recognition performance (Redrobe et al. 2012), deficits in PPI which are more enduring than caused by acute adult PCP treatment (Anastasio and Johnson 2008a; Takahashi et al. 2006), deficient social novelty discrimination (Harich et al. 2007) and a robust sensitization to subsequent PCP administration (Anastasio and Johnson 2008a; Boctor and Ferguson 2010). Similar treatment in mice revealed impaired spatial working memory, produced hyperlocomotion in response to a subsequent PCP challenge, and significantly reduced social interaction behaviours (Nakatani-Pawlak et al. 2009).

Apoptotic cell death induced by neurotoxicity in the frontal cortex may be partly responsible for the behavioural alterations seen (Wang et al. 2001; Wang and Johnson 2005), with four pro-apoptotic genes up-regulated and four anti-apoptotic genes down-regulated at PND12 by perinatal PCP treatment (Liu et al. 2010). This is accompanied by altered NMDA receptor expression in the frontal cortex (Anastasio and Johnson 2008b; Wang et al. 2001). A highly reproducible and enduring deficit in PV-positive GABAergic interneurons has also been observed in the cortex of rats following perinatal PCP treatment (Kaalund et al. 2013; Redrobe et al. 2012; Wang et al. 2008).

Whilst administration of PCP in early-life rather than adulthood maintains the vast majority of schizophrenia-like behaviours and neuropathology obtained by adult treatment, and adds a neurodevelopmental aspect to the model, it still produces false positive effects in drug reversal studies. Clozapine reverses impairments in social novelty (Harich et al. 2007) and social interaction tasks

(Nakatani-Pawlak et al. 2009), whilst it has little beneficial effect clinically on the cognitive or negative symptoms of schizophrenia (Keefe 2007).

Alternative approaches to using PCP to model schizophrenia utilise ketamine or MK-801. Whilst these compounds have similar modes of action to PCP and can produce deficits in spatial working memory, cognitive flexibility and attentional set-shifting (Stefani and Moghaddam 2005a; b), as well as impaired PPI performance (Cilia et al. 2007; Harris et al. 2003; Sabbagh et al. 2012; Uehara et al. 2009) they have some weaknesses. Acute ketamine treatment produces only small impairments in conditioned emotional response (Bolton et al. 2012), and repeated perinatal ketamine doesn't produce the impairment in spatial alternation caused by perinatal PCP (Boctor and Ferguson 2010). The potential for false positive effects of antipsychotics is also maintained, with clozapine able to reverse MK-801-induced NOD deficits in mice (Mutlu et al. 2011) and social memory deficits in rats (Shimazaki et al. 2010). Although hippocampal PV-positive interneurons are reduced in both ketamine (Keilhoff et al. 2004) and MK-801 (Braun et al. 2007) treated rats, MK-801 does not produce the same result in the PFC (Braun et al. 2007), a relative strength of PCP. These results support the use of PCP-based models of schizophrenia over other NMDA receptor antagonist models. The current thesis therefore also examines the combined effect of perinatal PCP and isolation rearing in the rat to attempt to produce more robust and relevant deficits than either treatment alone.

Table 1.1 – Table comparing the behavioural and neuropathological characteristics of the isolation rearing, GD17 MAM and perinatal PCP treatment models of 'schizophrenia-like' symptoms in rats

	Isolation Rearing	GD17 MAM	Perinatal PCP Treatment
"Positive" Symptoms	Hyperlocomotion in a novel arena[1,2]	Increased amphetamine-induced locomotion[18,19]	Increased amphetamine-induced locomotion[24]
"Negative" Symptoms	Increased social aggression[3]	Decreased social interactions[19]	No effect on social interaction[25]
Visual Learning and Memory	Novel Object Discrimination impairments[4,5]	Novel object discrimination impairments[20]	Novel Object Discrimination impairment[26]
Sensorimotor Gating	Prepulse Inhibition Impairments[6,7]	Prepulse Inhibition Impairments[18]	Prepulse Inhibition impairments[24]
Spatial Working Memory	Morris Water Maze Improvements[8] and Impairments[9]	Impaired Morris Water[20] and Y-maze[18] performance	Decreased acquisition of spatial alternation task[24]
Problem Solving	Impaired reversal learning[10]	Impaired reversal learning[18,20]	Impaired reversal learning[27]
Additional Behaviours	Impaired emotional conditioning[11]	-	Sensitization to NMDA receptor antagonists[28]
Neuro-pathology	Increased dopamine release in NAc after amphetamine[12] Altered dopamine receptor distribution[13,14] Loss of PV+ interneurons in hippocampus[15] Structural PFC deficits[16]	Loss of PV+ interneurons in hippocampus[21,22] Structural deficits in PFC[18] Elevated glutamate output from vHipp[23]	Altered NMDA receptor subunit expression[29] Loss of PV+ interneurons in cortex[26,30,31] Increased oxidative stress[32]
Relative Strengths	No confounding drug treatment Wide-ranging deficits	Strong neuropathology	Strong glutamate involvement
Relative Weaknesses	Susceptible to procedural differences Un-robust deficits[17]	Less complete symptom profile Limited reversal data	False-positive drug reversal

References: (Gentsch et al. 1988)[1], (Varty et al. 2000)[2], (Wongwitdecha and Marsden 1996b)[3], (Bianchi et al. 2006)[4], (Watson et al. 2012b)[5], (Cilia et al. 2001)[6], (Cilia et al. 2005)[7], (Wongwitdecha and Marsden 1996a)[8], (Hellemans et al. 2004)[9], (Jones et al. 1991)[10], (McIntosh et al. 2013)[11], (Jones et al. 1992)[12], (Hall et al. 1998)[13], (Gill et al. 2013)[14], (Harte et al. 2007)[15], (Schubert et al. 2009)[16], (Fone and Porkess 2008)[17], (Moore et al. 2006)[18], (Flagstad et al. 2004)[19], (Flagstad et al. 2005)[20], (Lodge et al. 2009)[21], (Penschuck et al. 2006)[22], (Lodge and Grace 2007)[23], (Wang et al. 2001)[24], (du Bois et al. 2008)[25], (Redrobe et al. 2012)[26], (Andersen and Pouzet 2004)[27], (Anastasio and Johnson 2008a)[28], (Anastasio and Johnson 2008b)[29], (Kaalund et al. 2013)[30], (Wang et al. 2008)[31], (Radonjic et al. 2010)[32]

1.3.4 Dual-Hit Models

In an attempt to overcome the weaknesses of preclinical models, emerging research focuses on combining two protocols to create 'dual-hit' models that may have improved robustness and face, construct and predictive validity.

In rats, much work has focused on the isolation rearing model, with its broad variety of reproducible behavioural deficits and relatively strong construct validity to the early-life adversity aetiology theory, combining it with pharmacological challenges. In almost all studies where the behaviour was assessed, isolation rearing induced baseline hyperlocomotion in a novel arena (Ashby et al. 2010; Gill et al. 2013; Hickey et al. 2012), but only twice was this effect enhanced by a second hit, subchronic or neonatal MK-801 (Lim et al. 2012; Simpson et al. 2010). Methylphenidate treatment did not affect anxiety caused by isolation rearing (Gill et al. 2013), and subchronic MK-801 did not affect isolation-induced polydipsia (Hawken et al. 2013) or emotional processing (Simpson et al. 2010). Subchronic methamphetamine produced similar deficits to isolation in PPI and social interaction studies, but when combined no additive effects were seen (Strauss et al. 2014). In contrast, combination of MK-801 treatment on PNDs 7-10 with isolation rearing produced consistent PPI and NOD deficits in Sprague-Dawley rats, where single treatments did not (Lim et al. 2012). These models, particularly those utilising NMDA receptor antagonists, could be considered to have greater construct validity due to the addition of glutamatergic hypofunction to the model, which isolation rearing alone lacks (Fone and Porkess 2008). Increased behavioural face validity is somewhat lacking in this approach, aside from the

work by Lim and colleagues, but some neurobiological effects of dual-hit treatment in rats have been observed. Subchronic MK-801 elevated $GABA_A$ receptor expression in the hippocampus of adult rats, coinciding with isolation-induced elevations in the frontal cortex, and increased GAT-1 expression in the frontal cortex and hippocampus (Hickey et al. 2012). Thus, combining perinatal NMDA receptor antagonists with isolation rearing appears a viable option for dual-hit treatment; but as discussed, PCP represents a potentially better pharmacological agent than MK-801. For this reason, work in this thesis will combine isolation rearing and perinatal PCP treatment.

Another approach used as one of two dual-hits in rodents is maternal immune activation through prenatal Poly(I:C) administration (Dalton et al. 2012; Deslauriers et al. 2013; Giovanoli et al. 2013; Richtand et al. 2012; Yee et al. 2011). This treatment has been successfully combined in mice with unpredictable mild stress (Giovanoli et al. 2013) or restraint stress (Deslauriers et al. 2013) to produce improved deficits in PPI paradigms, as well as increased sensitivity to acute MK-801 and amphetamine challenge. In rats, however, results have been mixed, with combined Poly(I:C) and adolescent stress only able to produce additional alterations in drug place-preference behaviour (Richtand et al. 2012), and not PPI or anxiety-related behaviour or physiology (Yee et al. 2011). The benefit of Poly(I:C)-based models is their very strong construct validity, given the available clinical odds ratio data (Sullivan 2005). Combining this model with stressor protocols may provide even greater validity, if more convincing behavioural data is forthcoming.

Additionally, Poly(I:C)-based models can be performed in mouse lines carrying a schizophrenia-relevant genetic alteration, including DISC-1 and Nurr-1 (Abazyan et al. 2010; Lipina et al. 2013; Vuillermot et al. 2012). These dual-hits have considerable construct validity, combining an underlying genetic predisposition with an obstetric complication, but as yet the face validity of these models is little better than single-hit treatments. Combining DISC-1 mutations with Poly(I:C) treatment has little additive effect on behaviours relating to the cognitive or positive symptoms of schizophrenia (Lipina et al. 2013), but there is some indication that negative symptom-like traits, such as social interaction and anxiety may be additionally impaired in dual-hit animals (Abazyan et al. 2010). Conversely, Nurr-1 mutants treated prenatally with Poly(I:C) show some potential additive deficits in cognition in latent inhibition and visual operant tasks (Vuillermot et al. 2012). A similar approach is to combine genetic knock-out models with stress protocols. DISC-1 mutations combined with isolation stress produced marked deficits in PPI and in a forced swim test (Niwa et al. 2013), whilst a combination with chronic social defeat produced little additional benefit (Haque et al. 2012). Similarly, neuregulin-1 knockouts did not present additional behavioural deficits having undergone stressor protocols (Chesworth et al. 2012; Desbonnet et al. 2012).

No publications have examined the role of prenatal MAM administration in a dual-hit model of schizophrenia. This thesis will therefore examine GD17 MAM in combination with post-weaning social isolation.

Chapter 2

Combined Gestational MAM Treatment and Post-weaning Social Isolation as a Model of 'Schizophrenia-like' Symptoms in the Rat

2.1 Introduction

Despite the wealth of preclinical models of schizophrenia available, the limitations are notable. The rodent paradigms of rearing rat pups in social isolation after weaning (Fone and Porkess 2008; Jones et al. 2011b) and prenatal treatment with the anti-mitotic agent methylazoxymethanol (Hradetzky et al. 2012; Lodge and Grace 2009; Moore et al. 2006), produce a variety of behavioural alterations including hyperactivity in a novel arena or increased amphetamine-induced activity, both thought to reflect 'positive-like' symptoms of schizophrenia (Table 1.1). However, any accompanying loss of working memory and negative symptoms may be more sensitive to procedural differences, such as the length of isolation rearing (Fone and Porkess 2008), or the gestational time of MAM administration (Jongen-Relo et al. 2004; Leng et al. 2005; Moore et al. 2006). Combining multiple interventions to attempt to produce more extensive and relevant abnormalities is a logical and worthy development, an approach not attempted until recently (Ashby et al. 2010; Hawken et al. 2013; Hickey et al. 2012; Simpson et al. 2010). Previous work combining isolation rearing and subchronic administration of the NMDA receptor antagonist MK-801 found only mild behavioural changes induced, with little or no evidence of additive or synergistic effects of combined administration (Hickey et al. 2012). However, a study utilising neonatal pharmacological challenge of the NMDA receptor with MK-801 in combination with isolation rearing did produce marked and robust deficits in

relevant behavioural paradigms (Lim et al. 2012), and so further investigation of combination models is warranted.

Based on their strength as pre-existing preclinical models of schizophrenia-like symptoms (see Table 1.1), and expertise in our lab, the combination of prenatal MAM treatment and subsequent rearing in social isolation post-weaning was examined in a variety of paradigms to mimic the deficits seen in schizophrenia. Secondly, the neurobiological outcome of this 'dual-hit' was examined with specific attention paid to the hippocampus, a key brain region in theories of schizophrenia aetiology. To ensure that this dual-hit did not produce severe deficits such as stereotypy in the home cage, a pilot study with six pregnant dams was undertaken, before an expanded twelve dam protocol. As this was the first time MAM administration had been used in our laboratory, the pilot study also allowed for refinement of the experimental techniques used. To best ensure a successful outcome, the well-characterised isolation rearing protocol previously utilised, and behavioural tests already validated in our laboratory, were used in the study. Furthermore, the two challenges used were presented both alone and in combination, giving four treatment groups (control, isolation, MAM, and isolation-MAM), to allow evaluation of the effectiveness of each individual treatment and any additive or synergistic effect of the combination. In keeping with previous isolation rearing studies, Lister-Hooded rats were selected since the behavioural and neurochemical changes evoked have been very well described in our laboratory (Bianchi et al. 2006; Jones et al. 2011a; McIntosh et al. 2013; Schubert et al. 2009), showing their relevance to 'schizophrenia-like' symptoms. Furthermore, the Lister-Hooded rat has good

visual acuity, making it ideal for visual attention tasks compared to albino strains (Broersen and Uylings 1999). However, no literature could be found on previous work assessing the effect of MAM in Lister-Hooded rats at any gestational day. Previous work with gestational MAM has been documented in Fischer 344 (Moore et al. 2006), Sprague-Dawley (Hradetzky et al. 2012; Lodge and Grace 2007), and Wistar (Flagstad et al. 2005) strains, and also in studies involving cognitive tasks like those reported herein. As there is a lack of consensus on the most suitable rat strain to utilise in prenatal MAM studies, the current study bases a new protocol on addition of prenatal MAM administration with isolation rearing in Lister-Hooded rats.

2.1.1 Hypothesis

Based on the previous literature detailing the extensive cognitive deficits observed in both isolation-reared and prenatal MAM-treated rats, combination treatment was hypothesised to produce a phenotype in adolescent rats that exhibited deficits in all cognitive paradigms investigated, and with greater robustness/reliability than previously reported in either preclinical model used alone. Furthermore, the 'dual-hit' protocol would produce significant neurobiological changes in the hippocampus of rats with some relevance to schizophrenia in patients.

2.2 Materials and Methods

2.2.1 Animals

Pregnant female Lister-Hooded rats (BMSU, University of Nottingham, Pilot study *n*=6, Charles River UK, Margate, Main Study *n*=12), were injected with methylazoxymethanol (MAM, 24mg/kg base weight, adjusted from 28mg/kg salt weight, i.p.; obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository, Midwest Research Institute, Kansas City, MO, USA) or saline vehicle (1ml/kg) as control, on gestational day 17 (Table 2.1). From the resulting litters, male pups (Pilot study *n*=30, Main Study *n*=63) were weaned on PND23 so that approximately equal numbers of each litter were housed in groups of 3-4 (32x51cm polycarbonate cages with metal grid lids), or placed into social isolation (25x42cm cages), giving four conditions; group-housed control (GH-Con), group-housed MAM-treated (GH-MAM), isolate control (Iso-Con) and isolate MAM-treated (Iso-MAM) (Table 2.1).

Rats were reared for 40 days post-weaning prior to beginning behavioural tests (Figure 2.1), housed on a 12h light-dark cycle (06:00-18:00 light), with food and water provided *ad libitum*, and the ambient temperature and humidity maintained (21±2°C and 45±15% respectively). All subsequent behavioural studies were performed such that the observer was unaware of rearing condition and all apparatus was cleaned with 20% w/v ethanol to remove odour cues between tests. All experiments complied with the UK Home Office Animals (Scientific Procedures) Act 1986, and were approved by the local ethical review committee and conform to the ARRIVE guidelines.

Table 2.1 Table to show the prenatal drug treatment and post-weaning housing condition of each pup in (A) the pilot study, and (B) the main study. Also shown, the behavioural tests undertaken, and the post-mortem analysis that was performed on tissue from each litter.

Dam	Treatment received	Dam Mass (g)	Gestation Length (days)	Litter		Male Assignments		Behaviours	Post-Mortem
				Male	Female	Group	Isolation		
1	MAM on GD17 (24mg/kg i.p.)	310	23	7	9	3	3	All animals performed LMA, NOD, PPI and CER	Total brain mass measured, hippocampus extracted, sectioned and volume estimated in all animals
2	Saline on GD17 (1ml/kg, i.p.)	289	22	2	3	0	2		
3	Saline on GD17 (1ml/kg, i.p.)	302	22	3	4	3	0		
4	Saline on GD17 (1ml/kg, i.p.)	297	22	3	5	0	3		
5	MAM on GD17 (24mg/kg i.p.)	354	22	10	7	5	5		
6	Saline on GD17 (1ml/kg, i.p.)	311	23	6	9	3	3		

Dam	Treatment received	Dam Mass (g)	Gestation Length (days)	Litter		Male Assignments		Behaviours	Post-Mortem
				Male	Female	Group	Isolation		
1	Saline on GD17 (1ml/kg, i.p.)	306	23	5	7	3	2	LMA, NOD, PPI, MWM	Hippocampus, striatum, frontal cortex and hypothalamus dissected and weighed. Tissue used for HPLC analysis of monoamine concentration.
2	Saline on GD17 (1ml/kg, i.p.)	329	23	7	6	3	4	LMA, NOD, PPI, MWM	
3	Saline on GD17 (1ml/kg, i.p.)	283	22	8	5	4	4	LMA, NOD, PPI, CER	
4	MAM on GD17 (24mg/kg i.p.)	297	22	5	8	3	2	LMA, NOD, PPI, CER	
5	MAM on GD17 (24mg/kg i.p.)	287	22	7	5	3	4	LMA, NOD, PPI, MWM	
6	MAM on GD17 (24mg/kg i.p.)	340	23	3	8	0	3	LMA, NOD, PPI, CER (x2), MWM (x1)	
7	MAM on GD17 (24mg/kg i.p.)	303	22	7	3	4	3	LMA, NOD, PPI, CER	
8	MAM on GD17 (24mg/kg i.p.)	295	23	1	3	0	1	LMA, NOD, PPI, CER, MWM	
9	MAM on GD17 (24mg/kg i.p.)	307	23	5	6	4	1	LMA, NOD, PPI, CER, MWM	
10	Saline on GD17 (1ml/kg, i.p.)	311	23	5	6	0	5	LMA, NOD, PPI, CER (x4), MWM (x1)	
11	Saline on GD17 (1ml/kg, i.p.)	296	22	5	4	3	2	LMA, NOD, PPI, CER, MWM	
12	Saline on GD17 (1ml/kg, i.p.)	283	23	5	3	3	2	LMA, NOD, PPI, CER	

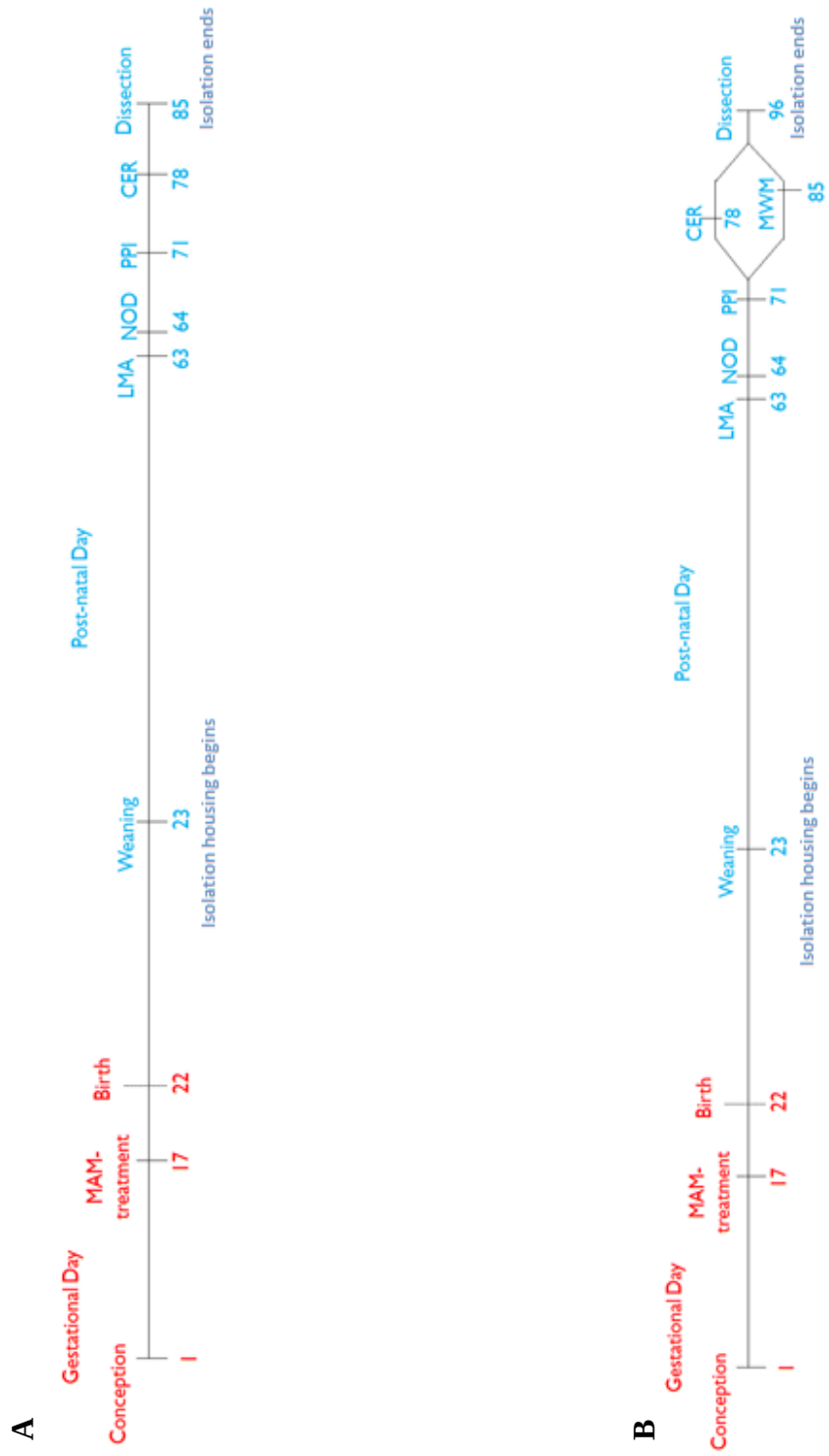

Figure 2.1 – Timestick representations of the protocols used in (A) the pilot and (B) main study. Protocols were identical until PND 78. In the pilot study, all rats performed the CER task, whereas in the main study, half were excluded from CER and performed the Morris Water Maze task from PND 85 onwards instead.

2.2.2 Locomotor Activity

To examine a motor response thought to be related to the positive aspects of schizophrenia, open field locomotor activity (LMA) testing occurred in twelve activity chambers, consisting of clear Perspex boxes (39 x 23.5 x 24.5cm), screened from their neighbours by white opaque sheeting. Rats were assigned to a chamber in a pseudo-random manner. Activity boxes were crossed by infrared beams 2cm above the floor (Photobeam Activity System, San Diego Instruments, USA) to measure ambulatory movements. The number of beams broken was counted in 5 min time bins for 1 h to measure horizontal ambulatory activity (a movement breaking two beams in a consecutive sequence (Bianchi et al. 2006; Hewitt et al. 2005; Jones et al. 2011b)) presented as locomotor counts distinct from stereotyped behaviour (repetitive breaking of one beam), which was not analysed.

2.2.3 Novel Object Discrimination

In order to assess visual learning and memory in a simple discrimination/ recognition task, novel object discrimination (NOD) was tested 24 h after the completion of locomotor activity, with rats assigned to the same chamber as during locomotor activity testing. The task was performed as described previously (Bianchi et al. 2006; King et al. 2004), but with some minor modifications. Briefly, rats were placed in a chamber for a 3 min acclimatisation period, before being returned to their home cage for 1 min, during which two identical objects (8cm high plastic bottles, 5cm diameter, filled with water and secured to the floor by blue-tac) were introduced to the

chamber. Rats were then returned to chamber, and exploration of each object recorded for 3 min (familiarisation, Trial 1). During a 2 h inter-trial interval the rats were returned to their home cage, and one object selected in a pseudo-random manner was switched for a visually distinct "novel" object (identical bottle covered in three rings of black 2cm wide tape) in each chamber. A second 3 min trial (choice, Trial 2) was performed, and object exploration (directional attention to each object with the nose ≤ 1cm away (sniffing with active vibrissae)) recorded using stop watches. Climbing on or chewing the object was not recorded as active exploration. Any rats that failed to explore both objects in trial 1 (minimum 5s per object), or failed to spend a total of 5s exploring both objects in trial 2 were judged to have not completed the task, and so excluded from analysis. Rats were also excluded where biting/chewing led to damage to the objects in the second, choice trial leading to sustained directed attention to the damage caused (loose tape, water leak etc.).

To analyse the results, a direct comparison between the time spent exploring the "novel" and "familiar" objects in the second, choice trial was made. A discrimination ratio (D1) was calculated by dividing the time spent exploring the novel object by the total (novel + familiar) object exploration time in the second choice trial, providing an index of preferential object exploration independent of total exploration. These D1 discrimination ratios were compared between treatment groups.

2.2.4 Prepulse Inhibition of Acoustic Startle

Rats were examined in a prepulse inhibition of acoustic startle test (PPI) to evaluate any change in sensorimotor gating, which is thought to map to the preattentional processing cognitive domain in man. Altered PPI is recognised to share a similar neurobiological basis to sensorimotor gating deficits seen in schizophrenia (Geyer et al. 2001). Prepulse inhibition of acoustic startle testing was performed in four computerized startle boxes (San Diego Instruments, San Diego, CA, USA) consisting of a clear Perspex tube attached to an accelerometer in a sound-proofed chamber containing a loudspeaker to administer the acoustic stimuli, as described previously (Jones et al. 2011a; Schubert et al. 2009). After acclimatisation to white background noise (65dB, 5mins), a series of 66 x 120dB tones (40ms in duration) were delivered, either alone, or preceded by a 20ms prepulse of 72, 76, 80 or 84dB, with an unpredictable inter-trial interval (10-20 seconds). The amplitude of the startle response to 120dB tone alone was compared to the final 120dB responses to calculate habituation, and compared to that produced by prepulse-pulse combinations to assess PPI, expressed as a percentage of the average pulse-alone startle amplitude. Previous studies by our group have shown that startle responses to 72dB trials fail to elicit attenuation of PPI, but exclusion of these trials leads to greater variation in response to other prepulse intensities, so the data from 72dB prepulse trials were collected, but excluded from analysis.

2.2.5 Conditioned Emotional Response

Rats were tested for their conditioned emotional response (CER) in a hippocampus- and amygdala-dependent contextual and cue-conditioned fear learning protocol (Anagnostaras et al. 1999; Fanselow 2000; Sigurdsson et al. 2007; Woods et al. 2012). Rats were placed in the light section of the partitioned CER chamber (510 (W) x 250 (D) x 240 (H) mm internal; 580 x 360 x 305 mm external, PanLab, LE 916) for 30 seconds, after which a centre door automatically opened and allowed the subject access to the dark part of the chamber. The latency for the rat to enter the dark chamber was recorded and the centre door closed. Following 30 seconds habituation, the rat was presented with a conditioned stimulus - a light and sound tone (5 sec, 40 Lux, 89 dB, 3 KHz, CS), and an unconditioned stimulus - an unavoidable foot shock (1 sec, 0.4 mA, US). This was followed by a 55 second consolidation period, after which the light/tone/shock sequence was presented once more. A further consolidation-CS+US-consolidation cycle was completed and the rat then returned to its home cage. Twenty-four hours later, the rats were returned directly to the dark side of the CER chamber with the centre door permanently closed. On this occasion, no stimuli were presented, but freezing, defined as a lack of movement for more than 1s (excluding respiration) was scored for 5 minutes. A further 24 hours later (48h after initial exposure) the rats were again returned to the dark side of the chamber. For 5 minutes, no stimuli were presented and freezing was scored. Following this, the CS was re-presented in absence of the US. Freezing was again scored for 5 minutes after the CS presentation and the rats then returned to their home cage.

2.2.6 Morris Water Maze

In order to assess spatial learning and memory, including a reversal learning aspect to examine cognitive flexibility, an in-house Morris Water Maze protocol was designed, based on concepts described previously (Morris 1984). Briefly, rats were taught to locate a hidden, submerged platform in a pool using visual cues positioned around the experimental arena by repeated exposure to the context.

Prior to the initiation of training, rats were exposed to the water maze (2m diameter, water depth of 80cm, made opaque with liquid latex added to the water) without the presence of the platform for a 60s habituation period. On experimental day one, rats were exposed to the water maze three consecutive times (trials) with the circular platform (10cm diameter, Atlantis Platform, Ugo Basile, Comerio, VA, Italy) always located in one of four fixed position: North, South, East, West (Figure 2.2). For each of the three trials, rats were placed at the edge of the maze, facing the wall, in one of the three other North/South/East/West locations, so that across the day, each location was represented. Upon placing in the maze, a trial lasted 90 s of free exploration or until the platform was located. If successful, the rat remained on the platform for 15 s to reinforce its location, before being removed from the arena and hand-dried prior to the next trial 1 min later. If unsuccessful after 90 s, the rat was removed from the water and immediately placed on the platform, where it was then left for the 15 s reinforcement period. If, during the reinforcement, the rat left the platform and did not immediately return, the rat was manually placed back on the platform to continue the reinforcement period.

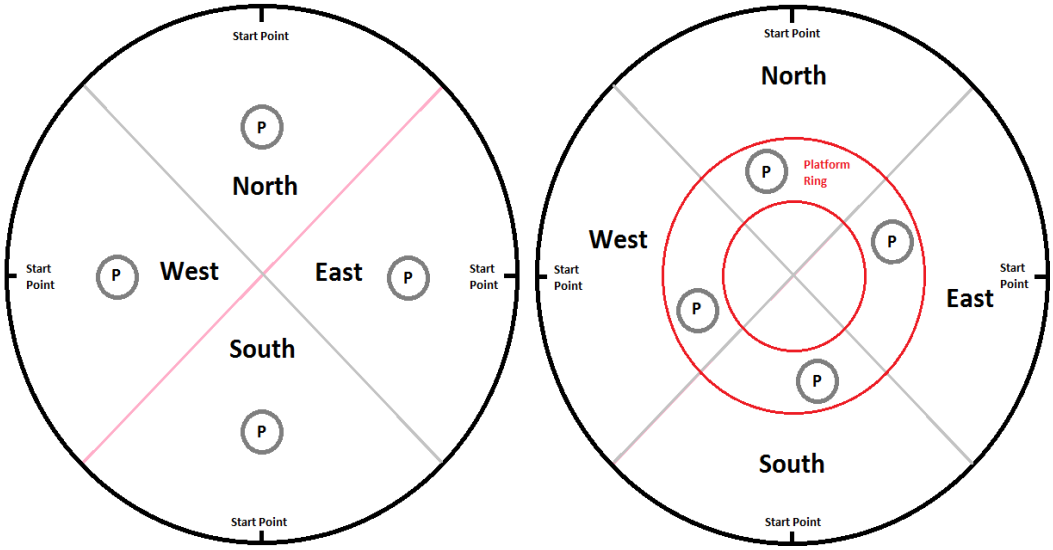

Figure 2.2 – Diagrammatic representation of the Morris Water Maze apparatus, split into 4 quadrants (North, South, East, West). The left panel shows the 4 positions rats were taught to locate a fixed-position hidden platform (P), as well as the four starting points which each trial began from in a pseudo-random order. The right panel shows the new locations of the platform for the reversal learning aspect of the protocol, as well as the "Platform Ring" area used during Probe 3 to assess exploration of the maze.

After the completion of three trials with reinforcement periods, rats were returned to their home cage. For 5 consecutive days, each rat was trained for 3 trials per day, with the platform in a fixed location, and the start point of each trial in a new pseudo random order. Following completion of trials 13, 14 and 15 on day five, a probe trial was performed. The platform was completely submerged and fixed and the bottom of the pool by electromagnet, making it undiscoverable by the rat. Rats were placed in the maze in one of the four start points (randomly assigned) and their exploration of the maze recorded for 60 s, after which the platform was released, allowing it to rise to the surface in its previously fixed position. The rat was then directed to the platform for a 15s reinforcement period, such that the probe trial did not cause extinction of the previously learned platform location.

Sixty hours (2.5 days) after probe 1, a second probe trial was performed, to assess the rats' retention of the task and platform location after an extended period away from the experimental context. This trial was identical to probe 1 and termed probe 2. Immediately after completion of probe 2, a reversal learning period was initiated with a rule change included, to assess cognitive flexibility. For this period, the platform was no longer in a fixed position, but located in one of four different positions around the maze at 60 cm from the arena wall, alternating for each individual trial, and never in the initially "correct" quadrant (Figure 2.2). This new rule forced the rats to adopt a different search strategy and abandon their previously learnt rule that the platform location was fixed. As before, each rat performed three trials per day, with the trial start points assigned randomly, never in the same maze quadrant

as the platform. In this study, rats were trained for three experimental days (nine trials), before being assessed in a final probe trial (probe 3), identical to those performed previously.

Throughout the experiment, all trials were recorded via a video camera fixed above the centre of the arena, with the rats' movements tracked and analysed by Ethovision software, at four images per second. For each training trial, the software recorded the latency for the rat to locate the platform, and during probe trials assessed what percentage of the 60s trial was spent in each area of the maze, indicating whether there was a preference for exploration in the "correct" quadrant in probes 1 and 2, as well as the time spent exploring in the "platform ring" in probe 3 – the circular zone around the maze at approximately 60cm from the walls where the four different platform locations were found.

2.2.7 Neuroanatomical Analysis

One week post-behaviour, rats were killed by concussion followed by immediate decapitation, and brains removed on a cold plate (4ºC). In the pilot study, whole brains were weighed and preserved in cooled OCT embedding matrix (VWR International Ltd, UK). OCT-coated brains were sliced using a Leica CM 100 CryoStat (Leica Microsystems, UK) into 75µm coronal sections in the region containing the hippocampus (approximately -1.8mm to -6.8mm from the bregma (Paxinos 1997)), mounted on 3-amino-propylmethoxysaline (APES)-coated slides and stained with cresyl violet to visualise internal structures. Cortical slices containing hippocampal cross-sections were imaged

electronically (AcquisBio, Synoptics Ltd., Cambridge, UK) and analysed for cross-sectional area (Scion Image, Scion Corp., Frederick, MD, USA). Using Cavalieri's principle (Volume of 3D structure = ∑ cross-sectional areas x distance between cross-sectional surfaces (Gundersen and Jensen 1987)), and systematic-random sampling of cross-sectional areas, an estimate of the total hippocampal volume was calculated. In the main study, the right hippocampus and frontal cortex (FC) were removed by blunt dissection and weighed before freezing in liquid nitrogen for future use (see below). The striatum and hypothalamus were also blunt dissected and weighed before being discarded.

2.2.8 HPLC Analysis

To each hippocampal and FC sample dissected, a perchloric acid (PCA) homogenisation solution was added (0.1M PCA, 0.02% w/v sodium metabisulfite, 0.01% w/v EDTA) at 1500µL per 100mg of tissue. Using a sonic probe (Soniprobe 150, Dawe Instruments Ltd, London, Output 20kHz), tissue was homogenised in the PCA solution and the resulting homogenate centrifuged at 17500g, 4ºC, for 20mins and the supernatant removed and stored at -80ºC. Before analysis, the samples were filtered through a 0.45µm PVDF 4mm syringe filter.

For HPLC analysis, a phenomenex sphereclone column (4.6x150mm, 5uM Octadecyl Saline (ODS(2))) was used with an initial mobile phase composition of 0.05M KH_2PO_4, 0.1mM EDTA, 0.16mM Octane Sulfonic Acid, 21% Methanol, adjusted to pH3 with orthophosphoric acid and a 0.8ml/min flow rate. A linearity check was performed to ensure the detector was responding in

a linear way across the concentrations of interest. Mixed standards of stock solutions of the monoamines of interest (dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin/5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and homovanillic acid (HVA)) at a range of $5x10^{-9}$ to $1x10^{-6}$M were prepared and analysed in triplicate. The peaks elicited were plotted against concentration to assess linearity, with $r^2>0.99$ required for continuation. Following a standard manual, full loop injection protocol per manufacturer's instructions, 60µL of sample was loaded and manually injected to the column. Using the mobile phase and column outlined above, the expected run time of the five monoamines was from 4mins for the earliest detectable peak (DA) to 21mins for the last peak (HVA). Sample peak heights were measured manually with a ruler, and compared to the peak heights of the known concentration reference samples to calculate the monoamine concentration within each sample (Equation 2.1).

$$\text{moles/mg tissue} = \frac{\text{pk ht}_{samp}\,(mm)}{\text{pk ht}_{std}\,(mm)} \times \text{std conc.(moles/L)} \times \frac{\text{homog. Vol (L)}}{\text{sample wt (mg)}} \times \frac{ATT_{samp}}{ATT_{std}}$$

Where pk ht = peak height in mm of std/sample
$ATT_{=}$ integrator attenuation setting
Std conc. = concentration of standard in moles/L

Equation 2.1 – Equation for the calculation of monoamine concentration in brain tissue samples via HPLC analysis

2.2.9 Statistical Analyses

Microsoft Excel 2010, GraphPad Prism v6 (GraphPad Software Inc., La Jolla, CA, USA), SPPS (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp) and InVivoStat (InVivoStat, Cambridge, UK) were used for statistical analyses. Three-way repeated-measures ANOVA (RM ANOVA), with isolation rearing and drug treatment as between subject factors, and time as the repeated measure, was used to analyse the LMA timecourse, whilst two-way ANOVA was used to compare total cumulative locomotor activity. Two-way ANOVA was further used to compare between exploration of the "novel" and "familiar" objects, and D1 discrimination ratios between treatment groups, in the NOD protocol, while three way ANOVA was used to assess the total object exploration time (novel+familiar) over the two NOD trials. Three-way RM ANOVA was used to analyse the effect of housing, drug treatment (between subject), and prepulse intensity (within subject) in PPI, with two-way ANOVA used to examine habituation and initial startle intensity to the 120dB tone. Two-way ANOVA assessed the effects of isolation and drug treatment on freezing at each consecutive time point in CER, as well as changes in latency to enter the CER chamber. Three-way RM ANOVA was used to analyse both acquisition phases of the MWM protocol, and two-way ANOVA to examine the exploration times in each probe trial. Finally, two-way ANOVA was used to compare hippocampal volume, frontal cortical wet weight, and monoamine concentrations calculated from HPLC analysis. For all experiments, multiple comparison tests and Bonferroni's post-hoc analysis were used following statistically significant ($p<0.05$) effects or interactions by ANOVA.

2.3 Results

2.3.1 Effect of isolation rearing and MAM treatment on locomotor activity

Pilot study: All rats showed the expected horizontal ambulations and progressively decreased locomotor activity, reflecting habituation to the initially novel arena (Figure 2.3), supported by a significant main effect of time by RM ANOVA [$F_{(11,286)}$=69.307, p<0.001] over the 60 min timecourse. There was, however, no significant main effect of housing [$F_{(1,26)}$=1.415, p=0.245] or of MAM treatment [$F_{(1,26)}$=0.018, p=0.893] by RM ANOVA, and there were no significant interactions between any of the factors.

Main study: Whilst all rats successfully habituated to the novel arena through the 60 minute protocol (significant effect of time, repeated-measures ANOVA [$F_{(11,649)}$=89.549, p<0.001], Figure 2.4), there was no significant effect of isolation [$F_{(1,59)}$=0.447, p=0.507], MAM [$F_{(1,59)}$=0.245, p=0.622], and no interaction between any of the factors by RM ANOVA. Of note the activity counts obtained from one of the 12 experimental chambers were significantly lower than those from the other chambers, regardless of treatment group. However removing these data points from the analysis did not affect the statistical outcome (data not shown).

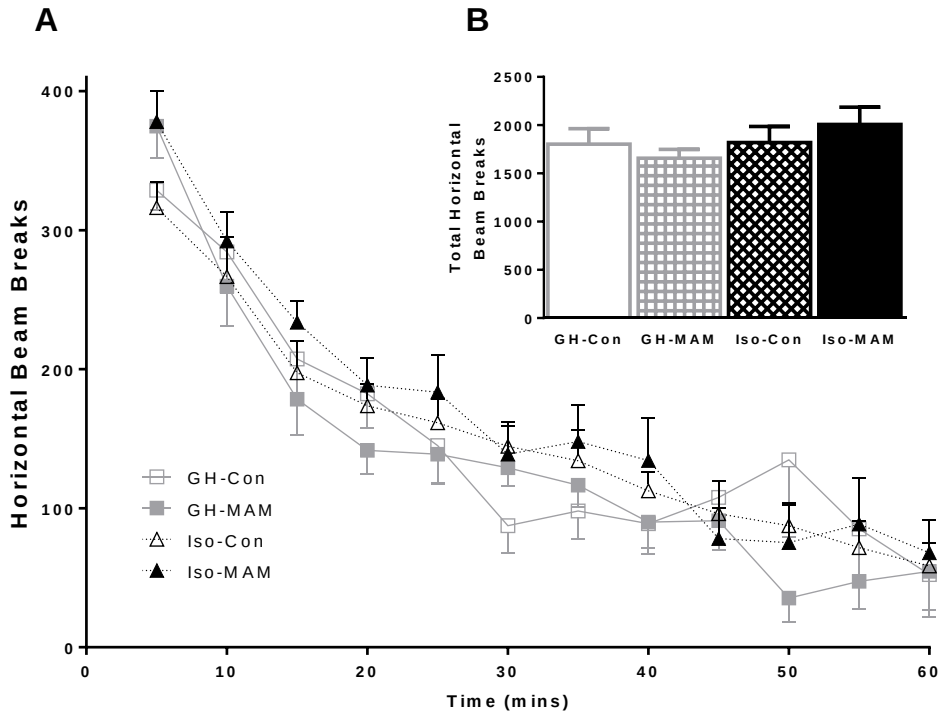

Figure 2.3 –A progressive decrease in locomotor activity during a 60 min exposure to a novel arena. (A) No significant main effect of isolation rearing (Iso) or prenatal MAM (MAM) treatment, compared with group-housed (GH) and vehicle-treated (Con) controls respectively, on horizontal beam breaks (mean ±SEM, *n*=6-8) per 5 min time bin reflecting habituation, supported by RM ANOVA [significant main effect of time, $F_{(11,286)}$=69.307, p<0.001]. (B) Total cumulative activity counts (mean ±SEM, *n*=6-8) over the 1h trial. Two-way ANOVA showed no significant effect of MAM, or isolation, and no interaction between the two treatments.

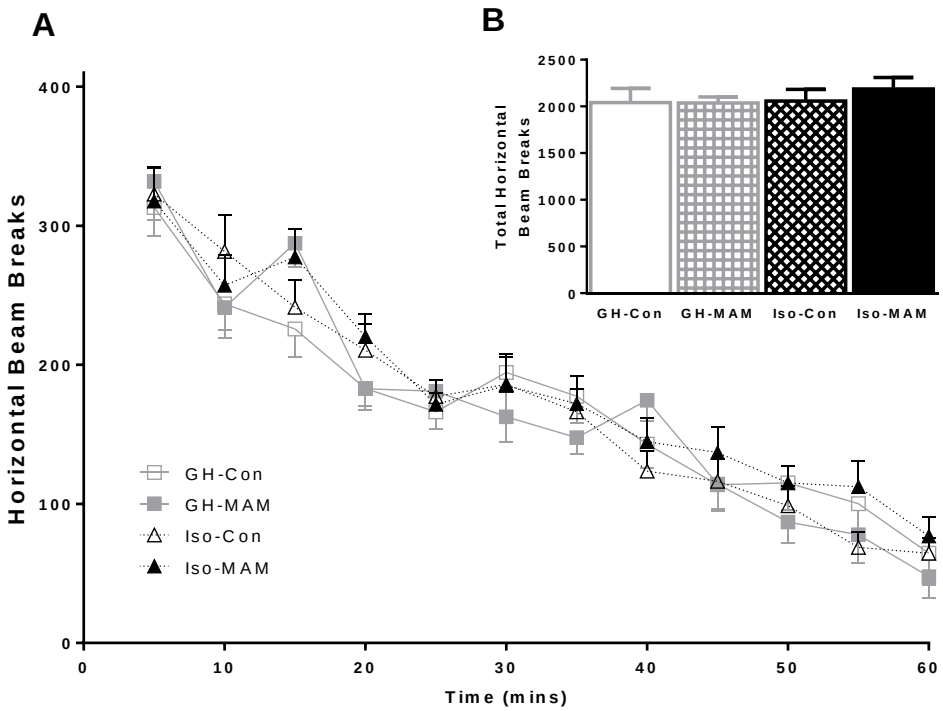

Figure 2.4 - A progressive decrease in locomotor activity during a 60 min exposure to a novel arena. (A) No significant main effect of isolation rearing (Iso) or prenatal MAM (MAM) treatment, compared with group-housed (GH) and vehicle-treated (Con) controls respectively, on horizontal beam breaks (mean ±SEM, *n*=14-19) per 5 min time bin. Overall reduction over time, reflecting habituation, supported by RM ANOVA [significant main effect of time, $F_{(11,649)}$=89.549, p<0.001]. (B) Total cumulative activity counts (mean ±SEM, *n*=14-19) over the 1h trial. Two-way ANOVA showed no significant effect of MAM, or isolation, and no interaction between the two treatments.

2.3.2 Effect of isolation rearing and MAM treatment on novel object discrimination

Pilot Study: During the second choice trial of the NOD test, rats explored the novel object significantly more than the familiar (Figure 2.5A, two-way ANOVA [$F_{(1,26)}$=29.62, p<0.0001]), with no main effect of treatment, nor an interaction. Bonferroni post-hoc analysis revealed that only GH-Con and GH-MAM groups explored the novel object significantly more (p<0.01) than familiar. Further analysis showed that the discrimination ratio for exploration of the novel and familiar objects was significantly affected by housing [two-way ANOVA, $F_{(1,26)}$=6.248, p=0.0191], but not by MAM-treatment [$F_{(1,26)}$=2.141, p=0.1554] and there was no housing x MAM interaction [$F_{(1,26)}$=0.1124, p=0.7401] (Figure 2.5B). There was no significant main effect of isolation rearing, prenatal MAM treatment, or of NOD trial on total object exploration (object 1 + object 2) by three way ANOVA (Figure 2.5C).

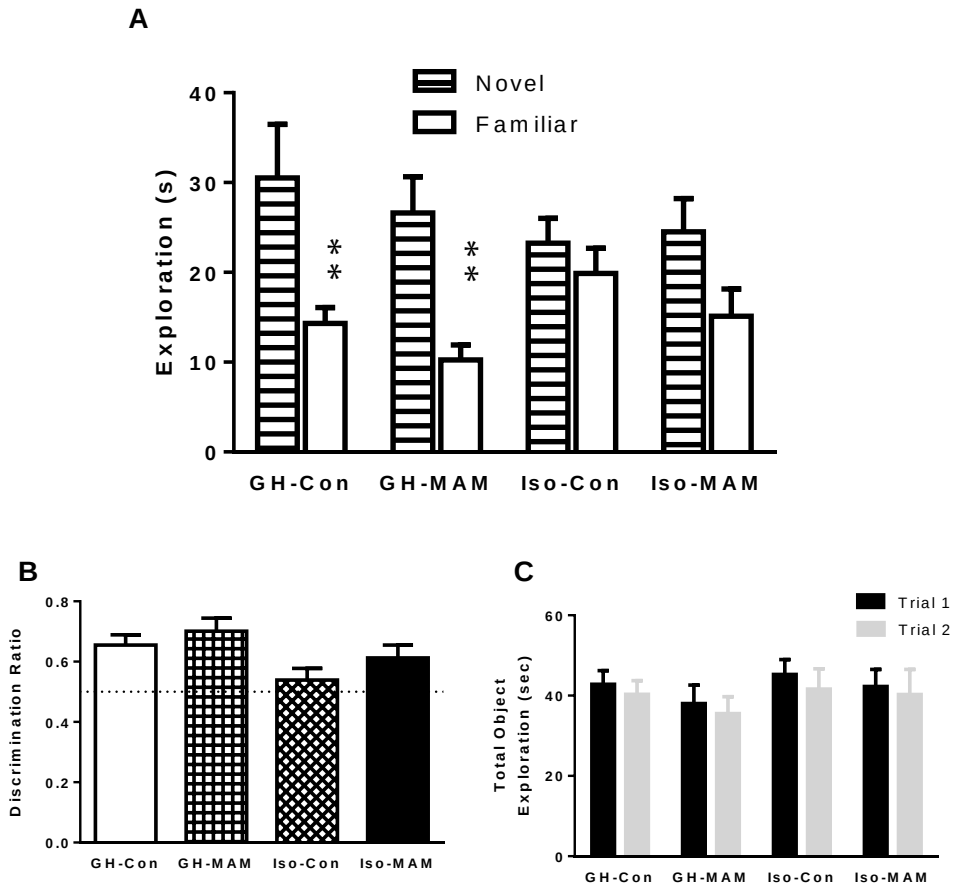

Figure 2.5 – Isolation rearing (Iso) caused impairment in novel object discrimination compared to group-housed (GH) controls, irrespective of prenatal MAM or vehicle control (Con) treatment. (A). Group-housed rats spent significantly more time exploring the novel object (s, mean±SEM, *n*=6-8), while isolation reared rats did not during the second choice trial of a two-trial novel object discrimination task [$F_{(1,26)=}29.62$, $p<0.0001$] $^{**}p<0.01$ vs. Novel object by Bonferroni post-hoc test following ANOVA. (B) The discrimination ratio (novel/novel+familiar object exploration, mean±SEM, *n*=6-8) during the second choice trial. Note a significant main effect of isolation rearing [ANOVA $F_{(1,26)}=6.248$, p=0.0191], but not MAM, and no interaction. (C) No significant main effects of isolation rearing, prenatal MAM treatment or of NOD trial were observed on total object exploration (object 1+ object 2) by three-way ANOVA.

Main Study: Rats preferentially explored the novel over the familiar object in the second choice trial of the NOD paradigm [two-way ANOVA, $F_{(1,56)}$=48, p<0.0001], indicative of object recognition memory, but there was no significant effect of treatment, and no treatment x object interaction (Figure 2.6). However, Bonferroni post-hoc analysis revealed that this significance was present in the GH-Con (p<0.01), GH-MAM (p<0.001) and Iso-MAM (p<0.05) groups, but not in the Iso-Con group (p>0.05). Analysis of the derived D1 discrimination ratio revealed that the effects of isolation rearing [$F_{(1,59)}$=3.040, p=0.0865], MAM treatment [$F_{(1,59)}$=3.649, p=0.0610], and the isolation x MAM interaction [$F_{(1,59)}$=0.1088, p=0.7427] all failed to reach significance by two-way ANOVA. There was no significant main effect of isolation rearing, prenatal MAM treatment, or of NOD trial on total object exploration (object 1 + object 2) by three way ANOVA, despite a trend towards a decrease in exploration time in the Iso-Con group (Figure 2.6C).

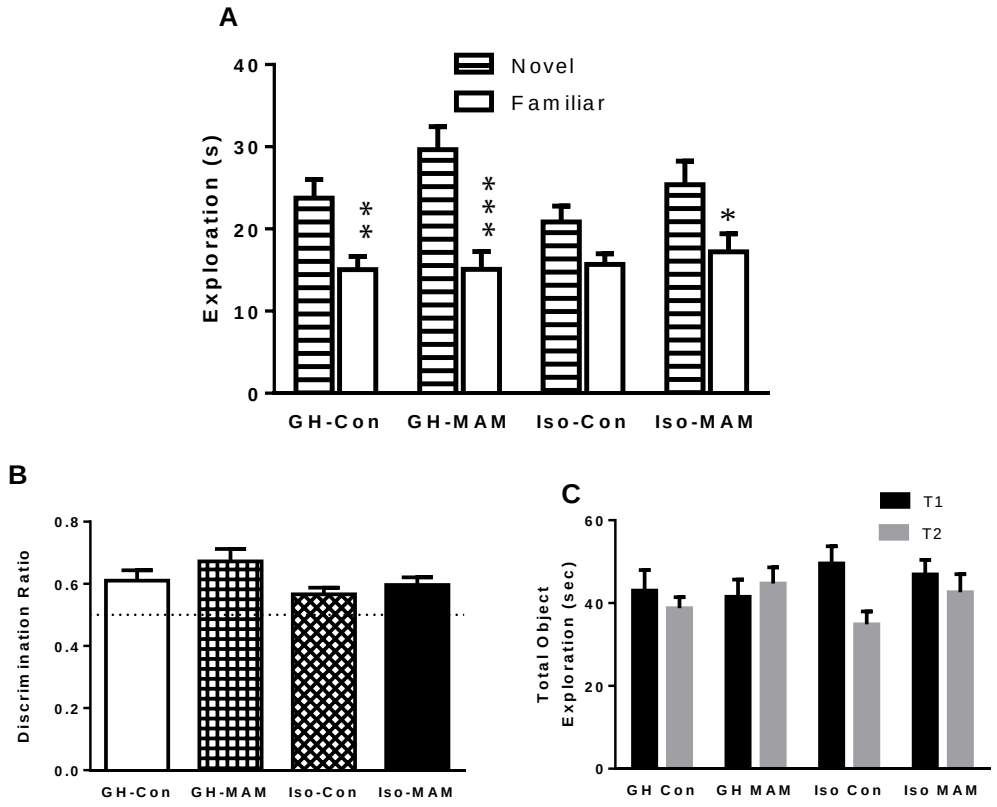

Figure 2.6 – Preferential exploration of the novel over familiar object in the second, choice trial of a two-trial NOD paradigm. (A) Significantly higher exploration (s, mean±SEM, *n*=14-19) of the novel vs. the familiar object regardless of treatment [$F_{(1,56)}$=48.00, p<0.0001]. Post-hoc significance observed in group-housed vehicle-treated (GH-Con), group-housed MAM-treated (GH-MAM) and isolation-reared MAM-treated (Iso- MAM), but not isolation-reared vehicle-treated (Iso-Con), indicating a loss of discrimination in this group alone. *p<0.05, **p<0.01, ***p<0.001 vs. Novel object by Bonferroni post-hoc test following ANOVA (B) The discrimination ratio (novel/novel+familiar object exploration, mean±SEM, *n*=14-19) during the second choice trial was unaffected by isolation rearing or MAM treatment, and there was no between-factor interaction. (C) No significant main effects of isolation rearing, prenatal MAM treatment, or of NOD trial were observed on total object exploration (object 1+ object 2) by three way ANOVA, despite a trend towards decreased exploration in trial 2 in the Iso-Con group.

