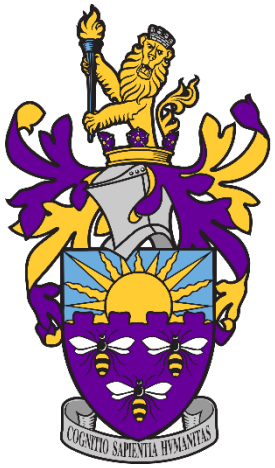
Investigation into effects of chronic treatment with antipsychotics using a combination of behavioural and electrophysiological techniques



A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Biology, Medicine and Health

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Abstract

Schizophrenia is a chronic and severe psychiatric disorder that follows a remitting and relapsing course of action. Impaired cognitive functions are a core feature of schizophrenia, which persist throughout the patients' life despite life-long treatment with antipsychotics (APs). While the neurocognitive effects of long-term AP treatment remain unclear, several lines of evidence point towards its detrimental impact on cognition, accompanied by structural brain alterations in patients with schizophrenia. Pre-clinical models provide a platform for systematic investigation into the neurocognitive effects of long-term AP treatment. However, model results so far lack translational validity due to methodological limitations. Substantial evidence suggests that disruptions in the functional interaction between the hippocampal formation (HF) and medial prefrontal cortex (mPFC) contributes to the cognitive impairments associated with the disease. In particular, pre-clinical investigations emphasise a key role for the direct pathway from the ventral hippocampus (vHipp) to the mPFC in mediating higher-order cognitive functions; these include episodic memory, executive function and goal-directed behaviour, deficits in which are well documented in patients with schizophrenia. Timely transfer and accurate processing of information between brain regions is governed by processes of synaptic plasticity which are thought to be modulated by AP treatments. Modulations of synaptic plasticity in this pathway in response to long-term treatment with APs could advance current understanding of APs' mechanism of action on cognition and its neural correlates. Since studying processes of synaptic plasticity are challenging in humans, their examination in pre-clinical models is essential.

Using the well-validated sub-chronic (sc) phencyclidine (PCP; scPCP) model for cognitive impairments associated with schizophrenia, this project investigated the neurocognitive effects of long-term treatment with haloperidol and olanzapine to address some of the methodological issues associated with pre-clinical research in this field. Performance in two variations of the novel object recognition (NOR) task formed the primary measure of cognition in the studies reported here (**Chapters 3, 4** and **6**). The disrupted NOR (dNOR; classic one-trial NOR test) test was employed to examine the ability of long-term AP treatment to rescue scPCP-induced memory deficits. In contrast to dNOR, performance of scPCP-treated rats is intact in the continuous NOR (cNOR). Therefore, cNOR was employed to examine potentially negative effects of long-term treatment with APs. Furthermore, through *in vivo* electrophysiological recordings under anaesthetised conditions, this project characterised the synaptic properties (synaptic connectivity, short- and long-term synaptic plasticity) of the vHipp-mPFC pathway in the scPCP model for the first time (**Chapter 5**). This was followed by an investigation into the impact of long-term haloperidol treatment on synaptic properties of the vHipp-mPFC pathway in the scPCP model (**Chapter 6**).

Results presented in **Chapter 3** were inconclusive in determining the influence of 22 days of treatment with haloperidol (0.1 mg/kg/day, oral administration; p.o.) and olanzapine (1.5 mg/kg/day; p.o.) on dNOR performance. Performance was assessed once weekly on days 1, 8, 15 and 22 of AP treatment and at two

other time points during treatment washout period. In this study, the presence of a robust scPCP-induced dNOR deficit could not be confirmed. It was reasoned that this could have been caused by excess handling prior to scPCP dosing and during AP treatment period. Furthermore, high variability in the dNOR outcome rendered the findings of this study inconclusive. In **Chapter 4**, investigations were limited to haloperidol (0.5mg/kg/day), which was delivered via subcutaneous osmotic minipumps over 28 days. In addition to the dNOR test, the cNOR test was also introduced as a measure of cognition. Tests were repeated at 6 time points throughout the study (1 dNOR and cNOR assessment prior to osmotic minipump implant and 2 other dNOR and cNOR testing sessions post minipump implant). In addition to dNOR, the effectiveness of scPCP treatment in this study was also examined by assessment of its locomotor activity response to an acute dose of amphetamine (1mg/kg) administered intraperitoneally (i.p.). While results of the dNOR test (prior to implant) could not confidently confirm the effectiveness of scPCP treatment, findings of the locomotor activity in response to amphetamine suggested that the scPCP treatment had been effective. Overall, the behavioural findings of this study were also inconclusive due to performance variability. By limiting the number of NOR testing sessions, **Chapter 6**, following the same treatment plan as **Chapter 4**, showed that 28 days of treatment with haloperidol did not impair cognitive performance in the cNOR task in scPCP and control rats. Investigations into the synaptic properties of the vHipp-mPFC pathway (**Chapter 5**), showed a significant reduction in strength of glutamatergic synaptic connectivity from vHipp to the mPFC in scPCP treated rats, although the vHipp-mPFC pathway was still able to support synaptic facilitation and long-term potentiation in scPCP treated rats. The general pattern of the results points towards compromised inhibitory mechanisms manifested as hyper excitability/plasticity in this pathway in the scPCP model, which is consistent with studies in other animal models of the disease. Investigations in this chapter further suggested a significant reduction in the excitability threshold in the scPCP treated rats, an effect which might also involve disturbances in β-adrenoceptor-mediated effects. As presented in **Chapter 6**, 28 days of treatment with haloperidol appeared to have reduced the strength of synaptic connectivity in the sub-chronic Vehicle (0.9% saline; scVeh) treated rats. This effect did not reach statistical significance in comparison to the scVeh-control treatment group. This trend was absent in the haloperidol treated scPCP rats in comparison to its control. The amplitude of synaptic connectivity was, however, appeared to have been reduced (also not statistically significant) to the same extent in both scVeh and scPCP treatment groups in response to long-term haloperidol treatment. This may point towards the effect of haloperidol in increasing the activity of a subset of inhibitory mechanisms, which may be involved in regulating response strength and size. Interestingly, investigations into short- and long-term synaptic plasticity showed that long-term haloperidol treatment induced a state of hyper excitability/plasticity in the vHipp-mPFC pathway in both scVeh and scPCP rats, which was significantly more robust in the scPCP treatment groups. These results suggest that the observed hyper-excitability may be due to disruptions in $GABA_B$-D2-NMDA receptor interaction. Collectively, these results suggest that different inhibitory mechanisms may be involved in regulating the vHipp-mPFC responses, which may be differentially affected by haloperidol.

In conclusion, the behavioural studies presented in this thesis highlighted that the scPCP model is susceptible to the effects of handling, which can interfere with study outcome. In addition, these studies suggested, that repeated administration of dNOR and cNOR tests, results in pronounced performance variability, which leads to ambiguous findings. In spite of these challenges, the behavioural studies presented in this thesis were able to demonstrate that, for the duration studied, haloperidol did not impair performance on the cNOR task in the scPCP and scVeh treatment groups. Through the use of electrophysiological techniques, the studies presented in this thesis were able to investigate previously unexplored aspects of the scPCP model, which contributes to its validity with relevance to cognitive impairments associated with schizophrenia. Results of these studies demonstrated deficits in synaptic connectivity and highlighted a general reduction in inhibitory tone, manifested as hyper excitability/plasticity in the vHipp-mPFC pathway in scPCP-treated rats. These disturbances, which may be responsible for cognitive impairments in schizophrenia, were further exacerbated by long-term haloperidol treatment. Functional consequences of these disturbances were not reflected in the behavioural paradigms employed, as these tests do not depend on vHipp-mPFC interaction for successful performance. Further studies are required to determine the behavioural and cognitive impact of these synaptic alterations using more complex and sensitive behavioural paradigms, which engage the vHipp-mPFC pathway. In addition to its role in mediating cognitive processes, disruptions in the activity of the vHipp-mPFC pathway, specifically the hyperactivity of the vHipp, are also thought to be involved in psychosis. The hyper-excitability in the vHipp-mPFC pathway following long-term treatment with haloperidol may be indicative of the processes of dopamine super-sensitivity and AP-induced psychosis, instances of which are observed in the clinic. Therefore, investigations into synaptic properties of the vHipp-mPFC pathway may provide a platform for better understanding of disease processes and contribute to advancements in novel drug development with improved efficacy in treating positive symptoms and cognitive impairments associated with schizophrenia.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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I dedicate this thesis to my mother, Homeyra and my father, Hatef. Two angels who sacrificed everything so I can be here.

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“He is God! How can we render Thee thanks, O Lord? Thy bounties are endless and our gratitude cannot equal them. How can the finite utter praise of the Infinite? Unable are we to voice our thanks for Thy favours and in utter powerlessness we turn wholly to Thy Kingdom beseeching the increase of Thy bestowals and bounties. Thou art the Giver, the Bestower, the Almighy. “
Abdu’l-Baha

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In close, I would like to remember all the Bahai’ youth in Iran, who have been wrongfully deprived of their right to access higher education over the past four decades. I pray for the day that each and every one of us has equal opportunities to learn and fulfil their potential.

به پایان آمد این دفتر، حکایت همچنان باقیست ...

Preface

I. List of Publications

Sahin, C., Doostdar, N. & Neill, J.C. (2016). Towards the development of improved tests for negative symptoms of schizophrenia in a validated animal model. *Behavioural Brain Research,* 312,93-101.

Cadinu, D., Grayson, B., Podda, G., Harte, M. K., Doostdar, N. & Neill, J.C. (2017). NMDA receptor antagonist rodent models for cognition in schizophrenia and identification of novel drug treatments, an update. *Neuropharmacology.* 142,41-62.

Doostdar. N., Kim, E., Grayson, B., Harte, M.K., Neill, J.C., Vernon, A.C. (2018). Global brain atrophy accompanies cognitive dysfunction in the sub-chronic phencyclidine animal model for schizophrenia. *J. Psychopharmacology* (This paper is currently in review).

II. Prizes and Awards

President's Doctoral Scholar (PDS) Award: This is a prestigious and highly competitive scheme at the University of Manchester which provides funding opportunities for outstanding students. I was granted the President's Doctoral Scholar's award in 2015. This award paid for the tuition fees for the duration of this project.

Certificate in Non-clinical Psychopharmacology: This certificate was awarded by the British Association for Psychopharmacology upon attending the residential training course held in Cambridge March 2016.

Poster Prize: Winner of the BAP "Pre-clinical Poster Prize" in 2017, awarded for poster presented on the study described in **Chapter 5.**

III. Conference Attendance and Presentations

University of Manchester Pharmacy and Optometry show-case: 2016

Poster presentation at the British Association for Psychopharmacology (BAP): July 2017 – The abstract was selected for a short oral presentation at the same meeting.

Poster presentation at British Neuroscience Association (BNA): April 2017

List of Abbreviations

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid **(AMPA)**
AMPA Receptor **(AMPAR)**
Amphetamine **(Amph)**
Anterior to Bregma **(B+)**
Antipsychotic **(AP)**
Antipsychotics **(APs)**
Area Under the Curve **(AUC)**
Brain-derived Neurotrophic Factor **(BDNF)**
Calcium-calmodulin-dependent protein kinase II **(CaMKII)**
Chlorpromazine **(CLP)**
Clinical Antipsychotic Trials of Intervention Effectiveness **(CATIE)**
Clozapine **(Clz)**
Continuous Novel Object Recognition **(cNOR)**
D-2-amino-5-phosphonovalerate **(D-AP5)**
Discrimination Index **(DI)**
Disrupted in Schizophrenia 1 **(DISC-1)**
Disrupted Novel Object Recognition **(dNOR)**
Dopamine D2 Receptor **(D2R)**
Dorsal Hippocampus **(dHipp)**
Dorsolateral prefrontal cortex **(dLPFC)**
Dulbecco's Phosphate-Buffered Saline **(DPBS)**
Extrapyramidal Side-effects **(EPS)**
Field Excitatory post-synaptic potential **(fEPSP)**
First Episode psychosis **(FEP)**
Gamma-Aminobutyric Acid **(GABA)**
Haloperidol **(Hal)**
High-affinity D2R **($D2^{High}R$)**
High Frequency Stimulation **(HFS)**
Hippocampal Formation **(HF)**
Individually Ventilated Cages **(IVC)**
Infralimbic **(IL)**
Inhibitory post-synaptic potential **(IPSP)**
Input-Output **(I/O)**
Inter-Pulse Interval **(IPI)**
Inter-Trial Interval **(ITI)**
Interval Between Pairs **(IBP)**
Intraperitoneal injection **(i.p.)**
Lister Hooded **(LH)**
Locomotor Activity **(LMA)**
Locus Coeruleus **(LC)**
Long-term synaptic Depression **(LTD)**
Long-term synaptic Potentiation **(LTP)**
Low Frequency Stimulation **(LFS)**
Measurement And Treatment Research to Improved Cognition in Schizophrenia **(MATRICS)**
MATRICS Consensus Cognitive Battery **(MCCB)**
Medial Prefrontal cortex **(mPFC)**
Medial-Lateral **(ML)**
Methylazoxymethanol **(MAM)**
N-methyl-D-aspartate **(NMDA)**
NMDA Receptor **(NMDAR)**
Noradrenaline **(NA)**
Not significant **(N.S.)**
Novel Object Recognition **(NOR)**
Nucleus Accumbens **(NAcc)**
Nucleus Reuniens **(RE)**
Olanzapine **(Olz)**
Oral administration **(p.o.)**
Paired-Associative Stimulation **(PAS)**
Pairs of Pulses **(PP)**
Paired-Pulse Depression **(PPD)**
Paired-Pulse Facilitation **(PPF)**
Paired-Pulse Index **(PPI)**
Paired-Pulse Stimulation **(PPS)**
Paraformaldehyde **(PFA)**
Parvalbumin **(PV)**
Perirhinal Cortex **(PRh)**

Perphenazine **(Phz)**

Phencyclidine **(PCP)**

Polyriboinosinic-polyribocytidylic acid **(Poly I:C)**

Positron Emission Topography **(PET)**

Posterior to Bregma **(B-)**

Post-synaptic density protein of 95 kDa **(PSD-95)**

Post-tetanic Potentiation **(PTP)**

Prefrontal Cortex **(PFC)**

Prelimbic **(Prl)**

Propranolol **(Pro)**

Protein Kinase A **(PKA)**

Protein Kinase C **(PKC)**

Proton Magnetic Resonance Spectroscopy **(H^+MRS)**

Quetiapine **(QT)**

Risperidone **(Ris)**

Serotonin Receptor **(5-HTR)**

Short-term synaptic plasticity **(STP)**

Sub-chronic **(sc)**

Sub-chronic Phencyclidine **(scPCP)**

Sub-chronic Vehicle **(scVeh)**

Subcutaneous Injection **(s.c.)**

Synaptosomal-associated protein of 25 kDa **(SNAP-25)**

Ultra-High Risk **(UHR)**

Ventral Hippocapus **(vHipp)**

Ventral Tegmental Area **(VTA)**

Ventrolateral prefrontal cortex **(vLPFC)**

Washout **(WO)**

Ziprasidone **(Zip)**

5-Choice Serial Reaction Time Test **(5CSRTT)**

4′,6-Diamidine-2′-phenylindole dihydrochloride **(DAPI)**

67 kDa isoform of glutamic acid decarboxylase **(GAD_{67})**

Chapter 1

General Introduction

1.1. Schizophrenia- An overview

Schizophrenia is a multifactorial and severely debilitating psychiatric disorder (Ibrahim and Tamminga, 2011). With higher prevalence in men, schizophrenia affects approximately 23 million people worldwide (McGrath et al., 2008; Ochoa et al., 2012; World Health Organisation, 2018). This disorder tends to manifest earlier in men (late teens to early twenties) than women (late twenties to early thirties) (Trivedi and Jarbe, 2011; Ochoa et al., 2012) and follows a chronic, relapsing and remitting course of action upon onset (Wong and Van Tol, 2003; Ochoa et al., 2012).