2.3.3 Effect of isolation rearing and MAM treatment on prepulse inhibition of acoustic startle

Pilot Study: Exposure to a sub-threshold acoustic pulse produced a significant attenuation of the acoustic startle response increasing with prepulse intensity (Figure 2.7). RM ANOVA revealed significant main effects of prepulse [$F_{(2,50)}$=51.084, p<0.001], and a MAM x isolation interaction [$F_{(1,25)}$=9.097, p=0.006], but there was no main effect of isolation rearing or prenatal MAM treatment alone. Post-hoc analysis showed the route of this interaction was a significant PPI decrease in Iso-MAM rats compared to the single hit treatment groups at the 80dB pulse level. Two-way ANOVA showed there was no effect of isolation rearing or prenatal MAM on initial startle intensity or habituation to the 120dB tone, nor an interaction in either measure (data not shown).

Main study: Isolation rearing had a significant effect on PPI such that RM ANOVA revealed significant main effects of housing [$F_{(1,59)}$=6.737, p=0.012] and prepulse level [$F_{(2,118)}$=117.593, p<0.001], but no main effect of MAM [$F_{(1,59)}$=0.385, p=0.537], and no interaction between any of the factors (Figure 2.8A). Post-hoc significance was seen due to isolation rearing at the 76dB prepulse level only. Two-way ANOVA revealed that there was a significant main effect of isolation rearing on initial startle intensity [$F_{(1,59)}$=14.615, p<0.001], but there was no effect of MAM [$F_{(1,59)}$=0.1052, p=0.747] and no isolation x MAM interaction [$F_{(1,59)}$=0.04980, p=0.8242] (Figure 2.8B). There was no significant effect on habituation to the 120dB tone by isolation or MAM, nor was there an interaction (data not shown).

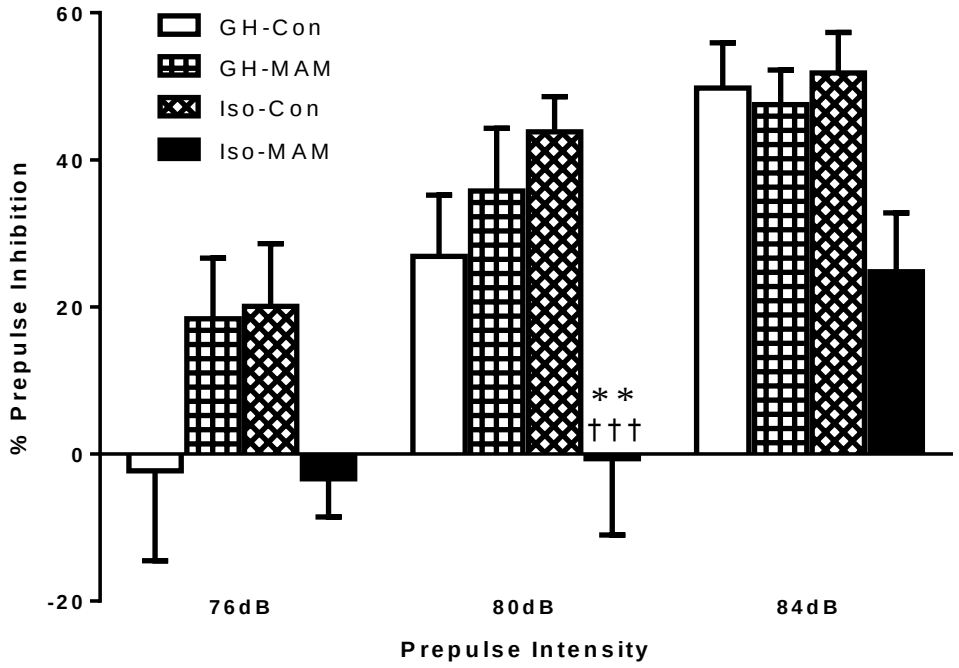

Figure 2.7 – A significant attenuation of the acoustic startle response by a combination of isolation rearing and prenatal MAM treatment. Despite a significant main effect of prepulse on percentage prepulse inhibition (mean±SEM, n=6-8) [$F_{(2,50)}$=51.084, p<0.001, RM ANOVA], no main effect of MAM treatment (MAM) or of isolation (Iso), either alone or combined, compared to vehicle-treated (Con) and group-housed (GH) rats was observed. There was a significant isolation x MAM interaction [$F_{(1,25)}$=9.097, p=0.006], with post hoc significance showing a decrease in PPI performance at the 80dB prepulse level. **p<0.01 isolation vs. MAM-matched group-housed controls, †††p<0.001 MAM-treatment vs. rearing-matched saline-treated control by Bonferroni post-hoc analysis following ANOVA.

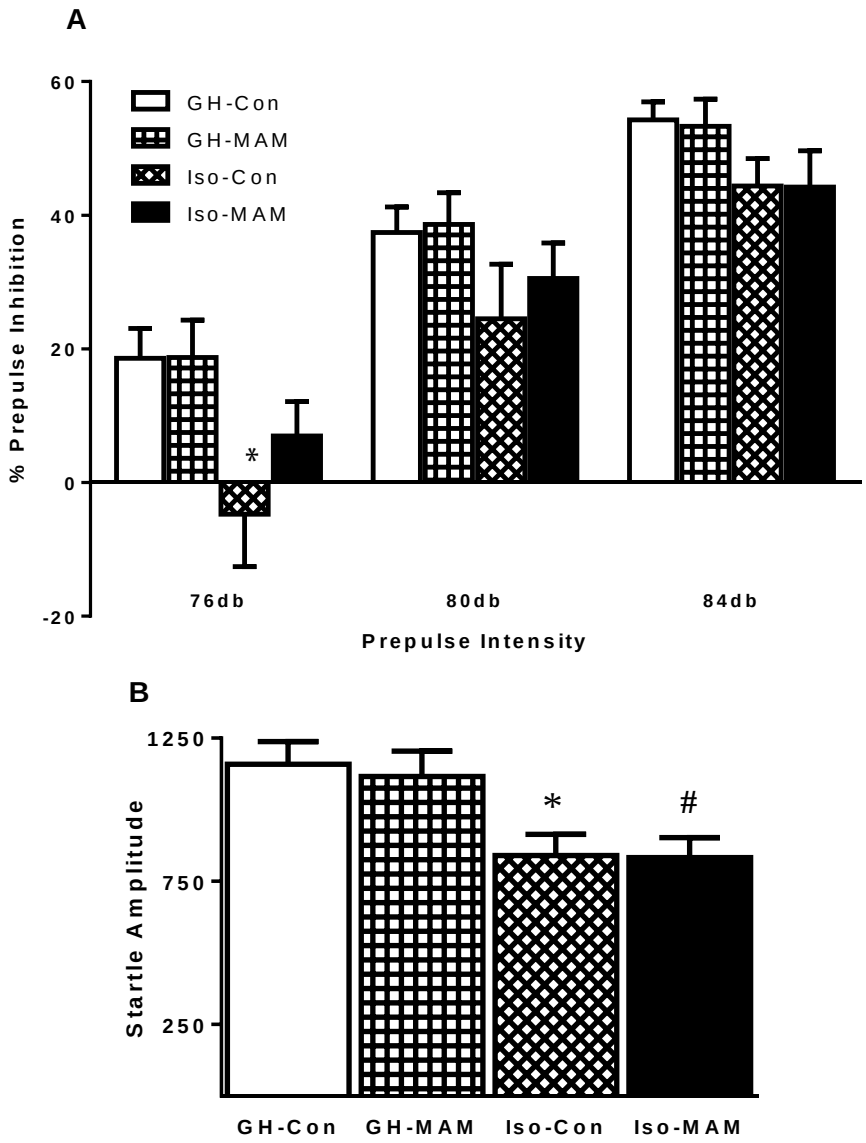

Figure 2.8 – A significant effect of isolation rearing on prepulse inhibition of acoustic startle. (A) The percentage PPI response (mean±SEM, *n*=14-19) was significantly altered by isolation rearing (Iso) [$F_{(1,59)}$=6.737, *p*=0.012] and prepulse intensity [$F_{(2,118)}$=117.593, *p*<0.001], but not prenatal MAM compared to group-housed (GH) and vehicle treated (Con) controls. There were no interactions between factors. **p*<0.05 isolation vs. MAM-matched group-housed controls by Bonferroni post-hoc analysis following ANOVA. (B) The initial startle amplitude (mean±SEM, *n*=14-19) elicited by 120dB tone was significantly altered by isolation rearing [ANOVA, $F_{(1,59)}$=14.615, *p*<0.001], but not MAM, with no interaction. **p*<0.05 isolation vs. MAM-matched group-housed controls, $^{\#}p$<0.05 isolation+MAM treatment vs. GH-Con absolute control by Bonferroni post-hoc following ANOVA.

2.3.4 Effect of isolation rearing and MAM treatment on a conditioned emotional response

Pilot Study: To examine contextual and conditioned emotional memory, processes involving input from the hippocampus and amygdala, behaviour was examined in an associative learning conditioned emotional response paradigm. There was a significant main effect of isolation rearing at 24h [$F_{(1,25)}$=16.58, p=0.0004] and 48h post-conditioning [$F_{(1,25)}$=21.15, p=0.0001] and following re-presentation of the CS alone [$F_{(1,25)}$=9.701, p=0.0046] by two-way ANOVA, with post hoc tests showing isolation significantly decreased freezing duration at all time points. Despite no effect of MAM at 24 or 48 h post-conditioning, a significant main effect occurred following presentation of the CS alone [$F_{(1,25)}$=7.322, p=0.0121]. There was a tendency for MAM-treatment to increase freezing response, but this failed to reach significance by post-hoc analysis. At none of the three time points was a significant isolation x MAM interaction observed (Figure 2.9).

Main Study: Half of the main study cohort was advanced into the CER paradigm (n=31 in total, n=7-10 per group). Freezing responses were decreased in isolates such that at all time points two-way ANOVA revealed a significant main effect of isolation rearing (24h: [$F_{(1,27)}$=9.852, p=0.0041], 48h: [$F_{(1,27)}$=13.64, p=0.001], Post-CS [$F_{(1,27)}$=15.72, p=0.0005]), with post hoc significance observed at the 24h and Post-CS time points. No alterations in freezing were observed due to MAM treatment, with no isolation x MAM interactions, and any stage of the protocol (Figure 2.10).

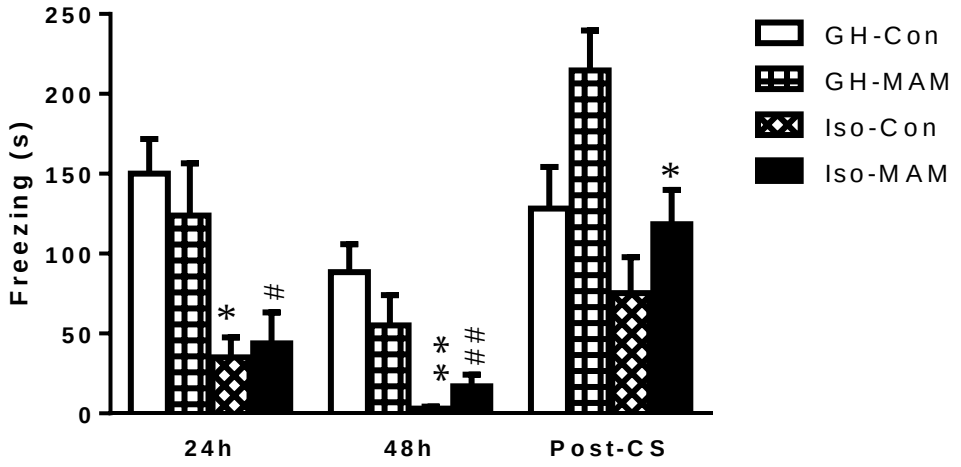

Figure 2.9 – Significant reduction in freezing time due to isolation rearing (Iso) compared to group-housed (GH) rats in a conditioned emotional response paradigm, irrespective of prenatal treatment with MAM (MAM) or saline control (Con). Two-way ANOVA revealed significant main effects of isolation rearing on freezing time (s, mean±SEM, n=6-8) at 24h [$F_{(1,25)}$=16.58, p=0.0004] and 48h [$F_{(1,25)}$=21.15, p=0.0001] post-conditioning to an aversive footshock (US) and a paired light-sound tone (CS), and following re-presentation of the CS alone (Post-CS) [$F_{(1,25)}$=9.701, p=0.0046]. ANOVA showed a main effect of prenatal MAM-treatment (24mg/kg, i.p.) on CS alone induced freezing [$F_{(1,25)}$=7.322, p=0.0121], but there was no isolation x MAM interaction. $^{**}p<0.01$ $^{*}p<0.05$ isolation vs. group-housing, $^{\#\#}p<0.01$ $^{\#}p<0.05$ isolation+MAM vs. group-housed control treated by Bonferroni post-hoc analysis following ANOVA. **Note that isolation rearing reduced freezing at all time points, but whilst there was a tendency for MAM to increase freezing on presentation of the CS, this did not reach post-hoc significance.**

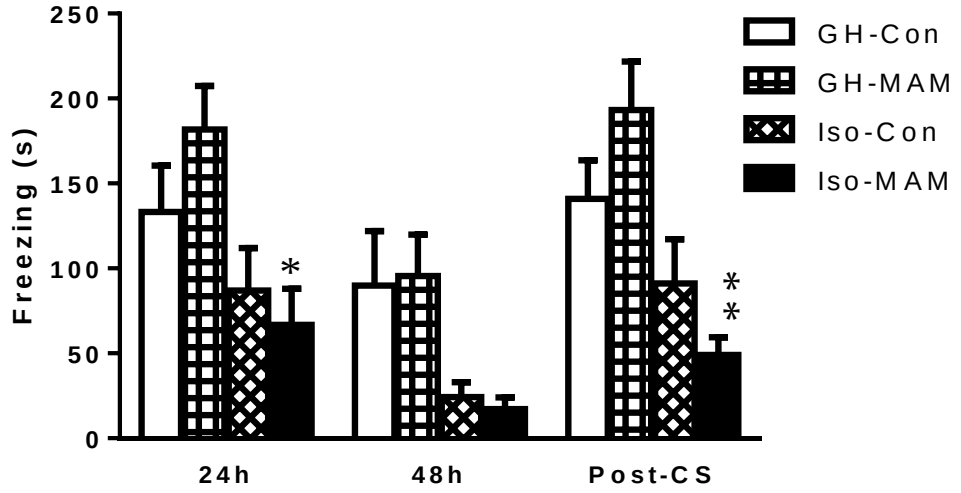

Figure 2.10 – Significant reduction in freezing time due to isolation rearing (Iso) compared to group-housed (GH) rats in a conditioned emotional response paradigm. Two-way ANOVA revealed a significant main effect of isolation rearing on freezing time (s, mean±SEM, n=7-10) at 24h [$F_{(1,27)}$=9.852, p=0.0041] and 48h [$F_{(1,27)}$=13.64, p=0.001] post-conditioning of an aversive footshock (US) and a paired light-sound tone (CS), and following re-presentation of the CS alone (Post-CS) [$F_{(1,27)}$=15.72, p=0.0005]. No significant main effect of prenatal MAM treatment (MAM, 24mg/kg, i.p.) compared to vehicle control (Con), nor was there an isolation x MAM interaction by ANOVA. **p<0.01 *p<0.05 isolation vs. group-housing by Bonferroni post-hoc following ANOVA. **Note that freezing decreases due to isolation rearing reached post-hoc significance at the 24h and Post-CS time points.**

.

2.3.5 Effect of isolation rearing and MAM treatment on Morris Water Maze performance

Main study: Rats which did not perform the CER paradigm advanced into the Water Maze task (total *n*=32). RM ANOVA revealed a highly significant main effect of trial on latency to platform [$F_{(14,392)}$=17.042, p<0.001], indicating that rats were able to successfully learn the position of the hidden platform and progressively find it more quickly (Figure 2.11A). Despite a trend, there was no significant main effect of isolation rearing or MAM, and no interaction.

Two-way ANOVA showed a significant rearing x MAM interaction on the time spent in the correct maze quadrant in probe 1 [$F_{(1,28)}$=4.962, p=0.0341], but no main effect of MAM or isolation alone. In Probe 2 there was a significant main effect of MAM on time in the correct quadrant [$F_{(1,28)}$=8.424, p=0.0071, two-way ANOVA], but no main effect of isolation and no interaction observed (Figure 2.12A and B).

During reversal learning training there was no significant effect of trial number on platform location latency by RM ANOVA [$F_{(8,224)}$=1.826, p=0.073], indicating that overall performance did not improve with training. There was no significant main effect of housing or MAM treatment, nor an interaction between the two (Figure 2.11B).

Two-way ANOVA indicated a significant MAM x isolation interaction [$F_{(1,28)}$=4.709, p=0.0386] on the time spent in the previous "correct" quadrant during Probe 3, but no main effect of housing or MAM treatment alone. There was no significant effect of any factor on the time spent swimming in the platform ring area in probe 3 (two-way ANOVA, Figure 2.12C and D).

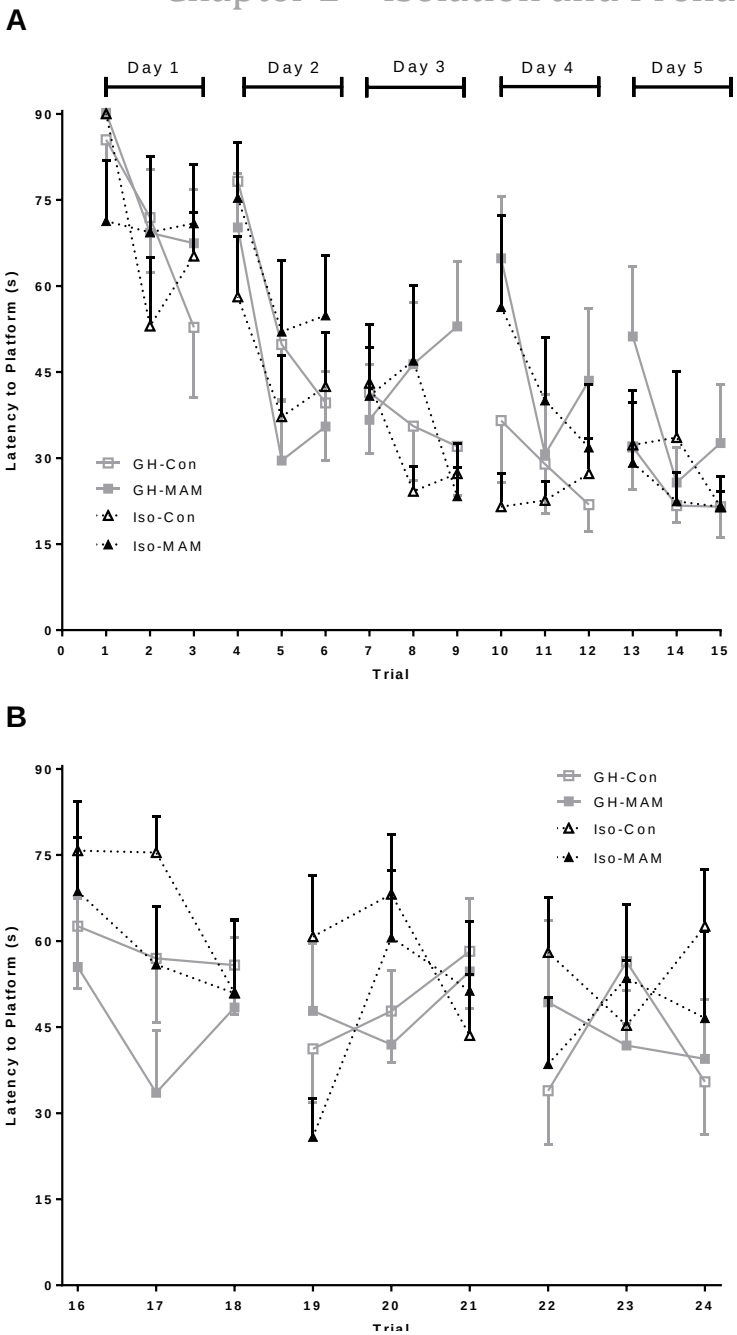

Figure 2.11 - Comparison of the effect of isolation rearing (Iso) and prenatal MAM treatment (MAM), both alone and in combination, with that of group-housed (GH) and vehicle treated (Con) controls, on the latency (s, mean±SEM, *n*=7-9) to find a hidden platform in the Morris Water Maze during (A) 15 consecutive learning trials, and (B) following a rule change, 9 further trials during a reversal learning period. Repeated-measures ANOVA showed a significant main effect of trial during the learning phase [$F_{(14,392)}$=17.042, p<0.001], but no main effect of isolation rearing or MAM treatment, and no interaction. There were no significant main effects of isolation rearing, MAM treatment, or trial by repeated-measures ANOVA during the reversal learning phase, and no interaction between any of the factors.

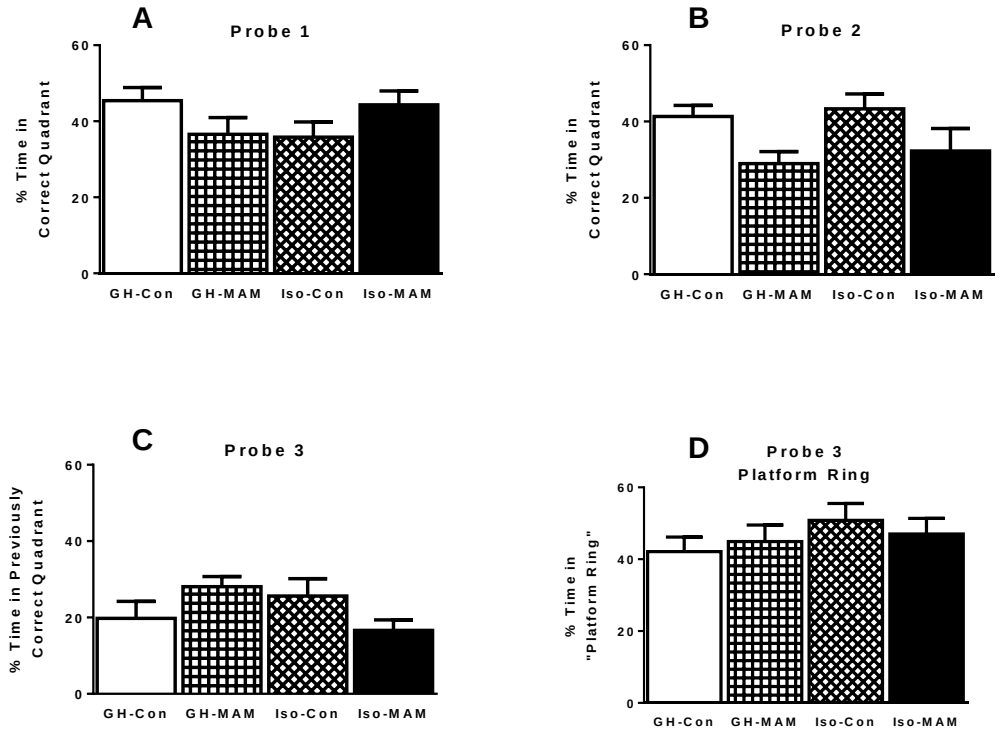

Figure 2.12 – Comparison of the effect of isolation rearing (Iso) and prenatal MAM treatment (MAM), both alone and in combination, compared with group-housed (GH) and vehicle treated (Con) controls, on the percentage time spent in the "correct" quadrant (mean ±SEM, *n*=7-9) in (A) Probe 1, (B) Probe 2, and (C) Probe 3 and in (D) the "Platform Ring" area in Probe 3. (A) A significant isolation x MAM interaction occurred by two-way ANOVA in Probe 1 [$F_{(1,28)}$=4.962, p=0.0341], but there was no effect of isolation or MAM alone. (B) In Probe 2 a significant effect of MAM treatment was seen by two-way ANOVA [$F_{(1,28)}$=8.424, p=0.0071], but no effect of isolation and no interaction. (C) A significant interaction occurred between isolation rearing and MAM treatment by two-way ANOVA in Probe 3 [$F_{(1,28)}$=4.709, p=0.0386], but no effect of isolation or MAM alone. (D) There was no effect on the percentage time spent in the "platform ring" area due to isolation rearing or MAM treatment, and no interaction occurred. No post-hoc significance was observed between any treatment groups in any measure examined.

2.3.6 Effect of isolation rearing and MAM treatment on hippocampal size

Pilot Study: Using Cavalieri's principle, hippocampal volumes were estimated to a mean of 30.48mm^3 across all treatment groups. Two-way ANOVA analysis revealed a significant main effect of MAM treatment [$F_{(1,25)}$=23.72, p<0.0001] which reduced the mean volume from 39.12 ±2.73 mm^3 in the GH-Con group, to 25.66 ±1.88 mm^3 in the Iso-MAM group, approximately 34% reduction, supported by Bonferroni post-hoc analysis revealing a significant reduction (p<0.01) in hippocampal volume in GH-MAM compared to GH-Con, and Iso-Con compared to Iso-MAM. There was no effect of rearing [$F_{(1,25)}$=1.387, p=0.25], and no rearing x MAM interaction [$F_{(1,25)}$=0.07599, p=0.7851] (Figure 2.13A). A significant main effect of MAM treatment was also seen on total brain mass [ANOVA, $F_{(1,25)}$=105.6, p<0.001], but again no effect of housing condition, and no between-factor interaction was observed (Figure 2.13B). The hippocampal volume to total brain mass ratio was also significantly affected MAM such that two-way ANOVA revealed a significant main effect of MAM [$F_{(1,25)}$=7.731, p=0.0102], but no effect of housing condition [$F_{(1,25)}$=0.9327, p=0.3434], nor any MAM x housing interaction [$F_{(1,25)}$=0.05366, p=0.8187]. Despite a trend towards reduced hippocampal volume:total brain mass ratio, post-hoc analysis revealed no significance between groups (Figure 2.13C).

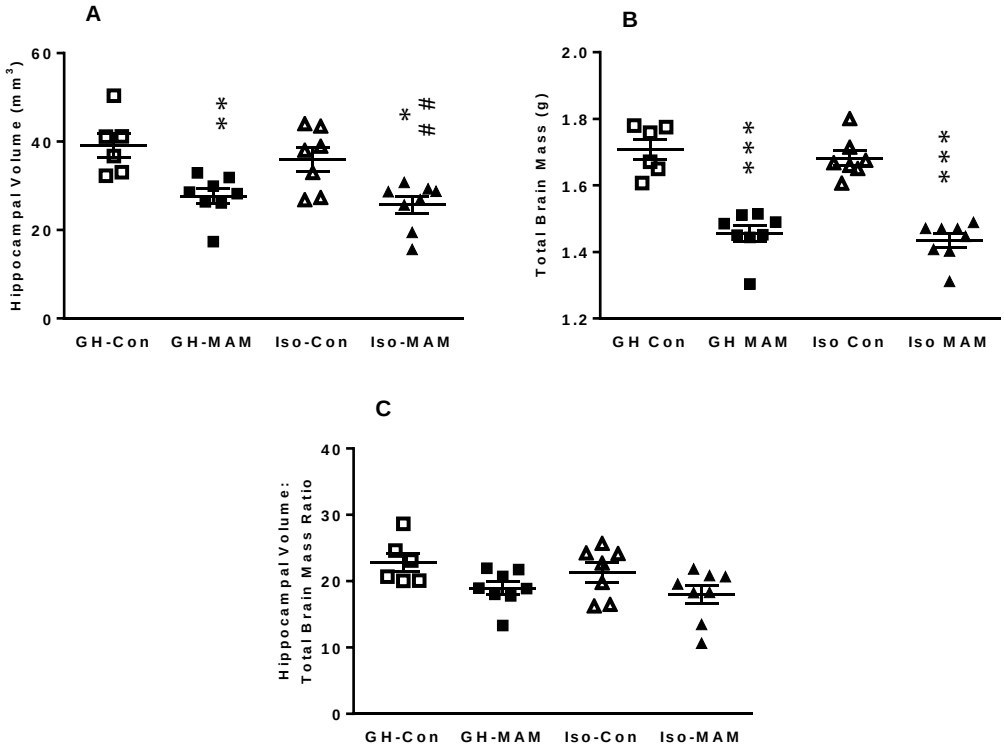

Figure 2.13 – Prenatal MAM treatment caused a significant reduction in brain mass and hippocampal volume. (A) Significant main effect of prenatal MAM treatment (MAM, 24mg/kg i.p.) compared to vehicle control (Con) on hippocampal volume (mm^3, bars = mean±SEM, n=6-8) by Cavalieri Principle, regardless of rearing in social groups (GH) or isolation (Iso) [two-way ANOVA, $F_{(1,25)}$=23.72, p<0.0001]. No main effect of isolation rearing, nor an isolation x MAM interaction. **p<0.01 *p<0.05 MAM-treatment vs. rearing-matched vehicle-treated controls, $^{\#\#}p$<0.01 isolation+MAM treatment vs. GH-Con absolute control by Bonferroni post-hoc test following ANOVA. (B) Significant main effect of prenatal MAM treatment on total brain mass (bars = mean±SEM) compared to vehicle controls [ANOVA, $F_{(1,25)}$=105.6, p<0.001]. No main effect of isolation rearing, and no rearing x MAM interaction. (C) Significant main effect of prenatal MAM treatment on hippocampal volume:total brain mass ratio (bars = mean±SEM) compared to vehicle controls [ANOVA, $F_{(1,25)}$=7.731, p=0.0102]. No main effect of isolation rearing, and no rearing x MAM interaction. Note a trend towards decreased ratio of hippocampal volume to total brain mass that failed to achieve post-hoc significance.

Main Study: Hippocampal wet mass was significantly altered by MAM-treatment [two-way ANOVA, $F_{(1,46)}$=12.49, p=0.0009] irrespective of whether rats were isolated or group-housed, such that the mass (mean±SEM) in the GH-MAM group (43.8 ± 2.5 mg) was reduced by 16% of control (52.1 ± 2.8 mg) (Figure 2.14A). The striatal wet mass was also significantly affected by MAM treatment by two-way ANOVA [$F_{(1,47)}$=4.688, p=0.0355], but unaffected by isolation rearing, and no interaction occurred (Figure 2.14B). Despite trends towards reduced volume in both regions, no post-hoc significance was observed in either region following ANOVA. There was no effect of MAM treatment or isolation rearing on frontal cortex or hypothalamus wet weight by two-way ANOVA, nor any interaction between factors.

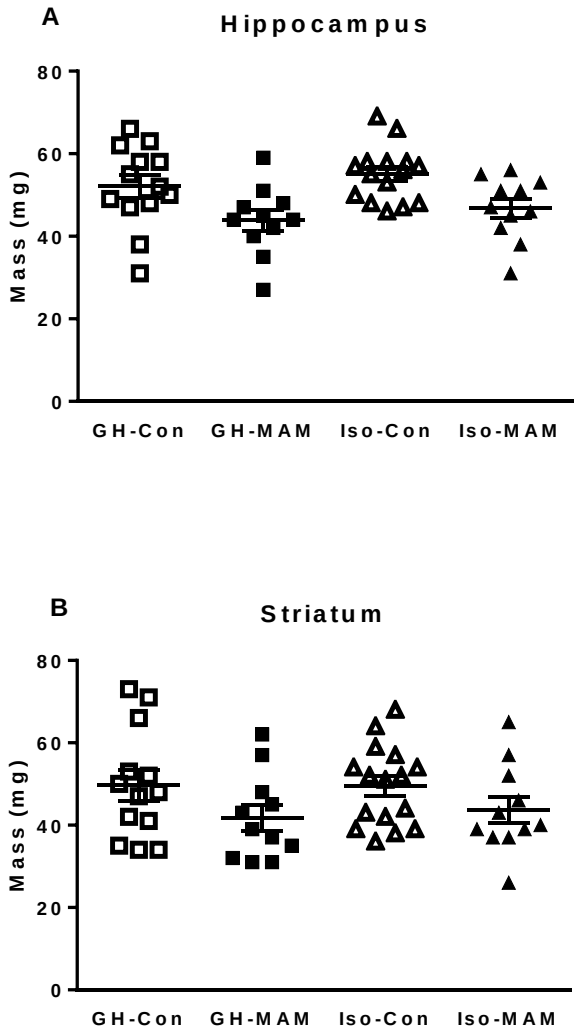

Figure 2.14 – Prenatal MAM treatment caused a significant change in hippocampal and striatal wet weights. There was a significant main effect of prenatal MAM treatment (MAM, 24mg/kg i.p.) on the wet weight (mg, mean±SEM, *n*=14-19) of (A) hippocampus [two-way ANOVA, $F_{(1,46)}$=12.49, *p*=0.0009] and (B) striatum [$F_{(1,47)}$=4.688, *p*=0.0355] compared to vehicle treated controls (Con), regardless of subsequent rearing in social groups (GH) or isolation (Iso). No main effect of isolation rearing or a rearing x MAM interaction in either region, with no post-hoc significance observed between any treatment group, despite a trend towards reduced hippocampal and striatal wet weights due to MAM.

2.3.7 Effect of isolation rearing and MAM treatment on neurochemistry

Main Study: HPLC analysis of the monoamine concentration in the frontal cortex and hippocampus revealed a significant main effect of MAM on dopamine concentration in the FC [$F_{(1,45)}$=6.031, p=0.0180], however Grubb's test for outliers (α = 0.0500) identified two data points as anomalous (one in each of the GH-MAM and Iso-MAM groups), and removal of these points removed this significance. There was no main effect of isolation rearing, nor a rearing x MAM interaction (two-way ANOVA, Figure 2.15A). A significant change in 5-HIAA concentration due to MAM was observed in the hippocampus [$F_{(1,44)}$=8.131, p=0.0066] by two-way ANOVA (Figure 2.15B), but no change in the 5-HT:5-HIAA ratio was seen, nor was there an effect of isolation rearing or a rearing x MAM interaction here also (Table 2.2). There was no effect of treatment or rearing, nor an interaction, on any other monoamine level tested in either structure (Table 2.2).

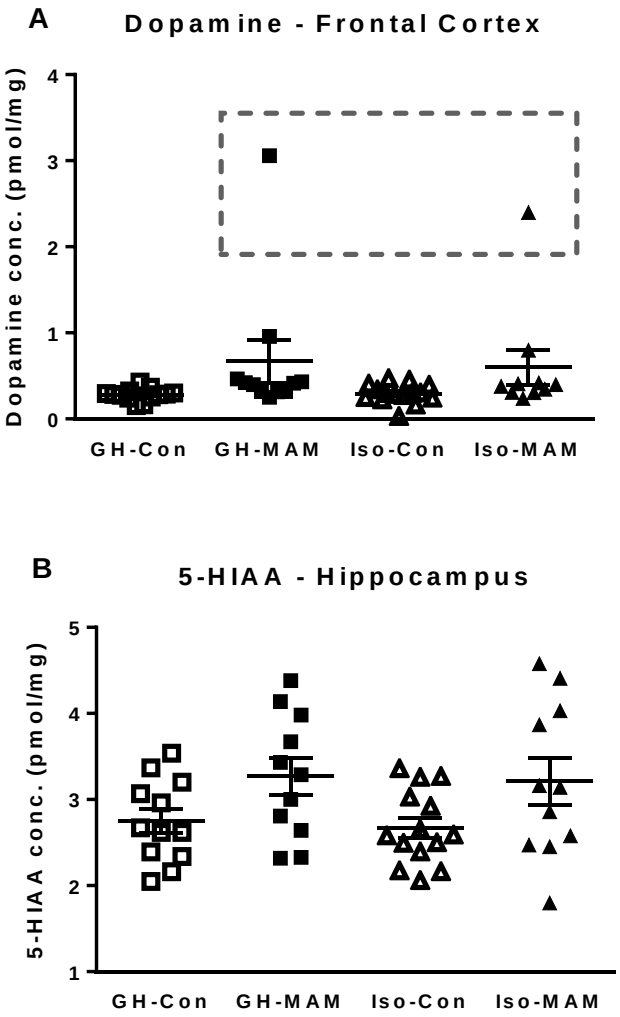

Figure 2.15 - Comparison of the effect of isolation rearing (Iso) and prenatal MAM treatment (MAM), both alone and in combination, with group-housed (GH) and vehicle treated (Con) controls, on (A) dopamine concentration in the post-mortem frontal cortex tissue via HPLC and (B) 5-HIAA concentration in post-mortem hippocampal tissue via HPLC (n=11-16, pmol/mg, bars: mean±SEM). (A) A significant main effect of MAM on dopamine levels [two-way ANOVA, $F_{(1,45)}$=6.031, p=0.0180], however removal of the highlighted anomalous data points removed this significance. There was no effect of isolation, nor an interaction between the two factors. (B) A significant effect of MAM on 5-HIAA levels [two-way ANOVA, $F_{(1,44)}$=8.131, p=0.0066] was observed, with no effect of isolation rearing, and no interaction observed. Despite a trend towards elevated 5-HIAA by prenatal MAM treatment, this effect failed to reach post-hoc significance.

Table 2.2 – Table to show the metabolite levels (pmol/mg) in post-mortem tissue of the the frontal cortex and hippocampus of vehicle and MAM treated rats, reared either in isolation or social groups, measured by HPLC analysis.

Frontal Cortex	GH Con		GH MAM		Iso Con		Iso MAM	
Monoamine	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
DA	0.282	±0.020	0.668	±0.246	0.294	±0.029	0.599	±0.205
DOPAC	0.310	±0.048	0.349	0±.062	0.344	±0.049	0.323	±0.070
5HIAA	2.571	±0.356	3.354	±0.421	2.567	±0.334	2.907	±0.396
5-HT	2.818	±0.399	3.300	±0.231	3.563	±0.404	3.405	±0.327
HVA	0.233	±0.022	0.311	±0.077	0.205	±0.024	0.244	±0.063
Turnover Ratio								
5-HT:5HIAA	1.344	±0.271	1.200	±0.200	1.680	±0.284	1.332	±0.188
DA:DOPAC+HVA	0.643	±0.087	0.979	±0.309	1.088	±0.427	1.010	±0.290

Hippocampus	GH Con		GH MAM		Iso Con		Iso MAM	
Monoamine	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
DA	0.164	±0.053	0.128	±0.042	0.196	±0.040	0.175	±0.047
DOPAC	0.113	±0.017	0.111	±0.012	0.138	±0.051	0.141	±0.026
5HIAA	2.749	±0.138	3.272	±0.216	2.674	±0.115	3.214	±0.271
5-HT	1.913	±0.226	1.736	±0.173	2.211	±0.356	1.904	±0.186
HVA	Could not be detected in any sample							
Turnover Ratio								
5-HT:5HIAA	0.747	±0.128	0.571	±0.082	0.861	±0.149	0.655	±0.094
DA:DOPAC	1.456	±0.042	1.156	±0.027	1.420	±0.046	1.241	±0.036

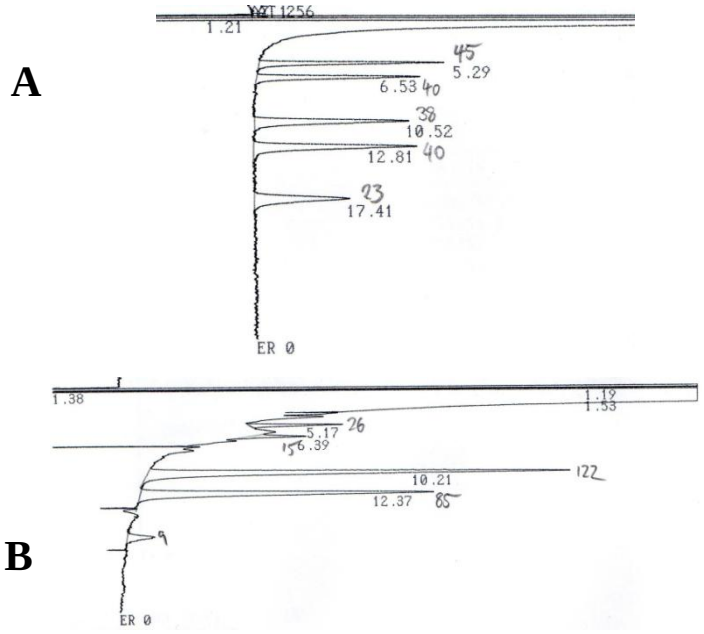

Figure 2.16 – Representative results from HPLC analysis of the hippocampus and frontal cortex in rats treated with prenatal MAM or vehicle control and subsequently reared in group or isolation. (A) A 1x10-7M mixed monoamine standard with peaks representing, from top to bottom, DA, DOPAC, 5-HT, 5-HIAA, HVA (B) Sample of frontal cortex with peaks for all 5 monoamines of interest identified, with peak run time (printed number, mins) and height measurements (mm) shown.

2.4 Discussion

The results obtained in these studies provide little support for the hypothesis that an isolation-MAM combination model may provide an improved model of 'schizophrenia-like' symptoms compared to either early-life perturbation alone, due to the lack of robust and reproducible behavioural deficits observed. Although in many of the behavioural paradigms assessed a significant effect was seen due to at least one of the treatments given, and reductions in hippocampal size were observed, these deficits were inconsistent and not reliably in line with previous publications.

Whilst the locomotor response of rats in both the pilot and main study displayed the expected habituation and reduced activity in consecutive epochs, no significant effects were observed due to isolation rearing or MAM treatment in either study. Elevations in locomotor activity in a novel environment, interpreted as an increased propensity to escape (neophobia) and/or attenuation of the ability to habituate to mildly aversive novelty (Fone and Porkess 2008; Jones et al. 2011b), have been reported in in up to 88% of cohorts where rats have been isolated for 5 weeks (Fone and Porkess 2008), and by multiple groups using both longer and shorter isolation periods than this study, as well as in various strains of rat (Bakshi and Geyer 1999; Fabricius et al. 2011; Schubert et al. 2009). An absence of this hyperlocomotion is therefore somewhat unexpected, and has only been previously reported as a result of changes in environmental enrichment (Schrijver et al. 2002). It was anticipated

that this behavioural deficit would be highly reproducible here, and was intended as a marker of model efficacy; however the lack of isolation-induced hyperactivity instead calls in to question whether the isolation procedure was successful at all. Previous publications have reported that MAM can enhance the locomotor stimulant effect of amphetamine or NMDA receptor antagonists such as PCP, and on occasion may enhance baseline locomotor activity (Le Pen et al. 2011). However, a number of studies have shown that GD17 MAM has no effect on baseline locomotor activity in a novel arena (Featherstone et al. 2009; Flagstad et al. 2004; Moore et al. 2006; Penschuck et al. 2006). Consistent with this, no main effect of MAM was seen in either cohort of this study.

Conversely, significance was observed due to one of the two treatments used in each cohort in each remaining behavioural paradigm. The ability for rats to discriminate between a novel and familiar object in the second choice trial of the NOD protocol was impaired by isolation rearing in the pilot study, where group-housed rats explored the novel object significantly more than the familiar, and those reared in isolation did not. This suggests that group-housed rats were more able to use working memory to remember previous attention to the object, and hence explore it significantly less on re-exposure. Furthermore, the D1 discrimination ratio of isolated rats was shown to be significantly lower than their group-housed counterparts, irrespective of prenatal treatment with MAM. As this test was performed just 24h after the LMA protocol and shows an isolation-induced deficit, it is difficult to conclude that the lack of

hyperactivity in the previous paradigm was due to a failure in producing isolation syndrome. The impairment of NOD in isolation-reared rats in the pilot study was as previously shown in the literature (Bianchi et al. 2006; McLean et al. 2010a; Neill et al. 2010). However, there is also a body of evidence to suggest that discrimination deficits similar to those observed here are produced in adolescent offspring from dams treated during gestation with MAM (Fiore et al. 1999; Lodge and Grace 2009), a finding not replicated herein. One potential explanation for the lack of effect of MAM is the use of Lister-Hooded rats in this study. As noted, there is no published data on the use of this strain of rats in studies where prenatal MAM is administered on GD17, and it is possible that strain sensitivity to MAM may account for the different results seen herein. In the main study, unlike the pilot, no significant effect was observed on the D1 discrimination ratio due to isolation rearing, and although rats in the Iso-Con group were identified as not exploring the novel significantly more than the familiar object, rats in the Iso-MAM group exhibited a preference for the novel object, indicating intact visual learning and memory at this time point. However, ANOVA of D1 data showed that there was no main effect of isolation or MAM alone, and there was no isolation x MAM interaction, which prevents the conclusion that the MAM treatment may have reversed a deficit induced by isolation. With inconsistent deficits produced by isolation rearing, and no MAM-induced deficits observed (whether due to a strain effect or not), results of the NOD protocol also undermine the reliability and viability of this dual-hit approach.

It is important to note that an additive or synergistic effect of combining isolation rearing and MAM treatment may be difficult to observe in this protocol due to a ceiling effect. Whilst an effect due to one treatment alone can be observed in one of the single treatment groups, as was seen in the Iso-Con group of both these cohorts, an inability to perform the task cannot be subsequently worsened by the addition of a second perturbation in the dual-hit group. This means any additive or synergistic effects of the dual-hit approach will be masked. Procedurally, the protocol could be made easier (shortening the inter-trial interval), so that rats in a single treatment group are still able to perform the task (potentially to a lesser extent), but a synergistic/additive effect of dual-hit treatment could produce an impairment in visual learning and memory.

A similar result to the NOD protocol was observed in the CER paradigm, where isolation rearing significantly reduced contextual and cue-mediated freezing responses following fear-motivated associative learning in both the pilot and main studies. MAM treatment alone had a significant main effect on freezing at the Post-CS time point of the pilot study, but no post-hoc significance was observed and the finding was not replicated in the main study. This paradigm may have particular relevance to psychiatric disorders, including schizophrenia, as previous reports indicate significant deficits in conditioned associative learning (Rushe et al. 1999) as well as the ability to maintain stimulus-reward relationships in memory over long delay periods in individuals with schizophrenia (Herbener 2009). The results observed due to

isolation rearing agree with previous literature including that of our laboratory. The CER protocol was extensively validated by the group and has been used in a number of studies relevant to cognition and schizophrenia, including a recent publication with the protocol showing sensitivity to serotonin mediation and NMDA receptor antagonism in rats exposed to the experimental conditions described here (Woods et al. 2012). The significant reduction in freezing response caused by isolation rearing in both cohorts here supports the findings of a number of other publications that observed decreased fear-induced freezing in a contextual fear conditioning paradigm (Weiss et al. 2004) and shorter retention latencies in a passive avoidance test (Del Arco et al. 2004) in isolation-reared Sprague-Dawley rats, as well as impaired context-induced freezing in mice reared in social isolation (Gresack et al. 2010).

The effect of prenatal MAM treatment on contextual fear-conditioning is less well characterised, but would be expected to be similar to that of isolation rearing since it also produces morphological deficits in the hippocampus. However, there is some evidence to suggest that despite marked reductions in entorhinal, prefrontal cortical and striatal volumes, MAM administration on GD12-15 produces no deficit in active avoidance or freezing paradigms (Leng et al. 2005). Whilst the neuroanatomical profile of GD12-15 rats is markedly different to GD17 rats, it does suggest that even with significant anatomical changes due to MAM, the functional effects of administration on freezing behaviour may be more difficult to highlight. The only main effect of MAM was observed at the post-CS time point of the pilot, but despite a trend towards increased freezing, this failed to achieve significance post-hoc. Previous

studies have identified specific, protocol-dependent effects of MAM treatment on contextual/cue conditioning (Gresack et al. 2010). A potential effect of MAM on cue-conditioning rather than contextual-conditioning may suggest an involvement of the amygdala, and whilst there is little published evidence on the specific effect of prenatal MAM treatment on the volume and organisation of the amygdala, in the rat GD17 is a key time point for neuronal development in regions including the amygdala (Bayer 1980). This would be consistent with neuronal development and/or organisation in this region being disrupted by MAM treatment at GD17, although this is mere speculation without statistically significant results to support the notion. Reduced cognitive flexibility, as seen previously with MAM treatment on GD17 (Gastambide et al. 2012) and GD15 (Leng et al. 2005), may also explain altered freezing following CS exposure. In this paradigm, a lack of flexibility may manifest as an inability to relearn that the CS is no-longer accompanied by an aversive footshock, and hence the rats continue to freeze for longer through the post-CS period than vehicle-treated controls. To assess cognitive flexibility, the Morris Water Maze paradigm was added to the behavioural battery (discussed below). However, the main study did not replicate the significance due to MAM in the pilot CER paradigm. A further lack of robustness for MAM-induced behavioural changes again weakens the basis for continued use in this dual-hit study. However, even if a significant increase in freezing due to MAM had been confirmed and replicated, this response is opposite to the effect of isolation rearing to reduce freezing. The effect of combining treatments would therefore cancel each other out, producing an inconclusive phenotype.

The Morris Water Maze test assessed visuo-spatial learning by requiring subjects to learn first the location of a hidden platform in a fixed position, and subsequently re-learn the location of the platform in differing positions on each trial requiring disregard of the previously learnt rule, and re-acquisition of a new rule in the same contextual setting. As expected, in the fixed location task rats progressively reduced their latency to reach the platform across the 15 training trials, demonstrating spatial learning using the visual cues provided around the arena. Despite an indication that MAM rats may have demonstrated an increased latency to reach the platform, particularly in trials 8-12, this was shown to be non-significant, supported by a lack of significance in Probe 1. However, following a 60 hour extinction period, MAM-treated rats spent significantly less time in the correct maze quadrant in the second probe trial, showing their retention of the task was impaired. Altered acquisition of a water maze task is not a novel finding in prenatal MAM models, having been reported on following MAM treatment on GD11 (Fiore et al. 2002), GD14 (Vorhees et al. 1984), and GD15 (Banfi et al. 1984), but only recently with GD17 (Snyder et al. 2013). Many prominent papers investigating MAM in the Morris Water Maze, however, have suggested the treatment is ineffective in impairing acquisition in the Morris Water Maze paradigm (Flagstad et al. 2005; Leng et al. 2005). Results presented herein suggest that MAM-treated rats were able to learn the task after completing 15 learning trials.

MAM-treated rats displayed impaired memory in Probe 2, 60h post-training, supporting previous work suggesting GD17 treatment impairs retention in the Morris Water Maze (Snyder et al. 2013) as well as other memory paradigms

(Gourevitch et al. 2004). There was no significant effect of isolation rearing on acquisition or retention of the task, in line with some previous work showing no deficit in performance (Schrijver et al. 2004), but not replicating previously observed improvements (Wongwitdecha and Marsden 1996a) or impairments (Hellemans et al. 2004; Lu et al. 2003) due to isolation.

In the subsequent reversal learning phase, performance in the task did not significantly improve across trials, irrespective of treatment group. Therefore, this reversal learning protocol may be too simple to demonstrate any deficit produced by isolation rearing and/or MAM treatment. A wide array of previous data suggests both prenatal MAM including GD17 injection (Featherstone et al. 2007; Flagstad et al. 2005; Gastambide et al. 2012), and rearing in social isolation (Li et al. 2007; Quan et al. 2010; Schrijver et al. 2004), causes deficits in reversal learning paradigms, including the Morris Water Maze, which reflects behavioural rigidity (Fone and Porkess 2008; Lodge and Grace 2009). Improvement in the performance of the control animals in this protocol by extending the number of trials may allow further evaluation of any deficits induced to better reflect the literature.

As changes in reversal learning were not significant, the results of probe 3 are less informative. There was no significant effect of isolation or MAM on the time spent exploring the "platform ring", but as rats did not significantly improve their performance over the course of the 9 trials, this would be expected. Of use from this probe is the ability to investigate how perseverative the rats are to explore the previously correct region of the maze. There was no main effect due to either isolation or MAM, but there was a significant

interaction between the two treatments. Whilst GH-MAM and Iso-Con groups showed a slight increase in time spent exploring the previously correct quadrant as you might expect in rats unable to reverse or "unlearn" a previous rule, rats in the Iso-MAM groups exhibited a slight decrease in time spent there. Whether this significance would persist with a fuller reversal learning protocol is unclear, however, as there was no significant effect of treatment on overall quadrant exploration in Probe 3 (data not shown).

Whilst the MWM protocol used was relatively successful, future improvements were clearly required to give more relevant results. An extension of the reversal learning protocol to a full 15 trials, as in the initial acquisition phase, would improve the statistical outcome.