The aetiology of schizophrenia is complex and far from being fully elucidated (Tandon et al., 2008). In the past two decades, the importance of environmental factors and prenatal events in elevating the risk of schizophrenia development is increasingly more understood (Brown, 2006; Kirkbride et al., 2010; Kirkbride et al., 2017). Amongst known risk factors, the significant role of genetic factors in schizophrenia development is most highlighted. Substantial evidence from familial and twin studies (Tiwari et al., 2010; Gejman et al., 2011) suggest that with an estimated mean heritability rate of 81%, schizophrenia is one of the most heritable psychiatric conditions (Gejman et al., 2011; Chen et al., 2015). So far, more than 108 genomic loci have been identified as risk factors for the development of schizophrenia. Many of these loci consist of genes that are involved in the regulation of glutamatergic and GABAergic neurotransmitter systems and regulation of synaptic and neuronal structure (Escudero and Johnstone, 2014; Tansey et al., 2015).

At the neurobiological level, schizophrenia is characterised by specific structural brain abnormalities (Connor et al., 2011; Haijma et al., 2013; van Erp et al., 2016; van Erp et al., 2018). These are accompanied by disturbances in major neurotransmitter systems including those responsible for glutamatergic (Javitt, 2012; Javitt et al., 2012; Moghaddam and Javitt, 2012), GABAergic (Taylor and Tso, 2015), dopaminergic (Howes and Kapur, 2009; Howes et al., 2015) and cholinergic neurotransmission (Bencherif et al., 2012). Such disturbances are thought to contribute to the manifestation of disorder symptoms (Wong and Van Tol, 2003; Rubio et al., 2012).

The symptoms of schizophrenia are diverse and are grouped into three separate domains (Tandon et al., 2009) . The positive symptoms are defined by their presence and include abnormal sensory experiences (hallucinations) and holding unusual beliefs despite evidence to contrary (delusions). The negative symptoms are defined by their absence and are manifested as deficits in emotional regulation, withdrawal from friends and society, blunted affect and lack of volition (Schultz et al., 2007; Newman-Tancredi and Kleven, 2011). The most debilitating symptomatic domain are those of cognitive impairments. These deficits span all measurable domains of cognition (Keefe and Harvey, 2012; Keefe et al., 2013; Tandon et al., 2013) and strongly compromise the quality of life and the functional outcome (occupation, relationships) of the patients (Green et al., 2000; Green et al., 2004; Kahn and Keefe, 2013; Green et al., 2015). Given the focus

of this thesis on the cognitive impairments associated with the disease, this symptomatic domain is discussed in more detail in **Section 1.2**.

Antipsychotic (AP) medications are the first line of treatment for the management of schizophrenia (NICE-Guidelines, 2014). The effectiveness of short-term treatment (up to two years) with typical and atypical APs (discussed in **Section 1.6**) in management of psychosis is well established (Goff et al., 2017). Based on the current guidelines, patients are recommended to remain on AP treatment over the long-term (NICE Guidelines 2014). Given the chronic nature of schizophrenia, treatment with these compounds tends to be life-long (Moncrieff, 2015; Goff et al., 2017). Whether prolonged treatment with antipsychotics (APs) is detrimental to the clinical outcome (remission from or relapse to psychosis) remains a matter of intense debate. Several lines of evidence from clinical studies suggest that prolonged AP treatment is associated with loss of treatment efficacy, resulting in increasing rate of relapse and poorer functional and symptomatic recovery (Leucht et al., 2012; Wunderink et al., 2013; Yin et al., 2017). Whilst pre-clinical studies have linked this effect to the phenomenon of dopamine D2 receptor (D2R) super-sensitivity (Samaha et al., 2007), the evidence for this in clinical studies is not compelling (Goff et al., 2017).

It is important to note that relapse rate in medicated patients is considerably lower than for patients who discontinue medication (Leucht et al., 2012; NICE-Guidelines, 2014; Goff et al., 2017). In case of relapse while on medication, patients are switched to a different AP and will be treated with higher doses of the drug (Yin et al., 2017). Clinical imaging studies have provided evidence that higher dose and longer duration of AP treatment reduces brain grey matter volume in patients with schizophrenia (Connor et al., 2011; Ho et al., 2011; Fusar-Poli et al., 2013; Haijma et al., 2013). Brain tissue loss is also associated with elevated risk of relapse (Andreasen et al., 2013; Walton et al., 2017) and cognitive decline (Andreasen et al., 2011; Ho et al., 2003; Jirsaraie et al., 2018; Dempster et al., 2017; Pol and Kahn, 2008) both of which have major implications on the functional outcome of the patients (Green et al., 2015). It has been suggested that gradual dose reduction and eventually treatment discontinuation might offer a better chance of recovery than treatment maintenance (Wunderink et al., 2013; Goff et al., 2017), however, evidence for this is still at its infancy. Goff and colleagues (2017) argue that a small population of patients may stay in remission for prolonged periods off medication or benefit from dose reduction. However, since there is no marker that can predict treatment response, maintenance on APs is safer when weighed against potential relapse.

It is noteworthy that the lifetime expectancy of patients with schizophrenia is almost 25 years shorter than for the general population (McGrath et al., 2008; Gejman et al., 2011; Tenback et al., 2012). Cardiovascular complications are the leading cause of death in patients with schizophrenia (Ringen et al., 2014; Olfson et al., 2015), which can be directly correlated with their high rate of smoking. Similarly, several lines of evidence support the negative impact of AP treatments on general health and life expectancy. It has been suggested that typical APs may promote smoking as a form of self-medication in patients to counteract treatment side effects (Tenback et al., 2012). Metabolic dysfunctions associated with atypical APs could also contribute to the development of cardiovascular complications (Ringen et al., 2014; Olfson et al.,

2015). Reports also suggest that treatment with haloperidol, risperidone and quetiapine is associated with higher rate of mortality in comparison to perphenazine, while this is significantly lower upon treatment with clozapine (Tiihonen et al., 2009). Evidence of reduced mortality upon AP treatment compared to no treatment has also been reported (Tiihonen et al., 2009). These reports in addition to the findings of reduced grey and white matter volume due to treatment with APs (Connor et al., 2011; Fusar-Poli et al., 2013) highlights the potentially damaging effects of long-term treatment with APs.

Evidence also supports the negative impact of brain volume change on cognition, suggesting that long-term treatment with APs may be detrimental to cognitive processing (Andreasen et al., 2011; Ho et al., 2003; Jirsaraie et al., 2018; Dempster et al., 2017; Pol and Kahn, 2008). Nevertheless, examining the neurocognitive effects of APs has proven difficult (discussed in **Section 1.7**) leaving treatment of this symptomatic domain a clinically unmet need. It is clear that there is a need to conduct further research into understanding the neurocognitive effects of long-term AP treatment. This process is aided by pre-clinical animal models for the disease, allowing for better understanding of disease process and development of new treatments for cognitive impairments associated with schizophrenia.

1.2. Symptoms of cognitive impairments

From the earliest conceptualisation of schizophrenia by Kraepelin and Bleuler deficits in cognitive performance were recognised as core features of the disease (Giuliano et al., 2012; Kahn and Keefe, 2013). Severe to moderate cognitive dysfunction is found in almost all of the measurable domains of cognition (Keefe et al., 2007b; Keefe and Fenton, 2007; Bortolato et al., 2015). This ranges from basic level perceptual functioning, including early visual processing (Symond et al., 2005) and early attentional processing (Jeon and Polich, 2003), to higher-order cognitive abilities (Dickinson and Harvey, 2009). Through careful factor analysis and expert consensus, the MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) launched by the National Institute of Mental Health has identified seven primary higher-order cognitive domains affected in schizophrenia (Green et al., 2014). These 7 domains include executive function, attention, working memory, verbal learning and memory, visual learning and memory, speed of processing and social cognition (Green, 2006; Keefe and Harvey, 2012).

In patients with schizophrenia, the mean score of cognitive performance falls almost 1 to 2 standard deviations below that of the normal population (Keefe and Fenton, 2007; Galderisi et al., 2009; Hill et al., 2010; Trampush et al., 2015). These deficits are reported to be largest in executive function, verbal and visual learning and memory (Bozikas et al., 2006) as well as attention (Sitskoorn et al., 2004). In comparison to other psychiatric disorders, such as bipolar disorder, all domain cognitive performance is more widely and profoundly disrupted in patients with schizophrenia. In these patients, the composite scores of cognitive performance are 0.5 standard deviations below the mean performance of patients with bipolar disorder and deficits are more pronounced in verbal fluency, executive function and working memory in patients with

schizophrenia in comparison to such patients (Krabbendam et al., 2005; Keefe and Fenton, 2007; Bora, 2015; Bortolato et al., 2015). It has been argued that these characteristic differences in the pattern of cognitive deficits in schizophrenia compared to other psychiatric disorders can be used for a more reliable and accurate diagnosis (Keefe and Fenton, 2007; Keefe and Harvey, 2012; Kahn and Keefe, 2013). However, in spite of their significance, symptoms of cognitive impairments are not included in disease diagnostic criteria (Kahn and Keefe, 2013; Tandon et al., 2013).

Much research has long focused on studying deficits in executive function associated with schizophrenia (Sullivan et al., 1994; Johnson-Selfridge and Zalewski, 2001). Executive function refers to a set of abilities that allow for objective planning, regulation of goal-directed behaviour, flexibility and adaptability of behaviour in response to changing relevant stimuli, problem solving and using past experiences to inform the decision-making process (Freedman and Brown, 2011; Orellana and Slachevsky, 2013). Patients with schizophrenia show poor performance on tasks which require cognitive flexibility. Cognitive flexibility is often assessed by the Wisconsin Card Sorting Test (WCST) (Berg, 1948), whereby participants are presented with a set of cards that can be sorted along three dimensions of symbol colour, shape and number. Participants will shift attention between these stimulus features in order to correctly identify the current sorting rule via experimenter feedback. The ability to inhibit a previously learnt sorting strategy when the rule changes and the inability to shift attention to relevant stimuli is severely compromised in patients with schizophrenia (Orellana and Slachevsky, 2013). Furthermore, patients with schizophrenia also show impairments in tasks that involve planning, including the Tower of London Task. A recent meta-analysis of studies assessing planning performance suggests that the performance of the patients deteriorates as a function of task difficulty (requiring minimum movement to a solution) (Knapp et al., 2017). Executive processes are heavily reliant on the prefrontal cortex (PFC) and subcortical brain regions, including the thalamus. This is supported by a meta-analysis of 41 neuroimaging studies, reporting activation of dorsolateral (dL) PFC, ventrolateral (vL) PFC, anterior cingulate cortex (ACC) and thalamus in both healthy participants and schizophrenia patients during tasks of executive function. However, this activity was significantly reduced in schizophrenic patients compared to controls (Minzenberg et al., 2009), suggesting that the ability of the PFC circuitry in top-down control of cognitive strategies and planning is compromised in schizophrenia.

In addition to impairments in executive function, deficits in episodic memory have also long been considered to be a core feature of schizophrenia (Aleman et al., 1999; Rushe et al., 1999). Episodic memory is defined as a conscious recollection of previous events and episodes in relation to their temporal and contextual setting (Tulving, 2002). The ability to recollect past experiences is essential to processes of planning and regulation of goal-directed behaviour. Deficits in episodic memory encoding and recall are well documented in patients with schizophrenia. Similar to deficits in executive function, impairments in episodic memory are also associated with PFC dysfunction, manifested as reduced activation of the dLPFC, vLPFC and ACC during encoding and retrieval of episodic memory (Ragland et al., 2009; Ragland et al.,

2015). Episodic memories include rich details about the context within which specific events have occurred. Substantial evidence suggests that contextual and spatial information is processed within the hippocampal formation (HF), which is then communicated to the PFC (Jin and Maren, 2015). Functional interaction between the HF-PFC is crucial for successful processing of episodic memories (Eichenbaum, 2017). This functional interaction is known to be dysfunctional in patients with schizophrenia (Meyer-Lindenberg et al., 2005; Bahner and Meyer-Lindenberg, 2017) and is thought to underlie episodic memory deficits associated with the disease. The HF-PFC interaction will be described in detail in **Section 1.9**.

1.2.1. Cognitive impairment and functional outcome

Symptoms of cognitive impairments play an important role in the functional outcome of patients, defined as success in everyday functioning, community life (work, education) and independent living, social problem solving, interpersonal relationships and acquisition of psychosocial skills (Green et al., 2000; Lin et al., 2013; Green et al., 2015). The relationship between neurocognitive performance and functional outcome is complex and influenced by factors such as social cognition (Fett et al., 2011; Schmidt et al., 2011; Green et al., 2015) and negative symptoms (Rabinowitz et al., 2012; Lin et al., 2013). Neurocognitive test performance acts as a predictor of functional outcome in the patients. Therefore, cognitive-enhancing drugs may improve functional outcomes and quality of life for the patients (Green et al., 2000; Schmidt et al., 2011). Determining the nature of cognition-functional outcome relationship and the mediating factors that influence this is essential for effective early cognitive interventions and therapy outcomes (Green et al., 2012; Green et al., 2015).

1.2.2. Developmental trajectory of cognitive impairments in schizophrenia

Substantial evidence supports the presence of cognitive impairments in individuals with ultra-high risk (UHR) of conversion to psychosis (Simon et al., 2007; Bora and Murray, 2014; Bora et al., 2014) as well as first episode psychosis (FEP) patients (Addington et al., 2005; Wood et al., 2007; Mesholam-Gately et al., 2009; Bora et al., 2014; Bora, 2015). Several studies have also reported that deficits in verbal memory, verbal reasoning and working memory (Reichenberg et al., 2010), intellectual abnormalities (Woodberry et al., 2008) and developmental abnormalities (language problems, reading and writing) are present in children and adolescents that go on to develop schizophrenia (Niemi et al., 2003; Arango et al., 2014). Nonetheless, the developmental trajectory of these deficits across disease stages (prodromal stage to onset of psychosis and chronic illness) remains unclear.

Cross-sectional studies suggest that cognitive impairments are more severe in individuals at later prodromal stages (UHR; representing stages before psychosis) compared to individuals at the early prodromal stages (basic symptom; self-limiting and sub-threshold symptoms) and less severe in FEP patients (Simon et al., 2007; Giuliano et al., 2012). Furthermore, cognitive performance is poorer in at-risk individuals who transit to psychosis compared to those who do not. This is supported by a recent meta-analysis of 19 cross-sectional studies of UHR individuals (Fusar-Poli et al., 2012) as well as two comprehensive reviews (Wood

et al., 2008; Freedman and Brown, 2011). Collectively these studies suggest that cognitive impairments are most severe in domains of verbal fluency, verbal and visual memory, working memory and executive function. These indices could serve as a predicting factor for conversion of UHR to psychosis and allow development of early preventative interventions.