In the prepulse inhibition of acoustic startle paradigm, results in the pilot and main studies differed. In the pilot study, a clear trend towards a significant decrease in PPI in the Iso-MAM group was noted at each of the three prepulse intensities, reaching post-hoc significance at prepulse intensity of 80dB. In the main study, a clear decrease in PPI due to isolation rearing was observed, with significance obtained post-hoc in at prepulse intensity of 76dB only. Previous work by numerous groups, including our own, has demonstrated impairments in prepulse inhibition in rats reared in social isolation (Cilia et al. 2001; Powell et al. 2002; Schubert et al. 2009), treated prenatally with MAM (Le Pen et al. 2006; Moore et al. 2006; Talamini et al. 1998) and in other rodent models of psychiatric disorder (Brody et al. 2003a; Li et al. 2011b). Such deficits are thought to have translational relevance to deficits in sensorimotor processing

and the ability to filter non-essential sensory information, symptoms observed in several neuropsychiatric disorders including schizophrenia (Braff and Geyer 1990). All of these deficits were observed in single-interference studies, yet a significant deficit due to MAM treatment alone was not observed in either study, with a main effect of isolation observed only the main cohort. Some previous reports have documented a lack of PPI deficit due to prenatal MAM treatment in rats (Jongen-Relo et al. 2004), but as this utilised earlier MAM administration the results cannot be directly compared to those herein. When combined with isolation rearing, MAM treatment in the pilot study showed a PPI deficit, suggesting the potential for a synergistic effect of the two perturbations. As the aim of this project was to develop a model with increased robustness, such a synergistic effect observed in the combined treatment is an important finding, however the results were not replicated in the main study.

One of the main motivations for developing a combined early-life manipulation model of 'schizophrenia-like' symptoms is the variability and lack of robustness of changes seen in a number of the most frequently used behavioural paradigms to assess change in performance in these models. PPI deficits are only reported in approximately 82% of isolation reared groups, falling to around 66% where shorter isolation periods have been utilised (Fone and Porkess 2008), but since this study further publications have also failed to observe a deficit in PPI following isolation rearing (Jones et al. 2011a; McIntosh et al. 2013). One small caveat to the isolation-induced PPI deficit seen here is the observation of a significant decrease in the initial startle amplitude of the isolation-reared rats compared to their group-housed

counterparts. Whilst this observation has also been reported previously in the absence of a PPI deficit (Jones et al. 2011a), meta-analyses concluded that isolation-induced PPI deficits were independent of startle reactivity (Cilia et al. 2001), so should not confound the interpretation of the significant effect of isolation on PPI here. A lack of MAM effect alone in either study may again undermine this dual-hit model, despite the potential synergistic effect of combination treatment in the pilot study. This outcome was a major motivation in conducting the main isolation-MAM combination study, but a failure to replicate the pilot data is a further negative result.

Consideration should be given to the fact that multiple behavioural tests were performed in the same animals in these studies, although there is little consensus over whether behavioural assessment may affect the outcome of subsequent tests. Some evidence suggests isolation-induced deficits may be lost due to previous testing (Domeney and Feldon 1998) or that the volume of rodent brain structures may be increased or decreased by repeated task training (Lerch et al. 2011), yet numerous publications, including those of our lab (McIntosh et al. 2013; Schubert et al. 2009), have shown schizophrenia-like deficits being produced in rodents undergoing numerous or repeated behavioural tests (Beninger et al. 2010; Moller et al. 2013; Nakatani-Pawlak et al. 2009). It is therefore unlikely that the repeated testing protocol used in this thesis is responsible for the lack of robust deficits observed.

MAM treatment significantly reduced the mean total brain weight, and reduced hippocampal volume by approximately 34% of control in the pilot study, and affected the wet weight of the hippocampus and striatum in rats from the main study (although this did not reach post-hoc significance). In contrast, isolation rearing had no similar effect on hippocampal volume or on total brain/hippocampal/striatal wet weight. The highly significant reduction in hippocampal volume due to GD17 has been seen consistently by many groups using a variety of measuring techniques, including magnetic resonance imaging (Chin et al. 2011), cross-sectional thickness (Moore et al. 2006) and area (Le Pen et al. 2006) measurement, and simple wet weight (Flagstad et al. 2004), with the extent of the reductions observed comparable to those seen here. Similarly, other groups have noted decreased wet weight of the dorsal striatum (Featherstone et al. 2007) in GD17 treated rats, to which our observation lends support. A decrease in striatal weight may be of significant interest in this model, as it is hypothesised that behaviours and key functions including reversal learning (Ragozzino 2003; Ragozzino et al. 2002a; Ragozzino et al. 2002b) and attentional processing (Rogers et al. 2001) are dependent on neural circuits involving the striatum. In contrast, whilst it is known that some structural alterations are observed in the hippocampi of rats reared in social isolation including reduced levels of synaptophysin (Varty et al. 1999) and altered dendritic properties in the pyramidal cells (Silva-Gomez et al. 2003), the effect of isolation rearing on overall hippocampal volume has produced both reductions (Fabricius et al. 2010) and a lack of significant changes (Schubert et al. 2009). Our results are in line with the findings of

Schubert et al, as no effects on volume or weight were observed in the hippocampus or striatum due to isolation rearing. Indeed, the results presented by Fabricius et al displayed changes in hippocampal volume following 15 weeks of isolation rearing, much longer than the period of single housing employed here. Interestingly, despite large (34%) MAM-induced reductions in hippocampal volume/weight, very few behavioural deficits occurred after MAM alone compared to the GH-Con group across the behavioural paradigms investigated. Previous work giving prenatal MAM on earlier gestational days (GD9-15) produces a much more severe reduction in brain weight and reduced prefrontal cortical and striatal volume, with little or no accompanying deficits in PPI, conditioned emotional freezing, or visual and spatial learning and memory (Jongen-Relo et al. 2004; Leng et al. 2005). These results indicate largely conserved cognitive function despite hippocampal abnormality. Consistent with this observation, ablation of discrete hippocampal regions has little or no effect on novel object discrimination (Lee et al. 2005; Sannino et al. 2012), or PPI (Bast and Feldon 2003; Pouzet et al. 1999), and cytotoxic lesions to the hippocampal poles (sparing the intermediate region) do not impair incremental learning in the Morris Water Maze (Bast et al. 2009). It is interesting to note that the highly significant decrease in total brain mass, accompanied by a limited profile of behavioural deficits, due to MAM treatment here most closely mirrors those observed previously with earlier prenatal treatments (Jongen-Relo et al. 2004; Leng et al. 2005). This may suggest a problem with the time of MAM administration used here, or may

indicate that in Lister-Hooded rats a different profile of deficits may be associated with particular time points of MAM treatment.

In contrast to the morphological findings, monoamine levels in the hippocampus and frontal cortex were largely unaltered in the main study. ANOVA revealed that there was a significant alteration in 5-HIAA concentration in the hippocampus of MAM-treatment animals, but this was the only significant effect once anomalies were removed from the data. No effect of isolation rearing was seen on any monoamine level in either region tested. Reported results have varied, but isolation rearing has been shown to increase basal PFC dopamine (Jones et al. 1992), yet decrease DA turnover in the mPFC together with a decrease in basal serotonin turnover in the NAc (Heidbreder et al. 2000). Furthermore, other studies have found no change in basal 5-HT, DA or glutamate in the NAc (Howes et al. 2000), and no change in 5-HT in the PFC (Dalley et al. 2002) in isolation reared rats compared to control. This lack of consensus on monoamine changes in isolates makes results herein difficult to conclusively interpret. In MAM-treated rats, some studies have shown increased hippocampal 5-HIAA levels in GD15 MAM (15mg/kg and above) treated rats which also had increased 5-HT levels in the cerebral hemispheres (Tamaru et al. 1988). Several groups have reported that MAM significantly effects dopamine signalling in the hippocampus (Lodge and Grace 2007; 2008; Tamaru et al. 1988), but this was not replicated in the current study.

The enduring impression obtained from these neuroanatomical results is that despite marked deficits in hippocampal formations due to prenatal MAM treatment, they did not translate to behavioural impairments, limiting the utility of this dual-hit model.

One of the biggest issues encountered in this study was identifying the time of conception, and hence accurately administering MAM on GD17. Whilst the mating period can be determined, the start of gestation can only be predicted by the discovery of the vaginal plug in the cage bedding, which although possible, can be difficult. Even in the event of the plug being found, this often occurred during morning inspection of the cages around 8am; as the cages were last checked the night before at approximately 6pm there is a 14 hour window in which gestation could have started. When attempting to accurately administer a drug on GD17, a 14 hour difference in injection time (3.4% of the gestational period to this point) could highly influence outcome. This is evident since injection of MAM on GD15 (36-48 hours earlier than GD17), has a drastically different effect on pup neurodevelopment, physiology and behaviour after birth (Balduini et al. 1991; Jongen-Relo et al. 2004; Leng et al. 2005). In order to counteract this problem in the pilot study, pregnant dams were sourced externally rather than bred internally, as this came with the guarantee of identical and specific gestational day across all dams. However, this adds further issues to the protocol, as consistency of genetic background, mating procedure and early gestational housing cannot be confirmed.

Furthermore, transportation of animals in early gestation may have adverse effects on the pups through maternal stress, potentially confounding results.

2.4.1 Conclusion

Taken individually, both of the studies presented above show interesting results towards the aim of combining two neurodevelopmental models of schizophrenia as a dual-hit treatment to produce a model of 'schizophrenia-like' symptoms. However, a striking a lack of consistent changes in any behavioural paradigm across the two studies, and no reproducible additive or synergistic effect of combined treatment, severely undermine the protocol and must lead to the hypothesis in this chapter being rejected. In the LMA, NOD and PPI paradigms there were no deficits caused by isolation or MAM that were consistent in both studies, with an isolation-induced reduction in freezing in the CER paradigm the only deficit seen in both cohorts. In contrast, neuroanatomical changes were consistently seen after MAM treatment only. These anatomical changes were not accompanied by consistent behavioural alterations, and so the conclusion must be that an isolation-MAM dual-hit treatment is not an improved model for 'schizophrenia-like' symptoms, and so will not be continued in this thesis. As a well-established model within the group, isolation rearing was continued as the basis for future dual-hit models, but with an alternative pharmacological challenge added in the place of prenatal MAM treatment (Chapter 3).

Chapter 3

Combined Rearing in Social Isolation and Perinatal Phencyclidine Treatment as a Model of 'Schizophrenia-Like' Symptoms in the Rat

3.1 Introduction

Following the conclusion drawn from the previous chapter to discontinue use of the gestational MAM model, an alternative 'dual-hit' strategy was developed to produce a more complete and robust animal model than isolation rearing alone. As outlined previously (see Chapter 1.3.3), administration of the NMDA receptor antagonist phencyclidine (PCP) has been used to model behavioural changes which resemble symptoms seen in schizophrenia by many other groups. In this chapter, perinatal exposure to PCP was combined with isolation rearing as an early-life adverse intervention that might elicit changes similar to those that could occur in schizophrenia development in humans. This programme of research intends to assess the predictive validity of a new 'dual-hit' preclinical model using existing antipsychotic and potentially novel compounds to treat schizophrenia. Since PCP treatment is during the perinatal period it avoids possible drug-drug interactions that could complicate interpretation of results in possible future reversal studies.

The specific protocol for PCP administration was modelled on a method previously published (Liu et al. 2010; Wang et al. 2001; Wang et al. 2008), utilising subcutaneous drug injection on PNDs 7, 9 and 11 at a dose of 10mg/kg. The theory was that this NMDA receptor antagonist treatment could cause a decrease in cortical GABAergic interneuron density as seen previously (McKibben et al. 2010; Radonjic et al. 2013), as well as alterations in gene expressions (Liu et al. 2010) and receptor densities (du Bois et al. 2009) that have validity to the pathology of schizophrenia. PCP was chosen over other

NMDA receptor antagonists, such as MK-801 which has been used previously by other groups (Ashby et al. 2010), due to its more extensive effects on GABAergic interneuron populations. Whilst MK-801 treatment mirrors that of PCP in reducing the number of PV-positive interneurons in the hippocampus, unlike PCP it does not have the same effect in the prefrontal cortex (Braun et al. 2007), where GABAergic interneuron loss has been seen in different groups of schizophrenia patients (Beasley and Reynolds 1997; Beasley et al. 2002).

3.1.1 Hypothesis

Considering the wealth of previous findings detailing the cognitive deficits induced in adult rats following isolation rearing and perinatal PCP treatment, combination treatment was hypothesised to produce a phenotype that displayed impairments in all behavioural paradigms assessed. Furthermore, based on the indication from the previous chapter that two treatments may have the potential to cause more marked deficits when used in combination, it was also hypothesised that an additive or synergistic effect of dual-hit treatment would be observed in some behavioural tests. Confirmation of this hypothesis would indicate a preclinical model with improved validity to schizophrenia in man.

3.2 Materials and Methods

3.2.1 Animals

Eight litters of three-day old, male, non-cross fostered Lister Hooded rat pups (Study 1 *n*=43, Study 2 *n*=43) were obtained from Charles River UK (CRUK, Margate, UK) accompanied by their natural mothers. On PND 7, pups were treated with phencyclidine hydrochloride (Sigma-Aldrich, Irvine, Scotland, UK) dissolved in saline (10mg/kg s.c.) or vehicle control (1ml/kg) following the protocol outlined previously (Wang et al. 2001). In order to avoid maternal rejection of the pups, hands were thoroughly washed and were rubbed in bedding material prior to handling individual pups. Photographic records of the pups were kept to avoid repeated individual marking. Pups were checked hourly following injection and returned to the nest if separated from the dam. This process was repeated on PND 9 and 11, with pups receiving the same drug treatment on each occasion. Further details of the perinatal PCP treatment procedure are attached (see Appendix). Pups were subsequently weaned on PND23 and housed in groups of 3-4 (32x51cm polycarbonate cages with metal grid lids) or in social isolation (25x42cm cages) to produce four treatment groups, with food and housing conditions as described in the previous chapter; group-housed control-treated (GH-Con), group-housed PCP-treated (GH-PCP), isolated control-treated (Iso-Con), and isolated PCP-treated (Iso-PCP) (Table 3.1).

Table 3.1 Table to show the perinatal drug treatment and post-weaning housing condition of each pup in (A) the pilot study, and (B) the main study. Also shown, the behavioural tests undertaken by each litter

A

Litter	No of Pups	Litter Assignments				Behaviours
		GH Saline	GH PCP	Iso Saline	Iso PCP	
1	6	1	2	1	2	All rats performed LMA, NOD, PPI, MWM, and PCP-induced LMA
2	7	1	2	2	2	
3	5	2	1	0	2	
4	3	0	0	2	1	
5	6	2	2	1	1	
6	6	1	2	2	1	
7	5	2	1	1	1	
8	5	1	2	1	1	

B

Litter	No of Pups	Litter Assignments				Behaviours
		GH Saline	GH PCP	Iso Saline	Iso PCP	
1	6	2	1	1	2	All rats performed LMA, NOD, PPI CER, MWM, and PCP-induced LMA
2	6	1	2	2	1	
3	5	2	1	1	1	
4	5	1	2	1	1	
5	5	2	1	1	1	
6	5	1	2	1	1	
7	4*	2	1	0	0	
8	5	0	0	2	3	

*One pup found dead on PND 8

Behavioural testing in the LMA, NOD, PPI and CER paradigms was identical to that detailed previously (see Chapter 2.2) and took place following the timeline showing in Figure 3.1. In the second cohort, half of each treatment group were assigned to the CER and half to the MWM behavioural tests to assess two different cognitive domains. Rats did not perform both tasks as they require considerable training, which could impact on subsequent behaviour if performed in the same animals. Modifications were made to the MWM protocol outlined below, and an additional acute response to PCP challenge paradigm was added to the battery.

3.2.2 Morris Water Maze Modifications

In modification of the previous MWM protocol (see Chapter 2.2.6), the reversal protocol was extended in this study to be the same length as the learning period – a 15 trial, 5 day protocol, following the same pseudo-random ordering as previously, to increase the available data. Probe 3, as described previously, was then performed at the cessation of reversal learning. An identical fourth probe test was conducted in the second cohort 60 h after probe 3, to objectively assess whether the rats had successfully re-acquired the new rule.

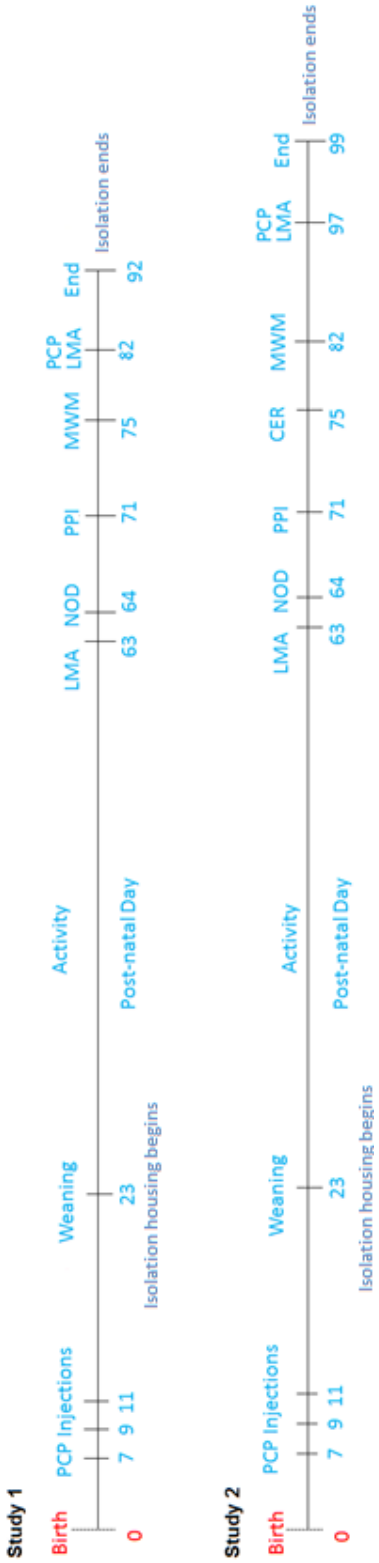

Figure 3.1 – Time stick representations of the PCP administration and behavioural testing protocols used in study 1 and study 2. Protocols were identical until PND 78, where in study 2 the CER protocol was reinstated to the behavioural battery.

3.2.3 Hyperactivity in Response to Acute PCP Treatment in an Open Field

The acute response to PCP-treatment was examined to assess any long-term changes in NMDA receptor function in the same open field Perspex chambers used in the initial locomotor and novel object discrimination tests. Rats were assigned in a pseudo-random manner to a different one of the 12 chambers used previously to ensure some contextual novelty, and testing took place 28 days after initial exposure to the context. Rats were placed in the chamber for 1h habituation, during which ambulations were measured by infra-red beam breaks as described previously. Following habituation, all rats received a single injection of phencyclidine hydrochloride (3.2mg/kg i.p.) dissolved in saline and were immediately returned to the test arena for a further 1h and ambulation recorded.

3. 3 Results

3.3.1 Effect of isolation rearing and perinatal PCP on locomotor activity

Study 1: Rats were assessed for neophobia to a novel arena by measuring locomotor activity in a closed environment. In keeping with previous results, rats exhibited gradual habitation to the novel arena such that RM ANOVA showed a significant main effect of time [$F_{(11,418)}$=69.977, p<0.001] but no significant main effect of housing [$F_{(1,38)}$=0.051, p=0.822] nor a main effect of PCP treatment [$F_{(1,38)}$=3.376, p=0.074]. There were no significant interactions between any of the three factors analysed by RM ANOVA (Figure 3.2). Two-way ANOVA analysis of the total locomotor activity across the entire protocol supported these findings, with no significant main effects of housing or PCP treatment, and no interaction. One rat which exhibited exploratory activity twice that of any other (more than two standard deviations from the mean) was removed from data analysis.

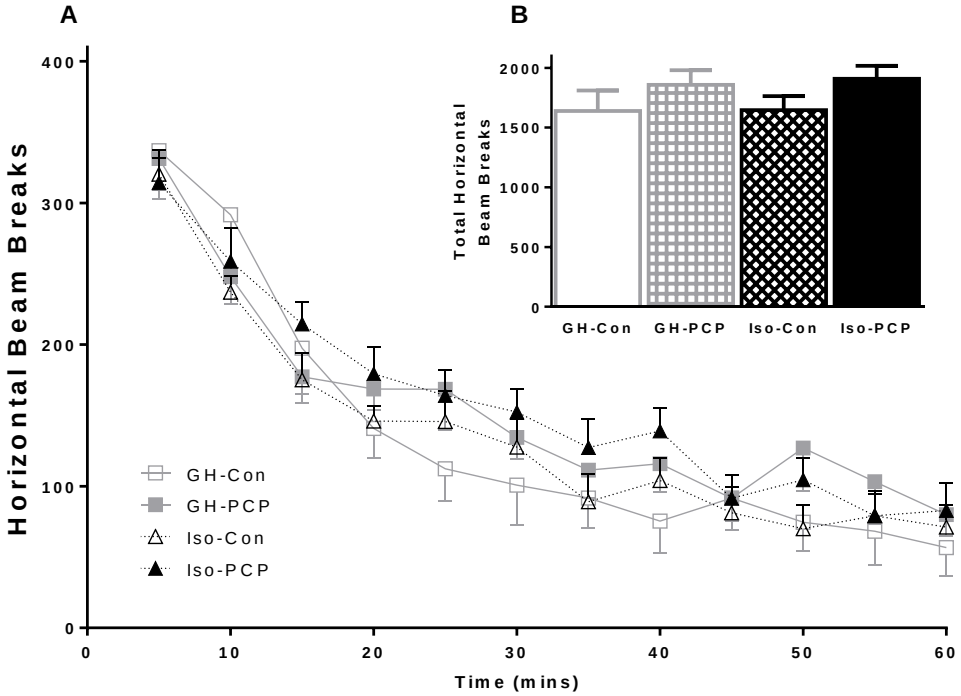

Figure 3.2 – Progressive decrease in locomotor activity during 60 min exposure to a novel arena. (A) Repeated-measures ANOVA showed a significant main effect of time [$F_{(11,418)}$=69.977, p<0.001] but no significant main effect of isolation rearing (Iso) or perinatal PCP treatment (PCP, 10mg/kg s.c. on PND 7, 9, 11), compared with group-housed (GH) and vehicle treated (Con) controls respectively, on horizontal locomotor response in 5 min epochs (horizontal beam breaks, mean ±SEM, n=6-8) for 1h. (B) Total cumulative activity counts (mean ±SEM) over the 1h trial showed no main effect of isolation rearing or perinatal PCP treatment, and no interaction between factors.

Study 2: Rats in all four treatment groups exhibited a progressive decrease in motor activity over time, reflecting habituation to the arena (Figure 3.3), and RM ANOVA analysis confirmed a significant main effect of time [$F_{(11,429)}$=109.676, p<0.001]. A significant main effect of rearing condition was also observed [$F_{(1,38)}$=7.893, p=0.008], with post-hoc data indicating that particularly later in the 60 min protocol, rats in the Iso-Con group displayed significantly elevated activity compared to GH-Con controls. There was no main effect of perinatal PCP treatment and no interactions between the factors over the timecourse. Two-way ANOVA analysis of the cumulative locomotor activity confirmed a significant main effect of isolation rearing [$F_{(1,38)}$=8.537, p=0.0058], and no main effect of PCP treatment nor an interaction between the two factors. Post-hoc tests following ANOVA showed that Iso-Con rats were significantly more active that GH-Con rats across the 1 h protocol (p<0.05).

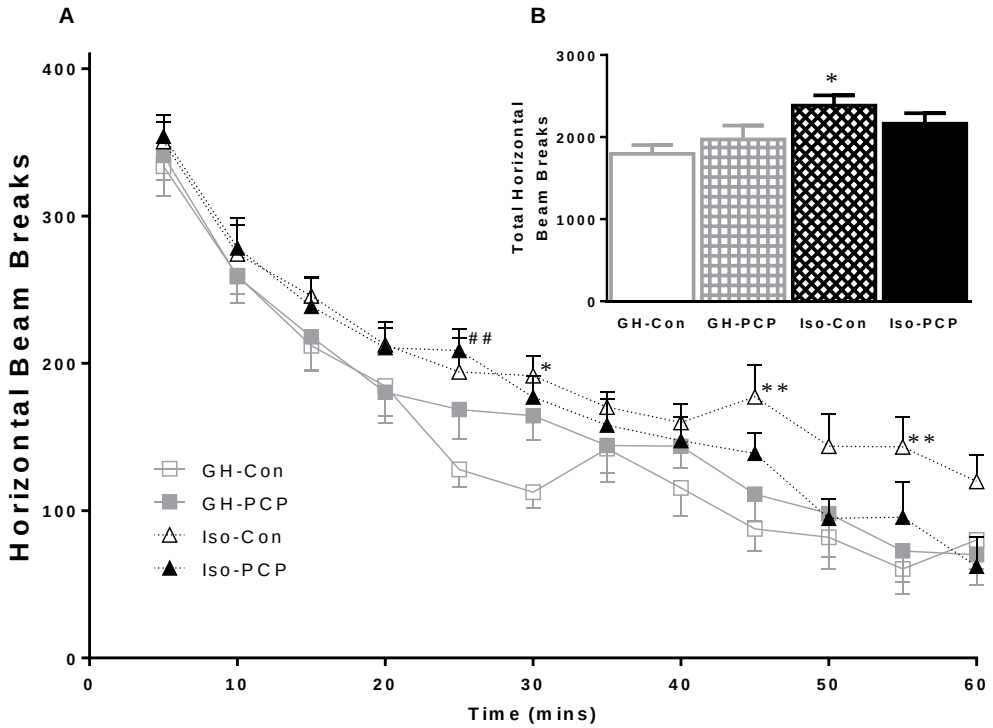

Figure 3.3 – Isolation rearing caused a significant elevation in locomotor activity. Significant effect of isolation rearing (Iso), but not perinatal PCP treatment (PCP), compared to group-housed (GH) and vehicle-treated (Con) controls respectively, on the locomotor response to a novel arena for a 1h test period by RM ANOVA. (A) Activity counts per 5 minute time bin (mean ±SEM, *n*=10-12) were significantly affected by time [$F_{(11,429)}$=109.676, p<0.001, RM ANOVA] and isolation rearing [$F_{(1,39)}$=7.893, p=0.008], but not perinatal PCP treatment (10mg/kg s.c. on PND 7, 9, 11) and there was no interaction. **p<0.01 *p<0.05 isolation vs. PCP-matched group-housed control, $^{\#\#}p$<0.01 isolation+PCP treatment vs. GH-Con absolute control group by Bonferroni post-hoc following ANOVA. (B) Total cumulative activity counts over the 1h test period (mean±SEM) were significantly affected by isolation rearing [$F_{(1,38)}$=8.537, p=0.0058] with no main effect of PCP treatment nor a between-treatment interaction. *p<0.05 isolation vs. PCP-matched group-housed control by Bonferroni post-hoc analysis following ANOVA.

3.3.2 Effect of isolation rearing and perinatal PCP on novel object discrimination

Study 1: To examine the effect of treatment on visual recognition memory, behaviour was examined in a two-trial novel object discrimination task. As expected, rats preferentially explored the novel over the familiar object such that two-way ANOVA of the second, choice trial showed a significant main effect of object [$F_{(1,36)}$=32.14, p<0.001] (Figure 3.4). Subsequent post-hoc analysis indicated that only GH-Con and GH-PCP groups (p<0.001 and p<0.01, respectively), spent significantly more time exploring the novel object, whilst Iso-Con and Iso-PCP groups were unable to differentiate the novel object over familiar. Further analysis revealed no significant effect of housing or PCP treatment, nor any interaction between the two, on the derived D1 discrimination ratio (two-way ANOVA, data not shown). There was no significant main effect of isolation rearing, prenatal MAM treatment, or of NOD trial on total object exploration (object 1 + object 2) by three way ANOVA (Figure 3.4B).

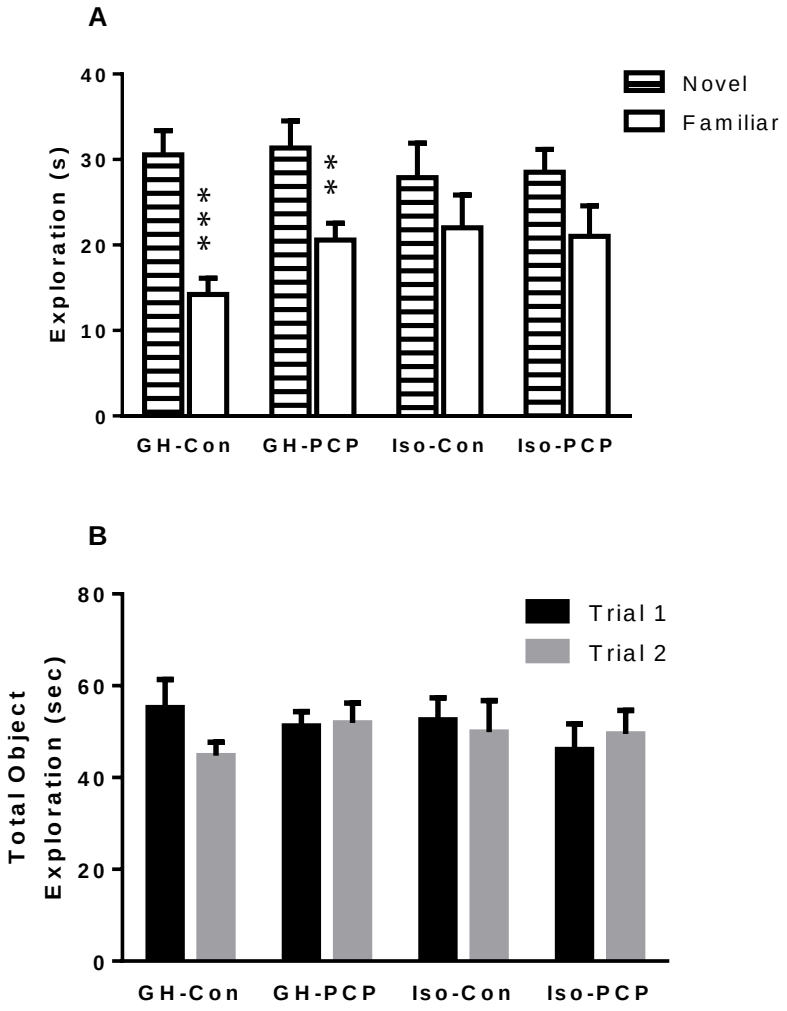

Figure 3.4 – Isolation rearing-induced impairment in visual reference memory in the novel object discrimination task. Group-housed rats (GH) spent significantly more time exploring the novel object than familiar (A) (s, mean±SEM, *n*=10-12) compared to isolation-reared controls (Iso), irrespective of perinatal PCP (PCP, 10mg/kg s.c., on PND 7, 9, 11) or saline (Con) treatment, during the second choice trial of a two-trial novel object discrimination task [Bonferroni post-hoc analysis following main effect of object by two-way ANOVA, $F_{(1,36)}$=32.14, p<0.001] ***p<0.001, **p<0.01 vs. novel object by Bonferroni post hoc analysis following ANOVA. (B) No significant main effect of isolation rearing, prenatal MAM treatment, or of NOD trial on total object exploration (object 1 + object 2) by three way ANOVA.

Study 2: Overall, rats preferentially explored the novel object during the choice trial of the NOD task [$F_{(1,37)}$=25.89, p<0.0001, ANOVA], which was particularly notable in GH-Con and GH-PCP rats, with post-hoc analysis confirming that only these groups (p<0.05 and p<0.001, respectively) and not the Iso-Con or Iso-PCP rats explored the novel significantly more than the familiar object (Figure 3.5A). A significant main effect of treatment was also seen [$F_{(3,37)}$=3.514, p=0.0245]. In support of this, the derived D1 discrimination ratio was significantly affected by housing by two-way ANOVA [$F_{(1,38)=}$5.380, p=0.0258], but there was no effect of PCP treatment, no interaction between the two factors, and no post-hoc significance observed (Figure 3.5B). There was no significant main effect of isolation rearing, prenatal MAM treatment, or of NOD trial on total object exploration (object 1 + object 2) by three way ANOVA, despite a trend towards increased exploration in isolation reared rats in both trial 1 and trial 2 (Figure 3.5C).

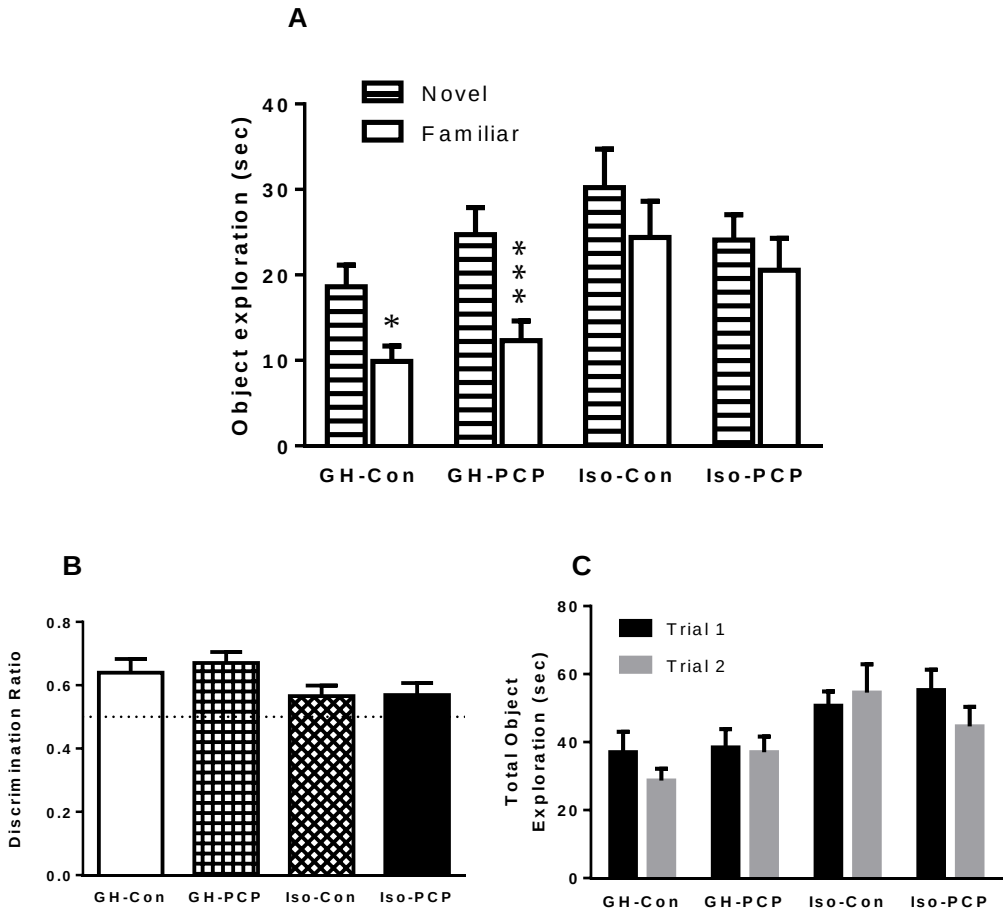

Figure 3.5 – Isolation rearing-induced impairment in visual reference memory in the novel object discrimination task. (A) Group-housed rats (GH) spent significantly more time exploring the novel object than familiar (s, mean±SEM, *n*=10-11) compared to isolation-reared controls (Iso), irrespective of perinatal PCP (PCP, 10mg/kg s.c., on PND 7, 9, 11) or saline (Con) treatment, during the second choice trial of a two-trial novel object discrimination task [Bonferroni post-hoc analysis following main effect of object $F_{(1,37)}$=25.89, p<0.0001 and treatment group $F_{(3,37)}$=3.514, p=0.0245 by two-way ANOVA] ***p<0.001, **p<0.01 vs. novel object by Bonferroni post hoc analysis following ANOVA. (B) Two-way ANOVA confirmed a significant main effect of isolation rearing [$F_{(1,38)=}$5.380, p=0.0258] on the derived discrimination ratio (mean±SEM, *n*=10-11), with no main effect of PCP treatment, and no between factor interaction. (C) No significant main effect of isolation rearing, prenatal MAM treatment, or of NOD trial on total object exploration (object 1 + object 2) by three way ANOVA.

3.3.3 Effect of isolation rearing and perinatal PCP on prepulse inhibition of acoustic startle

Study 1: Behaviour was examined in a prepulse inhibition of acoustic startle test to evaluate changes in sensorimotor gating, thought to map preattentional processing cognitive domain in man (Young et al. 2009). As expected, exposure of rats to a sub-threshold acoustic pulse produced a significant attenuation of the acoustic startle response which increased with prepulse intensity (Figure 3.6). RM ANOVA of prepulse inhibition revealed a significant main effect of prepulse [$F_{(2,78)}$=149.916, p<0.001] and of PCP treatment [$F_{(1,39)}$=4.730, p=0.036] but no main effect of housing [$F_{(1,39)}$=0.672, p=0.417] and no between-factor interactions. No post-hoc significance occurred. Observed effects were not due to an alteration in basal startle response or habituation of the groups to the 120dB startle tone, as two-way ANOVA analyses of both of these parameters revealed no significant main effect of housing or PCP treatment, and no interactions between the two.

Study 2: Prepulse inhibition was increased at ascending prepulse levels but was decreased by isolation rearing and PCP treatment, supported by RM ANOVA [significant main effects of prepulse level $F_{(2,78)}$=68.789, p<0.001, isolation rearing $F_{(1,39)}$=4.372, p=0.043, and PCP $F_{(1,39)}$=7.429, p=0.010]. Post-hoc significance was observed between the GH-Con and Iso-PCP dual-hit treatment groups only at 76dB (p<0.05) and 84dB (p<0.05) prepulse levels (Figure 3.7). Two-way ANOVA showed there were no effects of isolation rearing or PCP treatment on initial startle amplitude or habituation to the 120dB tone, and no between-factor interaction (data not shown).

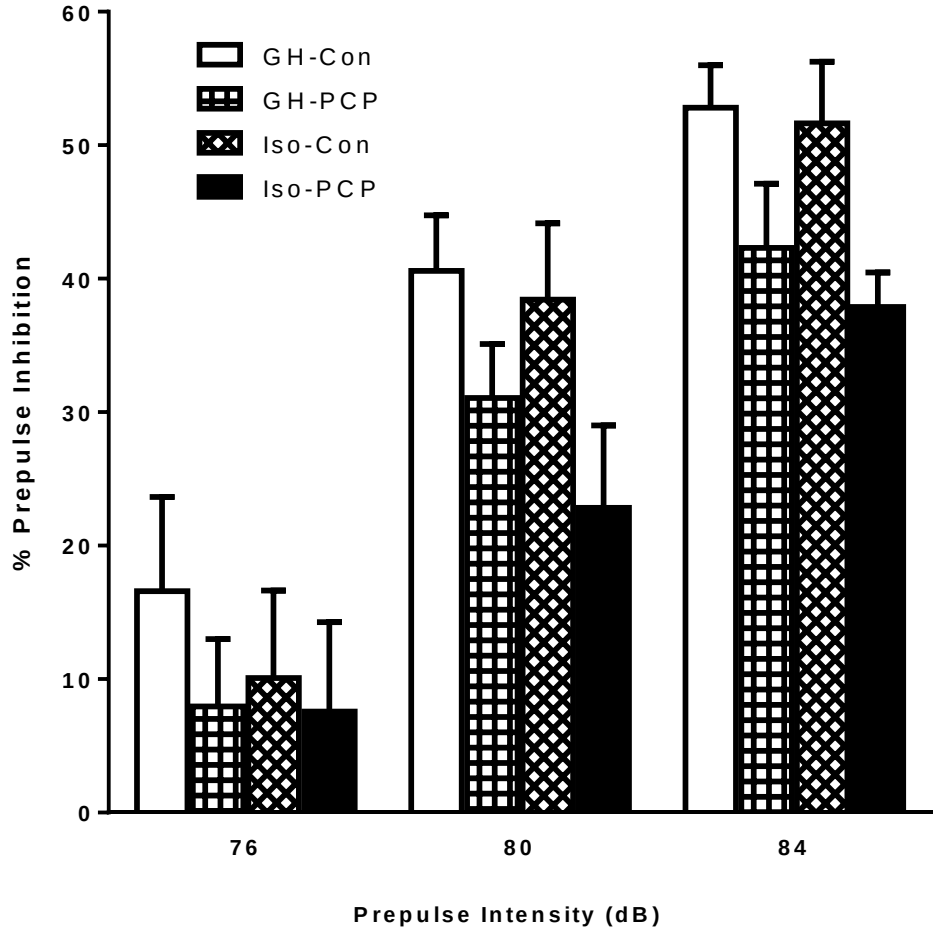

Figure 3.6 – Prepulse inhibition of acoustic startle was significantly affected by perinatal PCP treatment. A significant effect of perinatal PCP treatment (PCP, 10mg/kg s.c. on PND 7, 9, 11), but not isolation rearing (Iso), compared with group-housed (GH) and vehicle treated (Con) controls, on the percentage prepulse inhibition to a 120dB startle eliciting tone (mean±SEM, *n*=10-12) [main effects of perinatal PCP $F_{(1,78)}$=11.17, p=0.001, and prepulse level [$F_{(2,78)}$=149.916, p<0.001] by RM ANOVA]. There was no between factor interaction, and no post-hoc significance observed.

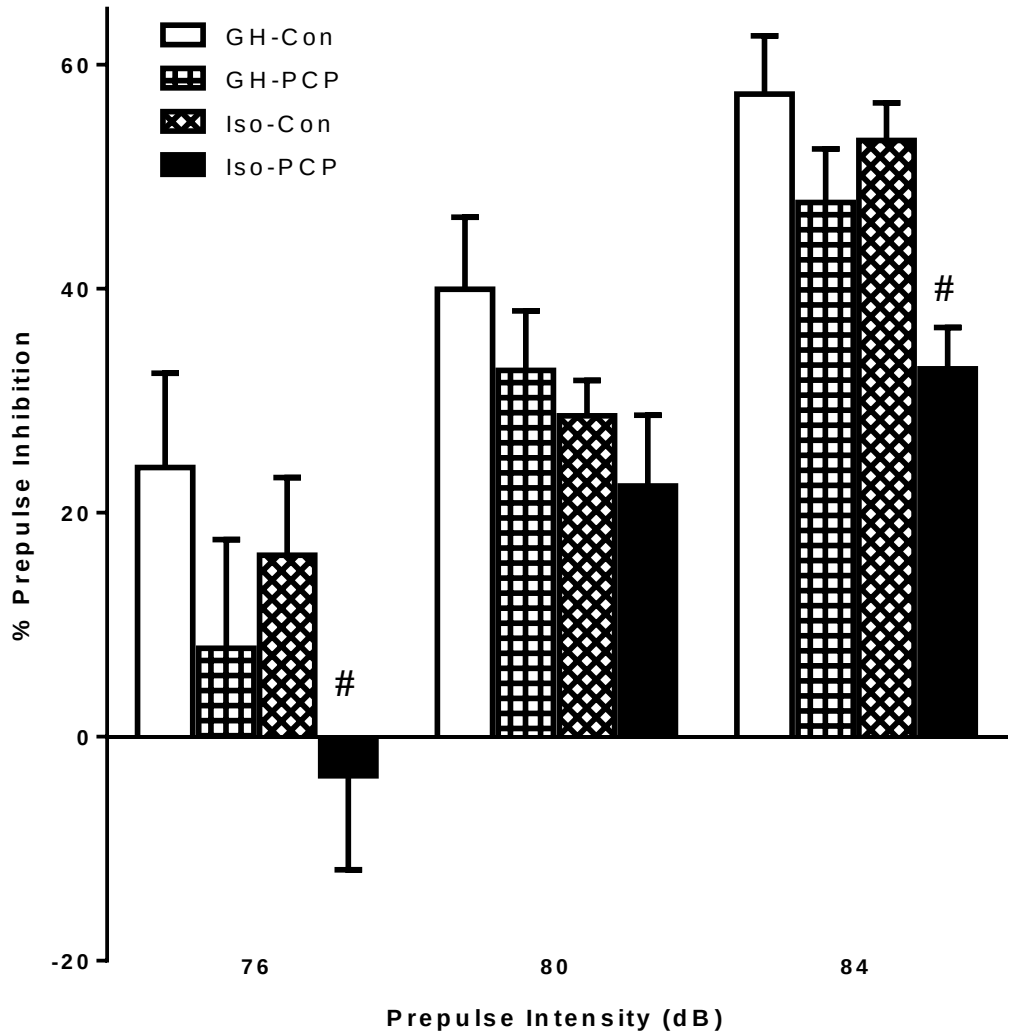

Figure 3.7 – Prepulse inhibition of the acoustic startle response was significantly reduced by combined isolation rearing and perinatal PCP treatment. Significant main effects of isolation rearing (Iso) and perinatal PCP treatment (PCP, 10mg/kg s.c. on PND 7, 9, 11), compared with group-housed (GH) and vehicle-treated (Con) controls, on the percentage prepulse inhibition of acoustic startle to a 120dB tone (mean±SEM, n=10-12). [main effects of prepulse level $F_{(2,78)}$=68.789, p<0.001, isolation rearing $F_{(1,39)}$=4.372, p=0.043, and perinatal PCP treatment $F_{(1,39)}$=7.429, p=0.010 by RM ANOVA]. $^{\#}p$<0.05 isolation+PCP treatment vs. GH-Con absolute control by Bonferroni post-hoc analysis following ANOVA.

3.3.4 Effect of isolation rearing and perinatal PCP on the conditioned emotional response

Study 2: To examine the impact of the early-life intervention on associative learning, contextual and conditioned emotional memory processes involving input from the hippocampus and amygdala were recorded in a conditioned emotional response paradigm (Woods et al. 2012). Freezing responses were reduced between the 24 and 48h time points, but were restored by presentation of the CS alone. Freezing responses were decreased in isolates at all time points, regardless of subsequent PCP treatment, such that two-way ANOVA revealed a significant main effect of isolation rearing at 24h [$F_{(1,38)}$=23.52, p<0.0001] and 48h [$F_{(1,38)}$=6.488, p=0.0150] post-conditioning, and following representation of the CS alone [$F_{(1,37)}$=22.35, p<0.0001]. Bonferroni post-hoc analysis revealed that the Iso-Con and the Iso-PCP treatment groups froze significantly less than their group-housed counterparts (GH-Con and GH-PCP, respectively) at both the 24h post-conditioning time point (p<0.05 and p<0.001) and following presentation of the conditioned stimuli alone (p<0.01 and p<0.05). The Iso-PCP combination group froze significantly less than the GH-Con absolute control group at all three time points (24h: p<0.001, 48h: p<0.05, Post-CS: p<0.001). Additionally, a main effect of perinatal PCP treatment was observed following CS presentation [$F_{(1,37)}$=8.654, p=0.0056], but there were no post-hoc effects of perinatal PCP treatment alone. No isolation x PCP treatment interaction was observed. (Figure 3.8).

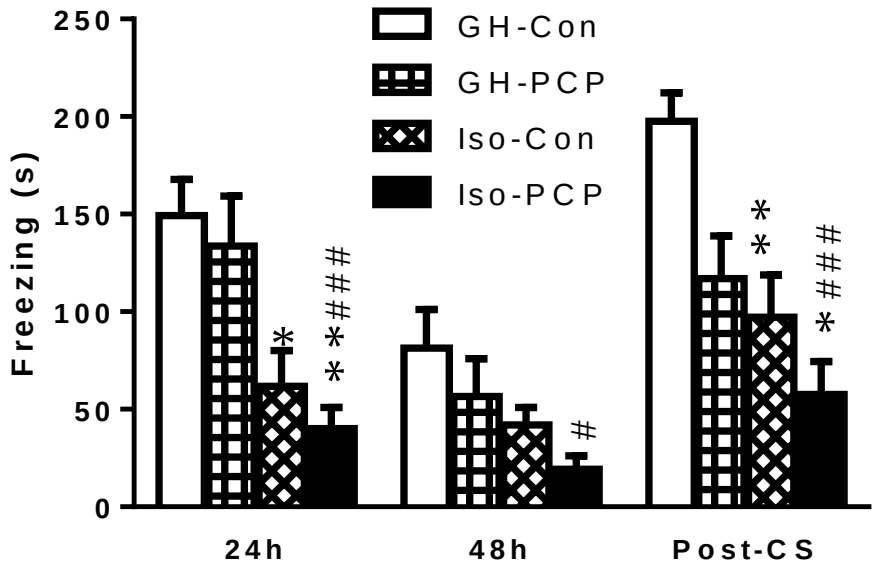

Figure 3.8 – Significant reduction in freezing time in a conditioned emotional response paradigm by isolation reared (Iso) compared to group-housed (GH) rats, regardless of perinatal PCP treatment (PCP) or vehicle control (Con). Two-way ANOVA revealed a significant main effect of isolation rearing on freezing time (mean±SEM, n=10-12) at 24h [$F_{(1,28)}$=23.52, p<0.0001] and 48h [$F_{(1,38)}$=6.488, p=0.0150] post-conditioning of an aversive footshock (US) and a paired light-sound tone (CS), and following re-presentation of the CS alone (Post-CS) [$F_{(1,37)}$=22.35, p<0.0001]. A significant main effect of perinatal PCP treatment (10mg/kg, s.c. on post-natal days 7, 9 and 11) was observed Post-CS only [$F_{(1,37)}$=8.654, p=0.0056], with no isolation x PCP interaction observed at any time. $^{**}p$<0.01 $^{*}p$<0.05 isolation vs. group-housing, $^{\#\#\#}p$<0.001 $^{\#}p$<0.05 isolation+PCP vs. group-housed control-treated by Bonferroni post-hoc analysis following ANOVA.

3.3.5 Effect of isolation rearing and perinatal PCP on Morris water maze performance

Study 1: Irrespective of treatment, all four groups of rats progressively decreased their latency to find the platform throughout the 15 trial acquisition phase, reflecting intact visuo-spatial learning. This was supported by RM ANOVA analysis, showing a highly significant main effect of trial [$F_{(14,546)}$=27.132, p<0.001], indicating that all rats successfully learned the task at an equivalent rate. ANOVA also revealed that the effect of housing just failed to reach significance [$F_{(1,39)}$=3.412, p=0.072], and there was no significant main effect of PCP treatment [$F_{(1,39)}$=0.239, p=0.628] nor any interaction (Figure 3.9).

Two-way ANOVA analysis of Probe 1, immediately following the completion of the learning phase in trial 15, revealed that the percentage of time spent exploring the "correct" quadrant during the 60s test was not significantly affected by perinatal PCP treatment [$F_{(1,39)}$=0.085, p=0.7715] or rearing in isolation [$F_{(1,39)}$=3.144, p=0.084], and there was no interaction between the two, confirming that the degree of learning after 15 trials was the same in all treatment groups (Figure 3.10A). Similarly, the time spent in the correct quadrant during Probe 2 performed 60 h later was unaltered by perinatal PCP [$F_{(1,39)}$=0.1533, p=0.6976] or isolation rearing [$F_{(1,39)}$=2.292, p=0.1381] and once again there was no interaction (Figure 3.10B).

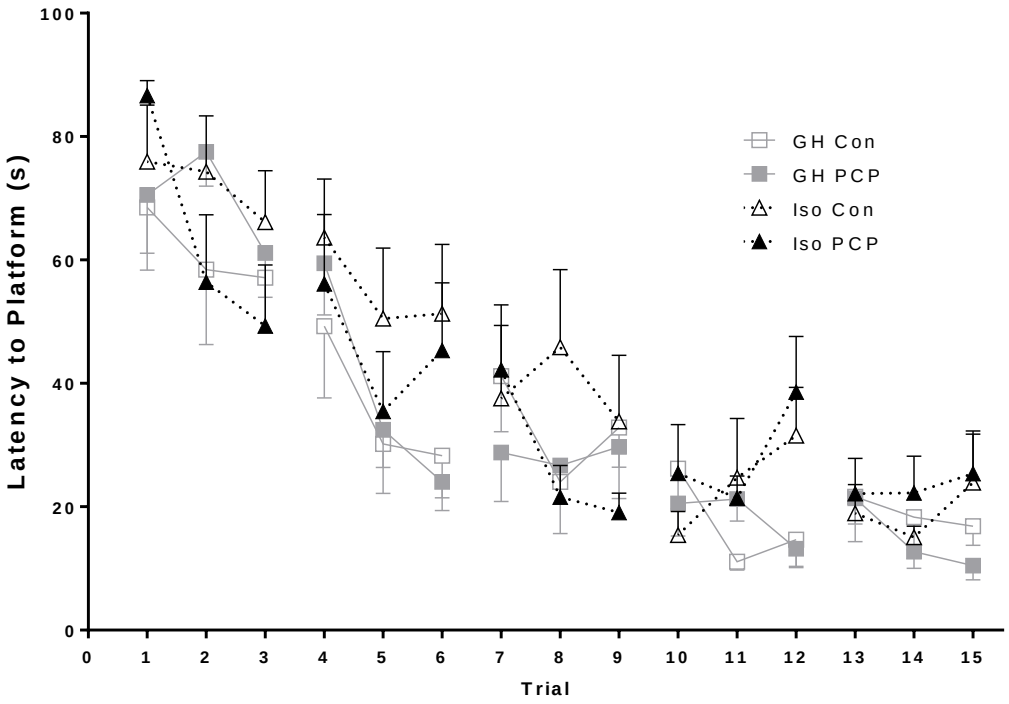

Figure 3.9 – Acquisition of the Morris Water Maze task was not altered by isolation rearing or perinatal PCP treatment. There were no main effects of isolation rearing (Iso) or perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), either alone or in combination, compared with group-housed (GH) and vehicle-treated (Con) controls, on latency to find a hidden platform in the Morris Water Maze during a 15 trial learning period (s, mean±SEM, n=10-12). Significant main effect of trial [$F_{(14,546)}$=27.132, p<0.001], but not of housing [$F_{(1,585)}$=9.18, p=0.003], or PCP [$F_{(1,39)}$=0.239, p=0.628] by RM ANOVA, reflecting a successful learning of the task by all rats.