Several cross-sectional studies suggest that transition to psychosis is associated with substantial cognitive decline (Fusar-Poli et al., 2012; Giuliano et al., 2012; Bora and Murray, 2014). Given the nature of cross-sectional design, direct comparison across various stages of the disease is not possible. Hence it cannot be concluded whether cognitive deficits are progressive (Bora and Murray, 2014). Longitudinal studies investigating this are sparse. One such study has shown that at psychosis onset, visual memory and attention are significantly deteriorated in patients. In this study, patients were treated with APs. Therefore, it remains unclear whether the observed deficits were due to the disease process and/or due to the influence of treatment on these patients (Wood et al., 2007).

Longitudinal studies ranging from 2 (Bombin et al., 2013) to 10 years of follow up period (Hoff et al., 2005) suggest cognitive deficit severity remains stable across the life of the patient after onset of psychosis. These findings are supported by recent meta-analysis of 25 longitudinal studies (1-5 year follow up period) investigating changes in cognitive performance before and after the onset of psychosis in UHR and/or FEP. Results showed no evidence of loss of previously acquired cognitive skills in FEP and UHR individuals over the follow up periods. In fact, the cognitive performance in FEP patients and UHR patients was significantly improved in the follow up period. The magnitude of these improvements was, however, non-significant in comparison to healthy controls suggesting the influence of practice effect (Bora and Murray, 2014).

Whether cognitive function progressively deteriorates after the onset of psychosis and throughout the chronic course of the disease remains to be investigated. Some evidence suggests that deficits in most cognitive domains remain relatively stable after psychosis onset. However, the cognitive domain of verbal memory seems to deteriorate across the course of the illness (Bozikas and Andreou, 2011). Similarly, meta-analysis of cross-sectional studies suggests that the extent of cognitive impairment in FEP patients is comparable to those at chronic states of the disease (Reichenberg et al., 2010; Irani et al., 2011). However, these findings must be interpreted with caution, since the potentially confounding effects of AP dose and treatment duration were not accounted for in these studies.

1.3. Behavioural tests of cognition

Validated and standardised tests of cognition are crucial for reducing confounding variables introduced to research outcomes by measurement and design inconsistencies (Green et al., 2004; Green, 2006; Kern et al., 2008; Green et al., 2014). With the primary aim of constructing a pathway for standardised testing for schizophrenia, the MATRICS consortium formulated the MATRICS consensus cognitive battery (MCCB). This battery consists of 10 tests for the seven impaired cognitive domains associated with schizophrenia

and was chosen on the basis of practicality (in terms of administration), high test-retest reliability, minimal practice effect, high tolerability for the patients and sensitivity to drug treatment from among 90 different cognitive tests (Nuechterlein et al., 2008). Correlation between individual tests and functional outcomes was also one of the most important criteria of inclusion in the MCCB (Green et al., 2004; Green, 2006; Young et al., 2009; Green et al., 2014). Recent multi-site clinical drug trials have confirmed the practicality, test-retest reliability and strong correlation between MCCB measures and functional outcomes (Keefe et al., 2011). Given the strong correlation between functional outcome and cognitive performance, the latter are a reliable measure for cognitive-enhancing effect of APs. However, many APs fail to improve functional outcomes to a clinically meaningful level (Harvey et al., 2005; Keefe et al., 2007a). Therefore, it has been suggested that improvement in functional outcome should be the index of efficacy of cognitive-enhancing compounds (Green et al., 2004).

Pre-clinical research plays an essential role in the process of drug development for schizophrenia. Therefore, establishment of a standardised battery of behavioural/cognitive tests is essential in the pre-clinical setting. Despite challenges of translation as well as face (the extent to which behavioural symptoms in an animal model mimic those of a human disease), construct (the extent to which a test measures the domains it is intended for) and predictive validity (the extent to which treatment outcome for the tested domain in the clinic can be predicted by its outcome in pre-clinical settings), several pre-clinical behavioural paradigms have been validated in accordance with MATRICS guidelines to test for disease-relevant cognitive impairments in animal models of schizophrenia (Young et al., 2009; Neill et al., 2010). For instance, the Novel Object Recognition (NOR) task is translatable to visual learning and memory (Young et al., 2009; Grayson et al., 2015b), while the attentional set shifting task (Tait et al., 2014) is analogous to tests of cognitive flexibility in clinical practice. Since this thesis has frequently employed the NOR test, a brief review of this task is provided below.

1.3.1. Novel Object Recognition (NOR) Task

The NOR task is an ethologically-valid test that exploits the natural tendency of animals to explore novelty (Ennaceur and Delacour, 1988). NOR is currently identified as the principal test for visual recognition memory by the MATRICS (Young et al., 2009), a cognitive domain which is impaired in patients with schizophrenia (Calkins et al., 2005). A single NOR trial consists of an acquisition phase and a retention phase separated by an inter-trial-interval (ITI) of varying duration (30 seconds to days). In the acquisition phase the animal is presented with two identical objects. Following the ITI, the rat is then presented in the retention phase with a replica of the familiar (acquisition) object and a novel object. Each phase can last from 3 to 10 minutes (Young et al., 2009). This test is particularly attractive as it does not require pre-training and does not rely on external sources of reinforcement to encourage task performance. Therefore, it provides a model of pure exploratory behaviour that is independent of the influence of training and reward (Dere et al., 2007; Winters et al., 2008; Antunes and Biala, 2012; Lyon et al., 2012; Ameen-Ali et al., 2015). In addition, NOR is relatively fast to administer and with manipulations of the ITI, a single trial can provide

enough information to study different stages of memory processing including encoding, consolidation and retrieval (Winters and Bussey, 2005; Winters et al., 2008; Bussey et al., 2013).

It has been argued that NOR provides a medium through which aspects of episodic memory can be studied (Ennaceur, 2010; Bussey et al., 2013). As such, the acquisition phase is an episode where the animal encodes specific object information used to distinguish between the novel and the familiar object in the retention phase (Ennaceur, 2010). This view is, however, a matter of controversy (Morici et al., 2015). Recognition memory requires judgement of previously encountered events on the basis of their relative familiarity (awareness of previous encounter of an event/object) or by recollection (conscious recall) of an event/object in association to its temporal and contextual component (Barker et al., 2007; Suzuki and Naya, 2014). The latter is a key feature of episodic memory. Since the classic one-trial NOR task (one-time exposure to the acquisition and retention phase), especially at short (<15 min) and intermediate (< 3h) delays does not involve the integration of contextual, spatial and temporal information, it can be argued that it relies on familiarity based rather than recollection-based strategies (Morici et al., 2015). This view is consistent with the distinct neural substrates that mediate familiarity and recollection processes.

The perirhinal cortex (PRh) plays a strong role in familiarly-novelty discrimination (Ennaceur et al., 1997; Barker et al., 2007; Barker and Warburton, 2011; Aggleton et al., 2010; Suzuki and Naya, 2014). Based on electrophysiological findings, PRh neurons respond rapidly to a novel stimulus while this response is significantly reduced upon repeated exposure (even upon single trial-learning) to the stimulus (Brown and Aggleton, 2001; Zhu et al., 1995). Excitotoxic lesion in the PRh severely impairs performance in the NOR task (Barker et al., 2007; Barker and Warburton, 2011). Pharmacological manipulation studies also signify the involvement of this region in encoding (acquisition), consolidation and retrieval of object memory (Winters and Bussey, 2005; Winters et al., 2008). As part of the neocortex, the PRh sustains glutamatergic (acting on ionotropic AMPA, NMDA and Kainate receptors as well as metabotropic receptors) (Winters and Bussey., 2005; Winters et al., 2008), dopaminergic (acting on D1, D2 and D4 receptors) (Pum et al., 2007; Balderas et al., 2013), serotonergic (5-HT_{1A} and 5-HT_2 receptors) (Pum et al., 2007; Kealy and Commins., 2011) and cholinergic (nicotinic and muscarinic receptors) (Warburton et al., 2003) transmission. GABAergic input to the PRh is lower compared to other neurotransmitter systems (Kealy and Commins., 2011). Substantial evidence from pharmacological manipulation studies have revealed that these neurotransmitter systems play an important role in mediating the processes of recognition memory in the PRh, by modulating learning-dependent synaptic plasticity in this region (Dere et al., 2007; Banks et al., 2012). These investigations further suggest that different receptors are differentially involved in processes of encoding, consolidation and retrieval of object recognition memory (Warburton et al., 2003; Winters and Bussey., 2005; Winters et al., 2008; Kealy and Commins., 2011; Banks et al., 2012). For instance, dopamine D1 receptor (D1R) mediate object recognition memory at long (24h) but not intermediate (1.5h) ITIs (Balderas et al., 2013). This is while muscarinic acetylcholine receptors appear to be involved in encoding and consolidation of object memory at short-intermediate but not long ITIs (Warburton et al.,

2003; Dere et al., 2007). Blockade of AMPAR receptors (AMPAR) by local PRh infusion of CNQX (AMPAR antagonist) prior to the acquisition phase of the NOR, impairs performance at both short (5 mins) and intermediate (3h) ITIs, while NMDA receptors (NMDAR) antagonist D-2-amino-5-phosphonovalerate (D-AP5) only impairs performance at longer delays (3h) (Winters and Bussey., 2005). CNQX or D-AP5 infusion, immediately after but not 40 mins post-acquisition phase, impairs performance following a 3h ITI, suggesting a role for both AMPA and NMDARs in consolidation of object memory (Winters and Bussey., 2005; see Banks et al., 2012 for a review on the role of NMDARs in recognition memory consolidation in PRh). This is while, CNQX but not D-AP5 infusion, immediately prior to retrieval, impairs performance following a 3h ITI, suggesting a role for AMPA but not NMDARs in object memory retrieval (Winters and Bussey., 2005).

Anatomically, the PRh is connected to the hippocampus and parahippocampal cortex, including the entorhinal and postrhinal cortices, in a reciprocal manner (Deacon et al., 1983; Jay and Witter., 1991; Naber et al., 1999; Kealy and Commins., 2011; Suzuki and Naya., 2014). Several lines of evidence from electrophysiological studies show that the PRh can influence and be influenced by hippocampal activity (Kealy and Commins., 2010; Kealy and Commins., 2011). However, involvement of the hippocampus in NOR is highly disputed (Bussey et al., 2013; Morici et al., 2015). Early reports suggested a delay-dependent impairment in NOR performance upon inactivation of the hippocampus in rodents. For instance, Clark et al (2000) reported impaired object recognition performance upon excitotoxic hippocampal lesion at intermediate to long ITI (15 mins to 24h) but not at shorter delays (10 s to 10 mins) (Clark et al., 2000). Similarly, injection of NMDAR antagonist D-AP5 into the dorsal hippocampus was found to impair NOR performance at intermediate (3h) but not short (5 minutes) ITIs (Baker and Kim, 2002). These findings were further confirmed by temporary lidocaine inactivation of the CA1 region of the dorsal hippocampus (dHipp) in mice. Hammond et al (2004) showed that while the cortical processing of object recognition was independent of hippocampal function at short delays (5 mins), the latter was essential for successful performance at longer delays (24 h) (Hammond et al., 2004). Intact NOR performance at short (2-15 mins) (Winters et al., 2004; Langston and Wood, 2010), intermediate (1-3 h) (Winters et al., 2004; Barker and Warburton, 2011) and long (24 h) delays (Winters et al., 2004; Barker and Warburton, 2011) have also been reported in rats with hippocampal lesion.

The PRh is also strongly connected to the mPFC, particularly to the prelimbic (Prl) and the infralimbic (IL) regions in a reciprocal manner (Burwell and Amaral., 1998; Agster and Burwell., 2009). Similar to the findings of hippocampus lesion studies, involvement of the mPFC in NOR is also controversial. Some evidence derived from lesion studies supports delay-dependent involvement of the mPFC in NOR. Collectively, these studies suggest that the mPFC may not be involved in NOR upon short (<15 mins) (Ennaceur et al., 1997; Barker et al., 2007) and intermediate delays (3h) but impairs performance at long delays (24h) (Akirav and Maroun, 2006). Several indirect lines of evidence, however, implicate mPFC function in NOR performance at short delays. For instance, a recent *in vivo* electrophysiology study

revealed an increase in the firing rate of the mPFC neurons in vehicle-treated rats during exploration of the novel object in comparison to the familiar (1 min ITI) (Asif-Malik et al., 2017). Furthermore, a recent microdialysis study revealed a significant increase in the mPFC dopamine levels of rats in the retention phase of the NOR paradigm following a 10 minute ITI (Mclean et al., 2017), further supporting the potential functional involvement of the mPFC in NOR performance.

Involvement of the hippocampus and the mPFC is confidently confirmed in recognition memory when the tasks cannot be solved by a single element. These include variations of the NOR test including the object-in-place, object-in-location, object-in-context and object recency tasks, which rely on integration of spatial, contextual and temporal cues (Barker et al., 2007; Barker and Warburton, 2011), task conditions that more in line with the definition of episodic memory and recollection-based strategies, even at short ITI. Collectively, it can be suggested that at short and intermediate delays (less than 1 h), the NOR performance is independent of mPFC and hippocampus and predominantly relies on familiarity based, rather than recollection-based, strategies.

In spite of its advantages, concerns have been raised with regards to the construct validity of the NOR test with relevance to schizophrenia. Emerging evidence suggests that not all aspects of recognition memory are equally affected in patients with schizophrenia (Libby et al., 2013; Wang et al., 2014). A recent meta-analysis suggested that patients are impaired in recognition memory when using recollection rather than familiarity-based strategies (Libby et al., 2013; Wang et al., 2014). In its classical format, the NOR is very sensitive to psychotomimetic agents such as phencyclidine (PCP). Sub-chronic treatment with PCP incudes a robust deficit in the performance of the NOR (Grayson et al., 2007; Neill et al., 2010; Grayson et al., 2014) which is manifested as the absence of novelty perference. In the classic NOR, (hereafter refered to as the disrupted NOR (dNOR)), the animal is removed from the testing arena during the ITI. Recent evidence suggests that rats sub-chronically treated with PCP are able to perform NOR at control levels when left undisturbed in the testing arena for the duration of the ITI (Grayson et al., 2014). This suggests that similar to patients with schizophrenia, the familiarity-based stategies are intact in the sub-chronic PCP (scPCP) treated rats and that these processes are susceptible to distraction (Cellard et al., 2007; Anticevic et al., 2011).

In recent years, there has been a substantial surge in the use of dNOR in schizohrenia research to investigate the influence of pharmacological agents. As elegantly reviewed by Lyon and colleagues (2012), an extensive number of AP agents have been shown to reverse the scPCP-induced dNOR deficit upon acute treatment (Neill et al., 2010; Lyon et al., 2012; Cadinu et al., 2017). These agents have been significantly less successful in improving cognitive impairments in patients with schizophrenia (discussed in **Section 1.7.2**), specifically upon long-term treatment. Therefore, while the NOR test offers a degree of face validity, concerns remain with regards to the predictive validity of this test. Nevertheless, NOR and its variants are amongst the most frequently employed tools in schizophrenia research and provide invaluable

information about the memory disturbances associated with the disease (Lyon et al., 2012).