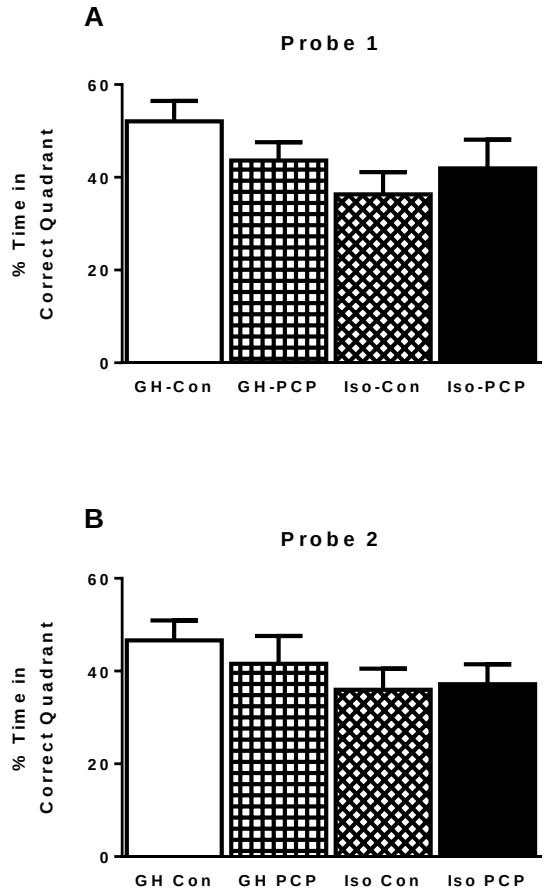

Figure 3.10 – Acquisition and retention of the Morris Water Maze task was not altered by isolation rearing or perinatal PCP treatment. There was no main effect of isolation rearing (Iso) or perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), either alone or in combination, compared with group-housed (GH) and vehicle-treated (Con) controls, on the percentage time spent in the "correct" quadrant in (A) Probe 1, and (B) Probe 2 (Mean Percentage ±SEM, *n*=10-12). In neither probe 1 nor probe 2 was a significant effect of housing or perinatal PCP treatment seen, nor was there an interaction between the two factors by two-way ANOVA.

Unlike previously (see Chapter 2.3.5), RM ANOVA analysis showed a highly significant main effect of trial on latency to find the platform in the reversal phase of the protocol [$F_{(14,546)}$=4.617, p<0.001], demonstrating that re-learning a new strategy as a result of a rule change occurred. There was no main effect of perinatal PCP treatment or isolation rearing on escape latency by RM ANOVA, with no observed interaction between any factors (Figure 3.11).

No between-group differences were observed due to PCP [$F_{(1,39)}$=2.337, p=0.1344] or housing [$F_{(1,39)}$=0.3378, p=0.5644] on time spent in the Platform Ring during Probe 3 by two-way ANOVA, and there was no interaction. Similarly, two-way ANOVA analysis of the time spent in the previously correct quadrant revealed no significant main effect of housing [$F_{(1,39)}$=0.76, p=0.3869], or perinatal PCP treatment [$F_{(1,39)}$=4.039, p=0.0514], and there was no interaction between the two [$F_{(1,39)}$=0.4006, p=0.5304] (Figure 3.12).

The mean latency to locate the platform consistently fell across the 3 trials within each test day for rats in the GH-Con group. Further analysis was performed to establish any difference in reversal learning strategy. The difference between the escape latency in trials 1 and 3 of each reversal learning day was calculated and compared between groups by three-way ANOVA. This analysis revealed significant main effects of isolation rearing [$F_{(1,195)}$=6.71, p=0.010] and perinatal PCP treatment [$F_{(1,195)}$=6.54, p=0.011], but no main effect of day, and no interaction between any parameters (Figure 3.13). Retrospective utilisation of this method on the main learning phase of the experiment revealed a significant main effect of day [$F_{(4,195)}$=3.69, p=0.006], but no comparable main effect of housing or PCP treatment (data not shown).

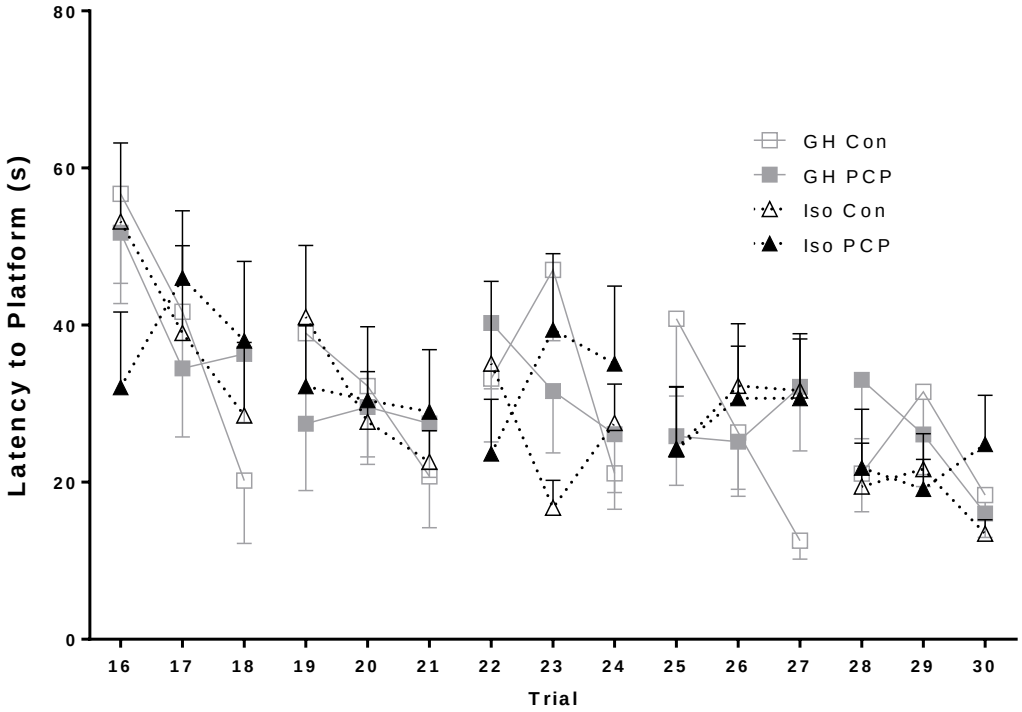

Figure 3.11 – Reversal learning in the Morris Water Maze task was unaffected by isolation rearing or perinatal PCP treatment. There was no main effect of isolation rearing (Iso) perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), either alone or in combination, compared with group-housed (GH) and vehicle-treated (Con) controls, on latency to find a hidden platform in the Morris Water Maze following a 15 trial reversal learning period (s, mean±SEM, n=10-12). Repeated measures ANOVA revealed a significant main effect of trial only [$F_{(14,585)}$=3.57, p<0.001], reflecting a significant improvement in acquisition of the task.

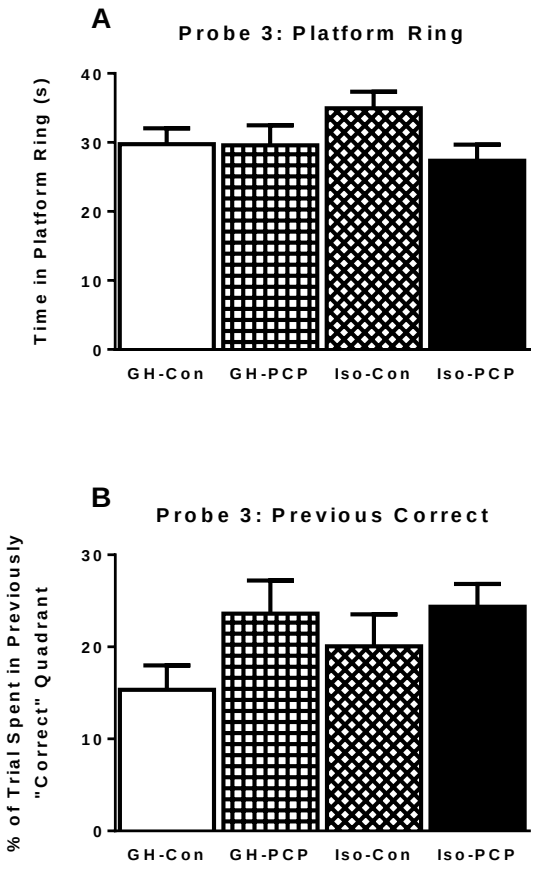

Figure 3.12 – Acquisition of a new rule and extinction of a previously correct rule was unaffected by isolation rearing or perinatal PCP treatment in the Morris Water Maze protocol. There was no main effect of isolation rearing (Iso) and perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), either alone or in combination, compared with group-housed (GH) and vehicle-treated (Con) controls, on the percentage time spent in (A) the Platform Ring area and (B) the previously "correct" quadrant during probe 3 (Mean Percentage ±SEM, *n*=10-12). In neither A, nor B, was a significant effect of housing or perinatal PCP treatment seen, nor was there an interaction between the two.

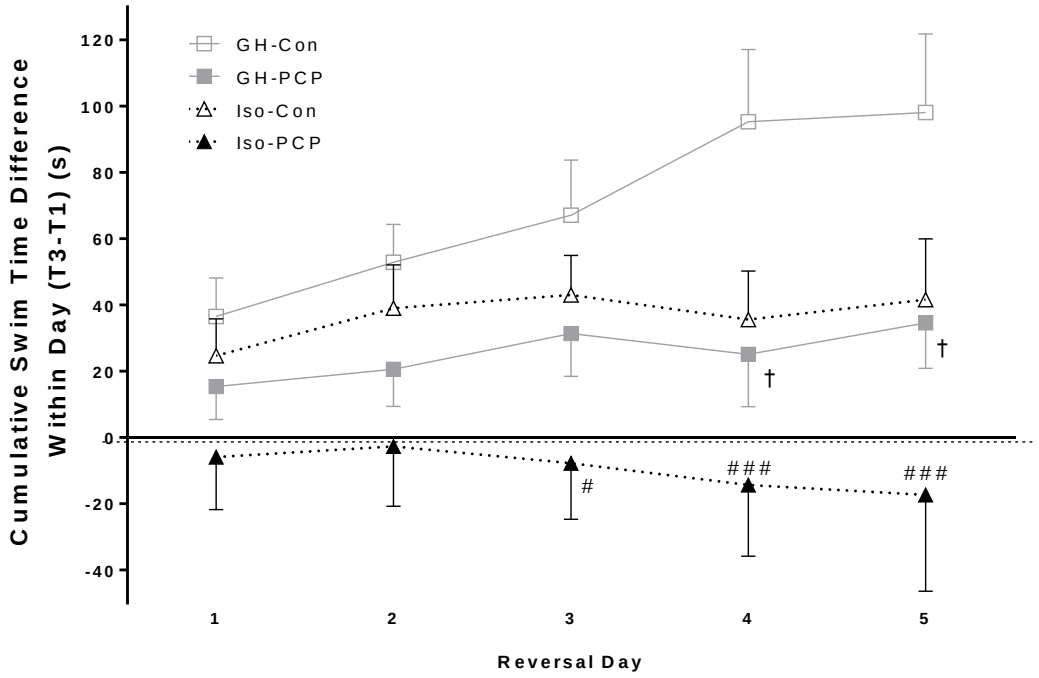

Figure 3.13 – A change in reversal learning strategy due to isolation rearing and perinatal PCP treatment in the Morris Water Maze protocol. Cumulative data representation of the "within-day" improvements in escape latency (s, mean±SEM, *n*=10-12) during each reversal learning day of the Morris Water Maze protocol, comparing the effect of isolation rearing (Iso) and perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), alone and in combination, with group-housed (GH) and vehicle-treated (Con) controls. Three-way ANOVA analysis of the raw data revealed a significant effect of both isolation [$F_{(1,195)}$=6.71, p=0.010] and perinatal PCP treatment [$F_{(1,195)}$=6.54, p=0.011] on within-day improvements, but no effect of test day. †p<0.05 perinatal PCP vs rearing-matched vehicle-treated control, $^{\#\#\#}p$<0.001 $^{\#}p$<0.05 isolation+PCP vs. GH-Con absolute control, Bonferroni post-hoc analysis following ANOVA.

Study 2: Initial observation demonstrated that the escape latencies in all groups progressively decreased to a comparable extent throughout the 15 trial learning period of this MWM experiment, showing successful task acquisition, supported by repeated measures ANOVA which showed a significant main effect of trial on escape latency [$F_{(14,546)}$=15.511, p<0.001] (Figure 3.14). Similar to the previous cohort, there was no significant main effect of housing [$F_{(1,39)}$=0.315, p=0.578], nor any significant main effect of perinatal PCP treatment [$F_{(1,39)}$=3.266, p=0.078] and no interaction.

In probe 1, PCP-treated animals performed no worse than saline treated rats; but ANOVA revealed a significant main effect of housing [$F_{(1,38)}$=4.399, p=0.0427] and a significant interaction between isolation and PCP treatment [$F_{(1,38)}$=5.527, p=0.024]. No post-hoc significance was observed (Figure 3.15A).

In contrast, in Probe 2 performed 60 h later, two-way ANOVA analysis revealed no significant effect of isolation rearing, or perinatal PCP treatment on time spent exploring the correct maze quadrant, nor was there an interaction between the two (Figure 3.15B).

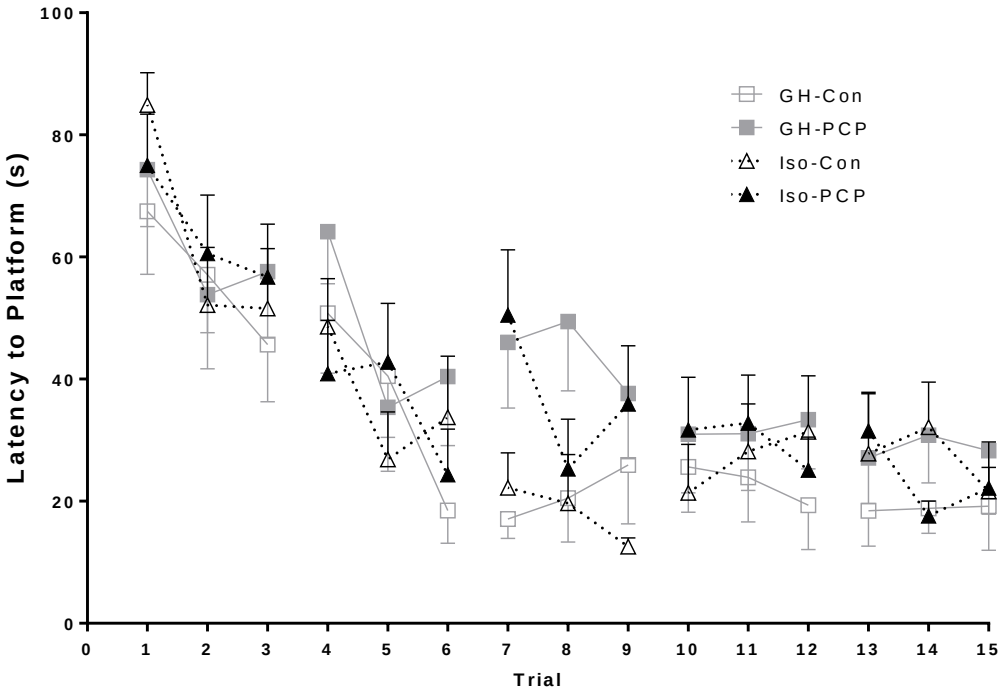

Figure 3.14 – Acquisition of the Morris Water Maze task was not altered by isolation rearing or perinatal PCP treatment. There were no main effects of isolation rearing (Iso) or perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), both alone and in combination, compared with group-housed (GH) and vehicle-treated (Con) controls, on latency to find a hidden platform in the Morris Water Maze during a 15 trial learning period (s, mean±SEM, n=10-11). Significant main effect of trial only [$F_{(14,546)}$=15.511, p<0.001] with no effect of isolation rearing or PCP treatment by repeated measures ANOVA.

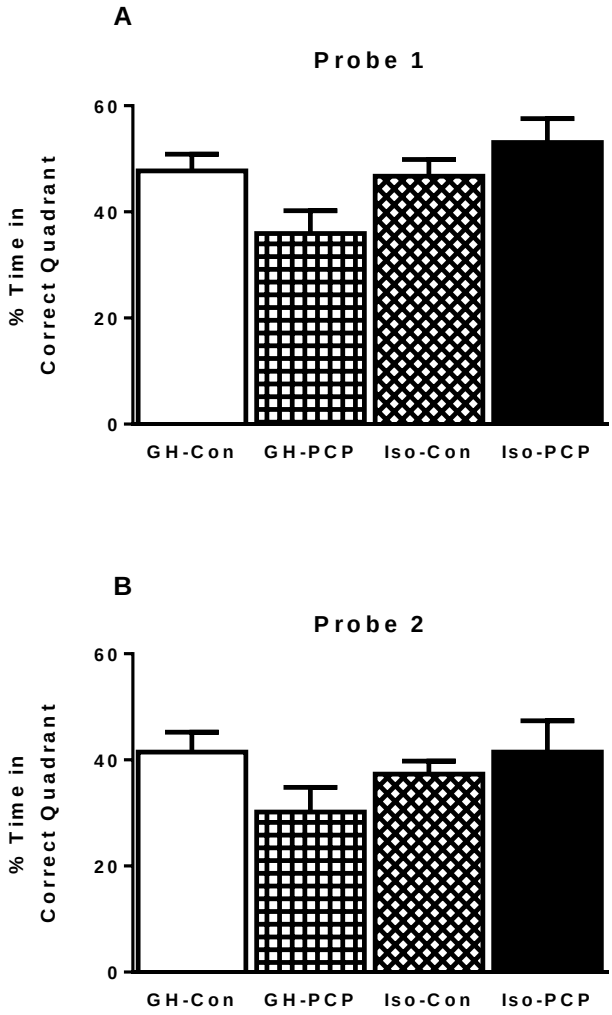

Figure 3.15 – Comparison of the effect of isolation rearing (Iso) and perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), both alone and in combination, compared with group-housed (GH) and vehicle-treated (Con) controls, on the percentage time spent in the "correct" quadrant in (A) Probe 1, and (B) Probe 2 (Mean Percentage ±SEM, n=10-12). (A) Two-way ANOVA analysis revealed a significant main effect of housing [$F_{(1,38)}$=4.399, p=0.0427] and a significant interaction between isolation and PCP treatment [$F_{(1,38)}$=5.527, p=0.024], but no effect of PCP treatment alone in Probe 1, and no post-hoc significance was observed. (B) No main effect of isolation rearing or perinatal PCP treatment, nor a between factor interaction, was observed during Probe 2.

As in Study 1, RM ANOVA analysis of the reversal learning protocol revealed a significant main effect of trial [$F_{(14,546)}$=3.871, p<0.001], indicating acquisition of the new rule. However, RM ANOVA also revealed significant main effects of housing [$F_{(1,39)}$=5.774, p=0.021] and PCP treatment [$F_{(1,39)}$=5.234, p=0.028], but no interaction was seen between any factor (Figure 3.16).

Two-way ANOVA analysis of performance in Probe 3 revealed a significant effect of PCP treatment in exploration of the "platform ring" area [$F_{(1,38)}$=4.299, p=0.045], but no post-hoc significance, no significant main effect of isolation rearing, and no interaction between the two factors was observed (Figure 3.17B). Further ANOVA analysis showed that neither PCP treatment nor isolation rearing significantly affected the amount of time spent exploring the previously correct quadrant during Probe 3, and there was no interaction (Figure 3.17A).

Similarly in Probe 4, no effect of isolation rearing or PCP treatment was observed on the exploration of the previously correct quadrant (Figure 3.17D). It was also revealed that there were no significant effects of isolation or PCP, nor was there an interaction between the two, on exploration of the platform ring in Probe 4 (Figure 3.17C).

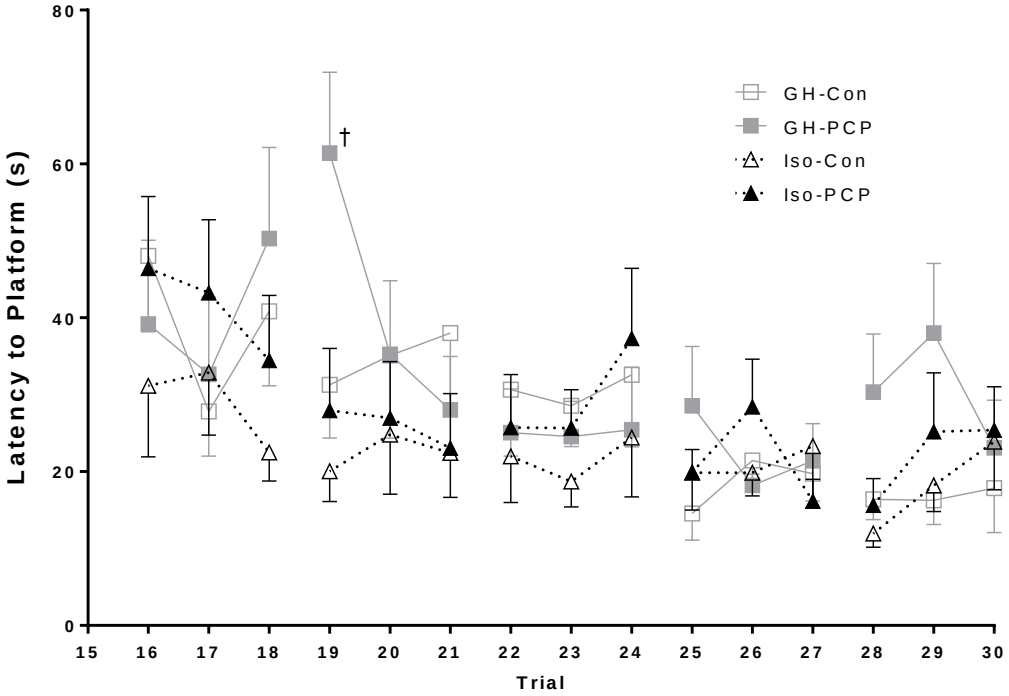

Figure 3.16 – Reversal learning in the Morris Water Maze protocol was significantly altered by isolation rearing and perinatal PCP treatment. Comparison of the effect of isolation rearing (Iso) and perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), both alone and in combination, compared with group-housed (GH) and vehicle-treated (Con) controls, on latency to find a hidden platform in the Morris Water Maze following a 15 trial reversal learning period (s, mean±SEM, n=10-12). RM ANOVA revealed significant main effects of trial [$F_{(14,546)}$=3.871, p<0.001], housing [$F_{(1,39)}$=5.774, p=0.021], and PCP treatment [$F_{(1,39)}$=5.234, p=0.028], but no interaction between any factor. †p<0.05 PCP treatment vs. rearing-matched vehicle-treated control by Bonferroni post-hoc analysis following ANOVA.

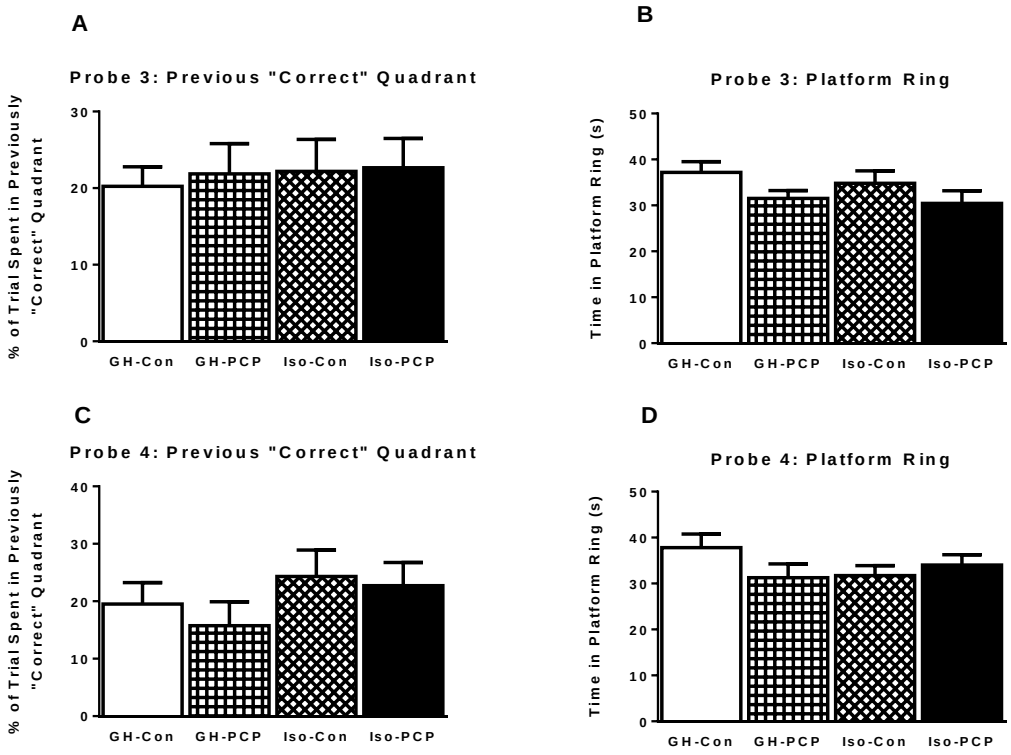

Figure 3.17 - Comparison of the effect of isolation rearing (Iso) and perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), both alone and in combination, compared with group-housed (GH) and vehicle-treated (Con) controls, on the percentage time spent in (A) the previously "correct" quadrant (Mean Percentage ±SEM, *n*=10-11) and (B) the Platform Ring area (s, mean±SEM) during Probe 3, and the same arenas during Probe 4 (C and D, respectively). Note a significant effect of perinatal PCP treatment on exploration of the Platform Ring area during Probe 3 [$F_{(1,38)}$=4.299, p=0.045] by two-way ANOVA (B), unaccompanied by post-hoc significance. No significant effect of isolation rearing or perinatal PCP treatment, or an interaction between the two, was seen in any other parameter.

As in the previous study, within-day improvement in learning for each rat was calculated for both the acquisition and reversal periods of the protocol. Three-way ANOVA revealed a significant main effect of day during the learning phase of the protocol [$F_{(4,190)}$=4.07, p=0.003] but no significant main effect of isolation rearing or perinatal PCP treatment on within-day improvement (data not shown). Furthermore, during the reversal learning portion of the protocol, none of the three factors assessed by three-way ANOVA (day, isolation, PCP) revealed a significant effect on within-day improvement in escape latency (Figure 3.18).

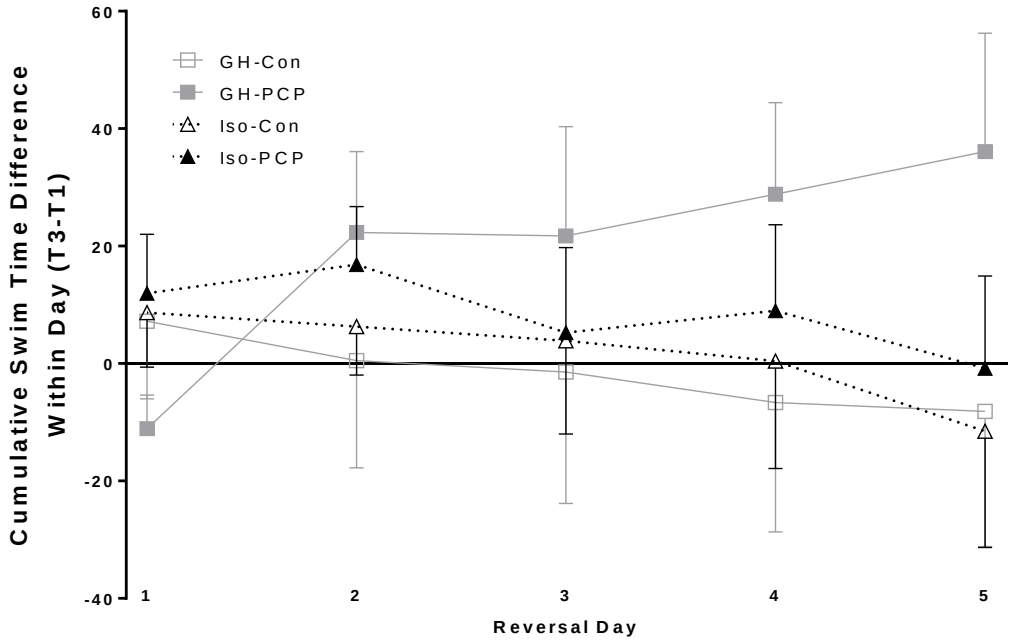

Figure 3.18 - Cumulative data representation of the "within-day" improvements in escape latency during each reversal learning day of the Morris Water Maze protocol. There was no main effect of isolation rearing (Iso) or perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), alone or in combination, compared with group-housed (GH) and vehicle-treated (Con) controls on "within-day" improvements in escape latency (s, mean±SEM, *n*=10-11) by ANOVA.

3.3.6 Effect of isolation rearing and perinatal PCP on locomotor response to acute PCP treatment

Study 1: To assess whether treatment caused hypersensitivity to acute NMDA receptor antagonist treatment, rats were tested in a locomotor response paradigm including an acute PCP challenge. As predicted, acute injection of PCP to all rats caused a spike in locomotor activity which decreased over time. RM ANOVA analysis of the 1 h period following injection revealed that this change in locomotion was significant [$F_{(11,429)}$=35.834, p<0.001], and there was also a significant change due to early-life PCP treatment [$F_{(1,39)}$=4.306, p=0.045], which reached post-hoc significance in one 5 min epoch (p<0.05). There was no main effect of housing [$F_{(1,39)}$=1.146, p=0.291], but a significant interaction between time and perinatal PCP treatment was revealed [$F_{(11,429)}$=2.353, p=0.008] (Figure 3.19).

Study 2: RM ANOVA of activity for the 1h period following acute PCP administration revealed a significant main effect of time [$F_{(11,429)}$=7.854, p<0.001], but no main effect of housing or PCP treatment. However a significant time x PCP interaction was observed [$F_{(11,429)}$=2.174, p=0.015], and post-hoc analysis revealed a significant increase in locomotion in the Iso-PCP dual-hit group compared to GH-Con controls in the 5 mins immediately following PCP injection (p<0.05). RM ANOVA of just the first 10 min following PCP injection revealed that the significant main effects of time and time x PCP interaction were maintained, with an additional effect of perinatal PCP treatment also observed [$F_{(1,39)}$=9.376, p=0.004] (Figure 3.20).

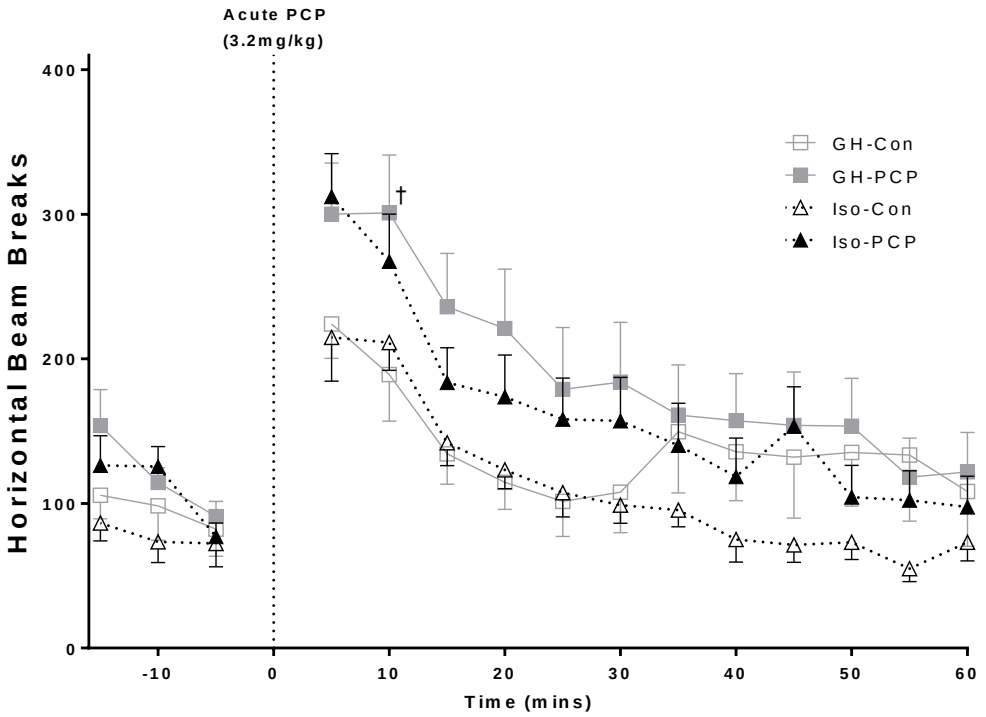

Figure 3.19 – Perinatal treatment with phencyclidine causes locomotor sensitisation to acute treatment in adult rats. A significant main effect of perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11) on locomotor response in 5 min epochs (horizontal beam breaks, mean±SEM, n=10-12,) over 1h to an acute injection of PCP (3.2mg/kg, i.p.), compared to animals treated perinatally with vehicle control (Con), regardless of subsequent rearing in isolation (Iso) or social groups (GH) [$F_{(1,39)}$=4.306, p=0.045] by RM ANOVA. There was also a significant main effect of time [$F_{(11,429)}$=35.834, p<0.001] and a significant time x PCP interaction [$F_{(11,429)}$=2.353, p=0.008]. Note three data points at -15 to -5 mins, showing baseline locomotor activity prior to acute PCP injection. †p<0.05 perinatal PCP treatment vs. rearing-matched vehicle-treated control by Bonferroni post-hoc analysis following ANOVA.

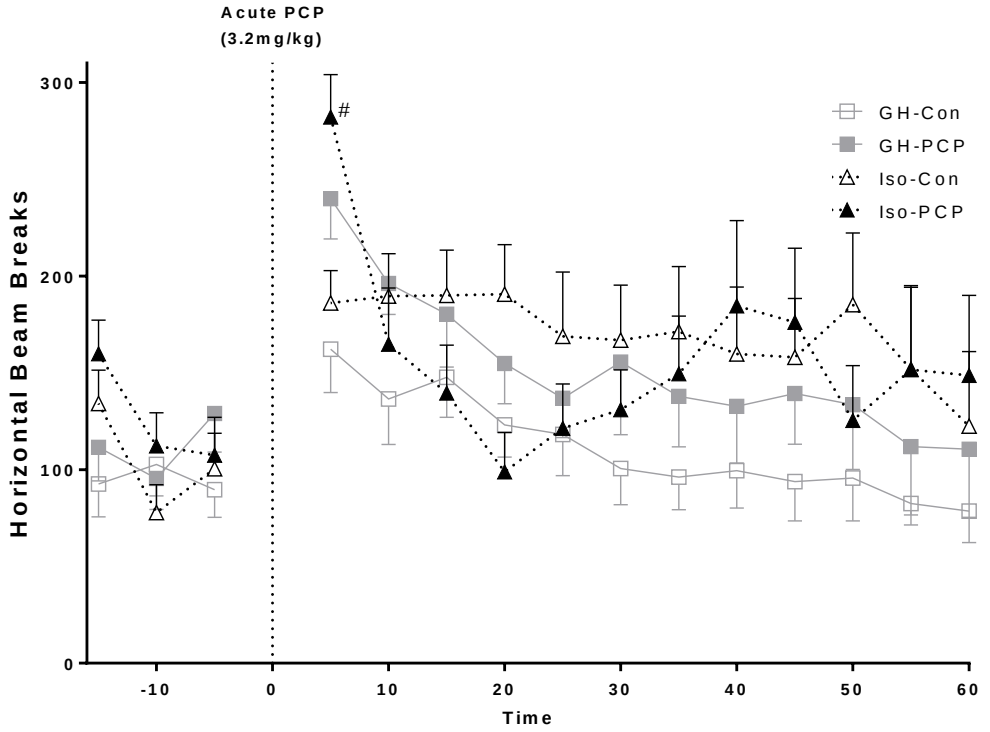

Figure 3.20 - Comparison of the effect of isolation rearing (Iso) and perinatal PCP treatment (PCP, 10mg/kg s.c., on PND 7, 9, 11), both alone and in combination, with group-housed (GH) and vehicle-treated (Con) controls, on the locomotor response to acute PCP injection (3.2mg/kg, i.p.) in an enclosed arena crossed with infra-red beam for a 1h trial. Repeated measures ANOVA revealed a significant main effects of time only [$F_{(11,429)}$=7.854, p<0.001], but perinatal PCP treatment was shown to have a significant main effect during the first 10 minutes only [$F_{(1,39)}$=9.376, p=0.004]. Note three data points at -15 to -5 mins, showing baseline locomotor activity prior to acute PCP injection. $^{\#}p$<0.05 isolation+PCP vs. GH-Con absolute control group by Bonferroni post-hoc analysis following ANOVA.

3.4 Discussion

Based on these results, perinatal PCP administration in combination with isolation rearing could be suggested to be a useful 'dual-hit' treatment for producing a translational model of 'schizophrenia-like' symptoms. The behavioural responses of rats in study 1 and 2 were again variable, but statistically significant effects were observed due to isolation rearing and/or perinatal PCP treatment in all of the paradigms assessed, so further investigation is warranted.

In the locomotor activity assessment, whilst no change was observed in study 1 as in the previous chapter, hyperlocomotion was seen in isolation-reared animals in study 2, irrespective of perinatal PCP treatment. It was expected that isolation rearing would induce an increase in locomotor activity in line with the majority of published work (detailed previously, see Chapter 1.3.1), but the lack of isolation-induced hyperlocomotion in the first cohort again highlights that these deficits are not 100% reproducible and an additive effect of dual-hit treatment is highly desirable. Acute PCP treatment has been consistently shown to cause increases in locomotor activity, however the effect of early-life treatment of rats with NMDA receptor antagonists has yielded varied results. Administration of PCP (10mg/kg, s.c.) to rat pups on PND 2-15 caused elevated locomotor activity on PND 30 in one study (Zhang et al. 2012b), but when Sprague-Dawley rats were treated on PND 7, 9 and 11, no baseline change in activity was seen on PND 42 compared to saline treated

counterparts (Boctor and Ferguson 2010); nor was increased activity seen following perinatal treatment with another NMDA receptor antagonist, ketamine (20mg/kg) (Boctor and Ferguson 2010). Subchronic treatment with MK-801 (0.5mg/kg, twice daily from PND52-70) did not induce baseline locomotor activity increases in a novel arena in rats, either alone or in combination with social isolation (Ashby et al. 2010). Neonatal MK-801 treatment (on PND 7, 9 and 11 at 0.2mg/kg) also did not affect locomotor activity in adulthood, but did produce hyperlocomotion when combined with isolation rearing (Lim et al. 2012). Thus, a lack of effect of perinatal PCP alone on locomotor activity is as expected, but an additive effect in combination with isolation rearing would have supported the recent findings of Lim et al.

Conversely, perinatal PCP treatment caused significant increases in locomotor response to acute PCP treatment in both of the cohorts tested herein. Acute challenge with PCP was specifically added to the current study due to the wealth of literature demonstrating sensitisation to the locomotor response after prior exposure to an NMDA receptor antagonist. Many studies have shown that pre-treatment with PCP, and other NMDA receptor antagonists, causes increased sensitivity to subsequent acute treatment of amphetamine (Ashby et al. 2010; Beninger et al. 2010) and PCP (Boctor and Ferguson 2010) in rats, as well as acute PCP treatment in mice (Nakatani-Pawlak et al. 2009). Results herein support the phenomenon of sensitisation to PCP, demonstrating a significant increase in the acute PCP locomotor response in rats exposed perinatally to PCP in both cohorts. Although the mechanistic basis of

sensitisation is unclear, importantly for the validity of the proposed new combination model, sensitization is regarded as a key index of psychosis, and movement and thought disorders, present in the human condition of schizophrenia (Robbins 1990), suggesting that the mechanisms responsible for sensitisation are preserved across species. Additionally, these results are the first to demonstrate that the sensitisation effect of perinatal NMDA receptor antagonists lasts 12 weeks post-treatment in rats. A consideration of the time point of behavioural testing post-PCP treatment is essential, as many previous studies have shown a transient nature of the deficits induced by NMDA receptor antagonism. Long-term effects of an early-life challenge are a vital component adding strength to the validity of any animal model, particularly considered in conjunction with the developmental aetiology theory of schizophrenia in humans. Therefore the results obtained in this study showing alterations in behaviour due to PCP on PND 98 are highly relevant.

Whilst acute PCP-induced hyperlocomotion is an interesting and relevant behaviour to assess in these dual-hit animals, it has little continuing utility beyond a proof of concept. Whilst exacerbation of symptoms in schizophrenia patients exposed to NMDA receptor antagonists is a well-established phenomenon, the need for acute drug treatment to induce the behavioural phenotype makes the paradigm less desirable for the assessment of antipsychotic compounds in the future. Drug-drug interactions may confound any results observed, and are less representative of the desired clinical outcome of antipsychotic drugs (to reverse baseline behavioural deficits, not drug-

induced deficits), and so this paradigm was not continued beyond this study now that the concept of PCP sensitisation has been proven.

Of far greater utility, hence its addition to the battery of tests in study 2 of this chapter, is the conditioned emotional response paradigm. As discussed previously, this paradigm uniquely includes an emotional substrate, which is combined with classical Pavlovian conditioning to assess cognitive performance relevant to both contextual and conditioned emotional cognition and memory. Both isolation rearing and perinatal PCP treatment had highly significant effects on these forms of learning and memory. Decreased freezing was observed in the Iso-PCP dual-hit treatment group at all three time points of the protocol. Whilst all four of the treatment groups demonstrated a decay in freezing response from the 24 to 48 hour time points as would be expected from extinction, as well as a marked elevation in freezing upon re-presentation with the conditioned stimuli, it is notable that at each of the three time points analysed the freezing response was greatest in the GH-Con group, with GH-PCP, Iso-Con and Iso-PCP groups all demonstrating decreased freezing to some extent. Based on work conducted over the past 15 years, a significant effect due to PCP treatment can be well justified. The neural circuits that underlie Pavlovian fear conditioning have come under great scrutiny and have been mapped (Maren 2001). In brief, conditioning relies strongly on activation of the hippocampus and the amygdala, and the molecular basis of this learning lies within activation of extracellular signalling-regulated kinases (ERKs) through NMDA receptors in the aforementioned regions (Athos et al. 2002;

Atkins et al. 1998; Coelho et al. 2013; Schafe et al. 2000). Hence, aberrant function of these receptors through treatment with NMDA receptor antagonists will alter the expected fear conditioning response (Bolton et al. 2012; Enomoto et al. 2005). Interestingly, although no significant interaction between housing and PCP treatment was seen, rats in the Iso-PCP group consistently froze less than those in either the GH-PCP or Iso-Con groups. From these results it is not possible to conclude that there was an additive effect of the two treatments, but the highly significant decrease in freezing between GH-Con and Iso-PCP animals could be utilised in future studies to aid the assessment of antipsychotic drugs. One major caveat of this, however, is the translational relevance of the CER paradigm to schizophrenia specifically. As behavioural tasks that rely on emotional processing and Pavlovian conditioning were not included in the MATRICs battery (Marder and Fenton 2004; Young et al. 2009), the significance of positive results in the CER paradigm is much lower than if they were achieved in the PPI paradigm, for instance.

Similar to the acute PCP locomotion test, in both cohorts prepulse inhibition of acoustic startle was significantly impaired by perinatal PCP treatment, without any accompanying changes in initial startle intensity or habituation to the 120dB tone alone. The results here support previous work that PPI impairments in NMDA receptor antagonist-treated animals, following not only PND 7, 9, 11 PCP administration (Anastasio and Johnson 2008a; Wang et al. 2001), but also after escalating and constant/sub-chronic treatment with PCP and MK-801 (Li et al. 2011b), and acute ketamine administration (Sabbagh et

al. 2012). Both the GH-PCP and Iso-Con groups herein exhibited marginally decreased PPI at all three analysed prepulse intensities in both cohorts which did not reach significance, whereas the Iso-PCP group had a significantly lower PPI response than GH-Con controls at two prepulse levels, i.e. a greater impairment, perhaps suggesting an additive effect of the 'dual-hit' treatment. The robustness of PPI in preclinical models of schizophrenia-like symptoms, particularly isolation reared deficits, has been somewhat called into question with a growing body of negative results (Cilia et al. 2001; Fone and Porkess 2008; McIntosh et al. 2013), so the fact that a significant effect of isolation rearing was only observed in the second of the two cohorts is not entirely surprising. However, that perinatal PCP treatment induced a deficit in both study 1 and 2, both in the presence (study 2) and absence (study 1) of a significant effect of isolation rearing, lends significant strength to this dual-hit model. The recent dual-hit model using isolation rearing and neonatal MK-801 combined also identified a significant effect of combining treatments on PPI deficits (Lim et al. 2012), supporting the results observed here. Although no interaction was seen between the isolation and perinatal PCP treatments in either study, suggesting no synergistic effects occurred, combining these two treatments ensured that a significant effect of treatment was seen in both cohorts with post-hoc significance in the Iso-PCP group in one.

In contrast to the PPI paradigm, results of the novel object discrimination task showed a highly significant impairment due to isolation rearing in both cohorts, in the absence of any effect due to perinatal PCP. As discussed in the

previous chapter, isolation rearing robustly and consistently produces deficits in novel object discrimination tasks similar to the one utilised in this study (Fone and Porkess 2008; McLean et al. 2010a), and so these results were as anticipated. Perhaps less expected, however, is the lack of any deficit induced by perinatal PCP treatment. Although the literature on the long-term effects of perinatal PCP treatment on visual learning and memory is sparse, evidence suggests that some PCP treatment protocols produce deficits in behaviour in non-spatial learning paradigm equivalent to the NOD paradigm used in mice (Hashimoto et al. 2005), as well as in visual reference memory in variations of a Morris Water Maze protocol (Andersen and Pouzet 2004; Sircar 2003). Most importantly, a recent paper utilising perinatal PCP treatment in Wistar rats, albeit at a dose higher than that used here (20mg/kg), did show PCP-induced deficits in novel object discrimination in adulthood (Redrobe et al. 2012). Whilst a lack of perinatal PCP-induced deficits in NOD does not support the aim of this study to produce additive effects of combined treatments (as has been seen in protocols using isolation rearing and neonatal MK-801 in combination (Lim et al. 2012)), the strong impairment in performance produced by isolation rearing here would prevent any additive effects being observed. Despite the reproducibility of the isolation-induced deficits in NOD, the translational relevance of the task must be considered. The task, recently extensively reviewed (Lyon et al. 2012), has many merits but also some drawbacks (see Chapter 1.2.3). The apparent lack of predictive validity the novel object discrimination task possesses is a major caveat. Whilst it has been shown to have some predictive validity (in so far as atypical antipsychotics

have greater efficacy in novel object tasks than their typical counterparts – similar to the effects in humans (Keefe 2007)), compounds including galantamine (Noda et al. 2010) and donepezil (Kunitachi et al. 2009) have produced strong reversals of NOD deficits, whilst producing little benefit on visual reference memory function in clinical trials (Buchanan et al. 2008; Keefe et al. 2008). This disparity between preclinical and clinical data is an important consideration when interpreting drug reversal of NOD deficits produced by dual-hit models of schizophrenia.

Whilst learning and memory in the NOD paradigm was shown to be impaired by isolation rearing, results of the Morris Water Maze protocols showed that visual and spatial reference memory were less affected. Indeed, neither cohort demonstrated any deficits in the MWM protocol due to isolation rearing. Previously, deficits in MWM learning protocols due to isolation rearing have been noted (Hellemans et al. 2004; Lu et al. 2003), whereas others have demonstrated an improvement in both spatial learning and reversal in isolation-reared rats compared to social controls, potentially through cholinergic mechanisms (Wongwitdecha and Marsden 1996a). Indeed, the results presented in the previous chapter showed no significant effects due to isolation rearing at any stage of the MWM protocol, so the outcome of these studies is not surprising and may suggest that isolation rearing is not a successful method for inducing deficits in spatial learning.

As stated previously, deficits in visual reference memory in variations of a Morris Water Maze protocol have been shown due to perinatal PCP treatment

(Andersen and Pouzet 2004; Sircar 2003), and to some limited extent these results were replicated in the second cohort presented here. Although neither cohort demonstrated impairments in spatial learning due to perinatal PCP treatment, cohort 2 did suggest impaired reversal learning, with a significant main effect of perinatal PCP on swim time in the "platform ring" area in Probe 3, but this failed to reach post-hoc significance. This is a fairly limited observation, however, considering the wealth of data that this paradigm produces, and that it was only evident in one of the two cohorts tested suggests it lacks the robustness for serious consideration as a deficit that may be targeted by putative antipsychotic compounds in future experiments.

The most striking observation from the MWM protocols in this study was from the reversal stage of the first cohort, where despite no apparent deficit due to either treatment during acquisition, an alteration in re-learning profile was identified. Analysis of the within-day improvements in escape latency revealed significant main effects of housing and PCP treatment, with post-hoc tests indicating lower within day improvements in GH-PCP and Iso-PCP rats, suggesting impaired working memory processes. As there were no deficits seen in Probe 3 of this protocol, however, a compensatory learning mechanism must have been utilised to improve escape latencies as the experiment progressed, masking the within-day learning deficit from having a significant observable effect on reversal learning as a whole. However, the changes in reversal learning profile seen in study 1 were not repeated in study 2, meaning that there was no correlation between the significant results seen between the two cohorts, another serious caveat to using this protocol in future studies.

3.4.1 Conclusion

This new ‘dual-hit’ model combines early-life developmental adversity through isolation rearing, with perinatal glutamatergic dysfunction by administering phencyclidine at a high dose, both separate risk factors for schizophrenia development. These studies show that combined perturbations produce a range of behavioural deficits with translational salience to the human condition of schizophrenia, although the robustness of the impairments does not completely meet the aims of the study. Furthermore, the behavioural effects of isolation rearing alone are not as robust as expected, even with the known limitations of this model (Fone and Porkess 2008), highlighting the possible need for a successful combination model of schizophrenia.

However, in all six of the behavioural paradigms examined, significant deficits were observed due to isolation rearing and/or PCP treatment in at least one cohort, and potential additive effects of the two treatments have been observed in PPI in the second cohort. These results provided enough support to continue evaluation the Iso-PCP model to examine reversal of the deficits with antipsychotic and novel therapeutic agents.

Chapter 4

Evaluating the Effect of Risperidone in the Iso-PCP Dual-Hit Model of 'Schizophrenia-Like' Symptoms in the Rat

4.1 Introduction

Risperidone is an atypical antipsychotic compound with high affinity and antagonist activity at 5-HT_{2A} serotonin and $D_{2/3}$ dopamine receptors (Shahid et al. 2009). Like many antipsychotic drugs it is relatively successful in the treatment of the positive symptoms of the disorder, however some evidence suggests efficacy in cognitive domains also (Bilder et al. 2002; Houthoofd et al. 2008). Preclinically, risperidone at relevant doses (0.1-1.0mg/kg) can produce marked improvement in a variety of behavioural paradigms with considerable face validity to the human condition, including in models and paradigms directly relevant to this study. Of particular note, the effect of risperidone in isolation-reared animals has been well-characterised, including ameliorating deficits in PPI (Cilia et al. 2001) and recognition memory (McIntosh et al. 2013), and reducing hyperlocomotion (Fabricius et al. 2011). Further work has also shown risperidone may be effective in ameliorating PCP-induced reversal learning deficits (McLean et al. 2010b), or preventing the induction of PPI and object recognition deficits and hyperlocomotion by PCP treatment (Anastasio and Johnson 2008a; McKibben et al. 2010). In rats treated perinatally with PCP (on PND 2, 6, 9, 12), chronic risperidone is also able to restore reduced glutathione levels and reverse alterations in antioxidant enzyme levels in a variety of brain structures including the hippocampus and cortex (Stojkovic et al. 2012). However, no previous studies have examined the effects of risperidone in a dual-hit model of schizophrenia-like symptoms.

4.1.1 Hypothesis

Based on previous evidence it was hypothesised that risperidone would be effective in reversing behavioural deficits induced by dual-hit isolation rearing-perinatal PCP treatment (hereupon denoted "IP") in rats at clinically relevant doses. However, as the dual-hit phenotype may have wider-ranging and differing deficits which have not previously been exposed to pharmacological challenge, the outcomes will be novel.

4.2 Materials and Methods

4.2.1 Animals

Three-day old, male, Lister-Hooded rat pups (*n*=48 from 8 litters in Study 1, *n*=50 from 5 litters in Study 2) were obtained from Charles River UK (CRUK, Margate, UK) accompanied by their natural dams, and underwent an early-life treatment protocol as described previously (see Chapter 3.2.1). In a change to earlier protocols, all animals treated perinatally with vehicle were subsequently housed in groups of 3/4 (GV, *n*=21 in Study 1, *n*=20 in Study 2), whereas all animals that received perinatal PCP were housed in isolation (IP, *n*=27 in Study 1, *n*=30 in Study 2). Rats were reared for forty days post-weaning in previously described ambient conditions (see Chapter 2.2.1).

4.2.2 Drug Administration

Risperidone (Sigma-Aldrich, Irvine, Scotland, UK) was dissolved in minimal amounts of 1 M HCl, brought to volume with 0.154 M saline and adjusted to pH 7 using 0.1 M NaOH. Rats were subdivided into risperidone treatment groups, with IP-reared rats receiving either vehicle control, low dose risperidone (Study 1: 0.2mg/kg, Study 2: 0.1mg/kg) or high dose risperidone (Study 1: 0.5mg/kg, Study 2: 0.2mg/kg), and group-housed, perinatal saline-treated (GV) animals receiving either vehicle control or high dose risperidone only, creating five treatment groups as below.

S1: GV-V (*n*=11), GV-R0.5 (*n*=10), IP-V (*n*=9), IP-R0.2 (*n*=9), IP-R0.5 (*n*=9).

S2: GV-V (*n*=10), GV-R0.2 (*n*=10), IP-V (*n*=10), IP-R0.1 (*n*=10), IP-R0.2 (*n*=10) (Table 4.1).

Table 4.1 Table to show the perinatal drug treatment, post-weaning housing condition, and acute risperidone treatment of each pup in (A) study 1, and (B) study 2. Also shown, the behavioural tests undertaken by each litter.

A

Litter	Pup Numbers	Litter Assignments					Behaviours
		GV-V	GV-R0.5	IP-V	IP-R0.2	IP-R0.5	
1	6	2	1	1	1	1	All animals performed LMA, NOD, PPI, CER and MWM tests
2	7	1	2	1	1	2	
3	7	2	1	1	2	1	
4	5	1	2	1	0	1	
5	5	2	1	1	1	0	
6	5	1	2	0	1	1	
7	5	2	1	1	0	1	
8	4	0	0	1	2	1	
9	4	0	0	2	1	1	

B

Litter	Pup Numbers	Litter Assignments					Behaviours
		GV-V	GV-R0.2	IP-V	IP-R0.1	IP-R0.2	
1	10	2	2	2	2	2	All animals performed LMA, NOD, PPI and CER tests
2	10	2	2	2	2	2	
3	10	2	2	2	2	2	
4	10	2	2	2	2	2	
5	10	2	2	2	2	2	

Risperidone doses were selected based on literature showing efficacy in previous schizophrenia models whilst undertaking behavioural analysis, but without demonstrating significant sedative effects (Anastasio and Johnson 2008a; McIntosh et al. 2013; McKibben et al. 2010; McLean et al. 2010b). Furthermore, acute risperidone treatment at 0.5mg/kg has been shown to achieve clinically relevant levels of D_2 receptor occupancy in rats (65-80%) (Kapur et al. 2003). All treatments were administered by intraperitoneal injection (1 ml/kg), 30 min before the initiation of each behavioural test, except in the Morris Water Maze paradigm, when treatment was administered 30 min before the first trial of each training day.

4.2.3 Behavioural Testing and Statistical Analysis

Behavioural testing took place following protocols described previously, with no alterations from the previous use (see Chapter 2.2 and 3.2), following the timeline shown in Figure 4.1. The method of statistical analyses for this study was also as previously, with all unbalanced data analysed using SPSS software.

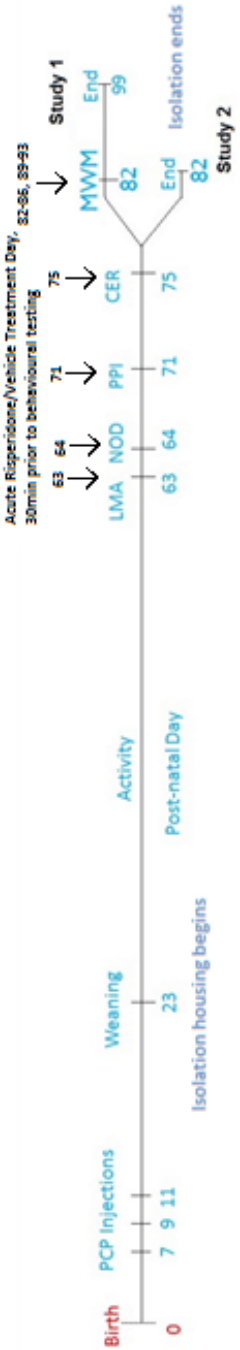

Figure 4.1 - Timestick representation of the protocols used in study 1 and 2 examining the effect of risperidone treatment (0.1, 0.2 and 0.5mg/kg, i.p.) on behaviour in the Iso-PCP dual-hit model of schizophrenia-like symptoms in rats. Protocol timings of study 1 and 2 were identical until the completion of the CER paradigm on PND78. In study 1, rats were then continued into the MWM protocol before completion of the experiment on PND99, whereas in study 2 the experiment was completed following the CER protocol with the final endpoint occurring on PND 82.

4.3 Results

4.3.1 Effect of risperidone on IP-rearing induced changes in locomotor activity

Study 1: A progressive decrease in locomotion was seen during the 60 min protocol, reflecting habituation to the arena as seen previously. RM ANOVA revealed a highly significant main effect of time [$F_{(11,462)}$=46.830, p<0.001] supporting this observation, with a marked reduction in activity in rats treated with risperidone, regardless of rearing condition. This was supported by a main effect of risperidone treatment by RM ANOVA [$F_{(2,41)}$=30.995, p<0.001] and subsequent post-hoc analysis revealed highly significant locomotor reductions in GV-R0.5 and IP-R0.5 groups (p<0.05) compared to respective vehicle-treated controls over the initial 40 minutes of the protocol. ANOVA also identified a significant IP-rearing x risperidone interaction [$F_{(1,42)}$=4.089, p=0.049], but a significant main effect of IP-rearing was seen across the first 20 min of the protocol only [$F_{(1,42)}$=4.558, p=0.039] (Figure 4.2A).

Two-way ANOVA of the total locomotor counts over the 1 h protocol supported the findings of RM ANOVA, with a significant main effect of risperidone [$F_{(2,41)}$=30.995, p<0.001] and an IP-rearing x risperidone interaction [$F_{(1,42)}$=4.089, p=0.049] (Figure 4.2B). Bonferroni post-hoc analysis notably revealed significant differences between the GV-V and GV-R0.5 groups (p<0.001), and between the IP-V and IP-R0.5R groups (p<0.01).

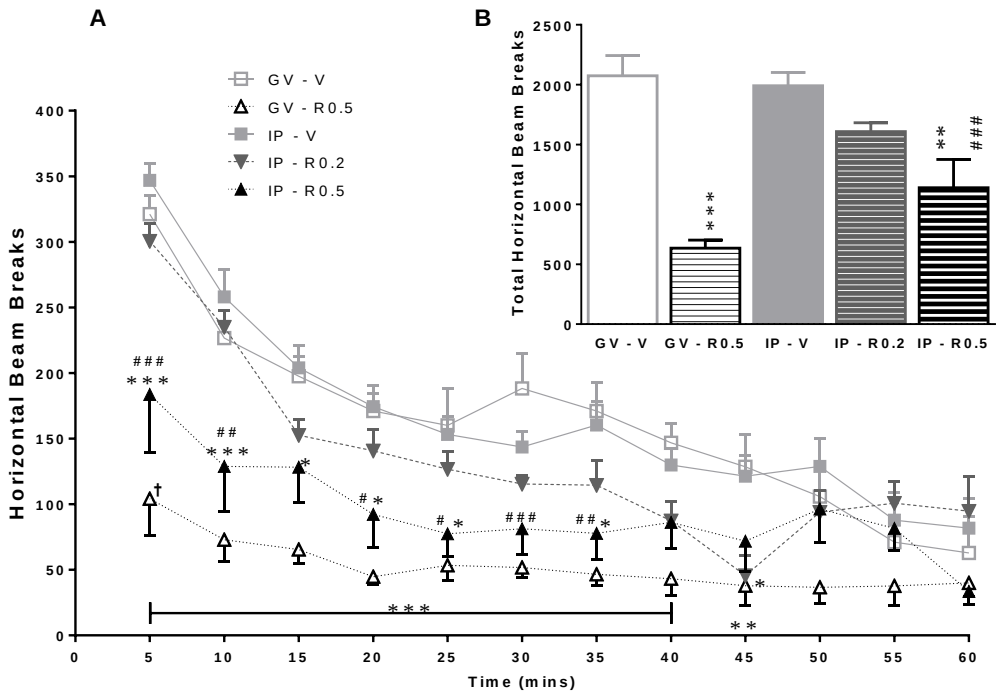

Figure 4.2 – Reduction in locomotor activity due to acute risperidone treatment in a 60 min LMA protocol. (A) Horizontal beam breaks (mean±SEM, *n*=9-11) decreased over a 60 min LMA protocol, demonstrating habituation, and were significantly reduced by acute risperidone treatment 30 min before testing (0.2mg/kg (R0.2) and 0.5mg/kg i.p. (R0.5)) compared with acute vehicle control (V) in IP-reared (IP) and group-housed, vehicle-treated (GV) rats. Main effects of time [$F_{(11,462)}$=46.830, p<0.001] and risperidone [$F_{(2,41)}$=30.995, p<0.001], plus IP-rearing x risperidone interaction [$F_{(1,42)}$=4.089, p=0.049] by RM ANOVA. ***p<0.001 **p<0.01 *p<0.05 risperidone vs vehicle control, †p<0.05 IP-rearing vs. GV-control, $^{###}p$<0.001 $^{##}p$<0.01 $^{#}p$<0.05 IP-rearing+risperidone vs. GV-V absolute control by Bonferroni post-hoc following ANOVA.

(B) Total cumulative horizontal beam breaks (mean±SEM, *n*=9-11) were significantly reduced by risperidone treatment [$F_{(2,41)}$=30.995, p<0.001] with an IP-rearing x risperidone interaction observed [$F_{(1,42)}$=4.089, p=0.049]. ***p<0.001 **p<0.01 risperidone vs. rearing-matched vehicle-treated control, $^{##}p$<0.01 IP-rearing+risperidone vs. GV-V absolute control by Bonferroni post-hoc following ANOVA.

Study 2: As previously, locomotor activity progressively decreased across the 1 h protocol reflecting habituation, such that RM ANOVA revealed a highly significant main effect of time [$F_{(11,473)}$=86.840, p<0.001]. A decrease in horizontal beam breaks was again noted due to risperidone treatment, supported by ANOVA [$F_{(2,43)}$=15.492, p<0.001] and post-hoc significance showing that both GV and IP rats treated with high dose, 0.2mg/kg risperidone recorded significantly fewer beam breaks than their respective saline-treated counterparts at numerous points throughout the protocol, most notably during the first 10 minutes of activity. A significant effect of IP-rearing was also observed [$F_{(1,43)}$=6.375, p=0.015], as was a significant time x risperidone interaction [$F_{(22,451)}$=2.066, p=0.003], but no post-hoc effects (Figure 4.3A**Figure**).

Two-way ANOVA of total cumulative locomotor activity confirmed the significant main effects of risperidone treatment [$F_{(2,43)}$=15.492, p<0.001] and IP-rearing [$F_{(1,43)}$=6.375, p=0.015], as well as the IP-rearing x risperidone interaction [$F_{(22,451)}$=2.066, p=0.003] (Figure 4.3B). Bonferroni post-hoc analysis identified between group differences between GV-V and GV-R0.2 (p<0.05) and the IP-V and IP-R0.2 (p<0.05) groups. Two rats were excluded from analysis due to faults with the apparatus leading to incorrect data acquisition.

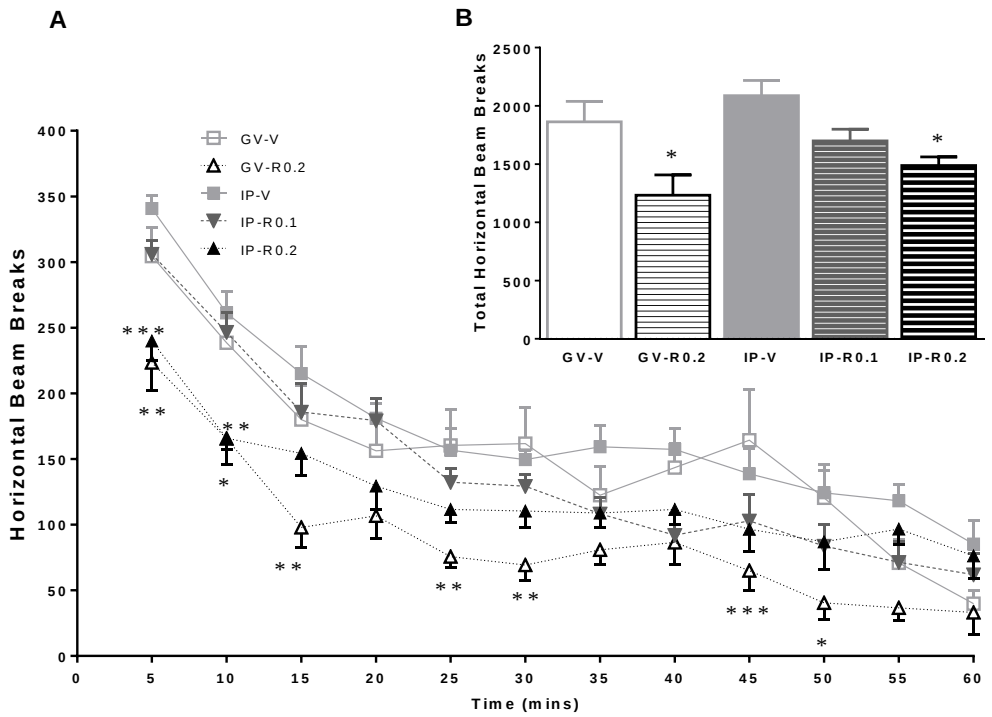

Figure 4.3 - Reduction in locomotor activity due to acute risperidone treatment in a 60 min LMA protocol. A reduction in horizontal beam breaks (mean±SEM, *n*=9-10) due to acute 0.2mg/kg i.p. risperidone (R0.2), but not 0.1mg/kg (R0.1), regardless of previous dual-hit isolation rearing-perinatal PCP (IP) treatment or group-housed perinatal vehicle (GV) treatment, over the 60 min time course of an LMA protocol, (A) supported by RM ANOVA [$F_{(2,41)}$=15.492, p<0.001]. Also noted were a significant main effects of time [$F_{(11,462)}$=86.840, p<0.001], and IP-rearing [$F_{(1,42)}$=6.375, p=0.015], with a significant IP-rearing x risperidone interaction [$F_{(22,451)}$=2.066, p=0.003] (RM ANOVA). ***p<0.001 **p<0.01 *p<0.05 risperidone treatment vs. rearing-matched vehicle control by Bonferroni post-hoc analysis following ANOVA.

(B) Total cumulative horizontal beam breaks (mean±SEM, *n*=9-11) over 60 min confirmed a significant main effect of IP-rearing [$F_{(1,43)}$=6.375, p=0.015], and a reduction by risperidone treatment [$F_{(2,43)}$=15.492, p<0.001], with an IP-rearing x risperidone interaction observed [$F_{(22,451)}$=2.066, p=0.003]. *p<0.05 risperidone treatment vs. rearing-matched vehicle-treated controls, by Bonferroni post-hoc analysis following ANOVA.

4.3.2 Effect of risperidone on IP-rearing induced changes in NOD

Study 1: As expected, rats in the GV-V group displayed in tact visual reference memory by exploring the novel over the familiar object in the second, choice trial of the NOD protocol. IP-V rats explored the two objects for similar amounts of time, reflecting impairment in this field of cognition. Bonferroni post-hoc analysis following a significant main effect of object [$F_{(1,37)}$=16.95, p<0.001, two-way ANOVA], and an interaction between object and treatment [$F_{(4,37)}$=2.700, p=0.0453], revealed a highly significant difference in exploration in GV-V rats (p<0.001), but not IP-V. Risperidone did not reverse the deficit, as no significant difference between exploration of the novel and familiar objects was noted in the IP-R0.2 or IP-R0.5 groups (p>0.05 for both). A reduction in discrimination was noted in GV animals treated with 0.5mg/kg risperidone (GV-R0.5), such that no significant difference between novel and familiar object exploration was observed (p>0.05) (Figure 4.4A). The discrimination ratio supported a change in exploration due to IP-rearing. A significant main effect of rearing condition was seen by two-way ANOVA [$F_{(1,37)}$=5.113, p=0.030] (no post-hoc significance), with no effect of risperidone, and no between-factor interactions (Figure 4.4B). A significant main effect of risperidone was seen on total exploration [$F_{(2,43)}$=5.561, p=0.007], with no main effect of rearing, trial, and no between factor interaction. Bonferroni analysis revealed that GV and IP-reared rats receiving 0.5mg/kg risperidone spent significantly less time exploring during Trial 1 than vehicle counterparts (p<0.01 and p<0.05, respectively) (Figure 4.4C). The scores of six rats had to be excluded for failing to complete the task.

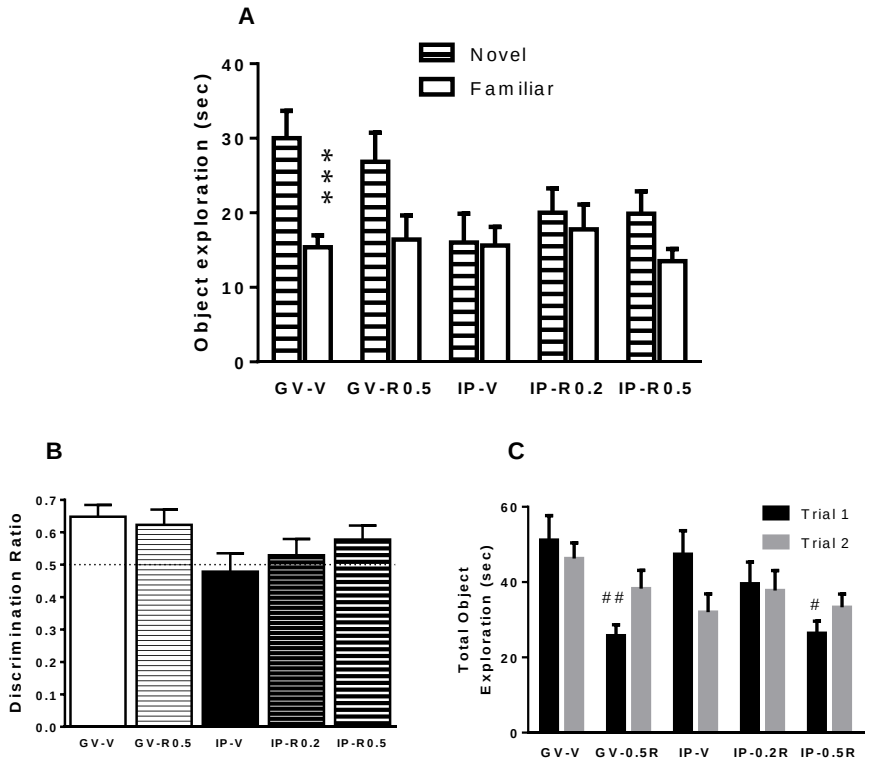

Figure 4.4 – A significant impairment in novel object discrimination caused by IP-rearing was not reversed by risperidone (A) Group-housed, perinatal saline treated animals that received vehicle prior to NOD (GV-V) spent significantly longer exploring the novel than familiar object during the second choice trial of the NOD protocol by Bonferroni post-hoc following ANOVA (s, mean±SEM, n=9-11) [treatment x object interaction, $F_{(4,37)}$=2.700, p=0.0453]; this was not observed in group-housed, perinatal saline treated animals receiving 0.5mg/kg risperidone (GV-R0.5), isolation-perinatal PCP reared animals given saline (IP-V), 0.2mg/kg risperidone (IP-R0.2) or 0.5mg/kg risperidone (IP-R0.5) ***p<0.001 Novel vs. Familiar by Bonferroni post-hoc test following ANOVA. (B) D1 discrimination ratio of object exploration during the choice trial was significantly affected by IP-rearing [$F_{(1,37)}$=5.113, p=0.030, two-way ANOVA], with no significant effect of risperidone**.** (C) High dose risperidone significantly decreased total object exploration during the familiarisation trial [$F_{(2,43)}$=5.561, p=0.007] with no effect of rearing condition $^{\#\#}p$<0.01, $^{\#}p$<0.05 Risperidone vs. rearing-matched vehicle control

Study 2: Whilst animals in the GV-V group displayed in tact visual reference memory, clearly exploring the novel object more than familiar during the second, choice trial of the NOD paradigm, this was not the case for any of the other four groups, suggesting that IP-rearing impaired performance, and that 0.2mg/kg risperidone had a negative effect in GV-reared rats. Following two-way ANOVA (significant main effects of object [$F_{(1,43)}$=25.93, p<0.001] and treatment group [$F_{(4,43)}$=2.636, p=0.0469], but no object x treatment group interaction [$F_{(4,43)}$=2.578, p=0.0508] (Figure 4.5A), Bonferroni post-hoc analysis of novel vs. familiar objects showed that there was a highly significant difference in exploration in the GV-V group alone (p<0.001). In contrast, analysis of the D1 discrimination ratio revealed no significant effect of IP-rearing, risperidone treatment, nor an interaction between the two factors by two-way ANOVA, suggesting no impairment due to either IP-rearing or risperidone treatment (Figure 4.5B). Analysis of the total exploration time revealed significant main effects of trial [$F_{(1,43)}$=4.646, p=0.037], and risperidone treatment [$F_{(2,43)}$=7.401, p=0.002], but not of rearing condition and no interactions, by three way RM ANOVA. Post-hoc analysis revealed that a decrease in object exploration occurred in the familiarisation trial due to 0.2mg/kg risperidone treatment in GV-reared animals only (Figure 4.5C).

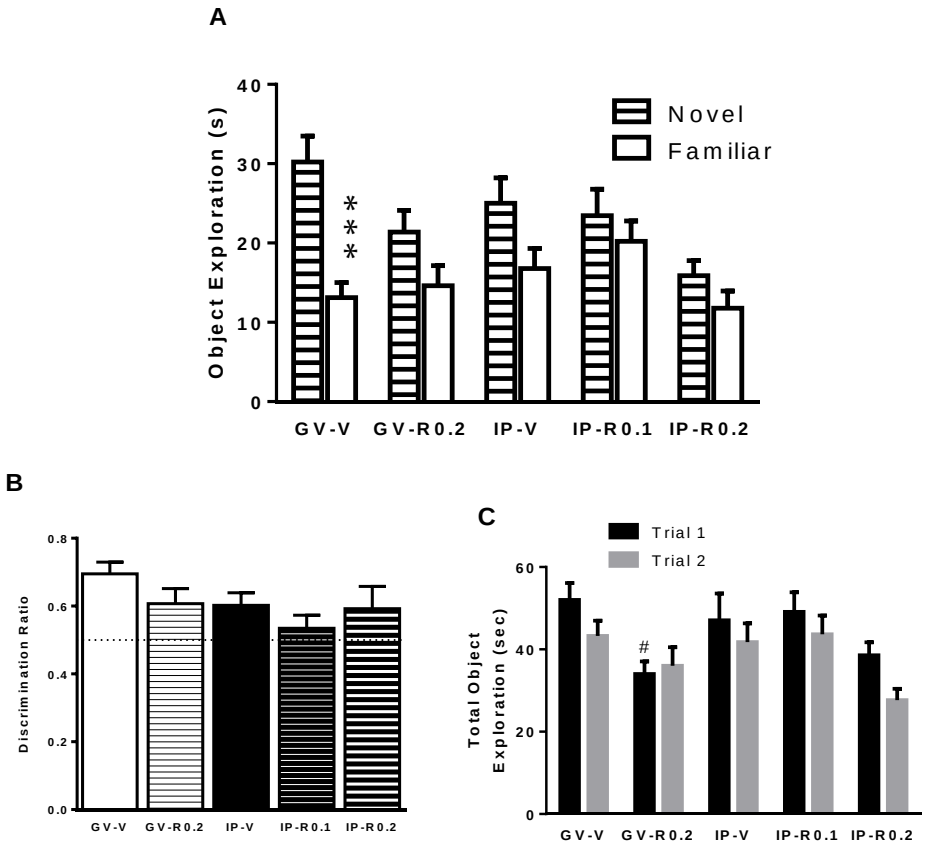

Figure 4.5 - Impairment in novel object discrimination caused by IP-rearing was not reversed by risperidone treatment at 0.2 or 0.1mg/kg. (A) GV-reared rats that received vehicle (GV-V) spent significantly longer exploring the novel than familiar object (s, mean±SEM, *n*=9-11) during the second, choice trial of the protocol by Bonferroni post-hoc following ANOVA, (significant main effect of object [$F_{(1,43)}$=25.93, p<0.001], and of treatment group [$F_{(4,43)}$=2.636, p=0.0469], but no interaction, ANOVA). GV-reared animals receiving 0.5mg/kg risperidone (GV-R0.5), or IP- reared animals receiving saline (IP-V), 0.2mg/kg risperidone i.p. (IP-R0.2) or 0.5mg/kg risperidone i.p. (IP-R0.5) did not discriminate. ***p<0.001 Novel vs. Familiar by Bonferroni post-hoc following ANOVA. (B) The D1 discrimination ratio of object exploration during the second choice trial was not significantly affected by IP-rearing or risperidone treatment by two-way ANOVA, nor was there an interaction observed**.** (C) 0.2mg/kg risperidone caused a significant decrease in object exploration in GV-reared animals only, following significant overall main effects of risperidone treatment [$F_{(1,43)}$=4.646, p=0.037] and trial [$F_{(2,43)}$=7.401, p=0.002] by ANOVA. $^{\#}p$<0.05 Risperidone vs. rearing-matched vehicle control

4.3.3 Effect of risperidone on IP-rearing induced changes in prepulse inhibition of acoustic startle

Study 1: The startle response to the 120dB tone was attenuated by addition of a sub-threshold prepulse as previously in all treatment groups, with increasing prepulse intensity causing greater reductions in the startle amplitude, supported by a highly significant effect of prepulse by RM ANOVA [$F_{(2,84)}$=95.562, p<0.001]. Although a significant main effect due to IP rearing [$F_{(1,42)}$=4.656, p=0.037] and a significant IP-rearing x risperidone interaction [$F_{(1,42)}$=50.53, p=0.030] were observed, this was due solely to elevation of PPI in GV-R0.5 rats only, confirmed by Bonferroni post-hoc tests showing a significant increase in PPI of this group compared to GV-V controls at 76dB (p<0.01) and 80dB (p<0.05) prepulse intensities. Furthermore, there was no main effect of risperidone [$F_{(2,41)}$=2.916, p=0.065] (Figure 4.6). There was no main effect of IP-rearing or risperidone treatment on either initial startle response amplitude or habituation to the 120dB tone across the protocol, nor was there an interaction between the two subject factors by two-way ANOVA (data not shown).

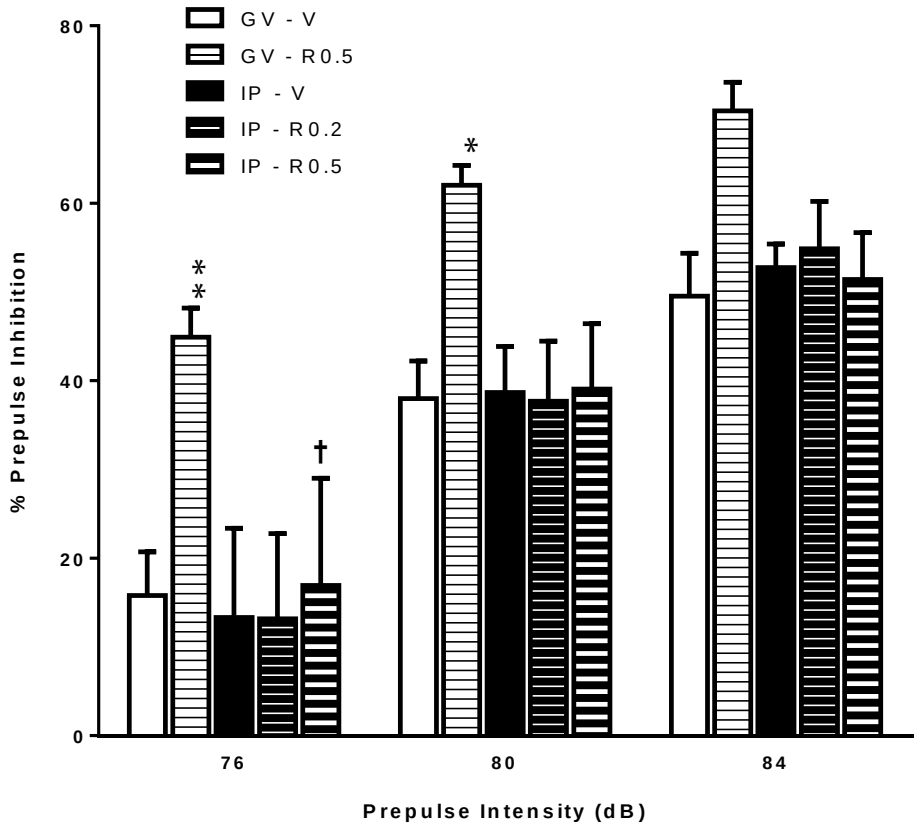

Figure 4.6 – Attenuation of the acoustic startle response to 120dB tone by sub-threshold prepulses was altered by IP-rearing in combination with acute risperidone treatment. Prepulse inhibition (mean percentage ±SEM, *n*=9-11) significantly increased with elevations in prepulse intensity [$F_{(2,86)}$=95.562, p<0.001, RM ANOVA]. Group-housed, perinatal vehicle treated animals receiving 0.5mg/kg i.p. risperidone (GV-R0.5) had significantly higher PPI responses than their acute vehicle treated counterparts (GV-V) following a significant IP-rearing main effect [$F_{(1,43)}$=4.656, p=0.037] and a rearing x risperidone interaction [$F_{(1,43)}$=50.53, p=0.030], but no main effect of risperidone treatment alone. IP-reared rats receiving acute vehicle (IP-V), 0.2mg/kg i.p. risperidone (IP-R0.2) or 0.5mg/kg i.p. risperidone (IP-R0.5) performed comparably to control (GV-V) at all prepulse levels. **p<0.01 *p<0.05 risperidone treatment vs. rearing-matched vehicle control, †p<0.05 IP-rearing vs. risperidone treatment-matched GV control by Bonferroni post-hoc test following ANOVA.

Study 2: As previously, attenuation of the startle response by presentation of increasing prepulse intensities was observed, supported by RM ANOVA with a highly significant main effect of prepulse intensity [$F_{(2,90)}$=125.129, p<0.001]. Despite a trend towards reduction in prepulse inhibition, ANOVA revealed no effect of IP-rearing [$F_{(1,45)}$=0.512, p=0.478], but an observable increase in percentage PPI due to risperidone was supported by a significant main effect by RM ANOVA [$F_{(2,45)}$=5.089, p=0.010] with Bonferroni post-hoc analysis confirming 0.2mg/kg risperidone caused a significant increase in PPI in IP-reared rats at the 76dB and 80dB prepulse levels. There were no interactions between any of the factors analysed (Figure 4.7). Two-way ANOVA analysis revealed no significant effects of IP-rearing or risperidone treatment on initial startle amplitude or habituation, with no interaction between the factors (data not shown).

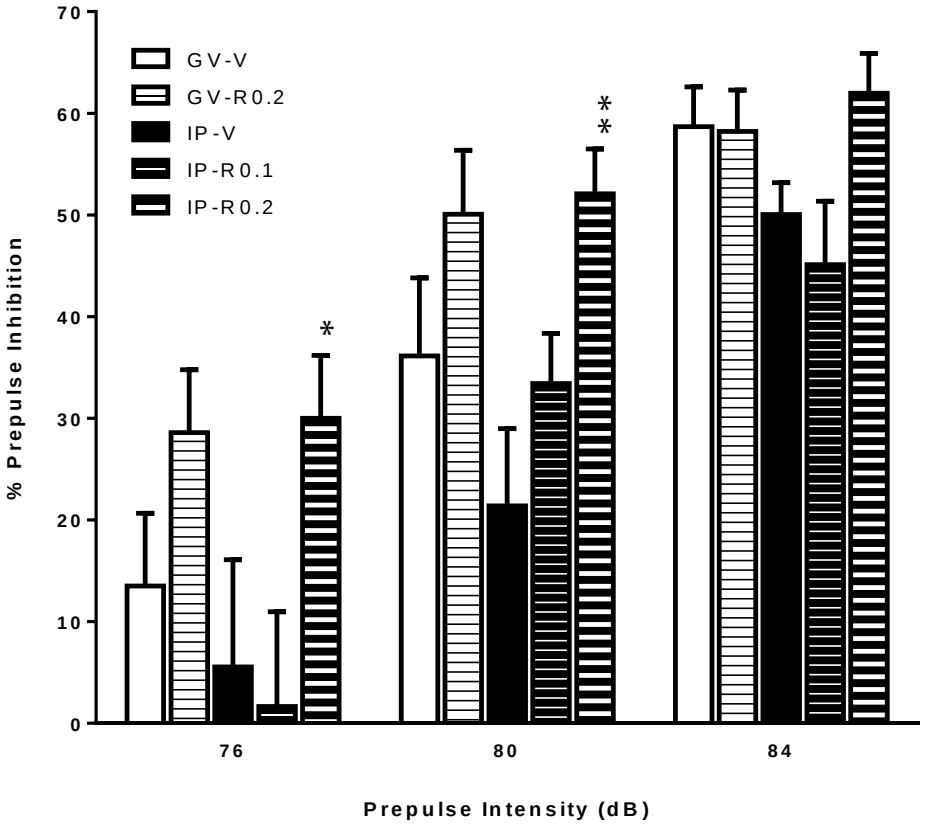

Figure 4.7 – Elevated prepulse inhibition of the acoustic startle response by risperidone pretreatment. Prepulse inhibition (mean percentage ±SEM, *n*=9-10) significantly increased with rising prepulse intensity [$F_{(2,90)}$=125.129, p<0.001]. PPI was significantly elevated by acute 0.2mg/kg i.p. risperidone treatment 30 min prior to testing to isolation-reared, perinatal PCP-treated rats (IP-R0.2), but less so at 0.1mg/kg to IP-reared rats (IP-R0.1) and 0.2mg/kg to GV-reared rats (GV-R0.2), compared to their acute vehicle-treated, rearing matched counterparts (IP-V and GV-V, respectively). There was no effect of IP-rearing, nor an IP-rearing x risperidone interaction by RM ANOVA. **p<0.01 *p<0.05 risperidone treatment vs. rearing-matched vehicle treated control by Bonferroni post-hoc test following ANOVA.

4.3.4 Effect of risperidone on IP-rearing induced changes in conditioned emotional response

Study 1: Animals which had received 0.5mg/kg risperidone did not display normal exploratory behaviour when placed in the CER chamber and appeared sedated. A general decrease in exploratory activity caused the latency to cross the chamber partition to dramatically increase, supported by two-way ANOVA which revealed a very highly significant effect of risperidone treatment [$F_{(2,41)}$=17.165, p<0.001], but no effect of IP-rearing and no interaction between the two factors (Figure 4.8A). Bonferroni post-hoc analysis showed that the route of this significance was specifically 0.5mg/kg risperidone treatment, which had a significantly increased entry latency compared to both saline and 0.2mg/kg risperidone treated animals (p<0.001).

With subsequent chamber exposures, rats in all treatment groups froze with decreasing duration, reflecting extinction of contextual conditioning. Upon re-presentation of the conditioned stimulus, freezing response was increased in all treatment groups, demonstrating successful acquisition of the cue-conditioning behaviour. During all testing phases, GV-V animals froze to a greater extent than all IP-rearing groups, replicating the IP-induced deficit seen previously (see Chapter 3.3.4). Two-way ANOVA supported this observation with highly significant effects of IP-rearing at 24h [$F_{(1,42)}$=4.748, p=0.035] and 48h post-conditioning [$F_{(1,42)}$=10.683, p=0.002], and following re-presentation of the CS [$F_{(1,42)}$=14.798, p<0.0001] (Figure 4.8B). Furthermore, freezing was decreased in group-housed animals that received 0.5mg/kg risperidone, reflected by a significant main effect of drug treatment at 24h [$F_{(2,42)}$=4.101, p=0.024] and

48h post-conditioning [$F_{(2,42)}$=4.504, p=0.017]. Significance was not reached following re-presentation of the CS alone, but a significant IP-rearing x risperidone interaction was observed at all three time points (24h: [$F_{(1,42)}$=11.903, p=0.00], 48h: [$F_{(1,42)}$=6.409, p=0.015], Post-CS: [$F_{(1,42)}$=6.830, p=0.012]). Bonferroni post-hoc analysis confirmed the decreases in freezing in GV-R0.5 and all IP-reared rats (regardless of risperidone treatment), with significance seen between GV-V and all four other groups at 24 and 48h post-training (p<0.01), and between GV-V and all IP-reared rats following re-presentation of the CS at the end of the protocol (p<0.05, p<0.01, p<0.001).

.

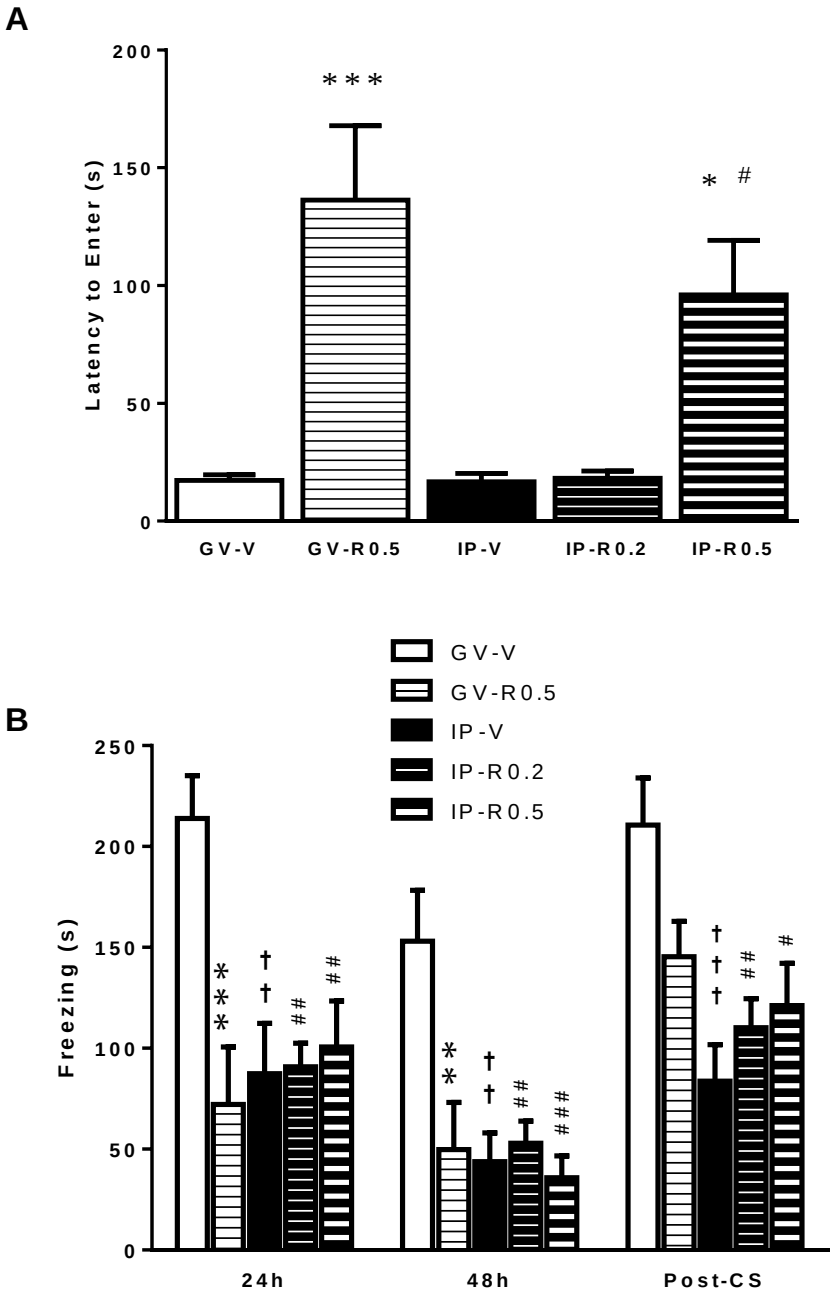

Figure 4.8 – see overleaf

Figure 4.8 – An IP-rearing induced reduction in freezing response to contextual and conditioned fear in a conditioned emotional response protocol was not reversed by risperidone treatment. (A) The entry latency (s, mean±SEM, *n*=9-11) to the CER chamber was significantly increased due to 0.5mg/kg risperidone, regardless of rearing condition (significant main effect of risperidone [$F_{(2,41)}$=17.165, p<0.001], two-way ANOVA). ***p<0.001 *p<0.05 risperidone vs. rearing-matched vehicle control, $^{\#}p$<0.05 IP-rearing+risperidone vs. GV-V absolute control by Bonferroni post-hoc test following ANOVA.

(B) Freezing response (s, mean±SEM, *n*=9-11) was reduced by combined isolation rearing and perinatal PCP treatment (IP) compared to vehicle-treated group-housed animals (GV) at 24h [$F_{(1,42)}$=4.748, p=0.035] and 48h [$F_{(1,42)}$=10.683, p=0.002] post-conditioning of an aversive footshock (US) and a paired light-sound tone (CS), and following re-presentation of the CS alone [$F_{(1,42)}$=14.798, p<0.0001] by two-way ANOVA. 0.2mg/kg (R0.2) and 0.5mg/kg i.p. risperidone (R0.5) 30 minutes before conditioning also altered freezing at 24h [$F_{(2,42)}$=4.101, p=0.024] and 48h post-conditioning [$F_{(2,42)}$=4.504, p=0.017] by ANOVA. An IP-rearing x risperidone interaction was also seen in all trials analysed (24h: [$F_{(1,42)}$=11.903, p=0.00], 48h: [$F_{(1,42)}$=6.409, p=0.015], Post-CS: [$F_{(1,42)}$=6.830, p=0.012]). ***p<0.001 **p<0.01risperidone vs. vehicle control, †††p<0.001 ††p<0.01 IP-reared vs. GV-controls, $^{\#\#\#}p$<0.001 $^{\#\#}p$<0.01 $^{\#}p$<0.05 IP-rearing+risperidone vs. absolute control GV-V by Bonferroni post-hoc following ANOVA.

Study 2: With lower doses of risperidone, the level of sedation observed in the first study was not repeated. No between group differences were observed in latency to enter the CER chamber, supported by non-significant effects of IP rearing and risperidone treatment by two-way ANOVA (Figure 4.9A). As previously, decreased freezing was observed at 48h compared to 24h post-training, with the response restored upon re-presentation of the CS, reflecting extinction of contextual conditioning and retention of cue-based conditioning. Freezing was markedly reduced by IP-rearing such that two-way ANOVA revealed significance at 24h [$F_{(1,45)}$=30.089, p<0.001], 48h post-conditioning [$F_{(1,45)}$=21.052, p<0.001] and following presentation of the CS alone [$F_{(1,44)}$=9.047, p=0.004]. Bonferroni post-hoc analysis showed that IP-V rats froze significantly less than GV-V rats at all the time points analysed (24h p<0.01, 48h: p<0.001, Post-CS: p<0.001) (Figure 4.9B). Despite minor increases in freezing response following risperidone treatment, this was non-significant by two-way ANOVA during all trials. However, ANOVA revealed a significant IP-rearing x risperidone interaction following presentation of the CS alone [$F_{(1,45)}$=9.187, p=0.004], reflecting an IP-induced reduction in freezing that was partially restored by risperidone treatment, confirmed by a lack of post-hoc significance between GV-V and IP-R0.2 groups at the Post-CS time point.

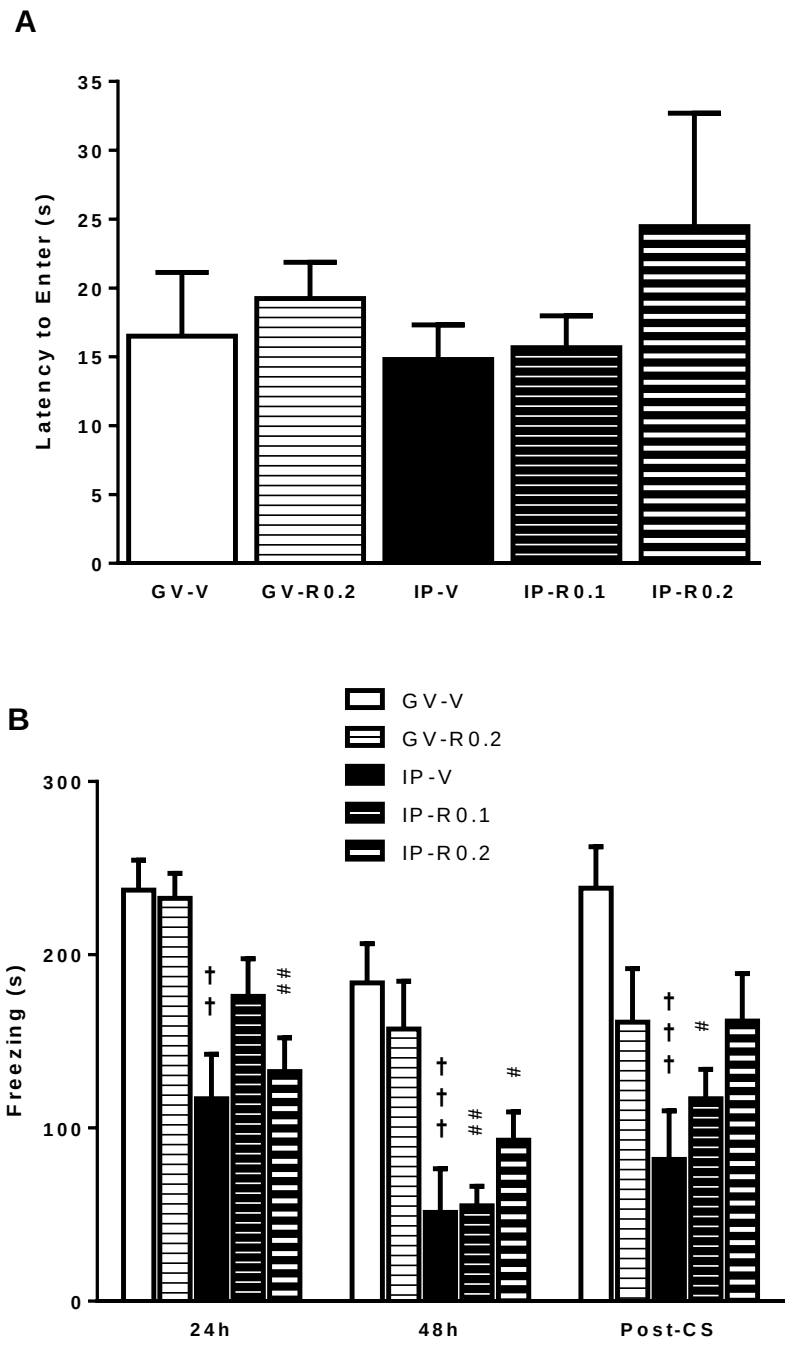

Figure 4.9 – see overleaf

Figure 4.9 - An IP-rearing-induced reduction in freezing response to contextual and conditioned fear in a conditioned emotional response protocol is partially reversed by acute risperidone treatment. (A) The entry latency (s, mean±SEM, *n*=10) to the CER chamber prior to acquisition was not significantly affected by risperidone treatment or rearing condition, and there was no interaction.

(B) Freezing response (s, mean±SEM, *n*=10) was reduced by isolation rearing and perinatal PCP treatment (IP) compared to vehicle-treated group-housed animals (GV) at 24h [$F_{(1,45)}$=30.089, p<0.001, two-way ANOVA] and 48h post-conditioning [$F_{(1,45)}$=21.052, p<0.001] of an aversive footshock (US) and a paired light-sound tone (CS), and following re-presentation of the CS alone [$F_{(1,44)}$=9.047, p=0.004]. There was no significant main effect of acute risperidone treatment at 0.1mg/kg i.p. (R0.1) or 0.2mg/kg i.p. (R0.2) 30 mins before conditioning compared to vehicle control (V) on freezing during any trial, however an IP-rearing x risperidone interaction was observed Post-CS [$F_{(1,45)}$=9.187, p=0.004], indicative of a partial reversal of the IP-induced deficit. †††p<0.001 ††p<0.01 IP-reared vs. GV-controls, $^{\#\#}p$<0.01 $^{\#}p$<0.05 IP-rearing+risperidone vs. GV-V absolute control by Bonferroni post-hoc following ANOVA.

4.3.5 Effect of risperidone on IP-rearing induced changes in Morris water maze performance

Study 1: For protocol viability, the IP-R0.2 group were excluded from the Morris Water Maze experiment. As in previous paradigms, 0.5mg/kg risperidone caused sedation, leading to markedly decreased swimming activity for much or all of the 90 second trials throughout the acquisition phase. RM ANOVA supported this observation, with a highly significant increase on latency to platform due to risperidone [$F_{(1,34)}$=55.869, p<0.001], and complete impairment of task acquisition. Vehicle-treated rats in both rearing groups did complete the task, improving with time, reflected by a significant main effect of trial [$F_{(14,476)}$=8.395, p<0.001]. A rearing x risperidone interaction was also observed [$F_{(1,34)}$=5.316, p=0.027], but there was no main effect of rearing alone [$F_{(1,34)}$=1.158, p=0.289], confounded by the impairment due to risperidone (Figure 4.10). Bonferroni post-hoc analysis supported the deficits produced by risperidone treatment, particularly notable during trials 9 to 15 (effect of risperidone p<0.001 in trials 9-14, p<0.01 in trial 15, GV-V vs. GV-R0.5).

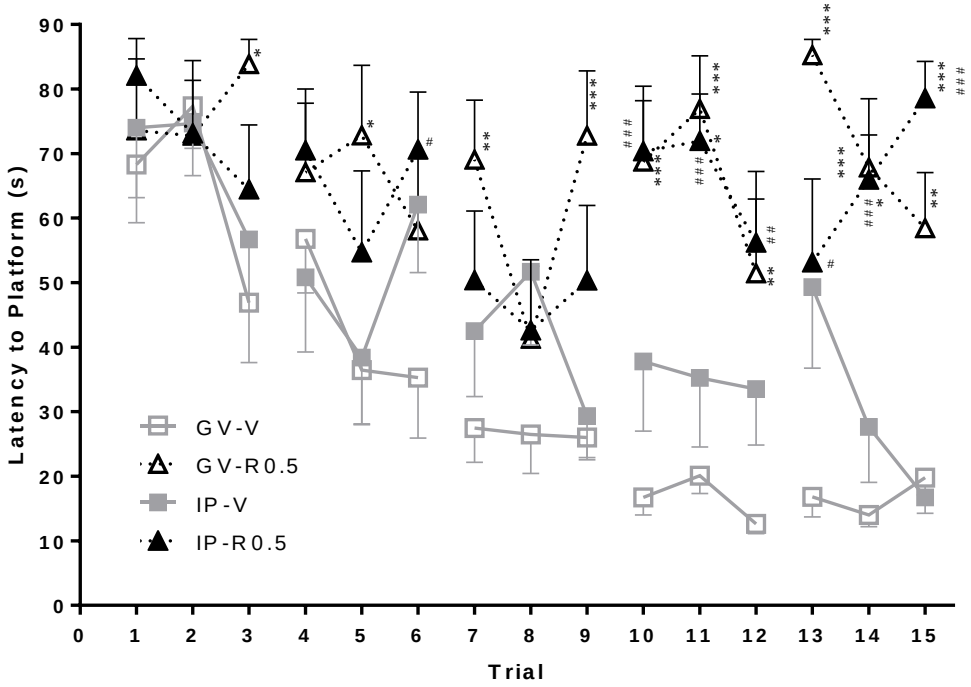

Figure 4.10 – Impaired acquisition of a Morris water maze task by pretreatment with risperidone. Latency to locate a hidden platform in a fixed location (s, mean ±SEM, *n*=9-11) in the Morris Water Maze paradigm was significantly increased by daily risperidone treatment (0.5mg/kg i.p. 30 min prior to training) in group-housed, perinatal saline-treated (GV-R0.5) and isolation-reared, perinatal PCP-treated (IP-R0.5) rats compared to respective acute vehicle controls (GV-V and IP-V). RM ANOVA of the 15 trial acquisition period revealed highly significant main effects of trial [$F_{(14,476)}$=8.395, p<0.001] and risperidone [$F_{(1,34)}$=55.869, p<0.001], plus an IP rearing x risperidone interaction [$F_{(1,34)}$=5.316, p=0.027], but no main effect of IP-rearing alone [$F_{(1,34)}$=1.158, p=0.289]. ***p<0.001, **p<0.01, *p<0.05 risperidone treatment vs. rearing-matched acute vehicle treatment, $^{###}p$<0.001, $^{##}p$<0.01 $^{#}p$<0.05 IP-rearing+risperidone treatment vs. GV-V absolute controls by Bonferroni post-hoc following ANOVA.

Following training, rats were tested in two unbiased probe trials to assess acquisition and retention levels. Probe 1 demonstrated that risperidone-treated animals spent significantly less time in the correct quadrant, reflecting an inability to learn the task [two-way ANOVA, $F_{(1,35)}=22.82$, $p<0.001$]. There was also a rearing x risperidone interaction [$F_{(1,35)}=10.75$, $p=0.0024$], but no main effect due to rearing, despite a clear trend towards decreased performance in the IP-V group compared to GV-V (Figure 4.11A). Bonferroni post-hoc analysis supported the previous finding that risperidone treatment impaired performance, with both GV-R0.5 ($p<0.001$) and IP-R0.5 ($p<0.01$) groups spending significantly less time in the correct quadrant than GV-V rats. Furthermore, IP-V rats spent a significantly decreased time in the correct quadrant compared to GV-V rats, suggesting IP-induced impairment in acquisition masked by risperidone effects in ANOVA ($p<0.05$).

Probe 2 demonstrated a similar rearing x risperidone interaction to Probe 1 [$F_{(1,35)}=5.882$, $p=0.0206$], but there was no significant main effect of IP-rearing or risperidone by two-way ANOVA. Performance of all rats fell reflecting extinction of the task (Figure 4.11B). Bonferroni post-hoc analysis revealed that GV-R0.5 rats still explored the correct quadrant significantly less than their vehicle treated GV-V counterparts ($p<0.05$).

Risperidone treated animals were removed from the experiment following completion of Probe 2, as their inability to learn the initial task would confound any reversal learning protocol findings.