1.4. Neurochemical hypotheses of schizophrenia

Dysregulation in several neurotransmitter systems is implicated in the pathophysiology of schizophrenia. Initially, significant attention was directed towards the dopamine neurotransmitter system. More recently, disturbances in amino acid neurotransmitter systems including glutamate and gamma-aminobutyric acid (GABA) have become the most prominent hypotheses of schizophrenia. Emerging evidence suggests that disturbances in these neurotransmitter systems are interrelated and collectively lead to manifestation of schizophrenia-like symptoms.

The first hypothesis of dopamine involvement in schizophrenia originated from indirect sources of evidence. A key finding was that administration of dopamine-releasing agents such as amphetamine (Amph), induced psychotic-like symptoms in healthy participants similar to those observed in patients with schizophrenia and exacerbated psychotic symptoms in patients (Lieberman et al., 1987). Further evidence came from the observation that reserpine reduced psychotic symptoms by inhibiting dopamine reuptake and depleting dopamine at synaptic terminals (Carlsson et al., 1957). This hypothesis was further advanced by the finding that chlorpromazine, the first AP agent discovered in the early 1950s, acted as an antagonist on the D2R (Carlsson and Lindqvist., 1963; Carlsson and Carlsson., 2006). In addition, findings that the clinical effectiveness of APs in treating positive symptoms was strongly correlated with their affinity for the D2R, further confirmed the involvement of dopamine in schizophrenia (Seeman and Lee, 1975; Kapur et al., 1996; Seeman, 2013; Howes et al., 2015). Collectively, these studies suggested that in schizophrenia, psychotic symptoms were associated with elevated levels of dopamine. At this time, however, the locus of this dopamine abnormality was unrecognised.

Advances in post-mortem and metabolite findings and imaging studies led to the understanding that dopamine transmission was differentially altered across various brain regions. Davis and colleagues (1991) drew on existing evidence at the time to suggest a mechanism of hypoactive dopamine transmission in the PFC and a hyperactive dopamine transmission in the sub-cortical regions in schizophrenia (Davis et al., 1991). This hypothesis has since been supported by imaging studies confirming hyperactive mesolimbic dopamine transmission (Breier et al., 1997; Abi-Dargham et al., 1998) and an increase in mesolimbic dopamine synthesis and release capacity (Howes et al., 2015; Weinstein et al., 2017) in patients with schizophrenia. Evidence for hypoactive dopamine transmission in the PFC came from studies that showed a strong correlation between reduced PFC blood flow (hypofrontality) and reduced dopamine metabolite levels in the cerebrospinal fluid (Weinberger et al., 1988; Davis et al., 1991; Howes and Kapur., 2009) in patients. Indeed, recent imaging techniques have provided conclusive proof of reduced dopamine release capacity in the PFC (Slifstein et al., 2015).

Consistent with the hypothesis proposed by Davis and colleagues (1991), several lines of evidence now support the involvement of hyperactive mesolimbic dopamine transmission in psychosis (Howes et al.,

2015). Furthermore, the significance of dopamine transmission in cognitive tasks mediated by the PFC is highlighted, suggesting that its reduced transmission in patients with schizophrenia may underlie cognitive deficits associated with the disease (Goldman-Rakic et al., 2004; Rao et al., 2018). In addition to reduced dopaminergic transmission, reduced frontal blood flow and glucose utilisation (hypofrontality) has been consistently associated with impaired cognitive performance in patients with schizophrenia (Buchsbaum et al., 1990; Andreasen et al., 1997; Barch and Ceaser., 2012**)**. Several lines of evidence also point towards a negative correlation between hypofrontality and striatal dopamine release. In these studies, the elevated mesolimbic dopamine transmittion has also been negatively correlated with cognitive performance (Meyer-Lindenberg et al., 2002; Howes et al., 2009; Fusar-Poli et al., 2010).

Collectively, these findings suggest that in addition to its involvement in psychosis, interruptions in mesolimbic dopamine transmission also contribute to the cognitive symptoms associated with schizophrenia (Howes et al., 2015). Whether hyperactivity of mesolimbic dopamine transmission is primary or secondary to hypoactivity of the mesocortical dopaminergic system is not clear. In recent years, hyperactivity of the hippocampal formation in patients with schizophrenia has been highlighted. Given its strong connections to the sub-cortical dopaminergic loci, it is thought that hippocampal hyperactivity has great implications in the emergence of psychotic symptoms and the hyperactive dopamine transmission in the mesolimbic area (Grace, 2010; Grace, 2016).

Expanding on the work of Davis et al (1991) and drawing on the available evidence, Howes and Kapur (2009) proposed a revised dopamine hypothesis known as the “final common pathway”. This hypothesis posits that presynaptic mesolimbic (striatal) hyper dopamine synthesis and release is the point of convergence for other dysfunctional neurotransmitter systems including glutamate and GABA. By shifting the focus from D2R to presynaptic events, this hypothesis also offers a new framework for the process of drug development. Importantly, this hypothesis suggests that the dopamine hypothesis is strongest when explaining psychosis in schizophrenia (as evident by high efficacy of APs to treat psychosis but not cognitive symptoms) rather than schizophrenia as a whole. This hypothesis implies the involvement of other neurotransmitter systems in generation of cognitive symptoms and negative symptoms associated with the schizophrenia which along with genetic and environmental factors, converge at the final common pathway to trigger the onset of psychosis.

Glutamate is the most abundant excitatory neurotransmitter in the brain. Cortical levels of this neurotransmitter and its metabolite, glutamine, have been positively correlated with cognitive performance in patients with schizophrenia (Bustillo et al., 2011). The first finding implicating glutamate dysregulation in schizophrenia was by Kim and colleagues (1980) who reported a significant reduction in glutamate levels in the cerebrospinal fluid of patients with schizophrenia (Kim et al., 1980). Other groups failed to replicate these findings, however, other aspects of glutamate transmission have been consistently implicated in disease pathology (Moghaddam and Javitt, 2012; Howes et al., 2015). Influential clinical findings and case observations dating back to 1950, have consistently revealed that single exposure to psychostimulants

such as phencyclidine (PCP) and ketamine in healthy individuals induces acute behavioural disruptions similar to the positive and negative symptoms observed in patients with schizophrenia (Luby et al., 1959; Krystal et al., 1994; Krystal et al., 2005) . Similarly, individuals with a history of chronic ketamine use show persistent negative symptoms and cognitive impairments similar to those in patients with schizophrenia (Morgan et al., 2010). The discovery that PCP acted as NMDAR non-competitive antagonist (Zukin and Zukin, 1979; Javitt and Zukin, 1991), implicated NMDAR hypofunction in the pathophysiology of schizophrenia.

Presynaptic and postsynaptic events can contribute to the emergence of NMDAR hypofunction. At a presynaptic level, reduced glutamate release (reduced excitatory tone) could be a potential cause for NMDAR hypofunction (Moghaddam and Javitt, 2012). Glutamine, produced by astrocytes from excess glutamate in the synaptic space, is considered an index of glutamatergic transmission (Bak et al., 2006). Proton magnetic resonance spectroscopy (1H-MRS) findings suggest an increase in the level of glutamate and glutamine in UHR, FEP and drug naïve patient populations (Poels et al., 2014). This is while the level of glutamate transmission indices appears either reduced (Natsubori et al., 2014; Marsman et al., 2013; Theberge et al., 2003), increased (Bustillo et al., 2014) or normal (Howes et al., 2015; Bustillo et al., 2011) in patients at chronic stages of the disease. Howes and colleagues (2015) argue that these inconsistencies could be due to differences in the 1H-MRS data acquisition and might reflect the confounding effect of AP treatment.

Functional NMDARs are heteromeric complex protein structures comprised of two obligatory GluN1 subunits with either two GluN2 (GluN2A-D) (of the same or difference subtype subunits) or a combination of GluN2 and GluN3 (GluN3A-B) subunits (Traynelis et al., 2010). Subunit composition of the NMDARs dictates their functional properties including permeability to calcium, channel gating dynamics and sensitivity to Mg^{2+} ion (Hanson et al., 2008; Traynelis et al., 2010). For instance, receptors containing GluN2A display faster activation and deactivation kinetics in comparison to GluN2B-containing NMDARs. GluN2C-containing receptors express low open probability and low sensitivity to Mg^{2+}, while GluN2D-containing receptors express very low decay times (Traynelis et al., 2010; Kehoe et al., 2013). At the postsynaptic level, one potential cause of NMDAR hypofunction could be reduced expression of NMDAR GluN1 and GluN2A-D subunit (Moghaddam et al., 2012). However, studies of NMDAR GluN1 and GluN2A-D subunit expression in post-mortem tissue of patients with schizophrenia have yielded inconsistent results (Rubio et al., 2012; McCullumsmith et al., 2012).

In recent years, the functional significance of GluN3 subunits has gained much attention. GluN3-containing NMDARs, express non-conventional properties which include significant reduction in calcium permeability and insensitivity to Mg^{2+} at hyperpolarised membrane potentials, resulting in reduced NMDAR-mediated current (Pachenregg et al., 2012; Kehoe et al., 2013). It can therefore be suggested that the GluN3 subunits act as dominant negative modulators of the NMDARs (Pachenregg et al., 2012; Kehoe et al., 2013). As such, a role for over-expression of GluN3 has been suggested in association to NMDAR hypofunction in

schizophrenia. This view is supported by a post-mortem study showing elevated levels of GluN3 mRNA in the dLPFC of patients with schizophrenia (Mueller and Meador-Woodruff., 2004). Contradictory evidence showing no change in GluN3 mRNA in patients compared to controls has also been reported (Henson et al., 2008).

In human brain, expression of GluN3 subunits is low during gestation, surges post-birth and progressively declines throughout adolescence and into adulthood (Henson et al., 2008). The time and activity-dependent removal of GluN3 subunits at critical developmental periods is essential for formation and maturation of glutamatergic synapses and dendritic spines (Pachenregg et al., 2012; Kehoe et al., 2013). Studies in mice overexpressing GluN3 (beyond normal developmental window) report a significant reduction in size, density and number of dendritic spines which is accompanied by deficits in learning and memory (Roberts et al., 2009). Indeed, reduced number and size of dendritic spines (which facilitate excitation) in layers II-III of the dLPFC (brain region associated with elevated GluN3 mRNA expression) has been reported in patients with schizophrenia (Mueller and Meador-Woodruf., 2004; Elsworth et al., 2011; Konopaske et al., 2014; Hoftman et al., 2017), further highlighting the potential significance of GluN3 in association with the disease.

In addition to altered subunit composition and expression, consistent evidence points towards changes in NMDAR trafficking machinery, which results in aberrant localisation of the receptor rather than its general elevated or reduced expression in the brain (Hammond et al., 2014). For instance, several studies have reported reduced expression of post-synaptic density-95 (PSD-95) in the anterior cingulate cortex (Funk et al., 2009; Funk et al., 2012) and reduced NMDAR-PSD-95 interaction in the dLPFC in patients with schizophrenia (Funk et al., 2012; Hammond et al., 2014). Aberrant localisation of NMDAR subunits has major implications in the functional integrity of NMDAR signalling cascades, consequently altering synaptic plasticity and signal transduction (Funk et al., 2012; Howes et al., 2015). Altered glutamate transmission in schizophrenia is also supported by genetic studies reporting polymorphisms in NMDAR subunits and molecular complexes mediating NMDA signalling (Ripke et al., 2014; Harrison, 2015). Alterations in glutamate transmission, particularly the activity of the NMDARs, has also been linked to dysregulation in dendritic spine formation and maintenance (Konopaske et al., 2014). Collectively, these findings suggest that glutamate transmission is severely dysregulated in schizophrenia.

GABAergic transmission is also heavily involved in the pathophysiology of schizophrenia. Findings of post-mortem studies have consistently reported reduced mRNA and protein expression levels of the 67 kDa isoform of glutamic acid decarboxylase (GAD_{67}) in the dLPFC, cingulate cortex, thalamus and the hippocampus of patients with schizophrenia (Hashimoto et al., 2008b; Lewis et al., 2012; Lewis, 2014; Murray et al., 2015). Reduction of GAD_{67}, which is essential for the synthesis of GABA, is most prominent in parvalbumin (PV)-containing GABAergic interneurons (Hashimoto et al., 2003; Gonzalez-Burgos and Lewis, 2008; Gonzalez-Burgos and Lewis, 2012). Reduced expression of PV mRNA and protein in the PFC (Beasley and Reynolds, 1997; Beasley et al., 2002) and hippocampus of patients with schizophrenia (Zhang

and Reynolds, 2002) is a hallmark of the disease. It is important to note that in addition to PV-containing interneurons, other populations of GABAergic interneurons also appear to be disturbed in schizophrenia. Reduced expression of somatostatin, neuropeptide Y (often co-expressed with the former), calbindin and cholecystokinin has been reported in the dLPFC (Morris et al., 2008; Hashimoto et al., 2008a; Sakai et al., 2008) and hippocampus (Konradi et al., 2011) of patients with schizophrenia while expression levels of calretinin appear to remain unchanged (Hashimoto et al., 2003; Sakai et al., 2008).

GABAergic inhibition is fundamental to the generation of several types of brain oscillations (Chen et al., 2014; Gonzalez-Burgos et al., 2015). Each subclass of GABAergic interneurons forms specific synapses with pyramidal neurons (Gonzalez-Burgos et al., 2008). As such, they have distinct effects on the activity of pyramidal neurons (Lewis and Sweet., 2009) and can generate distinct patterns of oscillatory activity in the local circuitry (Morris et al., 2008). For instance, the firing activity of the somatostatin-neuropeptide-Y containing interneurons is coupled with oscillatory activity in the theta range in hippocampus (Gonzalez-Burgos et al., 2008) and the mPFC (Morris et al., 2008). Altered expression these neuropeptides might offer some explanation for altered theta oscillatory activity during tasks of working memory (Schmiedt et al., 2005; Barch and Ceaser., 2012) and may have implications in episodic memory deficits associated with schizophrenia (Numan et al., 2015). In particular, the activity of basket cells, a sub-class of PV containing interneurons, has gained much attention with relevance to schizophrenia. Activity of these PV-containing fast-spiking interneurons is strongly linked to the generation of oscillations in the gamma frequency range (Gulyas et al., 2010; Rotaru et al., 2011). Basket cells predominantly target the perisomatic region of pyramidal neurons and synchronise neuronal output in the gamma range (Gonzalez-Burgos and Lewis, 2012) (**Figure 1.1 A**). In recent years, the significance of gamma oscillations in higher-order cognitive processing has been highlighted (Lisman and Jensen, 2013; Heusser et al., 2016; Cadinu et al., 2017). In agreement with the findings of PV abnormalities, several lines of evidence have consistently reported disruption of gamma oscillations in patients with schizophrenia at resting state (Andreou et al., 2015) and during performance of perceptual and cognitive tasks (Spencer et al., 2004; Ferrarelli et al., 2008; Minzenberg et al., 2010; Chen et al., 2014). Therefore, it is plausible that disturbances in GABAergic system could account for cognitive impairments associated with the disease (Cadinu et al., 2017).

Disturbances in GABAergic neurotransmission is thought to be secondary to NMDAR hypofunction (Rotaru et al., 2011; Gonzalez-Burgos and Lewis, 2012; Moghaddam and Javitt, 2012). Evidence for this came from animal studies reporting reduced expression of PV in the hippocampus (Abdul-Monim et al., 2007; Jenkins et al., 2010) and the medial prefrontal cortex (mPFC) (Cochran et al., 2003) upon sub-chronic treatment with NMDAR antagonists. Interestingly, acute systemic administration of NMDAR antagonists such as PCP and MK-801 increases the firing rate of pyramidal neurons in the mPFC (Suzuki et al., 2002; Jodo et al., 2005; Homayoun and Moghaddam, 2007). This is accompanied by an increase in efflux of glutamate (Adams and Moghaddam, 1998), which resembles the clinical finding of elevated excitatory neurotransmission in UHR, FEP and drug naïve patients (Poels et al., 2014). Substantial evidence suggests

that NMDAR antagonists preferentially act at the NMDARs located on fast-spiking GABAergic interneurons, leading to disinhibition of pyramidal neurons and excess glutamate release (Jodo, 2013; Homayoun and Moghaddam, 2007) (**Figure 1.1 B**). Increased glutamate efflux in response to NMDAR hypofunction, could trigger a compensatory reduction in the excitatory input. It can be hypothesized that prolonged reduction in excitatory input (reduced number and size of dendritic spines, NMDAR hypofunction) can lead to compensatory down-regulation of GABAergic tone, manifested as reduced expression of PV containing interneurons in schizophrenia. This might restore the inhibitory/excitatory balance in the circuitry, however, it weakens synchronicity and reduces power at gamma range (Gonzalez-Burgos et al., 2015) (**Figure 1.1 C**). Collectively, the process of disinhibition impairs the ability of the circuitry to synchronise its activity and regulate signal to noise ratio and differentiate between salient and non-salient stimuli (Moghaddam and Javitt, 2012). This may have implications for the emergence of positive (Jodo, 2013) and cognitive symptoms (Cadinu et al., 2017) associated with schizophrenia.

Accumulating evidence suggests that the NMDAR antagonist-induced disinhibition in the mPFC is not due to the direct action of the antagonist on mPFC circuitry but rather due to its action on remote structures with strong connections to the mPFC (Suzuki et al., 2002; Jodo et al., 2005). Unlike its systemic treatment, local infusion of MK-801 or PCP to the mPFC does not alter the activity of the neurons in this region. However, local infusion of the same compound in the vHipp induces similar effects to systemic administration (Jodo et al., 2005). This is further supported by findings that suggest minimal involvement of NMDAR in the activity of the fast-spiking PV interneurons in the mPFC (Rotaru et al., 2011).

The HF, particularly the ventral hippocampus (vHipp) and ventral subiculum, form strong excitatory connections to the mPFC (discussed in detail in **Section 1.9**). Disinhibition of pyramidal neurons in vHipp leads to dysregulation of pyramidal neuron firing in mPFC. Most importantly, vHipp plays a significant role in regulating the dopamine system through its connection to the ventral pallidum. When the vHipp is activated, it reduces the inhibitory tone of ventral pallidum on VTA, allowing for elevated dopamine release (Grace, 2010; Grace, 2016). This places vHipp at a very critical and pivotal node, whereby, deficits in glutamatergic and GABAergic transmission lead to hyperactivity of dopamine transmission. Given the critical involvement of HF in context-dependent stimulus appraisal and memory and its connection to the PFC, dysregulation in this region could not only account for the emergence of the positive symptoms but also highlights the neural correlates involved in cognitive impairments associated with the disease. Additionally, the vHipp and PFC form extensive bilateral connections with the amygdala, a brain region involved in emotional regulation (Goto and Grace., 2008; Jin and Maren., 2015; Esmaeili and Grace., 2016). It is suggested that dysregulation of the vHipp-amygdala projections could interfere with processing of affective information, leading to the development of the negative symptoms associated with schizophrenia (Grace., 2016). Dysregulation in this pathway also interferes with emotional learning mediated by the PFC-amygdala connections (Esmaeili and Grace., 2016) and the top-down control of PFC on affective processing (Milad et al., 2007) (**Figure 1.2**). It is noteworthy that the majority of evidence supporting the

involvement of vHipp hyperactivity in psychosis and cognition is based on studying the acute effect of NMDAR antagonists, which may not be a suitable model for schizophrenia. Limitations of acute NMDAR treatment as a valid animal model of the disease are discussed in **Section 1.5.3.**

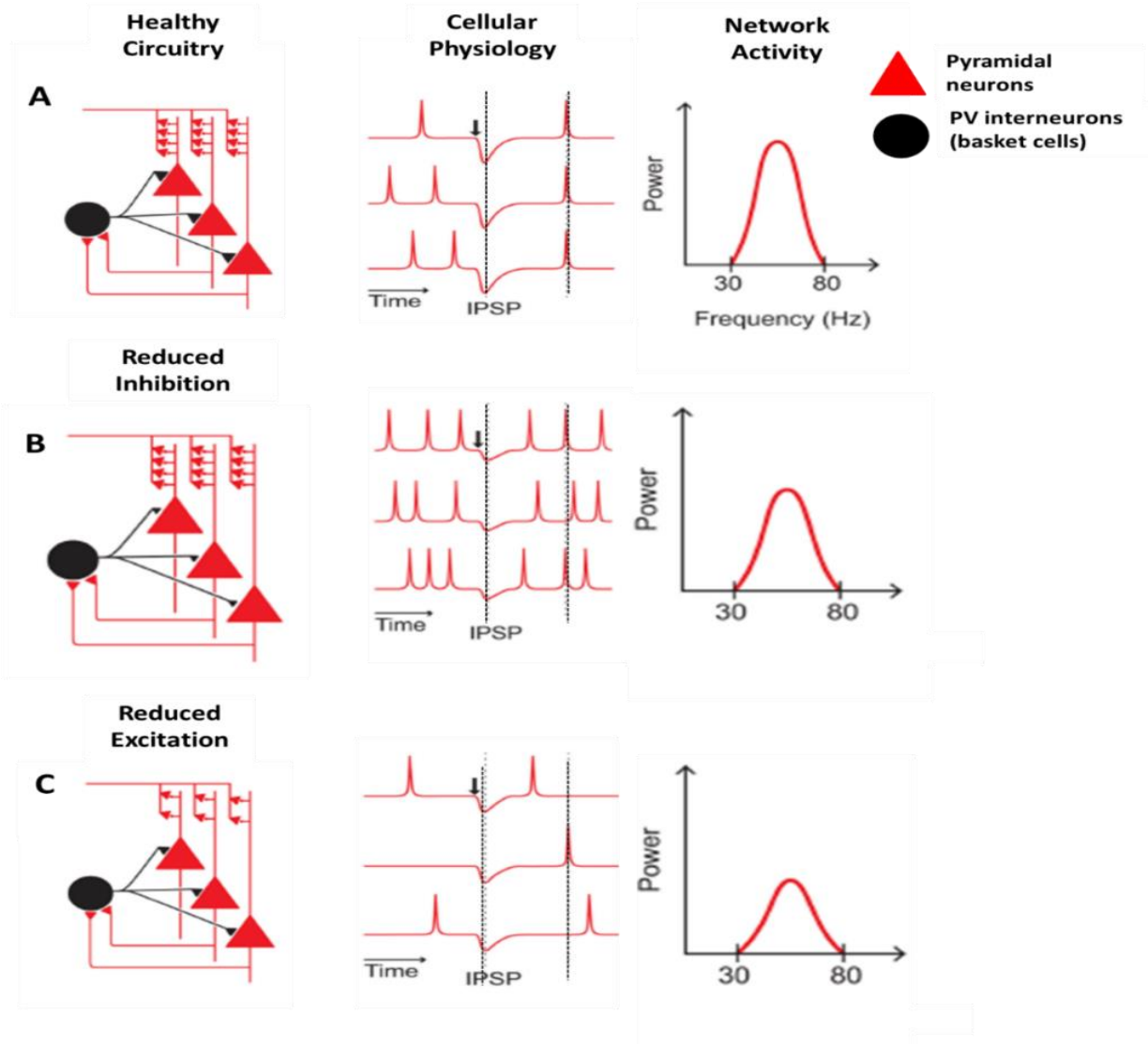

Figure 1.1. Interaction between pyramidal neurons and PV-interneurons in generating gamma oscillations. (A) Represents a basic circuitry at healthy state. Pyramidal neurons in the cortex or hippocampus release glutamate upon activation. Activation of pyramidal neurons recruits the GABAergic interneurons (primarily via AMPARs in the cortex and NMDARs in the hippocampus). After the inhibitory post-synaptic potential (IPSP) decays, the pyramidal neurons fire synchronously at gamma oscillation range and the cycle is repeated. (B) Represents a circuitry with reduced inhibition. Disinhibition due to blockade of NMDARs or reduced PV expression increases the firing rate of pyramidal neurons and results in their asynchronous activity and abnormalities in gamma oscillation regulations. (C) Represents a state of reduced excitation. Reduced excitatory input due to reduced number and size of dendritic spines results in compensatory down-regulation of inhibitory feedback. The combination of these factors results in weak synchronicity and reduced power of gamma oscillations. Figure adapted from Gonzalez-Burgos et al., 2015

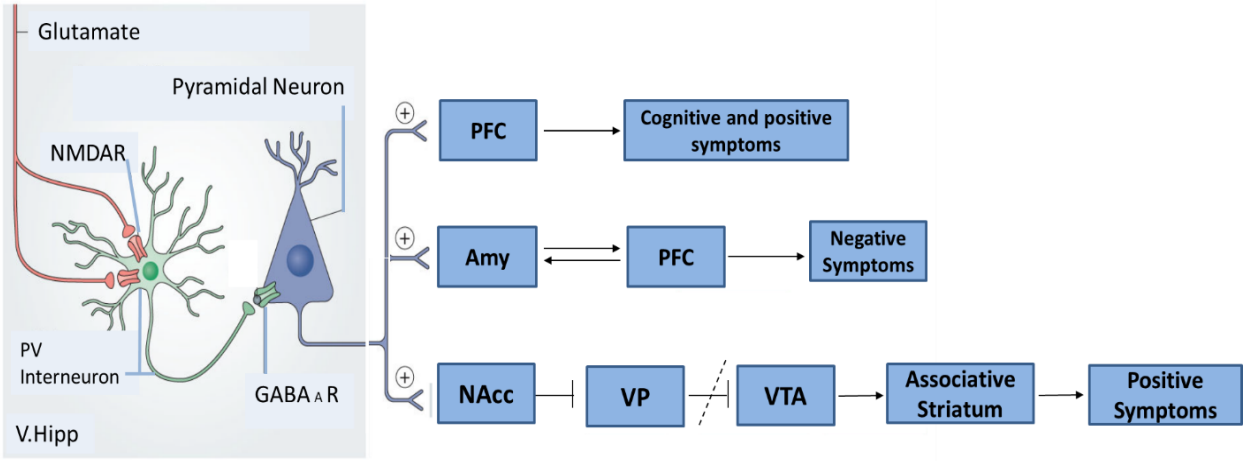

Figure 1. 2. Dysfunction in vHipp in relation to schizophrenia symptomatology. GABAergic interneurons in the vHipp are driven by NMDARs and exert a powerful inhibitory constraint on pyramidal neurons. In presence of NMDAR antagonist or in disease state, where the expression of PV is significantly reduced, pyramidal neurons become hyperactive and dysrhythmic. This leads to over-activation of the Nucleus Accumbens (NAcc), which inhibits ventral pallidum (VP). This reduces the inhibitory constraint of the VP on VTA, leading to hyperactive striatal dopaminergic transmission, which is proposed to be involved in emergence of positive symptoms associated with schizophrenia. Over-activation of the vHipp pyramidal neurons results in dysrhythmic activity of pyramidal neurons in the mPFC, which is implicated in cognitive impairments associated with the disease. Altered activity of the vHipp also interferes with activity of the amygdala. This, in turn, disrupts amygdala-mPFC activity and compromise regulation of emotional responses in the mPFC, potentially leading to emergence of negative symptoms. Figure adapted from Grace, 2016

1.5. Animal models for schizophrenia

Animal models of psychiatric disorders such as schizophrenia are crucial tools with which to examine the neurobiological complexities of the disease. They also play a pivotal role in development and testing of novel treatments with improved efficacy, prior to their administration to patients (Jones et al., 2011). The heterogenous nature of this human-unique disorder has posed a major challenge for developing well-validated disease models. These challenges include generating a model that faithfully reproduces schizophrenia's triad of symptoms (positive, negative, cognitive impairments) in animals with a simpler nervous system (face validity) (Trivedi and Jarbe, 2011). It is also challenging to generate a model that captures genetic and environmental risk factors associated with the disease (construct validity) (Moore, 2010). Much focus has also been placed on examining the predictive validity of the generated models (Jones et al., 2011; Neill et al., 2010) to evaluate the efficacy of novel compounds for treating the cognitive symptoms associated with the disease. Given the diversity of risk factors involved, it is impossible for one model to mimic the full spectrum of disease symptoms. Therefore, current animal model development has strived to represent individual aspects the disease using genetic, developmental (targeting neurodevelopmental aspects of the disease) and pharmacological manipulations to model various aspects of the disease (Jones et al., 2011).

1.5.1. Neurodevelopmental models

Evidence from epidemiological studies suggests that exposure to early life adversity at critical stages during pre and post-natal development increases the risk of schizophrenia development (Moller et al., 2015; Fatemi and Folsom, 2009; Reisinger et al., 2015). Therefore, factors such as maternal immune response to infection, stress and obstetric complications during the prenatal stages (Lewis and Levitt, 2002; Mittal et al., 2008) increase the risk of disease development. Additionally, genetic predisposition and exposure to post-natal adverse events could trigger a chain of neurobiological events which further increase the probability of disease development (Anglin et al., 2008; Jones et al., 2011; Mittal et al., 2008). Pre-clinical animal models investigating the neurodevelopmental aspects of the disease manipulate critical stages of development in pre and post-natal life to study its resulting symptomatology in association to schizophrenia (Jones et al., 2011).

Disruption of foetal neural development by administration of Methylazoxymethanol acetate (MAM), an anti-mitotic agent, to pregnant dams has been extensively explored as a possible neurodevelopmental model for schizophrenia (Jones et al., 2011). Influence of MAM on neural development and subsequent behavioural phenotypes depends on the gestational day of administration (Flagstad et al., 2004; Lodge and Grace., 2009). For instance, MAM treatment on gestational day 15 (the peak of cortical neurogenesis) is associated with gross anatomical abnormalities in brain structure including microencephaly, which are not comparable to observation in patients with schizophrenia (Lodge and Grace., 2009; Jones et al., 2011). In contrast, MAM treatment on gestational day 17 (corresponding to human late third trimester; MAM-17), which is associated with reduced cortical neural proliferation, results in brain structural abnormalities resembling those observed in schizophrenia patients (Jones et al., 2011) including reduced hippocampal and grey matter volume in the PFC and thalamus (Flagstad et al., 2004; Moore et al., 2006).Furthermore, MAM-17 leads to the emergence of schizophrenia-like pathophysiology that includes sub-cortical dopamine dysregulation (Flagstad et al., 2004) and reduced PV expression in the mPFC and vHipp (Lodge et al., 2009) as well as deficits in a range of cognitive domains including executive function and working memory (see Jones et al., 2011 for review). MAM-17 is also associated with disturbances in the functional interaction between the vHipp and mPFC, a pathway vital for higher-order cognitive processes. The offspring of MAM-treated rats show altered synchrony and neuronal spiking activity in the mPFC and vHipp (Goto and Grace, 2006; Lodge et al., 2009) as well as hypoactivity in the vHipp-mPFC pathway during a fear conditioning task (Lodge et al., 2009). Processes of synaptic plastcity, which are fundamental to cognition are also disturbed in this pathway in this model (Goto and Grace, 2006; Esmaeili and Grace, 2013). Collectively these findings point towards abnormal information processing which could account for the cognitive deficits found in this model.