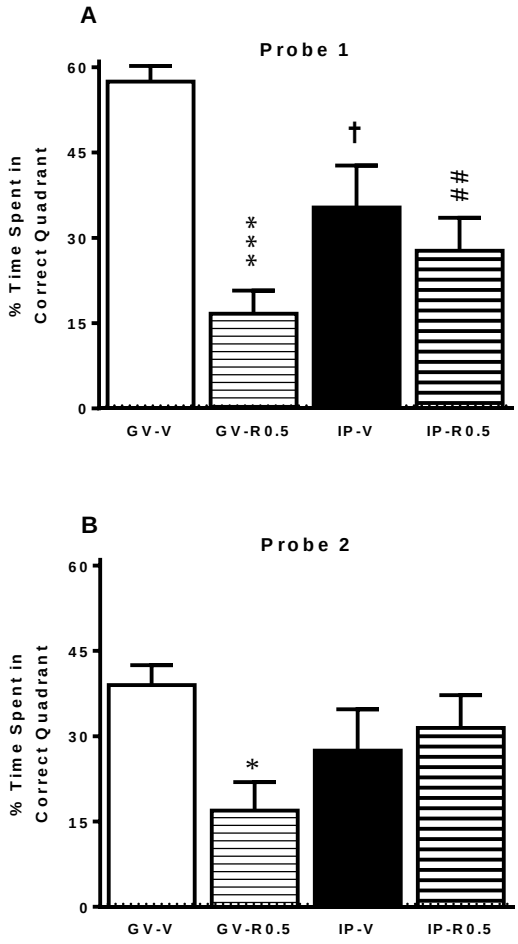

Figure 4.11 – Rats treated with risperidone (0.5mg/kg i.p.) during acquisition were significantly impaired in the MWM task, spending less time exploring the correct quadrant (mean percentage of time ±SEM, n=9-11) in (A) Probe 1 by two-way ANOVA [$F_{(1,35)}$=22.82, p<0.001]. A rearing x risperidone interaction was observed [$F_{(1,35)}$=10.75, p=0.0024], but no significant main effect of IP-rearing. Main significance was lost during Probe 2 (B), but a rearing x risperidone interaction was again observed [$F_{(1,35)}$=5.882, p=0.0206]. ***p<0.001 *p<0.05 risperidone treatment vs. rearing-matched acute vehicle control, † p<0.05 IP-rearing vs. risperidone-matched GV-control, $^{\#\#}p$<0.01 IP-rearing+risperidone vs. GV-V absolute control by Bonferroni post-hoc test following ANOVA.

The experiment was continued with just GV-V and IP-V rats in an attempt to confirm a deficit in reversal learning due to IP-rearing combination treatment. A trend towards increased latency to locate the platform in IP-V rats suggested a potential impairment in reversal learning performance, but RM ANOVA revealed that there was no main effect of IP-rearing (Figure 4.12). A significant main effect of trial was seen [$F_{(14,252)}$=2.4178, p=0.0034], as was a significant IP-rearing x trial interaction [$F_{(14,252)}$=1.797, p=0.0394], suggesting some trial-dependant impairment in reversal performance may have occurred, but this was not confirmed by post-hoc analysis. Probe 3 and Probe 4 revealed no significant differences in the time spent exploring the "platform ring" (Figure 2.2) or previously correct quadrant area between the GV-V and IP-V rats by *t*-test (Figure 4.13), further suggesting a lack of robust impairment by the combined IP-rearing.

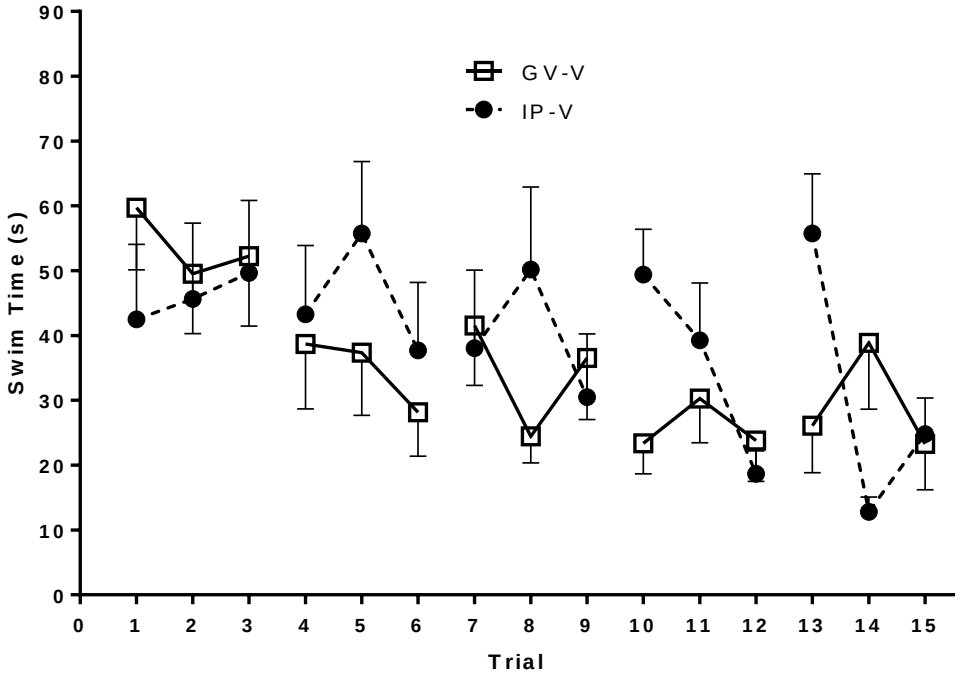

Figure 4.12 – No significant main effect of combined isolation rearing-perinatal PCP treatment (IP-V) compared to group-housed, saline treated controls (GV-V) on platform escape latency (s, mean±SEM, *n*=9-11) in a 15 trial reversal learning phase of a MWM protocol. A significant main effect of trial [$F_{(14,252)}$=2.4178, *p*=0.0034], and a significant IP-rearing x trial interaction [$F_{(14,252)}$=1.797, *p*=0.0394] were observed by RM ANOVA, with no post-hoc significance.

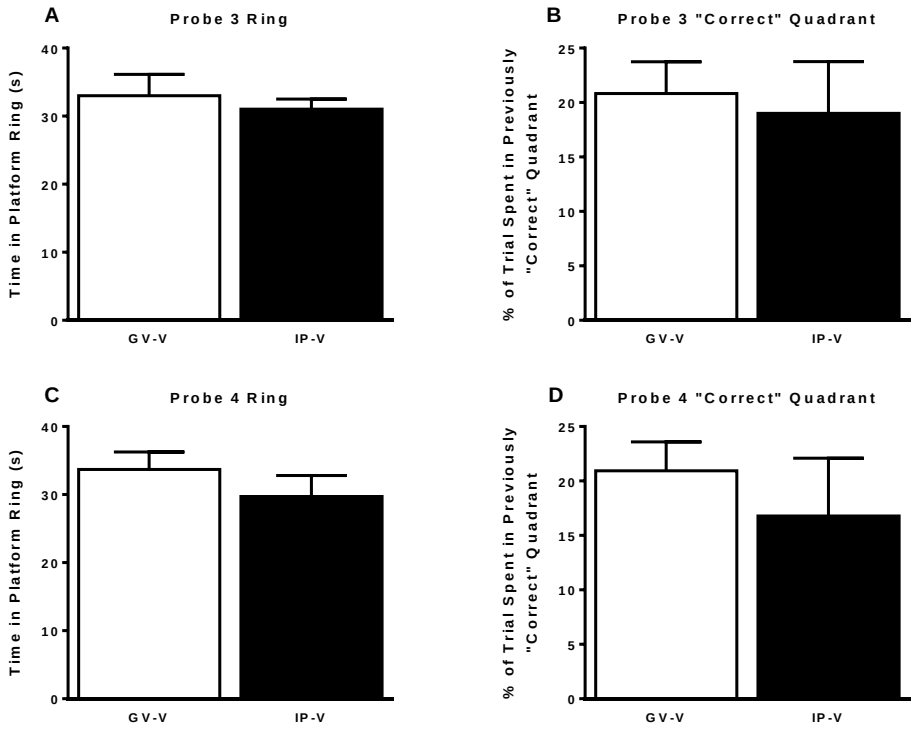

Figure 4.13 – No significant effects of combined isolation rearing-perinatal PCP treatment (IP-V) compared to group-housed, vehicle treated rats (GV-V) on time spent exploring the Platform Ring zone (s, mean±SEM) in Probe 3 (A) and Probe 4 (C), or on time spent exploring the previously "correct" quadrant (% time, mean±SEM, *n*=9-11) in Probe 3 (B) and Probe 4 (D) of the Morris Water Maze protocol.

4.4 Discussion

It is clear from the high dose risperidone study that 0.5mg/kg induced sedation, most notably in LMA, CER and MWM protocols which require active movement. Although sedative effects have not been seen at this dose when used previously in rats (Anastasio and Johnson 2008a; Fabricius et al. 2011; McLean et al. 2010b), including in recent work in our lab (McIntosh et al. 2013), behaviour was clearly compromised. In the LMA paradigm, risperidone produced very marked reduction in locomotor activity from the outset of the 1 h protocol that persisted throughout. In the CER paradigm, rats in the 0.5mg/kg group took up to 300 s to cross the box partition (only three rats receiving this dose took below 60 s), whilst no single rat that received vehicle control or low dose risperidone took more than 60 s. Although placing rats in the cool water of the MWM was anticipated to be mildly aversive and induce active swimming, rats placed in the maze following treatment exhibited little or no swimming activity and instead floated in the maze for much or all of the 90 s trial. Because of this sedation, risperidone-treated rats were severely impaired in acquisition of the MWM task, highlighted by their poor performance in Probe 1. Having failed to acquire the task successfully in the first place, rats in the 0.5mg/kg risperidone treatment groups (both GV and IP) were discontinued in the protocol, as any learning response would have been confounded by poor previous acquisition and continued sedation. Instead, GV-V and IP-V rats continued in the reversal learning phase to attempt confirmation of a significant IP-rearing-induced deficit. In line with earlier

findings, the IP-reared rats were not shown to be significantly impaired in reversal learning compared to controls either in latency to find the platform or maze exploration in Probe 3 and 4, suggesting no impairment in cognitive flexibility. The lack of consistent deficits observed in dual-hit animals throughout three cohorts indicates that this paradigm lacks the robustness required to be used as part of the test battery, and so it was discontinued.

The risperidone-induced sedation, which occurred regardless of rearing condition in study 1, also caused impairment in the NOD paradigm, where GV-R0.5 rats performed less well than vehicle treated controls (GV-V).

One paradigm where the GV-R0.5 rats performed comparably or better than controls was in the PPI protocol, where at each prepulse analysed, the percentage PPI exhibited by these rat was higher than those in the GV-V group. This is most likely also explained by the sedative effect of risperidone, reflecting a reduced physical response to the startling tones rather than an increased inhibition of the startle due to prepulse presentation.

One key observation from study 1 was that 0.2mg/kg produced little sedation. Based on these results, the second risperidone reversal study used this dose as "high" risperidone, with 0.1mg/kg used as the low dose. Although these doses have been commonly used in previous preclinical investigations, acute risperidone below 0.5-1mg/kg in rats has been shown to achieve a lower level of D_2 receptor occupancy than is seen with clinically relevant doses of the drug in patients (Kapur et al. 2003). However, with strong 5-HT_{2A} (Shahid et al. 2009) and recently identified voltage-gated sodium channel activity (Brauner

et al. 2014), occupancy of the D_2 receptor is unlikely to be the only factor governing the efficacy of risperidone in rodents or in man. In this second study, risperidone was shown to cause a significant reduction in locomotor activity in both GV and IP rats. This effect of risperidone reversing basal hyperlocomotion has been previously noted in isolation-reared rats, with different groups utilising doses from 0.08mg/kg to 0.5mg/kg to produce decreased locomotion in both isolates and controls (Fabricius et al. 2011; McIntosh et al. 2013). Furthermore, a single dose of 0.25mg/kg risperidone given as a pre-treatment before perinatal PCP administration has been shown to prevent the development of basal hyperlocomotion (Anastasio and Johnson 2008a). The results presented here support these published findings. As described previously (see Chapter 1.2.1), the locomotor activity paradigm is considered to have validity to the positive symptoms of schizophrenia in humans through aberrant dopamine activity, a symptom domain known to be well controlled by current antipsychotics including risperidone (Houthoofd et al. 2008). As elevated locomotor activity in isolates has recently been shown to be reversed by D_2 and D_3 dopamine receptor selective antagonists (Watson et al. 2012b), the activity of risperidone at these receptors likely underlies the decrease in locomotor activity observed here. This result lends weight to the use of the IP dual-hit protocol for modelling schizophrenia-like symptoms.

A similar reversal effect due to acute risperidone was observed in the PPI paradigm. Despite an inconsistent deficit due to IP-rearing, rats in the IP-R0.2 group were shown to have elevated prepulse inhibition compared to other IP-

reared animals, responding to a level comparable to GV-V and GV-R0.5 rats, and reflected by a statistically significant effect of risperidone in this paradigm. This effect was seen in the absence of any effect of risperidone on initial startle intensity or habituation to the 120dB startle-eliciting tone. These results also support the literature demonstrating that reversal of PPI deficits by antipsychotic drugs including risperidone is possible in preclinical models of schizophrenia, including subchronic PCP administration (Anastasio and Johnson 2008a; Li et al. 2011a), isolation rearing (Varty and Higgins 1995), hippocampal lesioning (Adams and van den Buuse 2011), and the administration of the NMDA receptor antagonist MK-801 (Varty and Higgins 1995). Much like locomotor activity, PPI is known to be affected by mesolimbic dopamine activity, and blockade of D_2 and D_3 dopamine receptors with the non-selective antagonist raclopride can reverse isolation-induced deficits (Geyer et al. 1993). With similarities between the receptor pharmacology of these two behaviours it is perhaps unsurprising that the beneficial outcome of risperidone treatment was seen in both. However, there was little effect of the 0.1mg/kg dose of risperidone in this paradigm, indicating that this dose may be below the therapeutic window. Considering the marked sedation that was previously observed with a dose of 0.5mg/kg risperidone, it could be suggested that Lister-Hooded rats may have heightened responsiveness to this drug over the Sprague-Dawley rats commonly used previously (Adams and van den Buuse 2011; Li et al. 2011a).

Whilst sensory gating in schizophrenia patients is consistently deficient compared to healthy controls, these deficits appear to be largely insensitive to

treatment with first- and second-generation antipsychotic drugs, notably including risperidone (Hong et al. 2009; Sanchez-Morla et al. 2009; Zhang et al. 2012a). Effective reversal of PPI deficits in this study may indicate that the problem of false-positive outcomes of antipsychotic drugs in PCP-based preclinical models persist in the IP-rearing dual-hit model, providing lower predictive validity to the human condition.

Risperidone treatment was less successful in reversing IP-induced deficits in the other two behaviours examined. In the novel object discrimination paradigm, risperidone treatment at both high and low doses did not reverse the IP-induced deficit in visual learning and memory. Evidence from the literature suggests that reversal of visual learning and memory impairments is a reasonably well characterised effect of risperidone in preclinical models of schizophrenia at a range of doses (Grayson et al. 2007; Jenkins et al. 2010a; McIntosh et al. 2013), although some prior work suggests risperidone is ineffective at preventing chronic PCP-induced recognition memory deficits in rats (McKibben et al. 2010). It has been noted that blockade of prefrontal D_3 dopamine receptors can reverse delay-induced deficits in NOD, whereas D_2 receptor antagonism impairs performance (Watson et al. 2012b). Given that risperidone is not selective between $D_{2/3}$ receptors, this may explain the lack of effect observed here. Clinically, risperidone has also produced mixed results in improving visual learning and memory in schizophrenic patients, with groups reporting both positive improvements (Harvey et al. 2005; Riedel et al. 2007) and lack of change (Purdon et al. 2000) due to the drug. The results presented

herein suggest that IP-induced novel object discrimination deficits are not susceptible to risperidone treatment at the doses used. Based on the level of sedation caused by the 0.5mg/kg dose in Study 1 it is unlikely that higher doses would yield more beneficial effects in these rats. Indeed, higher doses would yield elevated D_2 receptor occupancy, and based on evidence in man using aripiprazole, higher D_2 receptor occupancy may cause increased impairments in working memory, rather than improvement (Kim et al. 2013).

Although IP-induced deficits in the conditioned emotional response were not significantly reversed by risperidone overall, it could be speculated that a trend towards some increase in freezing time was observed. Previous work in our laboratory also found a trend towards improvement in this paradigm which failed to reach significance following 0.2mg/kg and 0.5mg/kg risperidone treatment to isolation-reared rats, supportive of findings presented here (McIntosh et al. 2013). However, another group suggested risperidone was unable to reverse deficits in contextual or cued fear-conditioning in rats with excitotoxin-induced hippocampal neuropathy, an alternative model of schizophrenia (Martin et al. 2005), suggesting the ability of risperidone to reverse such emotional memory-based deficits is dependent on the preclinical model being utilised. Clinically, deficits in emotional learning and conditioning are well characterised in schizophrenic patients (Herbener 2009; Rushe et al. 1999), but current evidence suggesting whether risperidone or other atypical anti-psychotic drugs are beneficial in treating these symptoms is mixed (Behere et al. 2009; Kucharska-Pietura et al. 2012; Woodward et al.

2005). Here, it can be suggested that the deficit induced by IP-rearing cannot be reversed by risperidone treatment.

4.4.1 Conclusion

The results presented herein are difficult to interpret due to a continuing lack of reliability in the deficits of the IP-dual hit model. Broadly, this study does not lend much support to suggest risperidone as an effective treatment of the cognitive symptoms of schizophrenia. Whilst study 1 demonstrated the sedative effect of risperidone, significant behavioural deficits due to IP-rearing were observed in the NOD and CER paradigms only. Further inconsistent results in the MWM supported the cessation of this test in future cohorts.

Improved performance of IP-reared rats following 0.2mg/kg risperidone was seen in LMA and PPI protocols, but no effect was seen on NOD or CER, suggesting a lack of effect of risperidone on cognition in this model. The IP dual-hit model is clearly amenable to pharmacological challenge, providing some support to its use. But with the effect of risperidone on cognition here not entirely in line with its effect in patients, it is hard to make inferences about the model's predictive validity for cognition in schizophrenia. However, as this clinical need is largely still unmet, a "positive control" to fully assess validity is unavailable.

Chapter 5

Evaluating the Effect of Lamotrigine in the Iso-PCP Dual-Hit Model of 'Schizophrenia-Like' Symptoms in the Rat

5.1 Introduction

The anticonvulsant drug lamotrigine is prescribed clinically for the treatment of epilepsy and bipolar disorder. The primary pharmacological target is thought to be blockade of voltage-gated sodium channels ($Na_v1.2$), preventing depolarization of neurones and reduction of synaptic release of excitatory amino acids including glutamate and aspartate (Leach et al. 1991). However, lamotrigine also has weaker inhibitory effects at the 5-HT_3, 5-HT_{1A} and nicotinic acetylcholine receptors among others (Bourin et al. 2005; GlaxoSmithKline 2007; Zheng et al. 2010). Although it is not approved for the treatment of schizophrenia, a number of clinical studies have noted that lamotrigine may have efficacy in treating some of the symptoms, particularly in patients who are resistant to the clozapine treatment (Goff 2009; Tiihonen et al. 2009). Notably, lamotrigine and related voltage-gated sodium channel blockers have been shown to have beneficial effects on, or block the induction of, cognitive deficits in rodent models of 'schizophrenia-like' symptoms, including visual and spatial learning (Celikyurt et al. 2012), reversal learning (Large et al. 2011) and prepulse inhibition of acoustic startle (Brody et al. 2003a; Nakato et al. 2010). The exact mechanisms of these effects are uncertain, however in line with the proposed contribution of altered function of glutamate signalling in the aetiology of schizophrenia it is plausible that lamotrigine could exert beneficial effects to negative and cognitive symptoms by attenuating cortical glutamate release. Furthermore, with the recent discovery that risperidone has activity at voltage-gated sodium channels also,

there is some suggestion that this pharmacology may underlie some of the beneficial effects of currently available antipsychotics (Brauner et al. 2014). Hence, evaluation of a more established sodium channel blocked in this model holds some value.

To date there have been few preclinical studies evaluating the effect of lamotrigine in rodent models of schizophrenia. However, as a compound which has already been shown to have preclinical efficacy in a number of the paradigms in which the IP model has been assessed, it was selected as good candidate for further examining the predictive validity of the model. Furthermore, lamotrigine lacks the dopaminergic activity held by risperidone and other clinically available antipsychotic agents. Examining whether the behavioural deficits induced by the IP dual hit model would be more susceptible to reversal through alternative mechanisms was considered a strong rationale for selecting lamotrigine for this study.

5.1.1 Hypothesis

Based on the preclinical results from the literature, it was hypothesised that lamotrigine would cause a dose-dependent reversal of the behavioural deficits induced by the IP-rearing dual-hit model of schizophrenia in the rat. In particular, the paradigms involving visual learning and memory (NOD) and sensorimotor gating (PPI) would be most susceptible to drug challenge, as these have been shown previously to be reversed by lamotrigine treatment.

5.2 Materials and Methods

5.2.1 Animals

Three day old, male, Lister Hooded rat pups (*n*=12 from 2 litters in the pilot test, *n*=38 from 5 litters in the full behavioural battery study) were obtained from Charles River UK (CRUK, Margate, UK) accompanied by their natural dams and underwent an identical early-life treatment protocol to that described previously (see Chapter 3.2.1). In the dose-response pilot study, all animals were treated perinatally with PCP and subsequently housed in isolation, giving IP-V (*n*=12). In the full behavioural battery study (FBBS), animals treated perinatally with vehicle were subsequently housed in groups of 3/4 (GV, *n*=15), whereas all animals that received phencyclidine were housed in isolation (IP, *n*=23). Rats were reared for 40 days post-weaning in previously described ambient conditions (see Chapter 2.2.1).

5.2.2 Drug Administration

Lamotrigine (Sigma-Aldrich, Irvine, Scotland, UK) is insoluble in H_2O or physiological saline (0.154 M) without reducing the pH to acidity unsuitable for systemic administration. Lamotrigine was therefore suspended in 50% w/v methyl cellulose, aided by the addition of a few drops 1M HCl before adjusting to pH 7 with 0.1M NaOH. Rats in the pilot study were placed on a within-subject protocol, receiving methyl cellulose vehicle control (2ml/kg), low dose lamotrigine (10mg/kg) or high dose lamotrigine (20mg/kg) once each on three occasions in a pseudorandom order. FBBS rats were subdivided into lamotrigine or control treatment groups, giving IP-reared animals receiving

either vehicle control (2ml/kg, IP-V, *n*=8), low dose lamotrigine (10mg/kg, IP-L10, *n*=8) or high dose lamotrigine (15mg/kg, IP-L15, *n*=7), and group-housed, perinatal saline-treated (GV) animals receiving either vehicle control (GV-V, *n*=8) or high dose lamotrigine only (15mg/kg, GV-L10, *n*=7). All treatments were administered by intraperitoneal injection (2 ml/kg), 60 min before the initiation of each behavioural test, based on the literature.

5.2.3 Behavioural Testing and Statistical Analysis

During the pilot study, rats were tested in the NOD protocol on three occasions. Rats were initially exposed to the test chamber for 60 mins exactly 24 hours before NOD testing was due to commence, and the NOD protocol was then performed as outlined previously (see Chapter 2.2.3). This was repeated with each rat assigned to a different test chamber and receiving a different pretreatment (vehicle control, L10 or L20) on two further occasions, such that each rat had been tested in the NOD protocol having received each drug treatment once, allowing within-subject statistical comparisons.

During the full study, behavioural testing took place following the LMA, NOD, PPI and CER protocols described previously (see Chapter 2.2), and following the timeline identified in Figure 5.1

Table 5.1 Table to show the perinatal drug treatment, post-weaning housing condition and acute lamotrigine treatment of each pup in the main study. Also shown, the behavioural tests undertaken by each litter

Litter	Pup Numbers	Litter Assignments					Behaviours
		GV-V	GV-L15	IP-V	IP-L10	IP-L15	
1	10	2	1	3	2	2	All animals performed LMA, NOD, PPI and CER tests
2	10	1	2	2	3	2	
3	7	2	1	1	1	2	
4	6	1	2	1	1	1	
5	5	2	1	1	1	0	

Figure 5.1 – Protocol Timeline used in the full behavioural test battery study examining the effect of lamotrigine treatment (10 and 15mg/kg, i.p.) on behaviour in the IP dual-hit model of schizophrenia-like symptoms in rats.

In an addition to the statistical analysis used previously, the D1 discrimination ratio derived from the NOD paradigm was also analysed by one sample *t*-test in the pilot study, comparing the D1 of each individual treatment group with a "chance" score of D1=0.5. Whilst this less stringent analysis does not compare between treatment groups, it does identify whether animals receiving a particular treatment have explored the two objects in a pattern that is significantly different to what would be expected by chance, and so some inference can be drawn about the efficacy of treatment.

5.3 Results

5.3.1 Pilot Study - Effect of lamotrigine on performance in novel object discrimination

As expected, vehicle-treated IP animals did not explore the novel more than the familiar object during the second choice trial, whereas those treated with lamotrigine did. However, two-way ANOVA of the NOD choice trial showed a significant main effect of object [$F_{(1,30)}$=37.93, p<0.0001] and a significant object x treatment interaction [$F_{(2,30)}$=3.404, p=0.0465] but no significant main effect of treatment alone [$F_{(2,30)}$=0.3023, p=0.7413]. Despite this, Bonferroni post-hoc analysis confirmed a highly significant difference in object exploration in both the L10 (p<0.001) and L20 (p<0.001) treatment groups which was absent in the vehicle control group (Figure 5.2A).

A trend towards dose-dependent improvement in discrimination due to lamotrigine was seen, but RM ANOVA analysis (one-way) failed to show a significant main effect of lamotrigine on D1 ratio [$F_{(2,18)}$=2.885, p=0.0819]. However, one sample *t*-test comparing each D1 ratio to chance (D1=0.5) demonstrated a significant effect of L10 and L20 (p<0.05 for both), not seen in vehicle-treated rats (Figure 5.2B).

Data from two rats was excluded from the analysis; one rat failed to complete the task in two of the three sessions and another had an unexpected adverse reaction to drug treatment. Although no adverse symptoms were noted in any other rats, a lower high dose of lamotrigine (15mg/kg) was used for the full behavioural battery study.

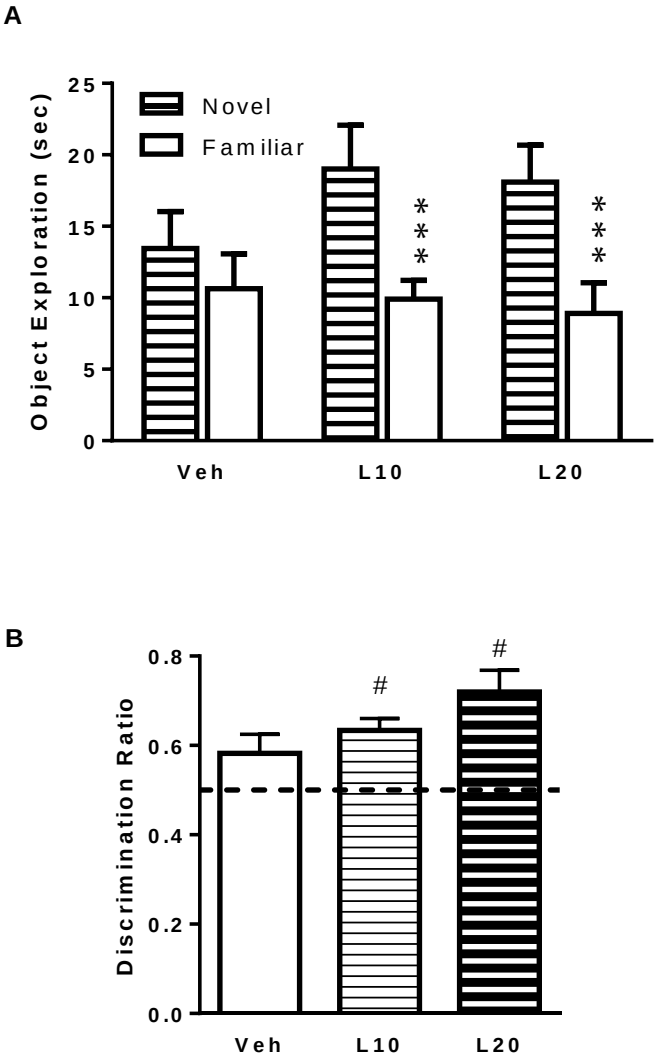

Figure 5.2 – An IP-rearing induced impairment in NOD performance was reversed by lamotrigine treatment at 10 and 20mg/kg. (A) Rats receiving vehicle (Veh) did not spend significantly longer exploring the novel than familiar object (s, mean±SEM, *n*=11) during the second choice trial of the NOD protocol by Bonferroni post-hoc following ANOVA, whilst those treated with lamotrigine at 10mg/kg (L10) or 20mg/kg (L20) did [treatment x object interaction, $F_{(2,30)}$=3.404, *p*=0.0465]. ***p<0.001 Novel vs. Familiar by Bonferroni post-hoc test following ANOVA.

(B) The D1 discrimination ratio (mean±SEM, *n*=11) during the second choice trial was not significantly increased by lamotrigine [$F_{(2,18)}$=2.885, *p*=0.0819, RM ANOVA]. L10 and L20 treated rats had a D1 ratio of significantly higher than chance, Veh did not. #p<0.05 vs. D1=0.5 ("chance") by one sample *t*-test.

5.3.2 Full Behavioural Battery Study - Effect of lamotrigine on IP-induced changes in locomotor activity

As expected, when placed in a novel arena, horizontal activity gradually decreased in all treatment groups during the LMA protocol, reflecting habituation to the mildly aversive arena, supported by a significant main effect of time by RM ANOVA [$F_{(11,352)}$=93.408, p<0.001]. Furthermore, lamotrigine pretreated rats showed decreased locomotion, reflected by a significant main effect of drug treatment [$F_{(2,31)}$=4.020, p=0.027] over the entire 60 min test period (RM ANOVA), although there was no main effect of rearing, and no rearing x lamotrigine interaction (Figure 5.3A). Post-hoc significance occurred during the 25-30 min epoch, where IP-L10 and IP-L15 rats were significantly less active than their IP-V control (p<0.01 for both), but no post-hoc significance by lamotrigine was observed in group-housed treated animals.

In contrast, two-way ANOVA analysis of total activity during the first 30 min (where activity differences were most marked) revealed a significant IP-rearing x drug interaction [$F_{(1,32)}$=6.719, p=0.014], but no main effect of either rearing condition or drug treatment alone (Figure 5.3B). This suggests that an increase in locomotor activity due to IP-rearing during in the first 30 min was reversed by lamotrigine, most notably at the highest dose, supported by Bonferroni post-hoc analysis revealing increase in locomotion in IP-V rats compared to control GV-V (p<0.05) and a significant reduction by 15mg/kg lamotrigine treatment (IP-L15) compared to IP-V (p<0.05).

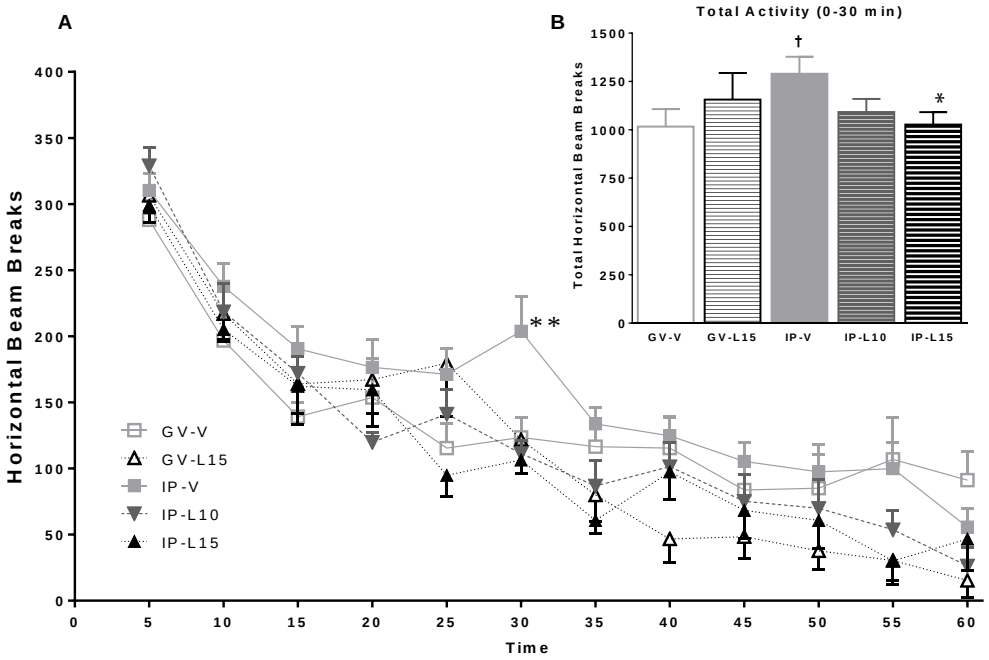

Figure 5.3 – Lamotrigine attenuated IP-rearing induced hyperlocomotion in a novel arena, without reducing horizontal activity in controls. (A) Locomotor beam breaks (mean±SEM, *n*=7-8) significantly decreased over 60 min, reflecting habituation to a novel arena [$F_{(11,352)}$=93.408, p<0.001, RM ANOVA]. Lamotrigine treatment at 10mg/kg i.p. (L10) and 15mg/kg i.p. (L15) 1 h before testing to IP-reared (IP) and control (GV) rats caused a significant alteration in activity compared to acute vehicle treatment (V, 2ml/kg) [$F_{(2,31)}$=4.020, p=0.027, RM ANOVA]. Although no main effect of IP-rearing occurred, there was a rearing x lamotrigine interaction during the first 30 minutes of the protocol [$F_{(1,32)}$=4.720, p=0.037], suggesting reversal of IP-induced hyperlocomotion by lamotrigine. **p<0.01 lamotrigine vs. rearing-matched group-housed control by Bonferroni post-hoc following ANOVA.

(B) Total locomotor counts during first 30min showed an elevation in locomotor activity in IP-rearing which was significantly attenuated by lamotrigine (15mg/kg), without any accompanying decrease in locomotion in GV-reared animals treated with the same dose [$F_{(4,33)}$=2.530, p=0.046, ANOVA]. †p<0.05 IP-rearing vs. lamotrigine-matched group-housed control, *p<0.05 lamotrigine vs. rearing-matched group-housed control by Bonferroni post hoc-test following ANOVA.

5.3.3 Effect of lamotrigine on IP-induced novel object discrimination deficits

Rats reared in social groups after perinatal vehicle injection were able to discriminate between objects during the second choice trial of NOD, such that they spent more time exploring the novel vs. familiar object (Figure 5.4A). Two-way ANOVA showed a main effect of object [$F_{(1,32)}$=64.72, p<0.0001], a significant object x treatment interaction [$F_{(4,29)}$=4.620, p=0.0045] but no main effect of drug [$F_{(4,29)}$=2.064, p=0.1081]. Bonferroni post-hoc showed GV-V and GV-L15 groups spent significantly longer exploring the novel over familiar object (p<0.001), while IP rats given vehicle (IP-V) or high dose lamotrigine (IP-L15) did not. In contrast, IP rats given lamotrigine at 10mg/kg (IP-L10) spent longer exploring the novel object (p<0.001). Thus low dose lamotrigine reversed an IP-rearing induced NOD impairment. Furthermore, two-way ANOVA analysis of the D1 ratio showed significant main effects of rearing [$F_{(1,31)}$=25.731, p<0.001] and drug treatment [$F_{(2,30)}$=9.795, p<0.001], but no interaction between the two [$F_{(1,31)}$=0.844, p=0.365]. Bonferroni post-hoc confirmed the findings, showing that D1 was significantly lower in IP-V than GV-V (p<0.01), and 10mg/kg lamotrigine (IP-L10) had a significantly higher D1 than IP-V rats (p<0.01, Figure 5.4B). Data from one animal was excluded from the analysis as it failed to perform the task, according to outlined criteria (see Chapter 2.2.3). A significant main effect of trial was observed on total object exploration [$F_{(1,33)}$=11.734, p=0.002], with a decrease in exploration shown by post-hoc analysis in IP animals treated with 15mg/kg lamotrigine (p<0.01). There was no overall main effect of rearing condition or lamotrigine treatment, with no between-factor interactions (Figure 5.4C).

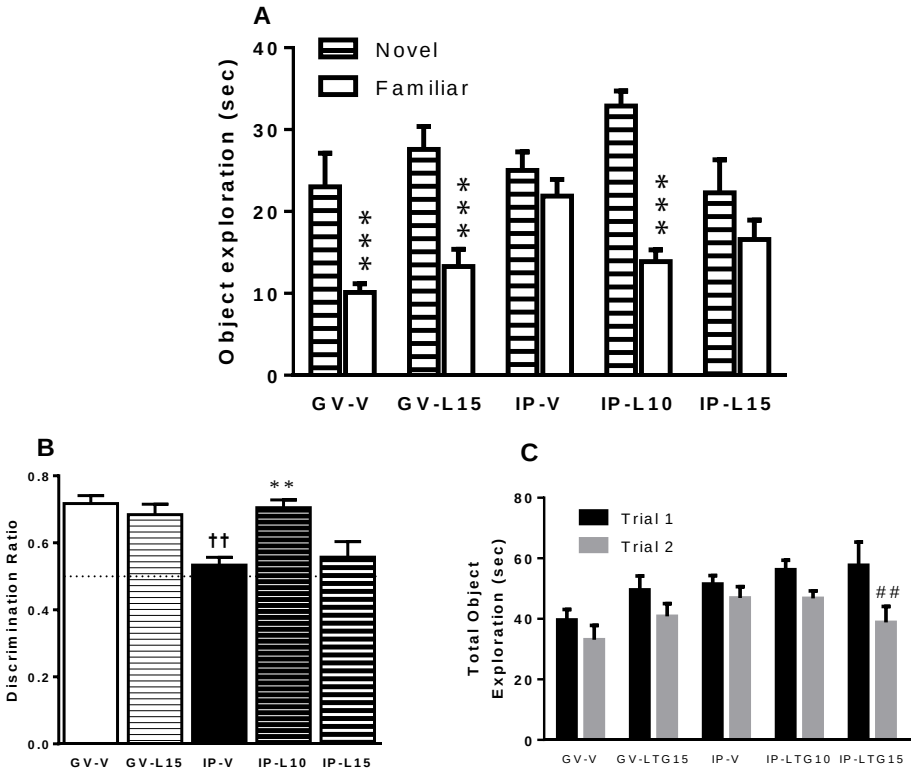

Figure 5.4 – A significant impairment in NOD discrimination due to IP-rearing is reversed by lamotrigine treatment at 10mg/kg. (A) GV-reared rats, regardless of acute treatment with 15mg/kg lamotrigine (GV-L15) or vehicle (GV-V), significantly discriminated between objects, exploring the novel over familiar (s, mean±SEM, *n*=7-8) by Bonferroni post-hoc during the second choice trial of the NOD protocol [significant object x treatment interaction by two-way ANOVA, $F_{(4,29)}$=4.620, *p*=0.0045]. IP-reared rats did not discriminate between objects when treated with vehicle (IP-V) or 15mg/kg lamotrigine (IP-L15). IP-reared rats receiving 10mg/kg lamotrigine (IP-L10) were able to discriminate between the two objects. ***p<0.001 Novel vs. Familiar by Bonferroni post-hoc test following ANOVA. (B) The D1 discrimination ratio (mean±SEM, *n*=7-8) was significantly affected by IP-rearing [$F_{(1,31)}$=25.731, p<0.001] and lamotrigine [$F_{(2,30)}$=9.795, p<0.001], with a significant impairment in IP-V rats reversed by 10mg/kg lamotrigine treatment **p<0.01 lamotrigine vs. rearing-matched vehicle-treated control, ††p<0.01 IP-rearing vs. lamotrigine-matched group-housed vehicle-treated control by Bonferroni post-hoc (C) A significant decrease in object exploration in trial 2 compared to trial 1 was confirmed by three way ANOVA [$F_{(1,33)}$=11.734, p=0.002] $^{\#\#}p$<0.01 Trial 2 vs. Trial 1 by post-hoc analysis following ANOVA

5.3.4 Effect of lamotrigine on IP-induced attenuation of prepulse inhibition of acoustic startle

As expected, inhibition of the acoustic startle progressively increased with increasing prepulse intensity in all five treatment groups. However, this was lower in IP-V rats at all prepulse intensities than in any other group such that RM ANOVA showed a significant main effect of prepulse [$F_{(2,64)}$=105.060, p<0.001] and rearing condition [$F_{(1,32)}$=4.447, p=0.043]. The effect of IP-rearing reached post-hoc significance at the 76dB prepulse level only (GV-V vs. IP-V, p<0.05). Despite a trend towards elevated PPI in the IP-L10 group compared to the IP-V, most notably at the 84dB prepulse intensity, the IP-induced impairment was not significantly reversed by lamotrigine [$F_{(2,33)}$=0.284, p=0.754], and there was no rearing x lamotrigine interaction by RM ANOVA [$F_{(1,32)}$=0.437, p=0.513] (Figure 5.5).

Two-way ANOVA analysis of the initial startle amplitude and habituation to the 120dB tone alone revealed no significant effect of either rearing condition or lamotrigine, nor any interaction between the two on either factor, confirming that the observed effects were not confounded by a non-specific effect on the startle response (data not shown).

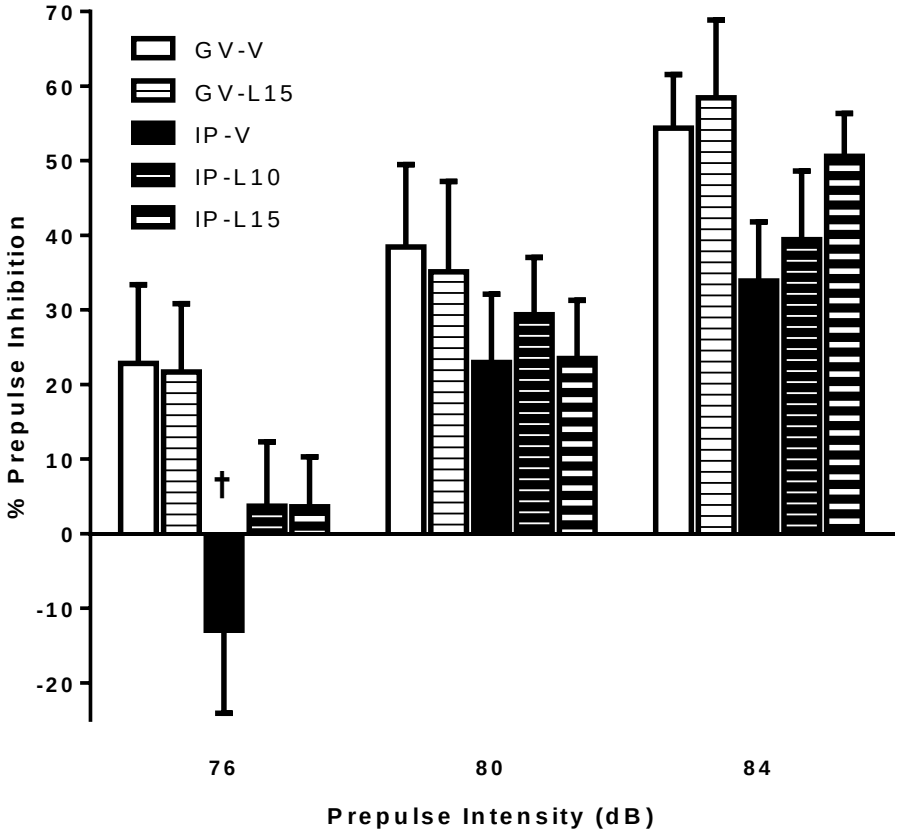

Figure 5.5 - A significant attenuation of the acoustic startle response by IP-rearing was not reversed by lamotrigine treatment. (A) IP-reared rats (IP) demonstrated a significant impairment in PPI response (mean±SEM, *n*=7-8) compared to group-housed, vehicle-treated controls (GV) following acute vehicle treatment (V) [$F_{(1,32)}$=4.447, *p*=0.043, RM ANOVA]. Impairment was not significantly reversed by lamotrigine treatment at either 10 (L10) or 15mg/kg (L15), and there were no between-factor interactions †*p*<0.05 IP-rearing vs. lamotrigine-matched group-housed control by Bonferroni post-hoc following ANOVA.

5.3.5 Effect of lamotrigine on IP-induced deficits in a conditioned emotional response

In order to assess any effect of lamotrigine on conditioned emotional learning, freezing responses were examined in a conditioning chamber. All rats froze as expected when returned to the chamber following conditioning, confirming that associative learning had occurred. Despite a trend towards decreased freezing in IP-V rats compared to GV-V, as has been seen previously, no significant decrease in freezing was observed due to IP-rearing by two-way ANOVA at 24h [$F_{(1,32)}$=3.066, p=0.090] or 48h post-conditioning [$F_{(1,32)}$=1.891, p=0.179], nor after re-presentation of the condition stimulus alone [$F_{(1,32)}$=1.173, p=0.287], and furthermore there was also no main effect due to lamotrigine at any time point, nor any IP-rearing x lamotrigine interaction observed (Figure 5.6).

Two-way ANOVA analysis of the latency to enter the CER chamber revealed no significant effect of rearing condition or lamotrigine, but there was a significant IP x lamotrigine interaction [$F_{(1,32)}$=4.629, p=0.039]. However, removal of a clear outlier in the IP-L15 group (more than 2 standard deviations from the mean of all groups) negated this interaction (data not shown).

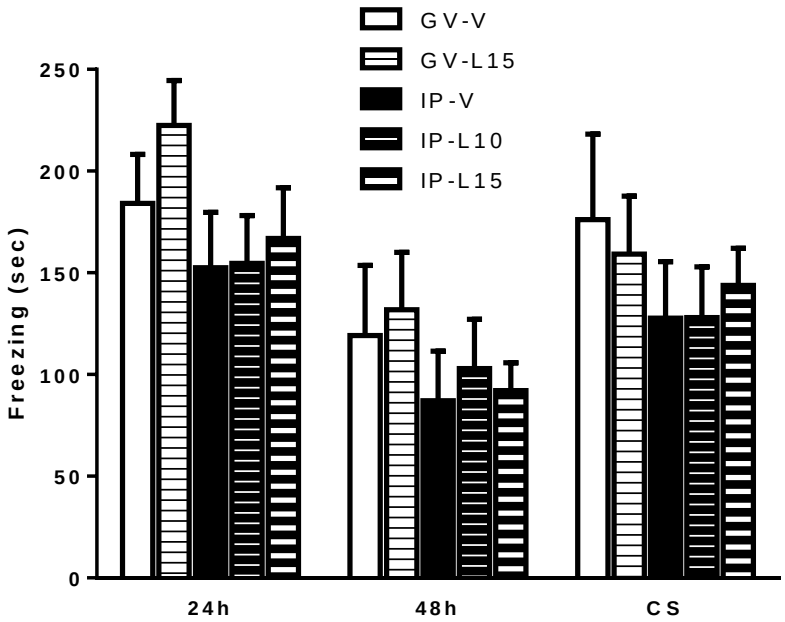

Figure 5.6 - A reduction in freezing response to contextual and conditioned fear in a CER protocol due to IP-rearing just failed to reach significance, and was not elevated by lamotrigine treatment. Freezing times (s, mean±SEM, *n*=7-8) were reduced at all time points by combined isolation rearing and perinatal PCP treatment (IP) compared to vehicle-treated group-housed animals (GV), but this failed to reach significance at any time point by two-way ANOVA. No significant increases in freezing were observed due to acute lamotrigine treatment at 10mg/kg i.p. (L10) or 15mg/kg i.p. (L15) 1h before conditioning compared to vehicle (V) at any time point, nor was an IP-rearing x lamotrigine interaction observed.

5.4 Discussion

As observed previously (see Chapter 3), the combination of isolation rearing and perinatal PCP treatment produced deficits in LMA, NOD and PPI performance, although the previously robust CER deficit was not replicated. Further issues with the reliability of this model make the effect of lamotrigine in the paradigms examined difficult to interpret. Whilst lamotrigine appeared to reverse a deficit in visual learning and memory in the NOD task and attenuated novelty-induced neophobia in the LMA protocol, it did not significantly restore the reduction in sensorimotor gating seen in PPI, nor did it increase freezing behaviour in the fear motivated associative learning paradigm. The results will now be considered in detail.

From the pilot dose-response NOD study, the potential for lamotrigine to exert a beneficial cognitive effect was evident. By using a within-subject evaluation of 10 and 20mg/kg lamotrigine, both doses were found to reinstate a preference for the novel object in the second choice trial. This suggested an improvement in IP-induced impairment in NOD, as has been previously reported by cognitive enhancing and antipsychotic drugs in isolation-reared rats by our group using this well-established method (Jones et al. 2011a; McIntosh et al. 2013; Watson et al. 2012a; Watson et al. 2012b), and in PCP-based models previously by others (Grayson et al. 2007; Hashimoto et al. 2005; McKibben et al. 2010; Nagai et al. 2009). Similarly in the FBBS study, lamotrigine reversed the neurodevelopmental deficit in NOD at 10mg/kg only. A lack of object discrimination following 15mg/kg lamotrigine may have been due to a

significant overall decrease in object exploration in trial 2. The preclinical evidence for a visual learning and memory effect of lamotrigine is very limited. Aside from the Morris Water Maze which also includes a significant spatial substrate (Celikyurt et al. 2012), no previous studies have reported an improvement in visual learning and memory after systemic lamotrigine as observed here. Furthermore, other groups focusing on the effects of antiepileptic drugs in the same or different class as lamotrigine showed that the drug did not cause a significant change in working memory performance (Shannon and Love 2004) or attention in a five-choice serial reaction time task in rats (Shannon and Love 2005), and in fact caused a significant impairment in learning as measured by a repeated acquisition of response sequences task (Shannon and Love 2007). However, there is conflicting evidence suggesting that reversal learning may be enhanced by lamotrigine (Large et al. 2011), as well as phenytoin and valproate, which like lamotrigine work through modulation of sodium ion channels (Idris et al. 2009). There are even fewer publications examining the effect of lamotrigine on cognition in the clinic, but currently there is little evidence to suggest any beneficial effect in limited patient groups (Eun et al. 2012; Ijff and Aldenkamp 2013; Vayisoglu et al. 2013). These studies combined suggest that the finding of a benefit of lamotrigine here in a specifically visual memory task is highly novel, and may reflect the problem of false-positive effects of compounds in PCP-based rodent models of schizophrenia (Chapter 1.3.3) and in novel object recognition-based tasks (Lyon et al. 2012).

Somewhat more evidence exists clinically in the reversal of positive symptoms of schizophrenia. Studies, including a large meta-analysis (Goff 2009; Tiihonen et al. 2009), have indicated the potential benefit of lamotrigine to treat psychosis and schizoaffective disorder (Erfurth et al. 1998), particularly in clozapine-resistant patients. It is important to balance these previously published results, however, with other studies that have found little or no supportive evidence for the use of lamotrigine in schizophrenia when reviewing the available literature (Reid et al. 2013; Sommer et al. 2012). In this study, lamotrigine significantly reduced the locomotor response elicited by placement in a novel arena selectively in IP rats but not in GH littermates. Importantly, this selectivity of action suggests that the suppression of hyperactivity seen was not due to sedation as observed with risperidone. Several previous studies have examined the effects of acute and perinatal lamotrigine on locomotor activity in adult rodents in various paradigms (Bourin et al. 2005; Forcelli et al. 2012; Kaur and Starr 1996; Quarta and Large 2010), but of particular interest, one study noted the effect of lamotrigine on acute PCP-induced hyperlocomotion in rats in combination with clozapine, which has direct relevance to its potential use in the clinic as an adjunct therapy alongside an antipsychotic (Williams et al. 2006). They noted that although lamotrigine enhanced acute PCP-induced hyperlocomotion when administered without clozapine, combined administration caused a highly significant decrease in locomotion in acute PCP treated rats, even below that of vehicle treated controls. This is consistent with some clinical data showing the potential benefit of combined clozapine and lamotrigine treatment (Tiihonen et

al. 2009). Mechanistically, lamotrigine has been shown to reduce excitability in striatal neurons *in vitro* (Calabresi et al. 1999) by preventing the release of neurotransmitters through pre-synaptic blockade of voltage-gated sodium channels (Leach et al. 1991). This activity could prevent the excessive levels of striatal dopamine release that have been correlated with hyperlocomotion in rodents (Paulson and Robinson 1995) and psychoses in schizophrenia patients (Abi-Dargham et al. 1998; Abi-Dargham et al. 2000; Laruelle et al. 1996), but without supporting evidence this is purely speculative.

Unlike each of the previous cohorts in this thesis, IP rats did not freeze significantly less than their control counterparts in the CER paradigm, indicating that the desired impairment in contextual and cue conditioned fear memory was not induced. This was despite the mean freezing time for each IP group (V, L10 and L15) being lower than both GV groups (V, L15) at each of the three examined time points. The lack of a significant IP-induced impairment makes it difficult to interpret the effect of lamotrigine in this paradigm, but there was no notable increase in freezing time in the IP-L15 group. Interestingly, some limited clinical evidence does suggest lamotrigine may affect conditioned emotional responses in humans (Haldane et al. 2008; Jogia et al. 2008). Despite a lack of significant behavioural effect, lamotrigine in these studies reduced elevated activity levels in the temporal regions around the left hippocampus/parahippocampus in patients with bipolar disorder. Furthermore, lamotrigine produced increased neural responses in regions of the prefrontal cortex involved in the regulation of emotion through innervation of

limbic regions, including the amygdala-hippocampus complex. The authors suggested that through these effects lamotrigine could potentially exert a 'normalising' effect on mood and emotional processing, which may be translatable from bipolar disorder to schizophrenia. Results presented herein fail to support or oppose those previously published, due to the unreliability of the model.

Positive effects of lamotrigine have been observed previously in preclinical PPI studies, with an impairment in PPI in mGluR1 knock-out mice reversed by 27mg/kg lamotrigine (Brody et al. 2003a). Another group demonstrated that 30mg/kg i.p. blocked the induction of PPI deficits by repeated methamphetamine (2.5mg/kg s.c.), and reversed methamphetamine-induced PPI deficits when given acutely (Nakato et al. 2010). Recent evidence also shows that unlike other antiepileptic drugs, lamotrigine does not induce sensorimotor gating deficits when administered on PNDs 7-13 to rats (20mg/kg i.p.) (Forcelli et al. 2012). The current results however do not replicate these reports since the IP-deficit in PPI was not elevated by lamotrigine. Nonetheless, not all groups have demonstrated an effect of lamotrigine on PPI. One group noted that oral administration was unable to reverse ketamine-induced PPI deficits (Cilia et al. 2007), and another showed it was unable to reverse either mGluR5 KO (Brody et al. 2004), or amphetamine induced deficits (Brody et al. 2003b), more closely resembling this data which shows no effect of lamotrigine on PPI in this dual-hit model.

5.4.1 Conclusion

The administration of lamotrigine to IP rats at 10 and 15mg/kg is somewhat effective at reversing specific behavioural deficits in IP-dual hit rats, and this may have some relevance to schizophrenia. However, the most notable effect of lamotrigine in this study was to reverse an NOD impairment known to be susceptible to false-positive reversals. Furthermore, lamotrigine has previously been shown to be ineffective at improving cognition in domains similar to those examined in NOD. Together, this suggests that rather than lamotrigine having therapeutic potential in treating the cognitive deficits of schizophrenia, in fact the IP dual-hit model is susceptible to producing false-positive drug reversals, again undermining the predictive validity of this model. In paradigms where a significant effect of lamotrigine was not seen (PPI and CER), questions still remain about the reproducibility of the IP model, making firm conclusions about the drug's efficacy in these domains difficult.

Chapter 6

General Discussion

6.1 Aims

The treatment of schizophrenia in the clinic is hindered by inadequate medication. Whilst "positive" symptoms of the disorder are relatively well understood and treated with available medications, symptoms in the "negative" and "cognitive" domains are not. Improved preclinical models of schizophrenia would aid research into its aetiology and lead to better therapies for antipsychotic-resistant patients.

Although current preclinical models of schizophrenia each have individual strengths, all also have weaknesses in procedure or validity. The main aim of this thesis was to produce, evaluate and validate a novel, "dual-hit" model of schizophrenia-like symptoms in the rat. Combining pre-existing preclinical models was intended to produce a phenotype that encompassed the relative strengths of each model whilst eliminating the weaknesses. The hypothesis was that such a combination model would be highly replicable and demonstrate increased robustness of behavioural and neurobiological changes salient to schizophrenia. Through this improved model, disease understanding and the evaluation of novel therapies could be improved.

6.2 Findings & Implications

Isolation rearing was selected as the foundation for this thesis, based on it being well-characterised and validated within the group. As expected, isolation rearing produced somewhat consistent changes in behaviour throughout the

experiments conducted, however it also demonstrated a lack of robustness, with individual deficits in LMA and PPI for example not occurring in every cohort. These findings demonstrated one of the major motivations behind developing a dual-hit model. Two challenges were selected for addition to the isolation rearing model, prenatal MAM and perinatal PCP treatment.

The addition of prenatal MAM on GD17 to the isolation rearing model (Chapter 2) was fraught with difficulties. Accurate identification of gestational day is vital to ensure correct administration of MAM, but the standard method for confirming conception, locating a vaginal plug in bedding material, is easily missed and inaccurate. Whilst this procedural challenge can be overcome by careful, continuous observation, the phenotype produced by the dual-hit treatment was largely inconsistent (Table 6.1). Despite isolation rearing-induced deficits in novel object discrimination, conditioned emotional response and prepulse inhibition paradigms, with a possible additional effect of isolation + MAM observed in one cohort, MAM was only successful in producing decreased retention of a visuo-spatial learning task in the Morris Water Maze. This shortage of effects was despite marked and replicated hippocampal and striatal pathology due to MAM treatment alone, suggesting that drug degradation or incorrect administration was not to blame.

Lister-Hooded rats were selected for this study based on their strong record of isolation rearing deficits, however with no literature published on the effect of prenatal MAM in this strain, it was concluded that different strain-sensitivity to MAM may be responsible for the behavioural results seen. Although the "isolation syndrome" was not fully induced (no locomotor hyperactivity was

Table 6.1 – Table showing the behavioural and neurobiological outcomes of combined isolation rearing-prenatal MAM treatment and isolation rearing-perinatal PCP treatment, as well as subsequent acute treatment with risperidone and lamotrigine

	Locomotor Activity	Novel Object Discrimination	Prepulse Inhibition of Acoustic Startle	Conditioned Emotional Response	Morris Water Maze	Neurobiology
Chapter 2 Isolation + MAM	No hyperlocomotion due to Iso or MAM	Iso-induced deficit, no effect of MAM	Iso-induced deficit, possible synergistic effect of MAM addition in one cohort	Robust Iso-induced deficit, possible improvement by MAM	Inconsistent retention deficit due to MAM, no effect on learning/reversal	MAM-induced decreases in hippocampal volume/mass No conclusive monoamine changes
Chapter 3 Isolation + Perinatal PCP	Iso-induced hyperlocomotion PCP-induced senzitization to acute PCP treatment	Robust Iso-induced deficit, no effect of PCP addition	Iso and PCP induced deficits, additive effect is one cohort only	Iso and PCP induced deficits in both cohorts	Inconsistent reversal learning deficits due to PCP, unreplicated change in learning profile by PCP+Iso	X
Chapter 4 Risperidone	High dose: sedation Low dose: reduced activity in IP-reared and control rats	No reversal of IP-induced deficits	High dose: sedation Low dose: Increased PPI in IP rats, although inconsistent deficits	High dose: sedation Low dose: Increased freezing in IP-reared rats	High dose: sedation Low dose: No notable effect, with no consistent deficit	X
Chapter 5 Lamotrigine	Selectively reversed IP-induced hyperlocomotion	Reversed IP-induced deficit	No reversal of IP-induced deficit	IP-induced changes lost, no effect of lamotrigine	X	X

observed in either cohort), the addition of MAM did not assist in ensuring a full profile of behavioural deficits occurred. On this basis the protocol failed to meet the aims of this thesis, and was discontinued.

In some ways the range and robustness of behavioural deficits induced by addition of perinatal PCP treatment to the isolation rearing procedure was increased, but not to such an extent to fully meet the aims of this thesis (Table 6.1). Hyperlocomotion in a novel arena was observed in only three of five cohorts due to isolation rearing, whilst PPI was only significantly impaired following dual-hit treatment in two cohorts. The variability in responses between cohorts was greater than expected, and it was hypothesised that combination treatment would eliminate this variability. Previous analyses have shown that the isolation rearing procedure does not produce a complete profile of behavioural deficits in all cohorts, even within the same group following an identical, repeated protocol (Fone and Porkess 2008). Despite stringent measures being put in place to ensure the isolation procedure was as identical as possible between cohorts here, there is a possibility that routine handling during cage changes may have negated the full induction of the isolation syndrome (Gentsch et al. 1988; Krebs-Thomson et al. 2001), or that the noise levels in the holding rooms may have been inconsistent, preventing identical auditory contact between isolates in individual cohorts. One suggested improvement to the isolation procedure could be to house the animals in individually ventilated cages or "IVCs". This system of housing, where each cage is connected to a continually changing air supply, would not only continue to prevent social contact between isolates, but would also limit

olfactory contact, and potentially auditory, depending on the background noise created by the ventilation system. These additional sensory isolations may, as has been seen previously with more austere housing (Greco et al. 1989; Holson et al. 1991), produce a more severe phenotype or one that is more robust than observed here. The addition of perinatal PCP to the protocol was not effective at eliminating the between-cohort differences in behaviour in these paradigms, and as such did not meet the thesis aim. Robust novel object discrimination and conditioned emotional response deficits were, however, seen due to isolation rearing with possible additional effects of adding perinatal PCP, but the magnitude of isolation rearing-induced deficits in these paradigms may have prevented additive effects of dual-hit treatment due to ceiling effects.

The translational relevance of the model to schizophrenia is also limited due to the fact that two of the least reliable paradigms assessed, locomotor activity and prepulse inhibition of acoustic startle, are seen as being some of the most relevant in modelling schizophrenia preclinically by the MATRICS initiative (Young et al. 2009). By contrast, the novel object discrimination task has the potential for false-positive compound reversals, and is less translatable to the human condition of schizophrenia (Lyon et al. 2012). Furthermore, the conditioned emotional response was not included in the MATRICS initiative, suggesting relevance to schizophrenia is limited, despite requiring brain regions potentially key in disease pathology (Maren 2001; Young et al. 2009).

Despite these limitations, the susceptibility of the IP-rearing model to pharmacological challenge was assessed with acute treatment of risperidone (Chapter 4) and lamotrigine (Chapter 5) immediately prior to behavioural

testing. Whilst 0.5mg/kg risperidone had a clear sedative effect, lower doses restored PPI responses and increased freezing behaviour in the CER paradigm in IP-reared rats specifically, as well as reducing locomotor activity across all treatment groups. Conversely, lamotrigine restored normal performance in the novel object discrimination paradigm and attenuated IP-induced hyperlocomotion, while having no beneficial effects in PPI or CER paradigms (Table 6.1). However, evidence for the efficacy of these drugs in treating particularly cognitive deficits in schizophrenia patients is mixed, with a number of recent studies suggesting that lamotrigine may have little future use in the disease (see Chapter 5.4). The ability for lamotrigine to reverse novel object deficits may therefore be indicative of the false positive drug reversal effects that are known to exist with NOD and paradigms and PCP-based models of schizophrenia alike. It is somewhat difficult to fully interpret the results showing that risperidone is able to restore PPI responses and increase CER freezing at low doses based on the unreliable nature of the IP deficits.

Compared to other, previously published dual-hit approaches, the isolation-PCP protocol has a number of relative strengths, although these may not have been strongly supported in this thesis. Isolation rearing has previously been combined with other NMDA receptor antagonists, most notably MK-801, with highly variable results (Ashby et al. 2010; Gilabert-Juan et al. 2013; Hawken et al. 2013; Hickey et al. 2012; Lim et al. 2012). As well as differing results due to isolation rearing alone, as has been seen here, most papers failed to note marked additive effects of the two hits combined. Lim et al were able to demonstrate that NMDA receptor antagonism in the early post-natal period

could produce PPI and NOD deficits in isolation reared animals that were more robust than where saline was administered instead. This paper is the closest published work to that presented in this thesis, as no other groups have yet attempted the isolation-PCP combination approach, and somewhat supports the decision to use perinatal PCP to augment the isolation rearing protocol.

Other strong lines of study using dual-hit protocols have focused on models utilising genetic and/or maternal immune activation to induce schizophrenia-like deficits (Abazyan et al. 2010; Deslauriers et al. 2013; Giovanoli et al. 2013; Lipina et al. 2013; Vuillermot et al. 2012), with some combining these challenges with developmental models such as isolation rearing (Ishihama et al. 2010; Jiang et al. 2013; Niwa et al. 2013). As discussed previously (Chapter 1.3), perturbations that hold a strong genetic link to schizophrenia or are associated with maternal infection have a high level of construct validity to schizophrenia (Sullivan 2005). This validity gains additional strength when combined with a developmental disruption model such as isolation rearing with a post-pubertal onset of phenotype. Hence, these models may have stronger validity than the Iso-PCP model presented here. However, no genetic x isolation dual-hit models have demonstrated cognitive deficits to date, and there is no published data on the effect of combining maternal immune activation with isolation rearing. Here, the isolation-PCP model displays a relative strength, with cognitive deficits observed reproducibly in the NOD paradigm. Despite this, and the face validity of NMDA receptor antagonism and glutamate hypofunction as a model of schizophrenia, the findings of this

thesis suggest that other dual-hit approaches are likely to yield more productive results than the Iso-PCP model presented herein.

6.3 Future Studies

The key long-term aim of this thesis was to produce a model that would enable better understanding of the aetiology of schizophrenia, as well as aid in the discovery of novel therapies. The proposed future studies below work towards these two goals by looking to improve the Iso-PCP model and better understand the neurobiology behind the behavioural deficits induced by both model hits.

It is clear that further work is required to make the Iso-PCP model a viable tool for use in future schizophrenia research. One approach could be to improve the constituent parts of the model individually, such that a combination of the two will automatically be strengthened. As mentioned above, more austere housing conditions could be used to induce a more severe isolation syndrome, and has been previously used to induce elevated basal corticosterone (Greco et al. 1989; Holson et al. 1991). The use of individually ventilated cages that limit the auditory and olfactory contact between isolates, could be compared to the housing conditions utilised here to evaluate whether enduring increases in corticosterone are coupled with more severe, robust or reproducible changes in behavioural relevant to schizophrenia. Additionally, routine handling to change cage bedding material could be reduced further to ensure isolates have as little physical contact as possible.

The administration of PCP perinatally could also be potentially improved. Other groups have utilised PCP administration on days 2, 6, 9 and 12 to induce oxidative stress in the frontal cortex and hippocampus through reduced expression and activity of antioxidant enzymes such as superoxide dismutase (Radonjic et al. 2010). Mounting evidence suggests that mitochondrial dysfunction leading to redox dysregulation may be a primary mechanism for neuronal damage in schizophrenia (Ballesteros et al. 2013; Do et al. 2009; Gubert et al. 2013; Wang et al. 2009), and markers of this pathology has also been observed following isolation rearing (Moller et al. 2011; Moller et al. 2013). Hence, using this alternative and early-starting PCP treatment regime may represent an improvement over the one utilised here, and could feasibly combine with isolation rearing in later life to produce marked dysregulation of antioxidant activity and hence neuropathology. Furthermore, improved cognitive performance has been seen with clozapine+NAC treatment in isolates (Moller et al. 2013), showing that a model based on oxidative stress may be susceptible to co-treatment with an anti-oxidant and antipsychotic.

To assess the impact of the isolation-PCP dual-hit treatment on neurobiology, *ex vivo* tissue could be examined for levels of parvalbumin-positive GABAergic interneurons by immunohistochemistry or microarray analysis. Aberrant PV-positive GABAergic interneuron function is now believed to be heavily implicated in schizophrenia (Chapter 1.1.2), with both isolation-reared and PCP-treated rats previously shown to have decreased levels of parvalbumin in the hippocampus and frontal cortex (Harte et al. 2007; Kaalund et al. 2013; McKibben et al. 2010; Wang et al. 2008). Preliminary data not

presented here has suggests that the Pvalb gene for parvalbumin may be down-regulated in the hippocampi of Iso-PCP treated rats, as well as the GAD1 gene encoding glutamate decarboxylase, responsible for the majority of neuronal GABA production. Confirmation of this down-regulation would add substantial weight to the face validity of the Iso-PCP model. Additionally, some evidence suggests that impaired antioxidant defence mechanisms are responsible for PV-positive interneuron pathology in isolation-reared animals (Jiang et al. 2013). Hence, further work to analyse the mechanisms by which parvalbumin-positive interneurons are lost in Iso-PCP rats could also contribute knowledge to the oxidative stress theory of the schizophrenia pathology.

6.4 Conclusions

This thesis sought to produce and characterise a new dual-hit model of schizophrenia-like symptoms, however the results obtained do not support the aim of creating a robust and reproducible model with significant benefits over the individual hits alone.

An initial protocol combining rearing in social isolation with prenatal MAM treatment did not produce robust behavioural deficits, despite marked neuropathology in relevant subcortical structures. The addition of perinatal PCP treatment to the isolation rearing model resulted in somewhat improved behavioural changes relevant to the positive and cognitive symptom domains of schizophrenia, although the reliability of the model is questioned due to

inconsistencies in the deficits observed. Furthermore, the behaviours in which robust deficits were seen, such as NOD and CER, are less translationally relevant to schizophrenia than other used and seen to be less reliable, such as LMA and PPI. Challenge with risperidone and lamotrigine was able to reverse some of the behavioural deficits observed in dual-hit animals, but the variability in behavioural data and the potential for false-positive reversal emerging from the NOD paradigm make interpretation of these results difficult. Overall, the model presented here shows some interesting traits but requires greater refinement before it will be a useful tool for on-going schizophrenia research.

Appendix

Perinatal PCP administration procedure

Great care was taken to prepare for perinatal PCP treatment, as the very early time point of the interference could lead to issues of maternal rejection. Pilot studies carried out within our lab (data not published) demonstrated that the administration of 10mg/kg PCP at this early stage of development produced visible effects on the pups, causing writhing, arching of the back, and stereotypic limb movement. PCP-treated pups commonly become separated from the nest. It is somewhat unclear whether the uncontrolled movement of PCP-treated pups causes them to make their way out of the nest, or whether dams choose to remove them from the nest due to their excessive movement (personal observation suggests the former). This departure from safety has a very direct impact on the pups, as they lose access to feeding from their mother and are exposed to the ambient temperature, which whilst comfortable for pups above approximately 13 days old (a point by which a significant amount of hair growth has occurred), is too low for the survival of younger pups. The first specific protocol consideration was to ensure that the pups obtained from Charles River UK for the study were from natural (non-cross-fostered) litters, limited to $n \leq 10$, with an experienced dam. This natural and manageably-sized litter ensured the greatest chance of maternal protection. The natural instinct of the dam is to protect her pups, and as was seen in this study, will briefly leave the nest to recover separated pups from the surrounding cage. This instinct may be reduced in a number of circumstances, and we hypothesised that non-natural pups introduced to the litter to make up numbers, or an inexperienced

dam, may not produce the strength of maternal instinct required. Further to this, the experimenter administering PCP (or saline) was required to thoroughly wash their hands between litters, as well as rubbing them in a small amount of home cage bedding material in between the handling of each pup. Both measures were intended to avoid transferring scents between each litter, or introducing external scents to pups, either of which may cause rejection of the pups or infanticide. This approach was successful in this study, as whilst pups were commonly noted to be out of the nest during hourly checks, in many cases closer inspection triggered the dam to recover the separated pups. Where pups were left exposed without recovery by the dam, the pups were manually returned to the nest by the experimenter. By following these self-imposed guidelines, only one pup in seven cohorts treated with perinatal PCP was lost prior to weaning.

References

Abazyan B, Nomura J, Kannan G, Ishizuka K, Tamashiro KL, Nucifora F, Pogorelov V, Ladenheim B, Yang C, Krasnova IN, Cadet JL, Pardo C, Mori S, Kamiya A, Vogel MW, Sawa A, Ross CA, Pletnikov MV (2010) Prenatal interaction of mutant DISC1 and immune activation produces adult psychopathology. Biol Psychiatry 68:1172-81.

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.

Abekawa T, Honda M, Ito K, Inoue T, Koyama T (2002) Effect of MS-153 on the development of behavioral sensitization to locomotion- and ataxia-inducing effects of phencyclidine. Psychopharmacology (Berl) 160:122-31.

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. Am J Psychiatry 155:761-7.

Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, Hwang DR, Keilp J, Kochan L, Van Heertum R, Gorman JM, Laruelle M (2002) Prefrontal dopamine D1 receptors and working memory in schizophrenia. J Neurosci 22:3708-19.

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. Proc Natl Acad Sci U S A 97:8104-9.

Abi-Dargham A, Xu X, Thompson JL, Gil R, Kegeles LS, Urban N, Narendran R, Hwang DR, Laruelle M, Slifstein M (2012) Increased prefrontal cortical D(1) receptors in drug naive patients with schizophrenia: a PET study with [(1)(1)C]NNC112. J Psychopharmacol 26:794-805.

Adams W, van den Buuse M (2011) Hippocampal serotonin depletion facilitates the enhancement of prepulse inhibition by risperidone: possible role of 5-HT(2C) receptors in the dorsal hippocampus. Neuropharmacology 61:458-67.

Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE, Jr., Jones EG (1995) Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. Arch Gen Psychiatry 52:258-66.

Akil M, Pierri JN, Whitehead RE, Edgar CL, Mohila C, Sampson AR, Lewis DA (1999) Lamina-specific alterations in the dopamine innervation of the prefrontal cortex in schizophrenic subjects. Am J Psychiatry 156:1580-9.

Akkerman S, Blokland A, Reneerkens O, van Goethem NP, Bollen E, Gijselaers HJ, Lieben CK, Steinbusch HW, Prickaerts J (2012) Object recognition testing: methodological considerations on exploration and discrimination measures. Behav Brain Res 232:335-47.

Anagnostaras SG, Maren S, Sage JR, Goodrich S, Fanselow MS (1999) Scopolamine and Pavlovian fear conditioning in rats: dose-effect analysis. Neuropsychopharmacology 21:731-44.

Anastasio NC, Johnson KM (2008a) Atypical anti-schizophrenic drugs prevent changes in cortical N-methyl-D-aspartate receptors and behavior following sub-chronic phencyclidine administration in developing rat pups. Pharmacol Biochem Behav 90:569-77.

Anastasio NC, Johnson KM (2008b) Differential regulation of the NMDA receptor by acute and sub-chronic phencyclidine administration in the developing rat. J Neurochem 104:1210-8.

Andersen JD, Pouzet B (2004) Spatial memory deficits induced by perinatal treatment of rats with PCP and reversal effect of D-serine. Neuropsychopharmacology 29:1080-90.

Andreasson S, Allebeck P, Engstrom A, Rydberg U (1987) Cannabis and schizophrenia. A longitudinal study of Swedish conscripts. Lancet 2:1483-6.

Arseneault L, Cannon M, Witton J, Murray RM (2004) Causal association between cannabis and psychosis: examination of the evidence. Br J Psychiatry 184:110-7.

Ashby DM, Habib D, Dringenberg HC, Reynolds JN, Beninger RJ (2010) Subchronic MK-801 treatment and post-weaning social isolation in rats: Differential effects on locomotor activity and hippocampal long-term potentiation. Behav Brain Res 212:64-70.

Athos J, Impey S, Pineda VV, Chen X, Storm DR (2002) Hippocampal CRE-mediated gene expression is required for contextual memory formation. Nat Neurosci 5:1119-20.

Atkins CM, Selcher JC, Petraitis JJ, Trzaskos JM, Sweatt JD (1998) The MAPK cascade is required for mammalian associative learning. Nat Neurosci 1:602-9.

Bakshi VP, Geyer MA (1999) Ontogeny of isolation rearing-induced deficits in sensorimotor gating in rats. Physiol Behav 67:385-92.

Bakshi VP, Swerdlow NR, Geyer MA (1994) Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. J Pharmacol Exp Ther 271:787-94.

Balduini W, Lombardelli G, Peruzzi G, Cattabeni F (1991) Treatment with methylazoxymethanol at different gestational days: physical, reflex development and spontaneous activity in the offspring. Neurotoxicology 12:179-88.

Ballesteros A, Summerfelt A, Du X, Jiang P, Chiappelli J, Tagamets M, O'Donnell P, Kochunov P, Hong LE (2013) Electrophysiological intermediate biomarkers for oxidative stress in schizophrenia. Clin Neurophysiol Epub ahead of print.

Banfi S, Dorigotti L, Abbracchio MP, Balduini W, Coen E, Ragusa C, Cattabeni F (1984) Methylazoxymethanol microencephaly in rats: neurochemical characterization and behavioral studies with the nootropic oxiracetam. Pharmacol Res Commun 16:67-83.

Bast T, Feldon J (2003) Hippocampal modulation of sensorimotor processes. Prog Neurobiol 70:319-45.

Bast T, Wilson IA, Witter MP, Morris RG (2009) From rapid place learning to behavioral performance: a key role for the intermediate hippocampus. PLoS Biol 7:e1000089.

Bayer SA (1980) Quantitative 3H-thymidine radiographic analyses of neurogenesis in the rat amygdala. J Comp Neurol 194:845-75.

Bayer SA, Altman, J. (1995) Neurogenesis and neuronal migration. In: Paxinos G (ed) The Rat Nervous System. Academic Press, San Diego, pp 1041-78

Beasley CL, Reynolds GP (1997) Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. Schizophr Res 24:349-55.

Beasley CL, Zhang ZJ, Patten I, Reynolds GP (2002) Selective deficits in prefrontal cortical GABAergic neurons in schizophrenia defined by the presence of calcium-binding proteins. Biol Psychiatry 52:708-15.

Behere RV, Venkatasubramanian G, Arasappa R, Reddy N, Gangadhar BN (2009) Effect of risperidone on emotion recognition deficits in antipsychotic-naive schizophrenia: a short-term follow-up study. Schizophr Res 113:72-6.

Benes FM, Kwok EW, Vincent SL, Todtenkopf MS (1998) A reduction of nonpyramidal cells in sector CA2 of schizophrenics and manic depressives. Biol Psychiatry 44:88-97.

Benes FM, Lim B, Matzilevich D, Walsh JP, Subburaju S, Minns M (2007) Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars. Proc Natl Acad Sci U S A 104:10164-9.

Beninger RJ, Beuk J, Banasikowski TJ, van Adel M, Boivin GA, Reynolds JN (2010) Subchronic phencyclidine in rats: alterations in locomotor activity, maze performance, and GABA(A) receptor binding. Behav Pharmacol 21:1-10.

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. Eur J Neurosci 24:2894-902.

Bilder RM, Goldman RS, Volavka J, Czobor P, Hoptman M, Sheitman B, Lindenmayer JP, Citrome L, McEvoy J, Kunz M, Chakos M, Cooper TB, Horowitz TL, Lieberman JA (2002) Neurocognitive effects of clozapine, olanzapine, risperidone, and haloperidol in patients with chronic schizophrenia or schizoaffective disorder. Am J Psychiatry 159:1018-28.

Blaha CD, Yang CR, Floresco SB, Barr AM, Phillips AG (1997) Stimulation of the ventral subiculum of the hippocampus evokes glutamate receptor-mediated changes in dopamine efflux in the rat nucleus accumbens. Eur J Neurosci 9:902-11.

Bloomfield C, French SJ, Jones DN, Reavill C, Southam E, Cilia J, Totterdell S (2008) Chandelier cartridges in the prefrontal cortex are reduced in isolation reared rats. Synapse 62:628-31.

Boctor SY, Ferguson SA (2010) Altered adult locomotor activity in rats from phencyclidine treatment on postnatal days 7, 9 and 11, but not repeated ketamine treatment on postnatal day 7. Neurotoxicology 31:42-54.

Bogerts B, Meertz E, Schonfeldt-Bausch R (1985) Basal ganglia and limbic system pathology in schizophrenia. A morphometric study of brain volume and shrinkage. Arch Gen Psychiatry 42:784-91.

Bolton MM, Heaney CF, Sabbagh JJ, Murtishaw AS, Magcalas CM, Kinney JW (2012) Deficits in emotional learning and memory in an animal model of schizophrenia. Behav Brain Res 233:35-44.

Bor D, Duncan J, Lee AC, Parr A, Owen AM (2006) Frontal lobe involvement in spatial span: converging studies of normal and impaired function. Neuropsychologia 44:229-37.

Bor D, Duncan J, Owen AM (2001) The role of spatial configuration in tests of working memory explored with functional neuroimaging. Scand J Psychol 42:217-24.

Bossong MG, Niesink RJ (2010) Adolescent brain maturation, the endogenous cannabinoid system and the neurobiology of cannabis-induced schizophrenia. Prog Neurobiol 92:370-85.

Bourin M, Masse F, Hascoet M (2005) Evidence for the activity of lamotrigine at 5-HT(1A) receptors in the mouse forced swimming test. J Psychiatry Neurosci 30:275-82.

Braff DL, Geyer MA (1990) Sensorimotor gating and schizophrenia. Human and animal model studies. Arch Gen Psychiatry 47:181-8.

Braff DL, Geyer MA, Swerdlow NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl) 156:234-58.

Braun I, Genius J, Grunze H, Bender A, Moller HJ, Rujescu D (2007) Alterations of hippocampal and prefrontal GABAergic interneurons in an animal model of psychosis induced by NMDA receptor antagonism. Schizophr Res 97:254-63.

Brauner JM, Hessler S, Groemer TW, Alzheimer C, Huth T (2014) Risperidone inhibits voltagqe-gated sodium channels. Eur J Pharmacol.

Breier A, Su TP, Saunders R, Carson RE, Kolachana BS, de Bartolomeis A, Weinberger DR, Weisenfeld N, Malhotra AK, Eckelman WC, Pickar D (1997) Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. Proc Natl Acad Sci U S A 94:2569-74.

Brody SA, Conquet F, Geyer MA (2003a) Disruption of prepulse inhibition in mice lacking mGluR1. Eur J Neurosci 18:3361-6.

Brody SA, Conquet F, Geyer MA (2004) Effect of antipsychotic treatment on the prepulse inhibition deficit of mGluR5 knockout mice. Psychopharmacology (Berl) 172:187-95.

Brody SA, Geyer MA, Large CH (2003b) Lamotrigine prevents ketamine but not amphetamine-induced deficits in prepulse inhibition in mice. Psychopharmacology (Berl) 169:240-6.

Broersen LM, Uylings HB (1999) Visual attention task performance in Wistar and Lister hooded rats: response inhibition deficits after medial prefrontal cortex lesions. Neuroscience 94:47-57.

Brown AS (2006) Prenatal infection as a risk factor for schizophrenia. Schizophr Bull 32:200-2.

Brown AS, Derkits EJ (2010) Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am J Psychiatry 167:261-80.

Brown AS, Schaefer CA, Quesenberry CP, Jr., Liu L, Babulas VP, Susser ES (2005) Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. Am J Psychiatry 162:767-73.

Brown RW, Maple AM, Perna MK, Sheppard AB, Cope ZA, Kostrzewa RM (2012) Schizophrenia and substance abuse comorbidity: nicotine addiction and the neonatal quinpirole model. Dev Neurosci 34:140-51.

Buchanan RW, Conley RR, Dickinson D, Ball MP, Feldman S, Gold JM, McMahon RP (2008) Galantamine for the treatment of cognitive impairments in people with schizophrenia. Am J Psychiatry 165:82-9.

Buka SL, Cannon TD, Torrey EF, Yolken RH (2008) Maternal exposure to herpes simplex virus and risk of psychosis among adult offspring. Biol Psychiatry 63:809-15.

Bullock WM, Bolognani F, Botta P, Valenzuela CF, Perrone-Bizzozero NI (2009) Schizophrenia-like GABAergic gene expression deficits in cerebellar Golgi cells from rats chronically exposed to low-dose phencyclidine. Neurochem Int 55:775-82.

Bullock WM, Cardon K, Bustillo J, Roberts RC, Perrone-Bizzozero NI (2008) Altered expression of genes involved in GABAergic transmission and neuromodulation of granule cell activity in the cerebellum of schizophrenia patients. Am J Psychiatry 165:1594-603.

Calabresi P, Centonze D, Marfia GA, Pisani A, Bernardi G (1999) An in vitro electrophysiological study on the effects of phenytoin, lamotrigine and gabapentin on striatal neurons. Br J Pharmacol 126:689-96.

Campbell LE, Hughes M, Budd TW, Cooper G, Fulham WR, Karayanidis F, Hanlon MC, Stojanov W, Johnston P, Case V, Schall U (2007) Primary and secondary neural networks of auditory prepulse inhibition: a functional magnetic resonance imaging study of sensorimotor gating of the human acoustic startle response. Eur J Neurosci 26:2327-33.

Carlsson A, Lindqvist M (1963) Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacol Toxicol (Copenh) 20:140-4.

Carlsson A, Lindqvist M, Magnusson T (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180:1200.

Cattabeni F, Di Luca M (1997) Developmental models of brain dysfunctions induced by targeted cellular ablations with methylazoxymethanol. Physiol Rev 77:199-215.

Celikyurt IK, Ulak G, Mutlu O, Akar FY, Erden F, Komsuoglu SS (2012) Lamotrigine, a mood stabilizer, may have beneficial effects on memory acquisition and retrieval in mice. Life Sci 91:1270-4.

Chesworth R, Yulyaningsih E, Cappas E, Arnold J, Sainsbury A, Karl T (2012) The response of neuregulin 1 mutant mice to acute restraint stress. Neurosci Lett 515:82-6.

Chin CL, Curzon P, Schwartz AJ, O'Connor EM, Rueter LE, Fox GB, Day M, Basso AM (2011) Structural abnormalities revealed by magnetic resonance imaging in rats prenatally exposed to methylazoxymethanol acetate parallel cerebral pathology in schizophrenia. Synapse 65:393-403.

Choi YK, Snigdha S, Shahid M, Neill JC, Tarazi FI (2009) Subchronic effects of phencyclidine on dopamine and serotonin receptors: implications for schizophrenia. J Mol Neurosci 38:227-35.

Cilia J, Hatcher P, Reavill C, Jones DN (2007) (+/-) Ketamine-induced prepulse inhibition deficits of an acoustic startle response in rats are not reversed by antipsychotics. J Psychopharmacol 21:302-11.

Cilia J, Hatcher PD, Reavill C, Jones DN (2005) Long-term evaluation of isolation-rearing induced prepulse inhibition deficits in rats: an update. Psychopharmacology (Berl) 180:57-62.

Cilia J, Reavill C, Hagan JJ, Jones DN (2001) Long-term evaluation of isolation-rearing induced prepulse inhibition deficits in rats. Psychopharmacology (Berl) 156:327-37.

Clark RE, Zola SM, Squire LR (2000) Impaired recognition memory in rats after damage to the hippocampus. J Neurosci 20:8853-60.

Clarke MC, Harley M, Cannon M (2006) The role of obstetric events in schizophrenia. Schizophr Bull 32:3-8.

Coelho CA, Ferreira TL, Soares JC, Oliveira MG (2013) Hippocampal NMDA receptor blockade impairs CREB phosphorylation in amygdala after contextual fear conditioning. Hippocampus 23:545-51.

Cohen BD, Rosenbaum G, Luby ED, Gottlieb JS (1962) Comparison of phencyclidine hydrochloride (Sernyl) with other drugs. Simulation of schizophrenic performance with phencyclidine hydrochloride (Sernyl), lysergic acid diethylamide (LSD-25), and amobarbital (Amytal) sodium; II. Symbolic and sequential thinking. Arch Gen Psychiatry 6:395-401.

Collip D, Myin-Germeys I, Van Os J (2008) Does the concept of "sensitization" provide a plausible mechanism for the putative link between the environment and schizophrenia? Schizophr Bull 34:220-5.

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 30:2135-43.

Cunningham C, Campion S, Teeling J, Felton L, Perry VH (2007) The sickness behaviour and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (poly I:C). Brain Behav Immun 21:490-502.

Curley AA, Eggan SM, Lazarus MS, Huang ZJ, Volk DW, Lewis DA (2013) Role of glutamic acid decarboxylase 67 in regulating cortical parvalbumin and GABA membrane transporter 1 expression: implications for schizophrenia. Neurobiol Dis 50:179-86.

Dalley JW, Theobald DE, Pereira EA, Li PM, Robbins TW (2002) Specific abnormalities in serotonin release in the prefrontal cortex of isolation-reared rats measured during behavioural performance of a task assessing visuospatial attention and impulsivity. Psychopharmacology (Berl) 164:329-40.

Dalton VS, Verdurand M, Walker A, Hodgson DM, Zavitsanou K (2012) Synergistic Effect between Maternal Infection and Adolescent Cannabinoid Exposure on Serotonin 5HT1A Receptor Binding in the Hippocampus: Testing the "Two Hit" Hypothesis for the Development of Schizophrenia. ISRN Psychiatry 2012:451865.

Davis KL, Kahn RS, Ko G, Davidson M (1991) Dopamine in schizophrenia: a review and reconceptualization. Am J Psychiatry 148:1474-86.

Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, Buxbaum J, Haroutunian V (2003) White matter changes in schizophrenia: evidence for myelin-related dysfunction. Arch Gen Psychiatry 60:443-56.

Day-Wilson KM, Jones DN, 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 141:1113-21.

Del Arco A, Ronzoni G, Mora F (2011) Prefrontal stimulation of GABAA receptors counteracts the corticolimbic hyperactivity produced by NMDA antagonists in the prefrontal cortex of the rat. Psychopharmacology (Berl) 214:525-36.

Del Arco A, Zhu S, Terasmaa A, Mohammed AH, Fuxe K (2004) Hyperactivity to novelty induced by social isolation is not correlated with changes in D2 receptor function and binding in striatum. Psychopharmacology (Berl) 171:148-55.

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

Desbonnet L, O'Tuathaigh C, Clarke G, O'Leary C, Petit E, Clarke N, Tighe O, Lai D, Harvey R, Cryan JF, Dinan TG, Waddington JL (2012) Phenotypic effects of repeated psychosocial stress during adolescence in mice mutant for the schizophrenia risk gene neuregulin-1: a putative model of gene x environment interaction. Brain Behav Immun 26:660-71.

Deslauriers J, Larouche A, Sarret P, Grignon S (2013) Combination of prenatal immune challenge and restraint stress affects prepulse inhibition and dopaminergic/GABAergic markers. Prog Neuropsychopharmacol Biol Psychiatry 45:156-64.

Deutsch SI, Rosse RB, Schwartz BL, Mastropaolo J (2001) A revised excitotoxic hypothesis of schizophrenia: therapeutic implications. Clin Neuropharmacol 24:43-9.

Didriksen M, Kreilgaard M, Arnt J (2006) Sertindole, in contrast to clozapine and olanzapine, does not disrupt water maze performance after acute or chronic treatment. Eur J Pharmacol 542:108-15.

Do KQ, Cabungcal JH, Frank A, Steullet P, Cuenod M (2009) Redox dysregulation, neurodevelopment, and schizophrenia. Curr Opin Neurobiol 19:220-30.

Domeney A, Feldon J (1998) The disruption of prepulse inhibition by social isolation in the Wistar rat: how robust is the effect? Pharmacol Biochem Behav 59:883-90.

Dong Z, Bai Y, Wu X, Li H, Gong B, Howland JG, Huang Y, He W, Li T, Wang YT (2013) Hippocampal long-term depression mediates spatial reversal learning in the Morris water maze. Neuropharmacology 64:65-73.

du Bois TM, Huang XF, Deng C (2008) Perinatal administration of PCP alters adult behaviour in female Sprague-Dawley rats. Behav Brain Res 188:416-9.

du Bois TM, Newell KA, Han M, Deng C, Huang XF (2009) Perinatal PCP treatment alters the developmental expression of prefrontal and hippocampal muscarinic receptors. Prog Neuropsychopharmacol Biol Psychiatry 33:37-40.

Duffy S, Labrie V, Roder JC (2008) D-serine augments NMDA-NR2B receptor-dependent hippocampal long-term depression and spatial reversal learning. Neuropsychopharmacology 33:1004-18.

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

Enomoto T, Noda Y, Mouri A, Shin EJ, Wang D, Murai R, Hotta K, Furukawa H, Nitta A, Kim HC, Nabeshima T (2005) Long-lasting impairment of associative learning is correlated with a dysfunction of N-methyl-D-aspartate-extracellular signaling-regulated kinase signaling in mice after withdrawal from repeated administration of phencyclidine. Mol Pharmacol 68:1765-74.

Erfurth A, Walden J, Grunze H (1998) Lamotrigine in the treatment of schizoaffective disorder. Neuropsychobiology 38:204-5.

Eun SH, Eun BL, Lee JS, Hwang YS, Kim KJ, Lee YM, Lee IG, Lee M, Ko TS, Kim JT, Eom S, Kim HD (2012) Effects of lamotrigine on cognition and behavior compared to carbamazepine as monotherapy for children with partial epilepsy. Brain Dev 34:818-23.

Fabricius K, Helboe L, Fink-Jensen A, Wortwein G, Steiniger-Brach B (2011) Pharmacological characterization of social isolation-induced hyperactivity. Psychopharmacology (Berl) 215:257-66.

Fabricius K, Helboe L, Steiniger-Brach B, Fink-Jensen A, Pakkenberg B (2010) Stereological brain volume changes in post-weaned socially isolated rats. Brain Res 1345:233-9.

Fanselow MS (2000) Contextual fear, gestalt memories, and the hippocampus. Behav Brain Res 110:73-81.

Featherstone RE, Burton CL, Coppa-Hopman R, Rizos Z, Sinyard J, Kapur S, Fletcher PJ (2009) Gestational treatment with methylazoxymethanol (MAM) that disrupts hippocampal-dependent memory does not alter behavioural response to cocaine. Pharmacol Biochem Behav 93:382-90.

Featherstone RE, Rizos Z, Nobrega JN, Kapur S, Fletcher PJ (2007) Gestational methylazoxymethanol acetate treatment impairs select cognitive functions: Parallels to schizophrenia. Neuropsychopharmacology 32:483-492.

Fendt M, Li L, Yeomans JS (2001) Brain stem circuits mediating prepulse inhibition of the startle reflex. Psychopharmacology (Berl) 156:216-24.

Fiore M, Korf J, Antonelli A, Talamini L, Aloe L (2002) Long-lasting effects of prenatal MAM treatment on water maze performance in rats: associations with altered brain development and neurotrophin levels. Neurotoxicol Teratol 24:179-91.

Fiore M, Talamini L, Angelucci F, Koch T, Aloe L, Korf J (1999) Prenatal methylazoxymethanol acetate alters behavior and brain NGF levels in young rats: a possible correlation with the development of schizophrenia-like deficits. Neuropharmacology 38:857-69.

Flagstad P, Glenthoj BY, Didriksen M (2005) Cognitive deficits caused by late gestational disruption of neurogenesis in rats: a preclinical model of schizophrenia. Neuropsychopharmacology 30:250-60.

Flagstad P, Mork A, Glenthoj BY, van Beek J, Michael-Titus AT, Didriksen M (2004) Disruption of neurogenesis on gestational day 17 in the rat causes behavioral changes relevant to positive and negative schizophrenia symptoms and alters amphetamine-induced dopamine release in nucleus accumbens. Neuropsychopharmacology 29:2052-2064.

Fletcher PJ, Tenn CC, Rizos Z, Lovic V, Kapur S (2005) Sensitization to amphetamine, but not PCP, impairs attentional set shifting: reversal by a D1 receptor agonist injected into the medial prefrontal cortex. Psychopharmacology (Berl) 183:190-200.

Floresco SB, Seamans JK, Phillips AG (1997) Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. J Neurosci 17:1880-90.

Floresco SB, Todd CL, Grace AA (2001) Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. J Neurosci 21:4915-22.

Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 6:968-73.

Fone KC, Shalders K, Fox ZD, Arthur R, Marsden CA (1996) Increased 5-HT2C receptor responsiveness occurs on rearing rats in social isolation. Psychopharmacology (Berl) 123:346-52.

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

Forcelli PA, Kozlowski R, Snyder C, Kondratyev A, Gale K (2012) Effects of neonatal antiepileptic drug exposure on cognitive, emotional, and motor function in adult rats. J Pharmacol Exp Ther 340:558-66.

Fulford AJ, Marsden CA (1998) Effect of isolation-rearing on conditioned dopamine release in vivo in the nucleus accumbens of the rat. J Neurochem 70:384-90.

Gastambide F, Cotel MC, Gilmour G, O'Neill MJ, Robbins TW, Tricklebank MD (2012) Selective remediation of reversal learning deficits in the neurodevelopmental MAM model of schizophrenia by a novel mGlu5 positive allosteric modulator. Neuropsychopharmacology 37:1057-66.

Gentsch C, Lichtsteiner M, Frischknecht HR, Feer H, Siegfried B (1988) Isolation-induced locomotor hyperactivity and hypoalgesia in rats are prevented by handling and reversed by resocialization. Physiol Behav 43:13-6.

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) 156:117-54.

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. Biol Psychiatry 34:361-72.

Gilabert-Juan J, Belles M, Saez AR, Carceller H, Zamarbide S, Molto MD, Nacher J (2013) A "double hit" murine model for schizophrenia shows alterations in the structure and neurochemistry of the medial prefrontal cortex and the hippocampus. Neurobiol Dis 59:126-40.

Gill KE, Beveridge TJ, Smith HR, Porrino LJ (2013) The effects of rearing environment and chronic methylphenidate administration on behavior and dopamine receptors in adolescent rats. Brain Res 1527:67-78.

Giovanoli S, Engler H, Engler A, Richetto J, Voget M, Willi R, Winter C, Riva MA, Mortensen PB, Schedlowski M, Meyer U (2013) Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. Science 339:1095-9.

Giusti-Rodriguez P, Sullivan PF (2013) The genomics of schizophrenia: update and implications. J Clin Invest 123:4557-63.

GlaxoSmithKline (2007) Lamictal Prescribing Information. Food & Drug Authority

Goff DC (2009) Review: lamotrigine may be an effective treatment for clozapine resistant schizophrenia. Evid Based Ment Health 12:111.

Golan H, Huleihel M (2006) The effect of prenatal hypoxia on brain development: short- and long-term consequences demonstrated in rodent models. Dev Sci 9:338-49.

Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams GV (2004) Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. Psychopharmacology (Berl) 174:3-16.

Gourevitch R, Rocher C, Le Pen G, Krebs MO, Jay TM (2004) Working memory deficits in adult rats after prenatal disruption of neurogenesis. Behav Pharmacol 15:287-92.

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. Behav Brain Res 184:31-8.

Greco AM, Gambardella P, Sticchi R, D'Aponte D, Di Renzo G, De Franciscis P (1989) Effects of individual housing on circadian rhythms of adult rats. Physiol Behav 45:363-6.

Gresack JE, Risbrough VB, Scott CN, Coste S, Stenzel-Poore M, Geyer MA, Powell SB (2010) Isolation rearing-induced deficits in contextual fear learning do not require CRF(2) receptors. Behav Brain Res 209:80-4.

Gubert C, Stertz L, Pfaffenseller B, Panizzutti BS, Rezin GT, Massuda R, Streck EL, Gama CS, Kapczinski F, Kunz M (2013) Mitochondrial activity and oxidative stress markers in peripheral blood mononuclear cells of patients with bipolar disorder, schizophrenia, and healthy subjects. J Psychiatr Res 47:1396-402.

Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, Grayson DR, Impagnatiello F, Pandey G, Pesold C, Sharma R, Uzunov D, Costa E (2000) Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch Gen Psychiatry 57:1061-9.

Gundersen HJ, Jensen EB (1987) The efficiency of systematic sampling in stereology and its prediction. J Microsc 147:229-63.

Haldane M, Jogia J, Cobb A, Kozuch E, Kumari V, Frangou S (2008) Changes in brain activation during working memory and facial recognition tasks in patients with bipolar disorder with Lamotrigine monotherapy. Eur Neuropsychopharmacol 18:48-54.

Hall FS, Wilkinson LS, Humby T, Inglis W, Kendall DA, Marsden CA, Robbins TW (1998) Isolation rearing in rats: pre- and postsynaptic changes in striatal dopaminergic systems. Pharmacol Biochem Behav 59:859-72.

Handford HA (1975) Brain hypoxia, minimal brain dysfunction, and schizophrenia. Am J Psychiatry 132:192-4.

Haque FN, Lipina TV, Roder JC, Wong AH (2012) Social defeat interacts with Disc1 mutations in the mouse to affect behavior. Behav Brain Res 233:337-44.

Harich S, Gross G, Bespalov A (2007) Stimulation of the metabotropic glutamate 2/3 receptor attenuates social novelty discrimination deficits induced by neonatal phencyclidine treatment. Psychopharmacology (Berl) 192:511-9.

Harris LW, Sharp T, Gartlon J, Jones DN, Harrison PJ (2003) Long-term behavioural, molecular and morphological effects of neonatal NMDA receptor antagonism. Eur J Neurosci 18:1706-10.

Harrison PJ (1999) The neuropathology of schizophrenia. A critical review of the data and their interpretation. Brain 122 (Pt 4):593-624.

Harte MK, Powell SB, Swerdlow NR, Geyer MA, Reynolds GP (2007) Deficits in parvalbumin and calbindin immunoreactive cells in the hippocampus of isolation reared rats. J Neural Transm 114:893-8.

Harvey PD, Rabinowitz J, Eerdekens M, Davidson M (2005) Treatment of cognitive impairment in early psychosis: a comparison of risperidone and haloperidol in a large long-term trial. Am J Psychiatry 162:1888-95.

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. Eur J Pharmacol 519:114-7.

Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z, Sampson AR, Lewis DA (2003) Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J Neurosci 23:6315-26.

Hawken ER, Delva NJ, Beninger RJ (2013) Increased drinking following social isolation rearing: implications for polydipsia associated with schizophrenia. PLoS One 8:e56105.

Hazane F, Krebs MO, Jay TM, Le Pen G (2009) Behavioral perturbations after prenatal neurogenesis disturbance in female rat. Neurotox Res 15:311-20.

Heckers S, Konradi C (2002) Hippocampal neurons in schizophrenia. J Neural Transm 109:891-905.

Heidbreder CA, Foxton R, Cilia J, Hughes ZA, Shah AJ, Atkins A, Hunter AJ, Hagan JJ, Jones DN (2001) Increased responsiveness of dopamine to atypical, but not typical antipsychotics in the medial prefrontal cortex of rats reared in isolation. Psychopharmacology (Berl) 156:338-51.

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 100:749-68.

Hellemans KG, Benge LC, Olmstead MC (2004) Adolescent enrichment partially reverses the social isolation syndrome. Brain Res Dev Brain Res 150:103-15.

Herbener ES (2009) Impairment in long-term retention of preference conditioning in schizophrenia. Biol Psychiatry 65:1086-90.

Hertzmann M, Reba RC, Kotlyarov EV (1990) Single photon emission computed tomography in phencyclidine and related drug abuse. Am J Psychiatry 147:255-6.

Hewitt KN, Shah YB, Prior MJ, Morris PG, Hollis CP, Fone KC, Marsden CA (2005) Behavioural and pharmacological magnetic resonance imaging assessment of the effects of methylphenidate in a potential new rat model of attention deficit hyperactivity disorder. Psychopharmacology (Berl) 180:716-23.

Hickey AJ, Reynolds JN, Beninger RJ (2012) Post-weaning social isolation and subchronic NMDA glutamate receptor blockade: effects on locomotor activity and GABA signaling in the rat suggest independent mechanisms. Pharmacol Biochem Behav 101:231-8.

Hirayasu Y, Tanaka S, Shenton ME, Salisbury DF, DeSantis MA, Levitt JJ, Wible C, Yurgelun-Todd D, Kikinis R, Jolesz FA, McCarley RW (2001) Prefrontal gray matter volume reduction in first episode schizophrenia. Cereb Cortex 11:374-81.

Holson RR, Scallet AC, Ali SF, Turner BB (1991) "Isolation stress" revisited: isolation-rearing effects depend on animal care methods. Physiol Behav 49:1107-18.

Hong X, Chan RC, Zhuang X, Jiang T, Wan X, Wang J, Xiao B, Zhou H, Jiang L, Weng B (2009) Neuroleptic effects on P50 sensory gating in patients with first-episode never-medicated schizophrenia. Schizophr Res 108:151-7.

Houthoofd SA, Morrens M, Sabbe BG (2008) Cognitive and psychomotor effects of risperidone in schizophrenia and schizoaffective disorder. Clin Ther 30:1565-89.

Howes OD, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A, Kapur S (2012) The nature of dopamine dysfunction in schizophrenia and what this means for treatment. Arch Gen Psychiatry 69:776-86.

Howes OD, Kapur S (2009) The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr Bull 35:549-62.

Howes SR, Dalley JW, Morrison CH, Robbins TW, Everitt BJ (2000) Leftward shift in the acquisition of cocaine self-administration in isolation-reared rats: relationship to extracellular levels of dopamine, serotonin and glutamate in the nucleus accumbens and amygdala-striatal FOS expression. Psychopharmacology (Berl) 151:55-63.

Hradetzky E, Sanderson TM, Tsang TM, Sherwood JL, Fitzjohn SM, Lakics V, Malik N, Schoeffmann S, O'Neill MJ, Cheng TM, Harris LW, Rahmoune H, Guest PC, Sher E, Collingridge GL, Holmes E, Tricklebank MD, Bahn S (2012) The methylazoxymethanol acetate (MAM-E17) rat model: molecular and functional effects in the hippocampus. Neuropsychopharmacology 37:364-77.

Ibi D, Nagai T, Koike H, Kitahara Y, Mizoguchi H, Niwa M, Jaaro-Peled H, Nitta A, Yoneda Y, Nabeshima T, Sawa A, Yamada K (2010) Combined effect of neonatal immune activation and mutant DISC1 on phenotypic changes in adulthood. Behav Brain Res 206:32-7.

Ibi D, Takuma K, Koike H, Mizoguchi H, Tsuritani K, Kuwahara Y, Kamei H, Nagai T, Yoneda Y, Nabeshima T, Yamada K (2008) Social isolation rearing-induced impairment of the hippocampal neurogenesis is associated with deficits in spatial memory and emotion-related behaviors in juvenile mice. J Neurochem 105:921-32.

Idris NF, Neill JC, Large CH (2009) Comparison of the efficacy of two anticonvulsants, phenytoin and valproate to improve PCP and d-amphetamine induced deficits in a reversal learning task in the rat. Front Behav Neurosci 3:8.

Ijff DM, Aldenkamp AP (2013) Cognitive side-effects of antiepileptic drugs in children. Handb Clin Neurol 111:707-18.

Ishihama T, Ago Y, Shintani N, Hashimoto H, Baba A, Takuma K, Matsuda T (2010) Environmental factors during early developmental period influence psychobehavioral abnormalities in adult PACAP-deficient mice. Behav Brain Res 209:274-80.

Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatry 148:1301-8.