Several other neurodevelopmental models have also been generated to explore the influence of maternal immune response as a risk factor associated with schizophrenia. Over the past several years, the polyriboinosinic-polyribocytidylic acid (poly I:C) model in particular has gained much attention. Injection of

poly (I:C) to dams on gestational day 15 induces a range of cognitive and behavioural phenotypes of relevance to schizophrenia in the offspring. These include imbalances in the dopaminergic transmission (manifested as heightened response to dopamine releasing agents such as Amph) as well as deficits in spatial memory, object recognition memory and social interaction (see Patterson, 2009; Meyer and Feldon, 2012 for review). In spite of growing interest in this model due to its face and moderate predictive validity (Meyer and Feldon, 2012; Reisinger et al., 2015), concerns have been raised with regards to the high variability in immune response between different batches of poly (I:C) and optimum gestational day for treatment and optimum poly (I:C) dose (Murray et al., 2018; Meyer and Feldon, 2012). Identifying the appropriate gestational day for poly (I:C) treatment is essential since each gestational day represents a different developmental stage. Therefore, this model, whilst attractive, is yet to be fully validated.

Post-natal treatment with PCP is another neurodevelopmental model that explores the influence of adverse early-life challenges as a risk factor associated with development of schizophrenia. Based on a recent review published from our laboratory (Grayson et al., 2015a), most published papers using a post-natal PCP dosing regimen have treated the animals at post-natal days 7, 9 and 11, which corresponds to the late third trimester and early days after birth in human pregnancy (Clancy et al., 2007), during which period brain development is susceptible to environmental and pharmacological challenges. Regardless of the time and duration of treatment, post-natal PCP treatment is associated with long-lasting neurobiological changes including reduced expression of PV in the mPFC (Kaalund et al., 2013), disrupted GABAergic transmission (Kjaerby et al., 2014) and deficits in cognitive domains including visual recognition memory (Lu et al., 2011) and attentional set-shifting (Broberg et al., 2009) which are manifested at adulthood. The full scope of the face and predictive validity of this model is also yet to be investigated.

The models described so far provide a platform with which to study the influence of pre-natal and early life adverse challenges as risk factors associated with schizophrenia. The period of adolescence represents another critical window of susceptibility to schizophrenia during which environmental challenges can significantly impact brain development and behaviour (Selemon and Zecevic, 2015). Rearing rats in isolation from weaning (post-natal day 23; early adolescence) represents a non-pharmacological intervention that explores the impact of adverse events during the later stages of neural development (Harte et al., 2007). Similar to models discussed, isolation rearing also induces long-lasting changes in mesocortical and mesolimbic dopamine regulation (Jones et al., 2011) and deficits in tasks of visual learning memory and executive function (McLean et al., 2010a; Li et al., 2007).

Consistent with the pathophysiology of schizophrenia, reduced PV expression has also been reported in the hippocampus of isolation-reared rats (Harte et al., 2007). A recent study, however, failed to replicate these findings (Kaalund et al., 2013). Thus far, a very limited number of studies have investigated the effectiveness of APs on rescuing isolation-induced cognitive deficits (Abdul-Monim et al., 2003; Li et al., 2007). Therefore, the predictive validity of this model remains to be explored. Furthermore, the cognitive deficits induced by isolation-rearing are fragile and can be readily reversed by repeated handling (Weiss et

al., 1999; Pritchard et al., 2013). As such, it is suggested that this model is best used in combination with others to create a multiple-hit paradigm (Jones et al., 2011; Grayson et al., 2015a).

1.5.2. Genetic models

One of the fastest growing domains of animal models with relevance to schizophrenia is the development of genetic models (Moore, 2010; Nestler and Hyman, 2010). Through genome-wide association studies over 108 risk-associated genomic loci have been identified in patients with schizophrenia (Ripke et al., 2014). Many of these consist of genes that are involved in regulation of synaptic plasticity, glutamatergic and dopaminergic transmission (Tansey et al., 2015) each of which could be manipulated and studied in animal. The disrupted in schizophrenia 1 (*DISC 1*), *Neurogulin 1* and its receptor *ErbB4*, catechol-o-methyltransferase (*Comt*) are just a few examples of genetic models generated based on most common genomic variations associated with schizophrenia. Description of these models is beyond the scope of the work presented here, however, they have been reviewed elsewhere in detail (Papaleo et al., 2012; Jones et al., 2011). It is noteworthy that while these models allow for targeted study of specific protein cascades, labelling them as animal models of schizophrenia should be done with caution. As with other animal models, there are certain challenges associated with creating genetic models for a given psychiatric disorder. Schizophrenia is a polygenic phenomenon; therefore, it is unlikely for one genetic mutation to encompass all schizophrenia-like phenotypes. Furthermore, human genes might create more isoforms than the equivalent genes in mice (species of choice for genetic studies), therefore, simply knocking-out genes in mice might not capture the relevant isoform while knocking-in human isoforms might not be compatible with mouse genome (Papaleo et al., 2012). Several lines of evidence also suggest that many of the genetic risk factors associated with schizophrenia are also associated with bipolar and autism spectrum disorder (Nestler and Hyman., 2010; Lichtenstein et al., 2014; Gaugler et al., 2014). The mechanisms by which the same genetic risk factor gives rise to various phenotypes may depend on its interaction with other genes and environmental factors (Nestler and Hyman., 2010). These are a few challenges that can potentially confound and limit the construct and face validity of genetic models for a given psychiatric disorder (Nestler and Hyman., 2010).

1.5.3 Pharmacological models: Sub-chronic phencyclidine

Influential clinical findings and case observations dating back to 1950, have consistently revealed that a single exposure to psychostimulants such as phencyclidine (PCP) in healthy individuals induces behavioural disruptions similar to the positive and negative symptoms and cognitive impairments observed in patients with schizophrenia (Luby et al., 1959; Krystal et al., 1994; Malhotra et al., 1996; Krystal et al., 2005). These findings led to the generation of a range of animal models using pharmacological agents such as PCP and similar molecules such as ketamine and dizocilpine (MK-801) to mimic schizophrenia-like symptoms.

PCP is a high-affinity non-competitive antagonist, binding to the NMDAR in a use-dependent manner (Roth et al., 2013). Although primarily acting on glutamatergic neurotransmission, PCP can also alter cholinergic neurotransmission by acting at nicotinic receptors (Morris et al., 2005). PCP also shows strong affinity for the sigma opioid receptor, however, its affinity for other receptors is less potent than its action on the NMDAR (Roth et al., 2013; Morris et al., 2005). Similar to clinical findings, substantial evidence suggests that acute treatment with PCP induces a range of behavioural and neurochemical imbalances in rodents. These include hyperlocomotor activity (Jentsch et al., 1998; Kalinichev et al., 2008; Janhunen et al., 2015), which is often considered an index with translational relevance to psychosis (Adams and Moghaddam, 1998; Janhunen et al., 2015; Jones et al., 2011). Acute treatment with PCP is also associated with deficits in a range of cognitive functions, including executive function and cognitive flexibility (Egerton et al., 2005), visual recognition memory (Grayson and Neill, 2004) and reversal learning of an operant task (Idris et al., 2005; Abdul-Monim et al., 2003).

As measured by microdialysis studies, acute treatment with PCP significantly increases glutamate and dopamine efflux in the mPFC and nucleus accumbens of rodents which is accompanied by behavioural disturbances including hyperlocomotor activity and impaired working memory performance (Adams and Moghaddam, 1998). Accumulating evidence also suggests that systemic acute administration of PCP significantly increases the firing rate and the activity of the mPFC pyramidal neurons (Suzuki et al., 2002; Jodo et al., 2005). Suzuki and colleagues (2002) further reported that the timing of this heightened activity corresponds to the observed behavioural disturbance, locomotor activity specifically, in the treated animals. Recent evidence also supports the relationship between potentiation of pyramidal neuron response and disturbances in executive function upon acute treatment with MK-801 (Blot et al., 2015). Interestingly, the behavioural and cognitive effects of acute treatment with NMDAR antagonist, return to baseline earlier (60-80 mins after acute NMDAR-antagonist treatment) than dopamine and glutamate concentrations in the mPFC and nucleus accumbens (160 mins after acute NMDAR-antagonist treatment) (Adams and Moghaddam, 1998; Moghaddam and Adams, 1998; Suzuki et al., 2002). Collectively these findings suggest that the behavioural effects of acute NMDAR antagonism are temporally dissociated from dopamine and glutamate levels (Adams and Moghaddam, 1998). Evidence also suggests that the elevated glutamate but not dopamine efflux (in both mPFC and nucleus accumbens) in response to acute PCP treatment, may be important in mediating its behavioural effects. This has been confirmed by Moghaddam and Adams (1998) who reported that when glutamate levels were normalised (by an acute pre-treatment with glutamate metabotropic receptor agonist) the behavioural effects of acute PCP treatment was ameliorated in spite of high dopamine levels in response to acute PCP treatment. Collectively, these findings suggest that elevated dopamine levels are not sufficient in maitaining behavioural effects of acute PCP treatment and that imbalances in glutamatergic neurotransmission may underlie these observed acute PCP-induced effects (Moghaddam and Adams, 1998; Jentsch and Roth, 1999; Jodo, 2013).

It is now well established that PCP preferentially acts at NMDARs located on the fast-spiking GABAergic interneurons (Homayoun and Moghaddam, 2007). These interneurons are crucial for regulating the firing rate and timing of activity of pyramidal neurons. Upon acute systemic treatment with PCP, GABAergic inhibitory tone on pyramidal neurons is significantly reduced, resulting in the heightened activity of those pyramidal neurons (Jodo et al., 2005; Homayoun and Moghaddam, 2007). As such, acute blockade of the NMDAR on GABAergic interneurons renders information processing less precise and fine-tuned leading to interruption in cognitive performance. Details of this phenomenon are beyond the scope of this thesis, but are thoroughly discussed in a recent review published from our laboratory (Cadinu et al., 2017). This is also briefly discussed in **Section 1.4**.

Although the behavioural and cognitive imbalances induced by acute PCP treatment represent a range of symptoms similar to the active stage of schizophrenia, this model fails to recapitulate disease related pathophysiology. For instance, acute PCP treatment is associated with an increase in corticofrontal blood flow (Gozzi et al., 2008), which contrasts the hypofrontality observed in schizophrenic patients (Buchsbaum et al., 1990; Andreasen et al., 1997; Janhunen et al., 2015). Furthermore, symptoms of cognitive impairments are persistent in patients with schizophrenia whilst the duration of acute NMDAR-antagonist induced cognitive impairment is less stable and readily reversible after treatment washout (Blot et al., 2015). However, in chronic abusers, withdrawal from PCP is associated with a range of persistent cognitive and behavioural abnormalities, resembling schizophrenia (Jentsch and Roth, 1999; Morgan et al., 2010). It is, therefore, suggested that repeated PCP administration might better represent the pathophysiology of the disease compared to acute exposure.

In sharp contrast with acute PCP exposure, its repeated administration, most commonly following the sub-chronic (sc) treatment regimen (scPCP; twice daily for 7 days (Grayson et al., 2007; Mclean et al., 2010b; Grayson et al., 2014; Jenkins et al., 2008; Jenkins et al., 2010; McKibben et al., 2010) or once daily for 5-14 days (Jentsch et al., 1997; Jentsch et al., 1998; Cochran et al., 2003; Fattorini et al., 2008; Dawson et al., 2014) followed by varying washout periods), significantly reduces dopamine utilisation (Jentsch et al., 1997; Jentsch et al., 1998), glucose utilisation (Cochran et al., 2003) and basal glutamate release in rats (Fattorini et al., 2008), which is consistent with evidence of hypofrontality in patients with schizophrenia (Buchsbaum et al., 1990; Andreasen et al., 1997). In addition, scPCP treatment induces a state of hypersensitisation to a subsequent PCP (Janhunen et al., 2015) or amphetamine (Jentsch et al., 1998) challenge, which is associated with a hyper-responsive sub-cortical dopaminergic system (Janhunen et al., 2015). Typical and atypical APs can attenuate psychostimulant-induced hyperlocomotor activity (Phillips et al., 2001; Jones et al., 2011; Samaha et al., 2007), which supports the predictive validity of this model for the positive symptoms associated with schizophrenia. ScPCP-induced PCP/amphetamine sensitisation is one of the most consistently replicated facets of this model. Hence, it is increasingly used as a test to ensure that the scPCP treatment has been effective (Janhunen et al., 2015).

Consistent with observations in patients with schizophrenia (Konopaske et al., 2014), sub-chronic treatment with PCP also significantly reduces the number of dendritic spines in the PFC (Elsworth et al., 2011). Reduced expression of parvalbumin (PV) is a hallmark of schizophrenia (Reynolds and Neill, 2016; Cadinu et al., 2017; Gonzalez-Burgos et al., 2015) and reduced PV expression in the hippocampus (Abdul-Monim et al., 2007; Jenkins et al., 2008; Jenkins et al., 2010) and the prelimbic (Prl) region of the medial prefrontal cortex (mPFC) (McKibben et al., 2010) has been reported 6 weeks following scPCP treatment. Reduced expression of PV mRNA in the Prl region of the mPFC has also been reported as early as 72 h post scPCP treatment (Cochran et al., 2003).

Due to their highly subjective nature, tests of negative symptoms have been difficult to develop and present findings on available measures are inconsistent (Jones et al., 2011). Snigdha and Neill (2008) reported a significant reduction in social behaviour (sniffing) (similar to social withdrawl, a negative symptom) 6 weeks post-PCP treatment (Snigdha and Neill, 2008). This is while Jenkins et al (2008) reported a significant increase in sniffing and following behaviour in rats 24 h to 6 weeks post-PCP treatment. Similarly, Lee et al (2005) reported a significant reduction in social contact behaviour (sniffing) and a significant increase in non-contact social behaviour (following) when tested 24 h post scPCP treatment (Lee et al., 2005). It has also been reported that scPCP treatment in rats fails to induce a deficit in sucrose intake, often used as a test with relevance to anhedonia (Jenkins et al., 2010). Nevertheless, we have been able to show scPCP-induced deficits in affective/mood and reward processing in our laboratory using the affective bias task and the sand paper paradigm (Sahin et al., 2016).

scPCP treatment is associated with enduring deficits in a range of cognitive functions, including deficits in visual learning memory (Grayson et al., 2007), executive function (Egerton et al., 2008; McLean et al., 2009; McLean et al., 2011), cognitive flexibility (Abdul-Monim et al., 2006; Abdul-Monim et al., 2007; McLean et al., 2012) and attention (Amitai et al., 2007). Cognitive deficits associated with this model are extensively reviewed by our team (Neill et al., 2010; Neill et al., 2014; Cadinu et al., 2017). Alterations in PV expression in the hippocampus and mPFC as well as the reduced functional interaction between these two regions (Dawson et al., 2014; Dawson et al., 2015), (latter also observed in patients with schizophrenia (Meyer-Lindenberg et al., 2005; Bahner and Meyer-Lindenberg, 2017)) may explain cognitive deficits associated with scPCP treatment. Whether behavioural paradigms and cognitive tests developed for pre-clinical research confidently target the complexity of cognitive processes in patients with schizophrenia remains a matter of debate (Janhunen et al., 2015; McAllister et al., 2015). Nonetheless, it can be argued that with high reproducibility, the scPCP model is perhaps the best validated animal model of cognitive deficits associated with schizophrenia. The predictive validity of this model is also extensively studied and reviewed elsewhere (Lyon et al., 2012; Janhunen et al., 2015; Cadinu et al., 2017).