Jenkins TA, Elliott JJ, Ardis TC, Cahir M, Reynolds GP, Bell R, Cooper SJ (2010a) Tryptophan depletion impairs object-recognition memory in the rat: reversal by risperidone. Behav Brain Res 208:479-83.

Jenkins TA, Harte MK, Reynolds GP (2010b) Effect of subchronic phencyclidine administration on sucrose preference and hippocampal parvalbumin immunoreactivity in the rat. Neurosci Lett 471:144-7.

Jentsch JD, Taylor JR, Roth RH (1998) Subchronic phencyclidine administration increases mesolimbic dopaminergic system responsivity and augments stress- and psychostimulant-induced hyperlocomotion. Neuropsychopharmacology 19:105-13.

Jentsch JD, Tran A, Le D, Youngren KD, Roth RH (1997) Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. Neuropsychopharmacology 17:92-9.

Jiang Z, Rompala GR, Zhang S, Cowell RM, Nakazawa K (2013) Social isolation exacerbates schizophrenia-like phenotypes via oxidative stress in cortical interneurons. Biol Psychiatry 73:1024-34.

Jogia J, Haldane M, Cobb A, Kumari V, Frangou S (2008) Pilot investigation of the changes in cortical activation during facial affect recognition with lamotrigine monotherapy in bipolar disorder. Br J Psychiatry 192:197-201.

Jones CA, Brown AM, Auer DP, Fone KC (2011a) The mGluR2/3 agonist LY379268 reverses post-weaning social isolation-induced recognition memory deficits in the rat. Psychopharmacology (Berl) 214:269-83.

Jones CA, Watson DJ, Fone KC (2011b) Animal models of schizophrenia. Br J Pharmacol 164:1162-94.

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. Pharmacol Biochem Behav 43:17-35.

Jones GH, Marsden CA, Robbins TW (1991) Behavioural rigidity and rule-learning deficits following isolation-rearing in the rat: neurochemical correlates. Behav Brain Res 43:35-50.

Jones MW, Wilson MA (2005) Theta rhythms coordinate hippocampal-prefrontal interactions in a spatial memory task. PLoS Biol 3:e402.

Jones P, Murray RM (1991) The genetics of schizophrenia is the genetics of neurodevelopment. Br J Psychiatry 158:615-23.

Jones RM, Lichtenstein P, Grann M, Langstrom N, Fazel S (2011c) Alcohol use disorders in schizophrenia: a national cohort study of 12,653 patients. J Clin Psychiatry 72:775-9; quiz 878-9.

Jongen-Relo AL, Leng A, Luber M, Pothuizen HH, Weber L, Feldon J (2004) The prenatal methylazoxymethanol acetate treatment: a neurodevelopmental animal model for schizophrenia? Behav Brain Res 149:159-81.

Joyce E (2005) Origins of cognitive dysfunction in schizophrenia: clues from age at onset. Br J Psychiatry 186:93-5.

Kaalund SS, Riise J, Broberg BV, Fabricius K, Karlsen AS, Secher T, Plath N, Pakkenberg B (2013) Differential expression of parvalbumin in neonatal phencyclidine-treated rats and socially isolated rats. J Neurochem 124:548-57.

Kaiser S, Foltz LA, George CA, Kirkwood SC, Bemis KG, Lin X, Gelbert LM, Nisenbaum LK (2004) Phencyclidine-induced changes in rat cortical gene expression identified by microarray analysis: implications for schizophrenia. Neurobiol Dis 16:220-35.

Kalinichev M, Robbins MJ, Hartfield EM, Maycox PR, Moore SH, Savage KM, Austin NE, Jones DN (2008) Comparison between intraperitoneal and subcutaneous phencyclidine administration in Sprague-Dawley rats: a locomotor activity and gene induction study. Prog Neuropsychopharmacol Biol Psychiatry 32:414-22.

Kapur S, Remington G (1996) Serotonin-dopamine interaction and its relevance to schizophrenia. Am J Psychiatry 153:466-76.

Kapur S, VanderSpek SC, Brownlee BA, Nobrega JN (2003) Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. J Pharmacol Exp Ther 305:625-31.

Kapur S, Zipursky RB, Remington G (1999) Clinical and theoretical implications of 5-HT2 and D2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. Am J Psychiatry 156:286-93.

Kaur S, Starr M (1996) Motor effects of lamotrigine in naive and dopamine-depleted mice. Eur J Pharmacol 304:1-6.

Keefe RS (2007) Cognitive deficits in patients with schizophrenia: effects and treatment. J Clin Psychiatry 68 Suppl 14:8-13.

Keefe RS, Malhotra AK, Meltzer HY, Kane JM, Buchanan RW, Murthy A, Sovel M, Li C, Goldman R (2008) 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 33:1217-28.

Kegeles LS, Abi-Dargham A, Frankle WG, Gil R, Cooper TB, Slifstein M, Hwang DR, Huang Y, Haber SN, Laruelle M (2010) Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. Arch Gen Psychiatry 67:231-9.

Keilhoff G, Becker A, Grecksch G, Wolf G, Bernstein HG (2004) Repeated application of ketamine to rats induces changes in the hippocampal expression of parvalbumin, neuronal nitric oxide synthase and cFOS similar to those found in human schizophrenia. Neuroscience 126:591-8.

Kestler LP, Walker E, Vega EM (2001) Dopamine receptors in the brains of schizophrenia patients: a meta-analysis of the findings. Behav Pharmacol 12:355-71.

Kim E, Howes OD, Turkheimer FE, Kim BH, Jeong JM, Kim JW, Lee JS, Jang IJ, Shin SG, Kapur S, Kwon JS (2013) The relationship between antipsychotic D2 occupancy and change in frontal metabolism and working memory : A dual [(11)C]raclopride and [(18) F]FDG imaging study with aripiprazole. Psychopharmacology (Berl) 227:221-9.

King MV, Sleight AJ, Woolley ML, Topham IA, Marsden CA, Fone KC (2004) 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation--an effect sensitive to NMDA receptor antagonism. Neuropharmacology 47:195-204.

Konradi C, Yang CK, Zimmerman EI, Lohmann KM, Gresch P, Pantazopoulos H, Berretta S, Heckers S (2011) Hippocampal interneurons are abnormal in schizophrenia. Schizophr Res 131:165-73.

Koola MM, McMahon RP, Wehring HJ, Liu F, Mackowick KM, Warren KR, Feldman S, Shim JC, Love RC, Kelly DL (2012) Alcohol and cannabis use and mortality in people with schizophrenia and related psychotic disorders. J Psychiatr Res 46:987-93.

Kovelman JA, Scheibel AB (1986) Biological substrates of schizophrenia. Acta Neurol Scand 73:1-32.

Krebs-Thomson K, Giracello D, Solis A, Geyer MA (2001) Post-weaning handling attenuates isolation-rearing induced disruptions of prepulse inhibition in rats. Behav Brain Res 120:221-4.

Kreczmanski P, Heinsen H, Mantua V, Woltersdorf F, Masson T, Ulfig N, Schmidt-Kastner R, Korr H, Steinbusch HW, Hof PR, Schmitz C (2007) Volume, neuron density and total neuron number in five subcortical regions in schizophrenia. Brain 130:678-92.

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. Arch Gen Psychiatry 51:199-214.

Kucharska-Pietura K, Tylec A, Czernikiewicz A, Mortimer A (2012) Attentional and emotional functioning in schizophrenia patients treated with conventional and atypical antipsychotic drugs. Med Sci Monit 18:CR44-49.

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 Res 1279:189-96.

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

Lapiz MD, 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. Neurosci Behav Physiol 33:13-29.

Large CH, Bison S, Sartori I, Read KD, Gozzi A, Quarta D, Antolini M, Hollands E, Gill CH, Gunthorpe MJ, Idris N, Neill JC, Alvaro GS (2011) The efficacy of sodium channel blockers to prevent phencyclidine-induced cognitive dysfunction in the rat: potential for novel treatments for schizophrenia. J Pharmacol Exp Ther 338:100-13.

Laruelle M, Abi-Dargham A, van Dyck CH, Gil R, D'Souza CD, Erdos J, McCance E, Rosenblatt W, Fingado C, Zoghbi SS, Baldwin RM, Seibyl JP, Krystal JH, Charney DS, Innis RB (1996) Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc Natl Acad Sci U S A 93:9235-40.

Le Pen G, Gourevitch R, Hazane F, Hoareau C, Jay TM, Krebs MO (2006) Peri-pubertal maturation after developmental disturbance: a model for psychosis onset in the rat. Neuroscience 143:395-405.

Le Pen G, Jay TM, Krebs MO (2011) Effect of antipsychotics on spontaneous hyperactivity and hypersensitivity to MK-801-induced hyperactivity in rats prenatally exposed to methylazoxymethanol. J Psychopharmacol 25:822-35.

Leach MJ, Baxter MG, Critchley MA (1991) Neurochemical and behavioral aspects of lamotrigine. Epilepsia 32 Suppl 2:S4-8.

Lecourtier L, Neijt HC, Kelly PH (2004) Habenula lesions cause impaired cognitive performance in rats: implications for schizophrenia. Eur J Neurosci 19:2551-60.

Lecourtier L, Saboureau M, Kelly CD, Pevet P, Kelly PH (2005) Impaired cognitive performance in rats after complete epithalamus lesions, but not after pinealectomy alone. Behav Brain Res 161:276-85.

Lee I, Hunsaker MR, Kesner RP (2005) The role of hippocampal subregions in detecting spatial novelty. Behav Neurosci 119:145-53.

Leng A, Jongen-Relo AL, Pothuizen HH, Feldon J (2005) Effects of prenatal methylazoxymethanol acetate (MAM) treatment in rats on water maze performance. Behav Brain Res 161:291-8.

Lerch JP, Yiu AP, Martinez-Canabal A, Pekar T, Bohbot VD, Frankland PW, Henkelman RM, Josselyn SA, Sled JG (2011) Maze training in mice induces MRI-detectable brain shape changes specific to the type of learning. Neuroimage 54:2086-95.

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.

Levinson DF, Duan J, Oh S, Wang K, Sanders AR, Shi J, Zhang N, Mowry BJ, Olincy A, Amin F, Cloninger CR, Silverman JM, Buccola NG, Byerley WF, Black DW, Kendler KS, Freedman R, Dudbridge F, Pe'er I, Hakonarson H, Bergen SE, Fanous AH, Holmans PA, Gejman PV (2011) Copy number variants in schizophrenia: confirmation of five previous findings and new evidence for 3q29 microdeletions and VIPR2 duplications. Am J Psychiatry 168:302-16.

Lewis DA, Hashimoto T, Volk DW (2005) Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci 6:312-24.

Li M, He E, Volf N (2011a) Time course of the attenuation effect of repeated antipsychotic treatment on prepulse inhibition disruption induced by repeated phencyclidine treatment. Pharmacol Biochem Behav 98:559-69.

Li M, He W, Chen J (2011b) Time course of prepulse inhibition disruption induced by dopamine agonists and NMDA antagonists: effects of drug administration regimen. Pharmacol Biochem Behav 99:509-18.

Li N, Wu X, Li L (2007) Chronic administration of clozapine alleviates reversal-learning impairment in isolation-reared rats. Behav Pharmacol 18:135-45.

Lim AL, Taylor DA, Malone DT (2012) A two-hit model: behavioural investigation of the effect of combined neonatal MK-801 administration and isolation rearing in the rat. J Psychopharmacol 26:1252-64.

Lipina TV, Zai C, Hlousek D, Roder JC, Wong AH (2013) Maternal immune activation during gestation interacts with Disc1 point mutation to exacerbate schizophrenia-related behaviors in mice. J Neurosci 33:7654-66.

Liu F, Zou X, Sadovova N, Zhang X, Shi L, Guo L, Qian F, Wen Z, Patterson TA, Hanig JP, Paule MG, Slikker W, Jr., Wang C (2010) Changes in gene expression after phencyclidine administration in developing rats: a potential animal model for schizophrenia. Int J Dev Neurosci 29:351-8.

Lodge DJ, Behrens MM, Grace AA (2009) A Loss of Parvalbumin-Containing Interneurons Is Associated with Diminished Oscillatory Activity in an Animal Model of Schizophrenia. Journal of Neuroscience 29:2344-2354.

Lodge DJ, Grace AA (2007) Aberrant hippocampal activity underlies the dopamine dysregulation in an animal model of schizophrenia. J Neurosci 27:11424-30.

Lodge DJ, Grace AA (2008) Hippocampal dysfunction and disruption of dopamine system regulation in an animal model of schizophrenia. Neurotox Res 14:97-104.

Lodge DJ, Grace AA (2009) Gestational methylazoxymethanol acetate administration: a developmental disruption model of schizophrenia. Behav Brain Res 204:306-12.

Lodge DJ, Grace AA (2011) Hippocampal dysregulation of dopamine system function and the pathophysiology of schizophrenia. Trends Pharmacol Sci 32:507-13.

Lu L, Bao G, Chen H, Xia P, Fan X, Zhang J, Pei G, Ma L (2003) Modification of hippocampal neurogenesis and neuroplasticity by social environments. Exp Neurol 183:600-9.

Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R (1959) Study of a new schizophrenomimetic drug; sernyl. AMA Arch Neurol Psychiatry 81:363-9.

Lyon L, Saksida LM, Bussey TJ (2012) Spontaneous object recognition and its relevance to schizophrenia: a review of findings from pharmacological, genetic, lesion and developmental rodent models. Psychopharmacology (Berl) 220:647-72.

MacMillan HL, Fleming JE, Streiner DL, Lin E, Boyle MH, Jamieson E, Duku EK, Walsh CA, Wong MY, Beardslee WR (2001) Childhood abuse and lifetime psychopathology in a community sample. Am J Psychiatry 158:1878-83.

Malhotra AK, Pinals DA, Adler CM, Elman I, Clifton A, Pickar D, Breier A (1997) Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics. Neuropsychopharmacology 17:141-50.

Malhotra D, Sebat J (2012) CNVs: harbingers of a rare variant revolution in psychiatric genetics. Cell 148:1223-41.

Marder SR, Fenton W (2004) Measurement and Treatment Research to Improve Cognition in Schizophrenia: NIMH MATRICS initiative to support the development of agents for improving cognition in schizophrenia. Schizophr Res 72:5-9.

Maren S (2001) Neurobiology of Pavlovian fear conditioning. Annu Rev Neurosci 24:897-931.

Maren S, Phan KL, Liberzon I (2013) The contextual brain: implications for fear conditioning, extinction and psychopathology. Nat Rev Neurosci 14:417-28.

Martin MV, Dong H, Bertchume A, Csernansky JG (2005) Low dose quetiapine reverses deficits in contextual and cued fear conditioning in rats with excitotoxin-induced hippocampal neuropathy. Pharmacol Biochem Behav 82:263-9.

Martinez ZA, Ellison GD, Geyer MA, Swerdlow NR (1999) Effects of sustained phencyclidine exposure on sensorimotor gating of startle in rats. Neuropsychopharmacology 21:28-39.

Matricon J, Bellon A, Frieling H, Kebir O, Le Pen G, Beuvon F, Daumas-Duport C, Jay TM, Krebs MO (2010) Neuropathological and Reelin deficiencies in the hippocampal formation of rats exposed to MAM; differences and similarities with schizophrenia. PLoS One 5:e10291.

Matsumoto H, Higa HH (1966) Studies on methylazoxymethanol, the aglycone of cycasin: methylation of nucleic acids in vitro. Biochem J 98:20C.

McCarthy SE, Makarov V, Kirov G, Addington AM, McClellan J, Yoon S, Perkins DO, Dickel DE, Kusenda M, Krastoshevsky O, Krause V, Kumar RA, Grozeva D, Malhotra D, Walsh T, Zackai EH, Kaplan P, Ganesh J, Krantz ID, Spinner NB, Roccanova P, Bhandari A, Pavon K, Lakshmi B, Leotta A, Kendall J, Lee YH, Vacic V, Gary S, Iakoucheva LM, Crow TJ, Christian SL, Lieberman JA, Stroup TS, Lehtimaki T, Puura K, Haldeman-Englert C, Pearl J, Goodell M, Willour VL, Derosse P, Steele J, Kassem L, Wolff J, Chitkara N, McMahon FJ, Malhotra AK, Potash JB, Schulze TG, Nothen MM, Cichon S, Rietschel M, Leibenluft E, Kustanovich V, Lajonchere CM, Sutcliffe JS, Skuse D, Gill M, Gallagher L, Mendell NR, Craddock N, Owen MJ, O'Donovan MC, Shaikh TH, Susser E, Delisi LE, Sullivan PF, Deutsch CK, Rapoport J, Levy DL, King MC, Sebat J (2009) Microduplications of 16p11.2 are associated with schizophrenia. Nat Genet 41:1223-7.

McClure MM, Romero MJ, Bowie CR, Reichenberg A, Harvey PD, Siever LJ (2007) Visual-spatial learning and memory in schizotypal personality disorder: continued evidence for the importance of working memory in the schizophrenia spectrum. Arch Clin Neuropsychol 22:109-16.

McGuffin P, Gottesman, II (1999) Risk factors for schizophrenia. N Engl J Med 341:370-1; author reply 372.

McIntosh AL, Ballard TM, Steward LJ, Moran PM, Fone KC (2013) The atypical antipsychotic risperidone reverses the recognition memory deficits induced by post-weaning social isolation in rats. Psychopharmacology (Berl) 228:31-42.

McKibben CE, Jenkins TA, Adams HN, Harte MK, Reynolds GP (2010) Effect of pretreatment with risperidone on phencyclidine-induced disruptions in object recognition memory and prefrontal cortex parvalbumin immunoreactivity in the rat. Behav Brain Res 208:132-6.

McLean S, Grayson B, Harris M, Protheroe C, Woolley M, Neill J (2010a) Isolation rearing impairs novel object recognition and attentional set shifting performance in female rats. J Psychopharmacol 24:57-63.

McLean SL, Neill JC, Idris NF, Marston HM, Wong EH, Shahid M (2010b) Effects of asenapine, olanzapine, and risperidone on psychotomimetic-induced reversal-learning deficits in the rat. Behav Brain Res 214:240-7.

Medoff DR, Holcomb HH, Lahti AC, Tamminga CA (2001) Probing the human hippocampus using rCBF: contrasts in schizophrenia. Hippocampus 11:543-50.

Melendez RI, Gregory ML, Bardo MT, Kalivas PW (2004) Impoverished rearing environment alters metabotropic glutamate receptor expression and function in the prefrontal cortex. Neuropsychopharmacology 29:1980-7.

Meyer U (2014) Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems. Biol Psychiatry 75:307-15.

Millstein RA, Ralph RJ, Yang RJ, Holmes A (2006) Effects of repeated maternal separation on prepulse inhibition of startle across inbred mouse strains. Genes Brain Behav 5:346-54.

Moller M, Du Preez JL, Emsley R, Harvey BH (2011) Isolation rearing-induced deficits in sensorimotor gating and social interaction in rats are related to cortico-striatal oxidative stress, and reversed by sub-chronic clozapine administration. Eur Neuropsychopharmacol 21:471-83.

Moller M, Du Preez JL, Viljoen FP, Berk M, Emsley R, Harvey BH (2013) Social isolation rearing induces mitochondrial, immunological, neurochemical and behavioural deficits in rats, and is reversed by clozapine or N-acetyl cysteine. Brain Behav Immun 30:156-67.

Moore H, Jentsch JD, Ghajarnia M, Geyer MA, Grace AA (2006) A neurobehavioral systems analysis of adult rats exposed to methylazoxymethanol acetate on E17: implications for the neuropathology of schizophrenia. Biol Psychiatry 60:253-64.

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11:47-60.

Moser E, Moser MB, Andersen P (1993) Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. J Neurosci 13:3916-25.

Muller-Vahl KR, Emrich HM (2008) Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia. Expert Rev Neurother 8:1037-48.

Murray RM, Jones P, O'Callaghan E (1991) Fetal brain development and later schizophrenia. Ciba Found Symp 156:155-63; discussion 163-70.

Mutlu O, Ulak G, Celikyurt IK, Akar FY, Erden F, Tanyeri P (2011) Effects of olanzapine, sertindole and clozapine on MK-801 induced visual memory deficits in mice. Pharmacol Biochem Behav 99:557-65.

Nagai T, Murai R, Matsui K, Kamei H, Noda Y, Furukawa H, Nabeshima T (2009) Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D1 and serotonin 5-HT1A receptors. Psychopharmacology (Berl) 202:315-28.

Nakatani-Pawlak A, Yamaguchi K, Tatsumi Y, Mizoguchi H, Yoneda Y (2009) Neonatal phencyclidine treatment in mice induces behavioral, histological and neurochemical abnormalities in adulthood. Biol Pharm Bull 32:1576-83.

Nakato Y, Abekawa T, Ito K, Inoue T, Koyama T (2010) Lamotrigine blocks the initiation and expression of repeated high-dose methamphetamine-induced prepulse inhibition deficit in rats. Neurosci Lett 481:183-7.

Need AC, McEvoy JP, Gennarelli M, Heinzen EL, Ge D, Maia JM, Shianna KV, He M, Cirulli ET, Gumbs CE, Zhao Q, Campbell CR, Hong L, Rosenquist P, Putkonen A, Hallikainen T, Repo-Tiihonen E, Tiihonen J, Levy DL, Meltzer HY, Goldstein DB (2012) Exome sequencing followed by large-scale genotyping suggests a limited role for moderately rare risk factors of strong effect in schizophrenia. Am J Hum Genet 91:303-12.

Neill JC, Barnes S, Cook S, Grayson B, Idris NF, McLean SL, Snigdha S, Rajagopal L, Harte MK (2010) Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. Pharmacol Ther 128:419-32.

Nelson MD, Saykin AJ, Flashman LA, Riordan HJ (1998) Hippocampal volume reduction in schizophrenia as assessed by magnetic resonance imaging: a meta-analytic study. Arch Gen Psychiatry 55:433-40.

Newell KA, Zavitsanou K, Huang XF (2007) Short and long term changes in NMDA receptor binding in mouse brain following chronic phencyclidine treatment. J Neural Transm 114:995-1001.

NICE (2009) Schizophrenia: Core interventions in the treatment and management of schizophrenia in adults in primary and secondary care In: NICE (ed) National Institute for Health and Clinical Excellence Clinical Guidelines, pp 1-41

Niwa M, Jaaro-Peled H, Tankou S, Seshadri S, Hikida T, Matsumoto Y, Cascella NG, Kano S, Ozaki N, Nabeshima T, Sawa A (2013) Adolescent stress-induced epigenetic control of dopaminergic neurons via glucocorticoids. Science 339:335-9.

Noda Y, Mouri A, Ando Y, Waki Y, Yamada SN, Yoshimi A, Yamada K, Ozaki N, Wang D, Nabeshima T (2010) Galantamine ameliorates the impairment of recognition memory in mice repeatedly treated with methamphetamine: involvement of allosteric potentiation of nicotinic acetylcholine receptors and dopaminergic-ERK1/2 systems. Int J Neuropsychopharmacol 13:1343-54.

Nordstrom AL, Farde L, Wiesel FA, Forslund K, Pauli S, Halldin C, Uppfeldt G (1993) Central D2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. Biol Psychiatry 33:227-35.

Nyberg S, Farde L, Halldin C, Dahl ML, Bertilsson L (1995) D2 dopamine receptor occupancy during low-dose treatment with haloperidol decanoate. Am J Psychiatry 152:173-8.

Olney JW, Newcomer JW, Farber NB (1999) NMDA receptor hypofunction model of schizophrenia. J Psychiatr Res 33:523-33.

Pacheco-Lopez G, Giovanoli S, Langhans W, Meyer U (2013) Priming of metabolic dysfunctions by prenatal immune activation in mice: relevance to schizophrenia. Schizophr Bull 39:319-29.

Paulson PE, Robinson TE (1995) Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: a microdialysis study in behaving rats. Synapse 19:56-65.

Paxinos G, Watson, C (1997) The Rat Brain In Stereotaxic Coordinates, 3rd edn. Acadmic Press, San Diego

Penschuck S, Flagstad P, Didriksen M, Leist M, Michael-Titus AT (2006) Decrease in parvalbumin-expressing neurons in the hippocampus and increased phencyclidine-induced locomotor activity in the rat methylazoxymethanol (MAM) model of schizophrenia. Eur J Neurosci 23:279-84.

Pletnikov MV, Ayhan Y, Nikolskaia O, Xu Y, Ovanesov MV, Huang H, Mori S, Moran TH, Ross CA (2008) Inducible expression of mutant human DISC1 in mice is associated with brain and behavioral abnormalities reminiscent of schizophrenia. Mol Psychiatry 13:173-86, 115.

Pouzet B, Feldon J, Veenman CL, Yee BK, Richmond M, Nicholas J, Rawlins P, Weiner I (1999) The effects of hippocampal and fimbria-fornix lesions on prepulse inhibition. Behav Neurosci 113:968-81.

Powell SB, Geyer MA, Preece MA, Pitcher LK, Reynolds GP, Swerdlow NR (2003) Dopamine depletion of the nucleus accumbens reverses isolation-induced deficits in prepulse inhibition in rats. Neuroscience 119:233-40.

Powell SB, Swerdlow NR, Pitcher LK, Geyer MA (2002) Isolation rearing-induced deficits in prepulse inhibition and locomotor habituation are not potentiated by water deprivation. Physiol Behav 77:55-64.

Proal AC, Fleming J, Galvez-Buccollini JA, Delisi LE (2014) A controlled family study of cannabis users with and without psychosis. Schizophr Res 152:283-8.

Purdon SE, Jones BD, Stip E, Labelle A, Addington D, David SR, Breier A, Tollefson GD (2000) Neuropsychological change in early phase schizophrenia during 12 months of treatment with olanzapine, risperidone, or haloperidol. The Canadian Collaborative Group for research in schizophrenia. Arch Gen Psychiatry 57:249-58.

Quan MN, Tian YT, Xu KH, Zhang T, Yang Z (2010) Post weaning social isolation influences spatial cognition, prefrontal cortical synaptic plasticity and hippocampal potassium ion channels in Wistar rats. Neuroscience 169:214-22.

Quarta D, Large CH (2010) Effects of lamotrigine on PCP-evoked elevations in monoamine levels in the medial prefrontal cortex of freely moving rats. J Psychopharmacol 25:1703-11.

Radonjic NV, Jakovcevski I, Bumbasirevic V, Petronijevic ND (2013) Perinatal phencyclidine administration decreases the density of cortical interneurons and increases the expression of neuregulin-1. Psychopharmacology (Berl) 227:673-83.

Radonjic NV, Knezevic ID, Vilimanovich U, Kravic-Stevovic T, Marina LV, Nikolic T, Todorovic V, Bumbasirevic V, Petronijevic ND (2010) Decreased glutathione levels and altered antioxidant defense in an animal model of schizophrenia: long-term effects of perinatal phencyclidine administration. Neuropharmacology 58:739-45.

Ragozzino ME (2003) Acetylcholine actions in the dorsomedial striatum support the flexible shifting of response patterns. Neurobiol Learn Mem 80:257-67.

Ragozzino ME, Jih J, Tzavos A (2002a) Involvement of the dorsomedial striatum in behavioral flexibility: role of muscarinic cholinergic receptors. Brain Res 953:205-14.

Ragozzino ME, Ragozzino KE, Mizumori SJ, Kesner RP (2002b) Role of the dorsomedial striatum in behavioral flexibility for response and visual cue discrimination learning. Behav Neurosci 116:105-15.

Rainey JM, Jr., Crowder MK (1975) Prolonged psychosis attributed to phencyclidine: report of three cases. Am J Psychiatry 132:1076-8.

Redrobe JP, Elster L, Frederiksen K, Bundgaard C, de Jong IE, Smith GP, Bruun AT, Larsen PH, Didriksen M (2012) Negative modulation of GABAA alpha5 receptors by RO4938581 attenuates discrete sub-chronic and early postnatal phencyclidine (PCP)-induced cognitive deficits in rats. Psychopharmacology (Berl) 221:451-68.

Reid JG, Gitlin MJ, Altshuler LL (2013) Lamotrigine in psychiatric disorders. J Clin Psychiatry 74:675-84.

Richtand NM, Ahlbrand R, Horn PS, Chambers B, Davis J, Benoit S (2012) Effects of prenatal immune activation and peri-adolescent stress on amphetamine-induced conditioned place preference in the rat. Psychopharmacology (Berl) 222:313-24.

Riedel M, Spellmann I, Strassnig M, Douhet A, Dehning S, Opgen-Rhein M, Valdevit R, Engel RR, Kleindienst N, Muller N, Moller HJ (2007) Effects of risperidone and quetiapine on cognition in patients with schizophrenia and predominantly negative symptoms. Eur Arch Psychiatry Clin Neurosci 257:360-70.

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kahler AK, Akterin S, Bergen SE, Collins AL, Crowley JJ, Fromer M, Kim Y, Lee SH, Magnusson PK, Sanchez N, Stahl EA, Williams S, Wray NR, Xia K, Bettella F, Borglum AD, Bulik-Sullivan BK, Cormican P, Craddock N, de Leeuw C, Durmishi N, Gill M,

Golimbet V, Hamshere ML, Holmans P, Hougaard DM, Kendler KS, Lin K, Morris DW, Mors O, Mortensen PB, Neale BM, O'Neill FA, Owen MJ, Milovancevic MP, Posthuma D, Powell J, Richards AL, Riley BP, Ruderfer D, Rujescu D, Sigurdsson E, Silagadze T, Smit AB, Stefansson H, Steinberg S, Suvisaari J, Tosato S, Verhage M, Walters JT, Levinson DF, Gejman PV, Laurent C, Mowry BJ, O'Donovan MC, Pulver AE, Schwab SG, Wildenauer DB, Dudbridge F, Shi J, Albus M, Alexander M, Campion D, Cohen D, Dikeos D, Duan J, Eichhammer P, Godard S, Hansen M, Lerer FB, Liang KY, Maier W, Mallet J, Nertney DA, Nestadt G, Norton N, Papadimitriou GN, Ribble R, Sanders AR, Silverman JM, Walsh D, Williams NM, Wormley B, Arranz MJ, Bakker S, Bender S, Bramon E, Collier D, Crespo-Facorro B, Hall J, Iyegbe C, Jablensky A, Kahn RS, Kalaydjieva L, Lawrie S, Lewis CM, Linszen DH, Mata I, McIntosh A, Murray RM, Ophoff RA, Van Os J, Walshe M, Weisbrod M, Wiersma D, Donnelly P, Barroso I, Blackwell JM, Brown MA, Casas JP, Corvin AP, Deloukas P, Duncanson A, Jankowski J, Markus HS, Mathew CG, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Spencer CC, Band G, Bellenguez C, Freeman C, Hellenthal G, Giannoulatou E, Pirinen M, Pearson RD, Strange A, Su Z, Vukcevic D, Langford C, Hunt SE, Edkins S, Gwilliam R, Blackburn H, Bumpstead SJ, Dronov S, Gillman M, Gray E, Hammond N, Jayakumar A, McCann OT, Liddle J, Potter SC, Ravindrarajah R, Ricketts M, Tashakkori-Ghanbaria A, Waller MJ, Weston P, Widaa S, Whittaker P, McCarthy MI, Stefansson K, Scolnick E, Purcell S, McCarroll SA, Sklar P, Hultman CM, Sullivan PF (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45:1150-9.

Robbins TW (1990) The case of frontostriatal dysfunction in schizophrenia. Schizophr Bull 16:391-402.

Robbins TW, Jones GH, Wilkinson LS (1996) Behavioural and neurochemical effects of early social deprivation in the rat. J Psychopharmacol 10:39-47.

Rogers RD, Baunez C, Everitt BJ, Robbins TW (2001) Lesions of the medial and lateral striatum in the rat produce differential deficits in attentional performance. Behav Neurosci 115:799-811.

Rosa ML, Silva RC, Moura-de-Carvalho FT, Brandao ML, Guimaraes FS, Del Bel EA (2005) Routine post-weaning handling of rats prevents isolation rearing-induced deficit in prepulse inhibition. Braz J Med Biol Res 38:1691-6.

Roseman AS, McGregor C, Thornton JE (2012) Estradiol attenuates the cognitive deficits in the novel object recognition task induced by sub-chronic phencyclidine in ovariectomized rats. Behav Brain Res 233:105-12.

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

Sabbagh JJ, Heaney CF, Bolton MM, Murtishaw AS, Kinney JW (2012) Examination of ketamine-induced deficits in sensorimotor gating and spatial learning. Physiol Behav 107:355-363.

Sams-Dodd F (1995) Distinct effects of d-amphetamine and phencyclidine on the social behaviour of rats. Behav Pharmacol 6:55-65.

Sams-Dodd F (1998a) Effects of continuous D-amphetamine and phencyclidine administration on social behaviour, stereotyped behaviour, and locomotor activity in rats. Neuropsychopharmacology 19:18-25.

Sams-Dodd F (1998b) A test of the predictive validity of animal models of schizophrenia based on phencyclidine and D-amphetamine. Neuropsychopharmacology 18:293-304.

Sanchez-Morla EM, Santos JL, Aparicio A, Garcia-Jimenez MA, Villanueva C, Martinez-Vizcaino V, Arango C (2009) Antipsychotic effects on auditory

sensory gating in schizophrenia patients. Eur Neuropsychopharmacol 19:905-9.

Sandyk R (1992) Pineal and habenula calcification in schizophrenia. Int J Neurosci 67:19-30.

Sannino S, Russo F, Torromino G, Pendolino V, Calabresi P, De Leonibus E (2012) Role of the dorsal hippocampus in object memory load. Learn Mem 19:211-8.

Savelyeva MV, Baldenkov GN, Kaverina NV (1988) Receptor binding potencies of chlorpromazine, trifluoperazine, fluphenazine and their 10-N-substituted analogues. Biomed Biochim Acta 47:1085-7.

Schafe GE, Atkins CM, Swank MW, Bauer EP, Sweatt JD, LeDoux JE (2000) Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of pavlovian fear conditioning. J Neurosci 20:8177-87.

Schenkel LS, Spaulding WD, DiLillo D, Silverstein SM (2005) Histories of childhood maltreatment in schizophrenia: relationships with premorbid functioning, symptomatology, and cognitive deficits. Schizophr Res 76:273-86.

Schmidt-Kastner R, van Os J, H WMS, Schmitz C (2006) Gene regulation by hypoxia and the neurodevelopmental origin of schizophrenia. Schizophr Res 84:253-71.

Schobel SA, Chaudhury NH, Khan UA, Paniagua B, Styner MA, Asllani I, Inbar BP, Corcoran CM, Lieberman JA, Moore H, Small SA (2013) Imaging patients with psychosis and a mouse model establishes a spreading pattern of hippocampal dysfunction and implicates glutamate as a driver. Neuron 78:81-93.

Schrijver NC, Bahr NI, Weiss IC, Wurbel H (2002) Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. Pharmacol Biochem Behav 73:209-24.

Schrijver NC, Pallier PN, Brown VJ, Wurbel H (2004) Double dissociation of social and environmental stimulation on spatial learning and reversal learning in rats. Behav Brain Res 152:307-14.

Schubert MI, Porkess MV, Dashdorj N, Fone KC, Auer DP (2009) Effects of social isolation rearing on the limbic brain: a combined behavioral and magnetic resonance imaging volumetry study in rats. Neuroscience 159:21-30.

Selten JP, Cantor-Graae E (2007) Hypothesis: social defeat is a risk factor for schizophrenia? Br J Psychiatry Suppl 51:s9-12.

Shahid M, Walker GB, Zorn SH, Wong EH (2009) Asenapine: a novel psychopharmacologic agent with a unique human receptor signature. J Psychopharmacol 23:65-73.

Shannon HE, Love PL (2004) Effects of antiepileptic drugs on working memory as assessed by spatial alternation performance in rats. Epilepsy Behav 5:857-65.

Shannon HE, Love PL (2005) Effects of antiepileptic drugs on attention as assessed by a five-choice serial reaction time task in rats. Epilepsy Behav 7:620-8.

Shannon HE, Love PL (2007) Effects of antiepileptic drugs on learning as assessed by a repeated acquisition of response sequences task in rats. Epilepsy Behav 10:16-25.

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

Shimazaki T, Kaku A, Chaki S (2010) D-Serine and a glycine transporter-1 inhibitor enhance social memory in rats. Psychopharmacology (Berl) 209:263-70.

Sigurdsson T, Doyere V, Cain CK, LeDoux JE (2007) Long-term potentiation in the amygdala: a cellular mechanism of fear learning and memory. Neuropharmacology 52:215-27.

Silva-Gomez AB, Rojas D, Juarez I, Flores G (2003) Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. Brain Res 983:128-36.

Simpson SM, Menard JL, Reynolds JN, Beninger RJ (2010) Post-weaning social isolation increases activity in a novel environment but decreases defensive burying and subchronic MK-801 enhances the activity but not the burying effect in rats. Pharmacol Biochem Behav 95:72-9.

Sircar R (2003) Postnatal phencyclidine-induced deficit in adult water maze performance is associated with N-methyl-D-aspartate receptor upregulation. Int J Dev Neurosci 21:159-67.

Smith MJ, Cobia DJ, Wang L, Alpert KI, Cronenwett WJ, Goldman MB, Mamah D, Barch DM, Breiter HC, Csernansky JG (2014) Cannabis-related working memory deficits and associated subcortical morphological differences in healthy individuals and schizophrenia subjects. Schizophr Bull 40:287-99.

Snigdha S, Neill JC (2008a) Efficacy of antipsychotics to reverse phencyclidine-induced social interaction deficits in female rats--a preliminary investigation. Behav Brain Res 187:489-94.

Snigdha S, Neill JC (2008b) Improvement of phencyclidine-induced social behaviour deficits in rats: involvement of 5-HT1A receptors. Behav Brain Res 191:26-31.

Snyder MA, Adelman AE, Gao WJ (2013) Gestational Methylazoxymethanol Exposure Leads to NMDAR Dysfunction in Hippocampus During Early Development and Lasting Deficits in Learning. Neuropsychopharmacology 38:328-40.

Sommer IE, Begemann MJ, Temmerman A, Leucht S (2012) Pharmacological augmentation strategies for schizophrenia patients with insufficient response to clozapine: a quantitative literature review. Schizophr Bull 38:1003-11.

Sorkin A, Peled A, Weinshall D (2005) Virtual reality testing of multi-modal integration in schizophrenic patients. Stud Health Technol Inform 111:508-14.

Sorkin A, Weinshall D, Modai I, Peled A (2006) Improving the accuracy of the diagnosis of schizophrenia by means of virtual reality. Am J Psychiatry 163:512-20.

St Clair D, Blackwood D, Muir W, Carothers A, Walker M, Spowart G, Gosden C, Evans HJ (1990) Association within a family of a balanced autosomal translocation with major mental illness. Lancet 336:13-6.

Stefani MR, Moghaddam B (2005a) Systemic and prefrontal cortical NMDA receptor blockade differentially affect discrimination learning and set-shift ability in rats. Behav Neurosci 119:420-8.

Stefani MR, Moghaddam B (2005b) Transient N-methyl-D-aspartate receptor blockade in early development causes lasting cognitive deficits relevant to schizophrenia. Biol Psychiatry 57:433-6.

Stojkovic T, Radonjic NV, Velimirovic M, Jevtic G, Popovic V, Doknic M, Petronijevic ND (2012) Risperidone reverses phencyclidine induced decrease in glutathione levels and alterations of antioxidant defense in rat brain. Prog Neuropsychopharmacol Biol Psychiatry 39:192-9.

Strauss L, Brink CB, Moller M, Stein DJ, Harvey BH (2014) Late-Life Effects of Chronic Methamphetamine Exposure during Puberty on Behaviour and Corticostriatal Mono-Amines in Social Isolation-Reared Rats. Dev Neurosci.

Sullivan PF (2005) The genetics of schizophrenia. PLoS Med 2:e212.

Sullivan PF (2013) Questions about DISC1 as a genetic risk factor for schizophrenia. Mol Psychiatry 18:1050-2.

Swerdlow NR, Geyer MA, Braff DL (2001) Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology (Berl) 156:194-215.

Swerdlow NR, Light GA, Cadenhead KS, Sprock J, Hsieh MH, Braff DL (2006a) Startle gating deficits in a large cohort of patients with schizophrenia: relationship to medications, symptoms, neurocognition, and level of function. Arch Gen Psychiatry 63:1325-35.

Swerdlow NR, Talledo J, Sutherland AN, Nagy D, Shoemaker JM (2006b) Antipsychotic effects on prepulse inhibition in normal 'low gating' humans and rats. Neuropsychopharmacology 31:2011-21.

Takahashi M, Kakita A, Futamura T, Watanabe Y, Mizuno M, Sakimura K, Castren E, Nabeshima T, Someya T, Nawa H (2006) Sustained brain-derived neurotrophic factor up-regulation and sensorimotor gating abnormality induced by postnatal exposure to phencyclidine: comparison with adult treatment. J Neurochem 99:770-80.

Talamini LM, Koch T, Ter Horst GJ, Korf J (1998) Methylazoxymethanol acetate-induced abnormalities in the entorhinal cortex of the rat; parallels with morphological findings in schizophrenia. Brain Res 789:293-306.

Tamaru M, Hirata Y, Nagayoshi M, Matsutani T (1988) Brain changes in rats induced by prenatal injection of methylazoxymethanol. Teratology 37:149-57.

Tamminga CA, Stan AD, Wagner AD (2010) The hippocampal formation in schizophrenia. Am J Psychiatry 167:1178-93.

Terry AV, Jr., Gearhart DA, Warner S, Hohnadel EJ, Middlemore ML, Zhang G, Bartlett MG, Mahadik SP (2007) Protracted effects of chronic oral haloperidol and risperidone on nerve growth factor, cholinergic neurons, and spatial reference learning in rats. Neuroscience 150:413-24.

Terry AV, Jr., Hill WD, Parikh V, Evans DR, Waller JL, Mahadik SP (2002) Differential effects of chronic haloperidol and olanzapine exposure on brain cholinergic markers and spatial learning in rats. Psychopharmacology (Berl) 164:360-8.

Terry AV, Jr., Hill WD, Parikh V, Waller JL, Evans DR, Mahadik SP (2003) Differential effects of haloperidol, risperidone, and clozapine exposure on cholinergic markers and spatial learning performance in rats. Neuropsychopharmacology 28:300-9.

Thomson DM, McVie A, Morris BJ, Pratt JA (2010) Dissociation of acute and chronic intermittent phencyclidine-induced performance deficits in the 5-choice serial reaction time task: influence of clozapine. Psychopharmacology (Berl) 213:681-95.

Thornton C, Rousset CI, Kichev A, Miyakuni Y, Vontell R, Baburamani AA, Fleiss B, Gressens P, Hagberg H (2012) Molecular mechanisms of neonatal brain injury. Neurol Res Int 2012:506320.

Tiihonen J, Wahlbeck K, Kiviniemi V (2009) The efficacy of lamotrigine in clozapine-resistant schizophrenia: a systematic review and meta-analysis. Schizophr Res 109:10-4.

Toua C, Brand L, Moller M, Emsley RA, Harvey BH (2010) The effects of sub-chronic clozapine and haloperidol administration on isolation rearing induced changes in frontal cortical N-methyl-D-aspartate and D1 receptor binding in rats. Neuroscience 165:492-9.

Tunstall B, Beckett S, Mason R (2009) Ultrasonic vocalisations explain unexpected effects on pre-pulse inhibition responses in rats chronically pre-treated with phencyclidine. Behav Brain Res 202:184-91.

Turetsky BI, Moberg PJ, Roalf DR, Arnold SE, Gur RE (2003) Decrements in volume of anterior ventromedial temporal lobe and olfactory dysfunction in schizophrenia. Arch Gen Psychiatry 60:1193-200.

Turnock-Jones JJ, Jennings CA, Robbins MJ, Cluderay JE, Cilia J, Reid JL, Taylor A, Jones DN, Emson PC, Southam E (2009) Increased expression of the NR2A NMDA receptor subunit in the prefrontal cortex of rats reared in isolation. Synapse 63:836-46.

Uehara T, Sumiyoshi T, Seo T, Itoh H, Matsuoka T, Suzuki M, Kurachi M (2009) Long-term effects of neonatal MK-801 treatment on prepulse inhibition in young adult rats. Psychopharmacology (Berl) 206:623-30.

Van Erp TG, Saleh PA, Rosso IM, Huttunen M, Lonnqvist J, Pirkola T, Salonen O, Valanne L, Poutanen VP, Standertskjold-Nordenstam CG, Cannon TD (2002) Contributions of genetic risk and fetal hypoxia to hippocampal volume in patients with schizophrenia or schizoaffective disorder, their unaffected siblings, and healthy unrelated volunteers. Am J Psychiatry 159:1514-20.

Varty GB, Higgins GA (1995) Examination of drug-induced and isolation-induced disruptions of prepulse inhibition as models to screen antipsychotic drugs. Psychopharmacology (Berl) 122:15-26.

Varty GB, Marsden CA, Higgins GA (1999) Reduced synaptophysin immunoreactivity in the dentate gyrus of prepulse inhibition-impaired isolation-reared rats. Brain Res 824:197-203.

Varty GB, Paulus MP, Braff DL, Geyer MA (2000) Environmental enrichment and isolation rearing in the rat: effects on locomotor behavior and startle response plasticity. Biol Psychiatry 47:864-73.

Vayisoglu S, Anil Yagcioglu AE, Yagcioglu S, Karahan S, Karci O, Gurel SC, Yazici MK (2013) Lamotrigine augmentation in patients with schizophrenia who show partial response to clozapine treatment. Schizophr Res 143:207-14.

Vollenweider FX, Barro M, Csomor PA, Feldon J (2006) Clozapine enhances prepulse inhibition in healthy humans with low but not with high prepulse inhibition levels. Biol Psychiatry 60:597-603.

Vorhees CV, Fernandez K, Dumas RM, Haddad RK (1984) Pervasive hyperactivity and long-term learning impairments in rats with induced micrencephaly from prenatal exposure to methylazoxymethanol. Brain Res 317:1-10.

Vuillermot S, Joodmardi E, Perlmann T, Ogren SO, Feldon J, Meyer U (2012) Prenatal immune activation interacts with genetic Nurr1 deficiency in the development of attentional impairments. J Neurosci 32:436-51.

Wang C, McInnis J, Ross-Sanchez M, Shinnick-Gallagher P, Wiley JL, Johnson KM (2001) Long-term behavioral and neurodegenerative effects of perinatal phencyclidine administration: implications for schizophrenia. Neuroscience 107:535-50.

Wang CZ, Johnson KM (2005) Differential effects of acute and subchronic administration on phencyclidine-induced neurodegeneration in the perinatal rat. J Neurosci Res 81:284-92.

Wang CZ, Yang SF, Xia Y, Johnson KM (2008) Postnatal phencyclidine administration selectively reduces adult cortical parvalbumin-containing interneurons. Neuropsychopharmacology 33:2442-55.

Wang JF, Shao L, Sun X, Young LT (2009) Increased oxidative stress in the anterior cingulate cortex of subjects with bipolar disorder and schizophrenia. Bipolar Disord 11:523-9.

Wang Z, Wang L, Yamamoto R, Sugai T, Kato N (2013) Role of the lateral habenula in shaping context-dependent locomotor activity during cognitive tasks. Neuroreport 24:276-80.

Watson DJ, Loiseau F, Ingallinesi M, Millan MJ, Marsden CA, Fone KC (2012a) Selective blockade of dopamine D3 receptors enhances while D2 receptor antagonism impairs social novelty discrimination and novel object recognition in rats: a key role for the prefrontal cortex. Neuropsychopharmacology 37:770-86.

Watson DJ, Marsden CA, Millan MJ, Fone KC (2012b) Blockade of dopamine D(3) but not D(2) receptors reverses the novel object discrimination impairment produced by post-weaning social isolation: implications for schizophrenia and its treatment. Int J Neuropsychopharmacol 15:471-84.

Weiss IC, Pryce CR, Jongen-Relo AL, Nanz-Bahr NI, Feldon J (2004) Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behav Brain Res 152:279-95.

Wible CG, Anderson J, Shenton ME, Kricun A, Hirayasu Y, Tanaka S, Levitt JJ, O'Donnell BF, Kikinis R, Jolesz FA, McCarley RW (2001) Prefrontal cortex, negative symptoms, and schizophrenia: an MRI study. Psychiatry Res 108:65-78.

Wicks S, Hjern A, Gunnell D, Lewis G, Dalman C (2005) Social adversity in childhood and the risk of developing psychosis: a national cohort study. Am J Psychiatry 162:1652-7.

Wiley JL, Buhler KG, Lavecchia KL, Johnson KM (2003) Pharmacological challenge reveals long-term effects of perinatal phencyclidine on delayed spatial alternation in rats. Prog Neuropsychopharmacol Biol Psychiatry 27:867-73.

Wilkinson LS, Killcross SS, Humby T, Hall FS, Geyer MA, Robbins TW (1994) Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent inhibition. Neuropsychopharmacology 10:61-72.

Williams HJ, Zamzow CR, Robertson H, Dursun SM (2006) Effects of clozapine plus lamotrigine on phencyclidine-induced hyperactivity. Prog Neuropsychopharmacol Biol Psychiatry 30:239-43.

Willner P (1984) The validity of animal models of depression. Psychopharmacology (Berl) 83:1-16.

Winters BD, Forwood SE, Cowell RA, Saksida LM, Bussey TJ (2004) Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. J Neurosci 24:5901-8.

Wolff AR, Bilkey DK (2010) The maternal immune activation (MIA) model of schizophrenia produces pre-pulse inhibition (PPI) deficits in both juvenile and adult rats but these effects are not associated with maternal weight loss. Behav Brain Res 213:323-7.

Wolkowitz OM, Pickar D (1991) Benzodiazepines in the treatment of schizophrenia: a review and reappraisal. Am J Psychiatry 148:714-26.

Wolpowitz D, Mason TB, Dietrich P, Mendelsohn M, Talmage DA, Role LW (2000) Cysteine-rich domain isoforms of the neuregulin-1 gene are required for maintenance of peripheral synapses. Neuron 25:79-91.

Wongwitdecha N, Marsden CA (1996a) Effects of social isolation rearing on learning in the Morris water maze. Brain Res 715:119-24.

Wongwitdecha N, Marsden CA (1996b) Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. Behav Brain Res 75:27-32.

Woo TU, Whitehead RE, Melchitzky DS, Lewis DA (1998) A subclass of prefrontal gamma-aminobutyric acid axon terminals are selectively altered in schizophrenia. Proc Natl Acad Sci U S A 95:5341-6.

Wood SJ, Yung AR, McGorry PD, Pantelis C (2011) Neuroimaging and treatment evidence for clinical staging in psychotic disorders: from the at-risk mental state to chronic schizophrenia. Biol Psychiatry 70:619-25.

Woods S, Clarke NN, Layfield R, Fone KC (2012) 5-HT(6) receptor agonists and antagonists enhance learning and memory in a conditioned emotion response paradigm by modulation of cholinergic and glutamatergic mechanisms. Br J Pharmacol 167:436-49.

Woodward ND, Purdon SE, Meltzer HY, Zald DH (2005) A meta-analysis of neuropsychological change to clozapine, olanzapine, quetiapine, and risperidone in schizophrenia. Int J Neuropsychopharmacol 8:457-72.

Wynn JK, Green MF, Sprock J, Light GA, Widmark C, Reist C, Erhart S, Marder SR, Mintz J, Braff DL (2007) Effects of olanzapine, risperidone and haloperidol on prepulse inhibition in schizophrenia patients: a double-blind, randomized controlled trial. Schizophr Res 95:134-42.

Xu X, Domino EF (1994) Asymmetric cross-sensitization to the locomotor stimulant effects of phencyclidine and MK-801. Neurochem Int 25:155-9.

Yee N, Ribic A, de Roo CC, Fuchs E (2011) Differential effects of maternal immune activation and juvenile stress on anxiety-like behaviour and physiology in adult rats: no evidence for the "double-hit hypothesis". Behav Brain Res 224:180-8.

Yoo HJ, Lee SA, Kim SY, Kang JG, Lee JG (2006) Compromised memory function in schizophrenia and temporal lobe epilepsy. J Neuropsychiatry Clin Neurosci 18:199-207.

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.

Zhang J, Abdallah CG, Wang J, Wan X, Liang C, Jiang L, Liu Y, Huang H, Hong X, Huang Q, Wu R, Xu C (2012a) Upregulation of adenosine A2A receptors induced by atypical antipsychotics and its correlation with sensory gating in schizophrenia patients. Psychiatry Res 200:126-32.

Zhang R, He J, Zhu S, Zhang H, Wang H, Adilijiang A, Kong L, Wang J, Kong J, Tan Q, Li XM (2012b) Myelination deficit in a phencyclidine-induced neurodevelopmental model of schizophrenia. Brain Res 1469:136-43.

Zhang ZJ, Reynolds GP (2002) A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. Schizophr Res 55:1-10.

Zhao X, Qin S, Shi Y, Zhang A, Zhang J, Bian L, Wan C, Feng G, Gu N, Zhang G, He G, He L (2007) Systematic study of association of four GABAergic genes: glutamic acid decarboxylase 1 gene, glutamic acid decarboxylase 2 gene, GABA(B) receptor 1 gene and GABA(A) receptor subunit beta2 gene, with schizophrenia using a universal DNA microarray. Schizophr Res 93:374-84.

Zhao X, Sun L, Jia H, Meng Q, Wu S, Li N, He S (2009) Isolation rearing induces social and emotional function abnormalities and alters glutamate and neurodevelopment-related gene expression in rats. Prog Neuropsychopharmacol Biol Psychiatry 33:1173-7.

Zheng C, Yang K, Liu Q, Wang MY, Shen J, Valles AS, Lukas RJ, Barrantes FJ, Wu J (2010) The anticonvulsive drug lamotrigine blocks neuronal {alpha}4{beta}2 nicotinic acetylcholine receptors. J Pharmacol Exp Ther 335:401-8.