1.6. Antipsychotics

Prior to the discovery of the first AP, management of schizophrenia was limited to prolonged hospitalisations and electroconvulsive therapy. Since the introduction of the first AP, chlorpromazine, in the 1950s, these compounds have become the cornerstone of schizophrenia treatment. Thus far, over 60 different APs have been developed which can be categorised into typical and atypical agents (Tandon et al., 2010). In spite of their distinct classification, blockade of D2R is a common feature of all APs (Meltzer, 2012; Meltzer, 2013).

1.6.1. Typical Antipsychotics

Chlorpromazine, haloperidol and perphenazine are a few examples of typical APs. The typical APs are particularly effective in the management of the positive symptoms of schizophrenia (Yin et al., 2017) and substantially reduce the risk of relapse for at least two years following continuous treatment (Leucht et al., 2012). The antipsychotic potency of the typical agents is strongly correlated with their affinity for the striatal D2R (Kapur et al., 1996). Substantial evidence derived from positron emission topography (PET) scanning studies report 60% to 75% D2R occupancy to be the optimum range for the efficacy of the typical APs to treat the positive symptoms (Uchida et al., 2011). Higher levels of D2R occupancy (above 80%) with typical agents is associated with severe extrapyramidal side effects (EPS) such as tardive dyskinesia, due to the interruptions in the nigrostriatal dopamine pathway (Uchida et al., 2011; Meltzer, 2013). Hyperprolactemia is also a side effect that can manifest due to the blockade of D2R in the tuberoinfundibular dopamine pathway (Montgomery et al., 2004).

Amongst typical APs, haloperidol has the highest discontinuation rate in comparison to perphenazine (Lieberman et al., 2005), chlorpromazine (Leucht et al., 2013) and atypical APs (Kahn et al., 2008). Discontinuation can be attributed to low tolerability and lack of efficacy (Kahn et al., 2008; Leucht et al., 2013; Zhang et al., 2013). In comparison to all other typical and atypical APs, haloperidol is associated with highest rate of EPS leading to its rapid discontinuation by the patients (Leucht et al., 2013). Haloperidol is also the most frequently used AP against which the neurocognitive effects of all other agents are measured. The neurocognitive effects of haloperidol are discussed in depth in **Section 1.7.** It suffices to note that controversies exist surrounding the beneficial effects of this compound, partly due to its general lack of tolerability. In the studies presented in this thesis, haloperidol was chosen as the representative typical AP agent and its neurocognitive effects upon long-term treatment was investigated in a well-validated animal model for cognitive impairments in schizophrenia.

1.6.2. Atypical Antipsychotics

In comparison to the typical agents, the atypical APs such as olanzapine, risperidone, quetiapine and clozapine have a more diverse pharmacology. All atypical APs act on one or more of the 14 serotonin

receptors (5-HTR) including the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_6 and 5-HT_7 receptors (Reynolds, 2004; Ishibashi et al., 2010; Meltzer et al., 2011; Meltzer, 2012). In addition to the 5-HTR, atypical APs also act as partial agonists or antagonists on D2R. However, their affinity for the D2R is much less potent than the typical APs (Meltzer, 2012). 5-HTR are abundant on pyramidal and GABAergic interneurons in the PFC as well as dopaminergic neurons in the VTA and substantia nigra (Alex and Pehek, 2007; Meltzer, 2012). Serotonin strongly modulates dopamine transmission in the brain (Alex and Pehek, 2007). Ample evidence suggests that atypical APs increase dopamine release in the PFC. This effect is thought to be induced by the combined antagonistic effect of APs such as olanzapine, clozapine, risperidone and ziprasidone on 5-HT_{2A}R and D2R. This is supported by studies that report no change in PFC dopamine concentration when 5-HT_{2A}R or D2R antagonist is administered alone (Assie et al., 2005; Alex and Pehek, 2007; Oyamada et al., 2015). This, in addition to the selective reduction of mesolimbic dopamine release underlies the mild neurocognitive effects and potent antipsychotic effect of atypical APs (Meltzer, 2012; Oyamada et al., 2015). In contrast to typical APs, the atypical APs are associated with significantly lower instances of EPS (Meltzer et al., 2011; Meltzer, 2012; Leucht et al., 2013). This is also associated with action of atypical agents 5-HT_{1A}R and 5-HT_{2C}R and their modulation of dopamine in the nigrostriatal dopamine pathway (Reynolds, 2004; Newman-Tancredi and Kleven, 2011; Meltzer, 2012).

In spite of lower instances of EPS, the atypical agents are associated with severe disruptions in lipid and glucose metabolism, potentially resulting in weight gain and diabetes (Freyberg et al., 2017; Szmulewicz et al., 2017). The mechanisms underlying these disruptions are not well understood, however, atypical AP-induced weight gain is strongly linked to the action of these compounds on the 5-HT_{1A}R and 5-HT_{2C}R. As 5-HT_{2C}R agonists reduce food-intake, it is not surprising that APs with higher instances of weight gain (such as olanzapine) are high-affinity 5-HT_{2C}R antagonists (Reynolds et al., 2006). AP-induced weight gain is the single most likely cause of treatment discontinuation in patients. Olanzapine is consistently reported to induce the highest instances of weight gain in patients (Leucht et al., 2013; Zhang et al., 2013), nevertheless, rate of treatment discontinuation for any cause is significantly lower for olanzapine and then clozapine in comparison to all other atypical and typical APs (Lieberman et al., 2005; Leucht et al., 2013). Contrary to this, Mustafa and colleagues (2018) reported no significant difference in all cause treatment discontinuation between atypical APs (Mustafa et al., 2018).

Current guidelines promote the use of atypical APs as the first line of treatment for the management of schizophrenia (NICE Guidelines, 2014; Mustafa et al., 2018). Although these compounds are effective in treating the positive symptoms associated with the disease, their neurocognitive effects upon long-term treatment remains a matter of debate. Since time to discontinuation appears longer upon treatment with olanzapine, studies presented in this thesis will investigate the neurocognitive effects of long-term treatment with this compound as a representative of atypical agents.

1.7. Influence of Antipsychotics on Cognition

1.7.1 Clinical Studies

For some years after the introduction of the atypical APs, this class of drugs was regarded superior to the typical agents in improving cognitive function in patients with schizophrenia (Keefe et al., 1999). For instance, in a sample of chronic stable schizophrenia patients (n=25), 8 weeks of treatment with quetiapine (468.2 mg/day) but not haloperidol (2-20 mg/day) was found to significantly improve verbal fluency and reasoning and visuospatial processing in comparison to baseline. Similar results emerged at a 6 month follow-up period where, in addition to the sustained improvement in the aforementioned cognitive domains, a trend towards improvement of executive function and immediate recall was also observed in patients receiving quetiapine but not haloperidol in comparison to baseline (Purdon et al., 2001). It is noteworthy that in this study only 3 patients remained in the haloperidol treatment group and 8 patients remained in the quetiapine treatment group at 6 month follow-up, which severely compromises the statistical power of the study. In a similar study of chronic schizophrenia patients (n=101), 14 weeks of treatment with risperidone (8 mg/kg/day) and olanzapine (20 mg/kg/day) was significantly superior to treatment with haloperidol (20 mg/kg/day) and clozapine (500 mg/kg/day) and baseline measures in improving global cognitive performance, executive function, attention and memory (Bilder et al., 2002). Apparent superiority of the atypical over typical APs was further supported by treatment switch studies. Velligan and colleagues (2002) obtained baseline cognitive measures from patients who were receiving haloperidol (<30 mg/day). Patients (n=58) were then randomised to receive quetiapine or remain on haloperidol treatment for the duration of the study. At 6 months follow up, treatment with high dose of quetiapine (600 but not 300 mg/day) significantly improved overall cognitive function, executive function and attention in comparison to treatment with haloperidol (12 mg/day) and baseline measures (Velligan et al., 2002). In a similar study, patients (n=13) were assessed at baseline while being treated with haloperidol (40 mg/day). After initial assessment, all patients were switched to risperidone (3-11 mg/day) treatment for 6 months. Results revealed a significant improvement in tests of selective attention in comparison to baseline (Stip and Lussier, 1996).

Several studies also suggested that typical APs were detrimental to cognitive performance. For instance, in a study of stable schizophrenia patients, 10-weeks of treatment with haloperidol (10-30 mg/day) was found to significantly impair performance on tests of executive function, verbal fluency and visuospatial processing in comparison to baseline. In the same study clozapine (200-600 mg/day) induced a trend towards improvement in performance on the same cognitive tests (Buchanan et al., 1994). A quasi-randomised study of FEP and chronic schizophrenia patients showed that in comparison to haloperidol (3-15 mg), risperidone (8-4 mg) and clozapine (200-400 mg) significantly improved executive function and fine motor skills. Performance was significantly poorer in chronic patients treated with haloperidol compared to clozapine and risperidone and their FEP counterparts. Performance in the haloperidol treatment group was also significantly poorer in comparison to unmedicated chronic and FEP. This study has design limitations

that must be considered when interpreting the relevance of the results. For instance, the recruitment criteria were poor, no baseline measures were obtained and the duration of the study was inappropriate and very short (7 days). Patients were included if they were on their current APs for a minimum of 7 days, which is inadequate for treatment stabilisation (Gallhofer et al., 1996).

Several methodological issues bring these findings into question. The majority of these studies were of inadequate sample size and the treatment history of the patient was not clear (Buchanan et al., 1994; Gallhofer et al., 1996). Therefore, the influence of APs during the course of the trial is confounded. In some studies, doses were gradually increased week by week. However, this was not accounted for in the statistical analysis (Buchanan et al., 1994). Short-term follow up period as well as lack of appropriate comparison groups was also common across these studies. Given the short test-re-test interval, much of the improvement observed with the atypical APs can be attributed to the practice effect. One major methodological obstacle was the inappropriate comparison between high doses of typical APs against low doses of atypical APs (Keefe et al., 2004). At high doses, haloperidol interferes with the practice effect by compromising procedural learning (Woodward et al., 2007). This is thought to be due to the severe side-effects associated with haloperidol treatment as well as the negative impact of adjunct treatment including anticholinergic drugs which have a detrimental impact on cognition (Keefe et al., 2007a; Eum et al., 2017).

Substantial evidence from methodologically modified studies now suggests that both typical and atypical APs significantly improve cognition and the magnitude of these improvements is similar for both agent categories. Keefe and colleagues (2004) showed a significant improvement in cognitive performance on tasks of vigilance and attention, verbal memory and fluency and working memory upon 3 months of treatment with haloperidol (5.5 mg/day) and olanzapine (10 mg/day) in comparison to baseline. In this sample of FEP (n=167), performance of the olanzapine treated group was significantly superior to the haloperidol treatment group in aforementioned cognitive domains at 3 month follow up. Furthermore, weighted composite scores (calculated from scores of each test) was significantly higher in the olanzapine treatment group, suggesting that overall, olanzapine may have been more beneficial that haloperidol. In the same sample, a similar pattern emerged at a 6 month follow-up when comparing weighted composite scores to baseline and between treatment groups. While maintaining performance improvement in comparison to baseline, there was no significant difference between the treatment groups in performance in individual cognitive domains or in weighted composite scores at 1 year and 2 year follow-up (Keefe et al., 2006).

Neurocognitive effects of risperidone in comparison to haloperidol also followed a similar pattern. In FEP (n=533), 3 months of treatment with risperidone (3.3 mg/day) and haloperidol (2.9 mg/day) significantly improved cognitive performance in tasks of episodic memory, vigilance and attention as well as visuospatial processing in comparison to baseline. However, risperidone showed a small advantage in improving executive function and verbal fluency (Harvey et al., 2005). While maintaining the findings of significant improvement at the aforementioned cognitive domains, treatment with risperidone and haloperidol also

improved sustained attention and verbal learning and memory with no significant difference between the treatment groups at 1 (Crespo-Facorro et al., 2009), 2 (Green et al., 2002) and 3 years (Ayesa-Arriola et al., 2013) follow-up points. In the study reported by Crespo-Facorro and colleagues (2009), 15 patients from the haloperidol treatment group switched to other treatments at study midpoint. Authors report no significant difference in the cognitive performance of the on-going haloperidol treatment group and the haloperidol-switch treatment group. Results of these studies are reinforced by others reporting no significant difference in the neurocognitive effects of risperidone (0.5-4 mg/kg), olanzapine (2.5-20 mg/kg) and quetiapine (100-800 mg/kg) in the FEP upon 3 months to 1 year of treatment (Keefe et al., 2007a).

Short-term clinical trials in chronically ill patients also point towards findings similar to the FEP. Perhaps the best examples of such studies are the US Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE). These non-industry funded studies included chronic schizophrenia patients with an average of 14 years of prior AP treatment. Patients were randomised to receive atypical APs including olanzapine, risperidone, quetiapine and ziprasidone or the typical AP perphenazine, which followed a flexible dosing regimen (Keefe et al., 2007b; Lewis and Lieberman, 2008). Results revealed that all treatments induced a small but significant improvement in the composite scores derived from 11 neurocognitive tests at 2 and 6 month follow-up. A similar pattern emerged at 18 months, however, the neurocognitive effects of perphenazine was moderately higher than for the other APs (Keefe et al., 2007b). Generally, findings of these studies have been confirmed by meta-analyses suggesting an improvement in cognitive performance with both typical and atypical APs (Woodward et al., 2005; Woodward et al., 2007; Desamericq et al., 2014). Details and results of these studies are summarised in **Table 1.1.**

The significance of these neurocognitive effects in terms of clinical and functional improvement of the patients remains to be determined. The effect sizes of the neurocognitive improvements reported in these studies are relatively small and can be explained by changes in clinical symptoms in studies of both FEP and chronic patients (Keefe et al., 2007b; Keefe et al., 2007a; Keefe and Harvey, 2012). In studies that include healthy participants as controls, the extent of neurocognitive improvement in patients treated with atypical antipsychotics is similar to healthy controls, which signifies the effect of the practice effect (Crespo-Facorro et al., 2009; Ayesa-Arriola et al., 2013). It has also been suggested that haloperidol (independent of dose) might impede this practice effect (Woodward et al., 2007), hence, haloperidol appears less effective in improving cognitive performance. This might explain the apparent superiority of atypical AP in studies with short follow-up, which is eliminated when the test-retest interval is prolonged. In randomised studies of chronic patients, in addition to the practice effect, factors such as duration of illness, duration of un-treated psychosis (Chang et al., 2013), hospitalisation period, poly-pharmacy (Hori et al., 2006) and treatment discontinuation confound the interpretation of the findings.

Based on current guidelines, patients are recommended to remain on APs long-term (Moncrieff, 2015). Given the recurrent and chronic nature of the disease, treatment with APs tends to be life-long. It can be argued that the duration of AP treatment in the studies reported above are relatively short and may not be

representative of chronic treatment. A recent naturalistic study showed that in comparison to controls, higher life-time accumulative dose of AP was associated with poorer baseline performance in tasks of verbal learning and memory in chronically ill patients (mean illness duration of 16.5 years). Performance in this domain of cognition had significantly declined at the 9-year follow-up as a function of higher accumulative AP exposure (Husa et al., 2014). Similarly, higher AP exposure was significantly negatively associated with scores of global cognitive performance, obtained from 9 neurocognitive tests (Husa et al., 2017).

Naturalistic studies provide a more realistic setting for studying the influence of long-term AP treatment on neurocognitive functioning. While randomised clinical trials are well equipped with examining treatment efficacy and its side effects, they are not feasible for studies of long-term treatment effects (Young et al., 2015). Longitudinal imaging studies report a significant negative association between higher accumulative AP dose and brain volume (Fusar-Poli et al., 2013). Furthermore, imaging studies have provided evidence both for (Ho et al., 2003; Dempster et al., 2017; Jirsaraie et al., 2018) and against (Veijola et al., 2014; Heinrichs et al., 2017) a relationship between brain cortical and white matter reduction and cognition. Since symptoms of cognitive impairments associated with schizophrenia severely impacts patients' functional outcome and quality of life, determining the influence of long-term treatment with APs is essential. Thus far, understanding these effects has proven difficult, leaving development of effective treatment an unmet need in clinical practice (Neill et al., 2010; Neill et al., 2014).

Authors	Patients	Treatment (mg/day)/Design	Cognitive Assessment	Outcome	Limitations /Extra detail
Buchanan et al (1994)	Stable schizophrenia **(n=38)**	Randomised, 10 weeks follow up **Hal (10-30)** **Clz (200-600)** (After 10 weeks, patients in Hal group were switched to open label Clz for 1 year follow up)	Executive function and verbal fluency Visuospatial processing Memory	At 10 weeks, Hal impaired performance on executive function, verbal fluency and visuospatial processing. Clz had no significant effect. At 1-year, significant improvement was present at all measured domains. Improvement unrelated to change in clinical symptoms.	- Small sample size - Lack of control group - High dose of Hal - Patient treatment history not provided
Gallhofer et al (1996)	FEP, chronic schizophrenia and unmedicated counterparts **(n=78)** (patients stable on their medication for at least 7 days)	Patients remained on the APs regimen, not randomised **Hal (3-5)** **Ris (4-8)** **Clz (200-400)**	Executive function and motor learning	Overall performance on measured tasks was significantly improved in patients treated with Ris and Clz compared to Hal. Performance was significantly poorer in chronic patients treated with haloperidol compared to clozapine and risperidone and their FEP counterparts. Performance in the haloperidol treatment group was also significantly poorer in comparison to unmedicated chronic and FEP	- Heterogeneous sample - High dose of Hal - No baseline measures - Short treatment duration
Stip and Lussier (1996)	Schizophrenia **(n=13)** All patients were on Hal (appx. 40 mg/day) at baseline	Not randomised, All patients switched to **Ris (3-11)** Follow up at 6 months	Selective attention	Ris treatment significantly improved performance in both tasks	- No control groups - Small sample size - Disease-stage unspecified - Positive-correlation between-cognitive improvement-and other-clinical symptoms. - Limited cognitive battery
Purdon et al (2001)	Stable schizophrenia **(n=25)**	Randomized, Follow up at 8 weeks and 6 months **QT (468.2)** **Hal (2-20)**	Attention Verbal reasoning visuospatial processing Executive function Immediate recall	At 8 weeks QT but not Hal improved verbal reasoning, fluency and visuospatial processing compared to baseline. At 6 months also, a trend towards improvement in executive function, verbal reasoning and immediate recall was reported with QT but not Hal compared baseline	- High haloperidol dose - Small Sample size - By 6 months follow up only 3 patients remained in the Hal and 8 in the QT treatment groups, hence study is statistically under powered
Bilder et al (2002)	Chronic schizophrenia **(n=101)**	Randomised, 14 weeks follow up **Clz (500)** **Ris (8)** **Olz (20)** **Hal (20)**	Attention Memory Global cognition Executive function Motor learning	Global cognitive scores and executive function were significantly higher in Olz and Ris group compared to Hal and Clz and in comparison to baseline. Olz specifically improved attention and Ris specifically improved memory while Clz improved motor function only when compared to baseline	- High haloperidol dose - No control groups - Suggests that each AP may be effective in improving specific cognitive domains - Effect sizes for Hal were small

Velligan et al (2002)	Stable schizophrenia **(n=58)** All patients were on Hal (<30 mg/day) at baseline	Randomized, follow up at 6 months **QT (300 and 600)** **Hal (12)**	Verbal memory Visual memory Cognitive flexibility Executive function Selective attention	High but not low doses QT significantly improved overall cognitive performance, executive function and attention compared to Hal and baseline	- Disease-stage unspecified - Fixed dose regimen - High-dropout rate
Green et al (2002)	FEP **(n=62)**	Randomised, follow up at 4,24,48,74 and 104 weeks **Hal (4.5-5.2)*** **Ris (5.7-61)***	Executive function Cognitive flexibility Visual learning/memory Verbal learning/memory Attention Speed of processing	No significant difference in global or individual tests across treatment groups. Neurocognitive effects of treatment with haloperidol were most prominent at early time points but then stabilized. Effect of Ris was more gradual.	* Values represent average dose range. Flexible dosing was used in this study, with doses decreasing every 6-12 months. - Neurocognitive benefits possibly due to practice effect given the short test-re-test interval - No control groups - Patient treatment history not reported
Keefe et al (2004)	FEP, Schizoaffective or schizoform disorder **(n=167)**	Randomised, follow up at 3 months **Hal (5.5)** **Olz (10)**	Compete battery of cognitive tests at Baseline and follow-up (weighted and unweighted composite scores calculated)	Improvement on tasks of vigilance and attention, verbal memory and fluency upon 3 months of treatment with Hal and Olz. Olz showed a small but significant improvement in weighted composite scores compared to Hal	- Heterogenous samples. - Treatment history varied between zero to 16 weeks previous exposure to AP - For Hal but not Olz cognitive improvement was associated with improvement in clinical symptoms. - Practice effect due to short test-re-test duration cannot be ruled out.
Harvey et al (2005)	FEP **(n=533)**	Randomised, follow up at 3 months **Ris (3.3)** **Hal (2.9)**	Executive function Episodic memory Visuospatial processing Vigilance and attention Cognitive flexibility	Both treatments significantly improved performance in tasks of episodic memory, vigilance and attention and visuospatial processing. However, Ris showed a small advantage in improving executive function and verbal fluency	- High drop-out rate (359 at 12 weeks) - For Hal but not Ris cognitive improvement was associated with improvement in clinical symptoms. - Practice effect due to short test-re-test duration cannot be ruled out.
Keefe et al (2006)	Same cohort as Keefe et al (2004) **(n=123)**	Randomised, follow up at 6 months, 1 and 2 years **Hal (5.5)** **Olz (10)**	Compete battery of cognitive tests at Baseline and follow-up (weighted and unweighted composite scores calculated)	Similar pattern of results as Keefe et al (2004) emerged at 6 months follow up. But there was no significant difference between treatment groups at 1 and 2 year follow up.	- At 2 years only 26 patients completed the tests - Improvement in cognitive performance with Hal but not Olz was modestly correlated with improvement in clinical symptoms and reduced side-effects. This was significantly different from Olz treatment.

Keefe et al (2007a)	FEP with less than 5 years disease duration **(n=400)**	Randomised, follow up at 12 weeks and 1 year **Ris (0.5-4)** **Olz (2.5-20)** **QT (100-800)**	Complete battery of cognitive tests (as designed by CATIE trials)	Significant improvement with modest effect sizes in all domains tested across all treatment groups at 12 week and 1 year compared to baseline. There was no significant difference between treatment groups at either time point when comparing composite scores. Pairwise comparisons showed a small but significant advantage for QT over Ris and Olz in tests of speed of processing at 12 weeks only.	- 224 patients completed test at 12 weeks and only 81 at 1 year follow up. - A negative correlation (small effect size) between cognitive improvement and clinical symptoms. - A significant relationship between cognitive improvement and functional outcome. But this was not significant when controlling for symptom change
Keefe et al (2007b)	Chronic Schizophrenia **(n=817)**	Randomised, follow up at 2,6 and 18 months **Olz (7.5)*, Ris (1.5)*, QT (200)*** **Zip (40)*, Phz (8)***	Complete battery of cognitive tests (as designed by CATIE trials)	Small but significant improvement across individual tested domains and composite scores at 2 and 6 months with no difference between treatment groups. At 18 months, small but significant advantage in Phz treatment compared with all others.	* Values represent treatment dose contained within one capsule. Patients were treated with 1-4 capsules/day as advised by physicians. - 303 patients completed 18 month follow up only - A small negative correlation between cognitive improvement and clinical symptoms and treatment side-effects.
Crespo-Facorro et al (2009)	FEP **(n=174)** and controls **(n=37)**	Randomised, follow up at 6 months and 1 year **Olz (5-20)** **Ris (3-6)** **Hal (3-9)**	7 cognitive tasks targeting executive function, cognitive flexibility and speed of processing	Significant improvement in all cognitive measures in all treatment groups at follow up time points. No significant difference between treatment groups.	- Cognitive improvement in FEP and controls were similar, suggesting the strong effect of practice effect. - High drop-out rate (79 FEP at 1 year follow up)
Ayesa-Arriola et al (2013)	FEP **(n=79)** and controls **(n=41)**	Treatment and design same as Crespo-Facorro et al (2009), follow up at 3 years	7 cognitive tasks targeting executive function, cognitive flexibility and speed of processing	Same as Crespo-Facorro et al (2009). Slight advantage for Olz in verbal learning and memory	- Olz-specific cognitive improvement attributed to lower instances of cholinergic inhibitors for treating EPS. - The extent of neurocognitive improvements in patients receiving APs were similar to improvements associated with practice effect in healthy controls.

Table 1.1. Summary of clinical studies investigating the influence of AP treatment on cognition. High dose of Haloperidol and lack of healthy control groups is a common limitation in early clinical studies. Effect sizes of neurocognitive improvement with APs are small. As evident by more recent trials including healthy controls in the study, the neurocognitive improvement in patients is similar to the healthy controls. Hal: Haloperidol; Phz: Perphenazine; Ris: Risperidone; Olz: Olanzapine; Clz: Clozapine; Zip: Ziprasidone; QT: Quetiapine.

1.7.2. Pre-clinical Studies

The influence of typical and atypical AP on cognition has been widely studied in neurodevelopmental (Li et al., 2007; Nagai et al., 2011) and pharmacological animal models (Paine and Carlezon, 2009; Hill et al., 2010; McLean et al., 2011; Ozdemir et al., 2012) of cognitive impairments associated with schizophrenia. In the majority of these studies, acute treatment with atypical AP is superior to acute treatment with the typical agents in rescuing the manipulation-induced cognitive impairments. For example, acute treatment with olanzapine (1.5 mg/Kg; intraperitoneal injection; i.p.), clozapine (5 mg/Kg; i.p.), ziprasidone (2.5 mg/Kg; i.p.) (Abdul-Monim et al., 2006), cariprazine (0.1 and 0.25 mg/kg; oral administration; p.o.) and risperidone (0.1 mg/kg; i.p.) (Neill et al., 2015) but not haloperidol (0.05 mg/Kg; i.p.) and chlorpromazine (2 mg/Kg; i.p.) (Abdul-Monim et al., 2006) significantly ameliorated the scPCP induced deficits in the reversal phase of an operant reversal-learning paradigm. Acute treatment with clozapine (1.0 and 5 mg/kg; i.p.) but not haloperidol (0.05 and 0.075 mg/kg; i.p.) reversed the scPCP induced deficits in the retention phase of the NOR task (Grayson et al., 2007). Similarly, acute treatment with olanzapine (1-2 mg/kg; i.p.) and meloprone (1-3 mg/kg; i.p.) but not haloperidol (0.05-0.1 mg/kg; i.p.) rescued the scPCP-induced deficit in the NOR task (Snigdha et al., 2010). Hashimoto et al (2005) confirmed haloperidol's (0.1 mg/kg; i.p.) ineffectiveness in rescuing scPCP-induced NOR deficit in mice and further reported that clozapine (5 mg/kg; i.p.) also failed to rescue this deficit. At low doses (0.05 mg/kg; i.p.), risperidone also fails to rescue the scPCP-induced NOR deficit (Grayson et al., 2007; Snigdha et al., 2010), at higher doses (0.1 and 0.2 mg/kg; i.p.) evidence for its effectiveness in rescuing scPCP-induced deficit is contradictory (Grayson et al., 2007; Snigdha et al., 2010).

Acute treatment with olanzapine (0.3 and 1.0 mg/kg; p.o.) and aripiprazole (1.0 and 3.0 mg/kg; p.o.) but not haloperidol (0.1 mg/kg; p.o.) can also reverse the CPP (NMDA receptor competitive antagonist) induced deficits in performance accuracy in the 5-choice serial reaction time task (5-CSRTT), a measure of sustained and divided attention (Carli et al., 2011). This is while acute treatment with haloperidol (0.016 mg/kg; subcutaneous injection; s.c.) and clozapine (0.32 mg/kg; s.c.) failed to ameliorate the sub-chronic MK-801-induced (scMK-801) (0.5 mg/kg for 12 days; i.p.) deficit in 5-CSRTT (Paine and Carlezon, 2009). Acute treatment with risperidone (0.2 mg/kg; i.p.), clozapine (2.5 mg/kg; i.p.) but not haloperidol (0.05 mg/kg; i.p.) rescues the scPCP-induced deficit in the ability of rats to shift attention between different sorting categories in the attentional set shifting paradigm (McLean et al., 2008). Rodefer et al (2008), however, showed that these atypical APs, in addition to olanzapine (1.5-3 mg/kg; p.o.), were ineffective in rescuing scPCP-induced deficit in treated rats. This study further showed that, at high doses, sertindole (1.3 and 2.5 mg/kg; p.o.) was able to rescue this deficit (Rodefer et al., 2008). Differences in the findings of the last two studies could be explained by differences in rat strain, sex and the scPCP dosing regimen between studies. Details of these studies are summarised in **Table 1.2.**

Many studies have also investigated the influence of acute AP treatment on cognition in physiologically healthy animals. In these investigations, treatment with APs, typical and atypical, tends to impair cognitive

performance. Acute doses of clozapine (1.25 and 2.5 mg/kg; s.c.) but not haloperidol (0.04 mg/kg; s.c.) and risperidone (0.05 mg/kg; s.c.) significantly impaired response accuracy (increased errors) in the radial arm maze task, a measure of spatial working memory. All treatments also significantly increased response latency (Addy and Levin, 2002). In a similar study, acute treatment with olanzapine (2.5, 5 and 10 mg/kg; i.p.) and clozapine (5, 10, 20 and 40 mg/kg; i.p.) induced a dose-dependent reduction in correct performance in the 8-arm radial maze task. This could be due to the robust dose-dependent sedation associated with these APs (Ortega-Alvaro et al., 2006). Acute treatment with high dose of risperidone (1 but not 0.25 and 0.5mg/kg; i.p.), clozapine (3 but not 1 and 2 mg/kg; i.p.) and quetiapine (7.5 but not 2.5 and 5 mg/kg, i.p.) but not haloperidol (0.0125, 0.025 and 0.05 mg/kg; i.p.) significantly impaired attention (but not impulsivity) as measured by reduced percentage correct response in the 5-CSRTT (Amitai et al., 2007). Similar results were reported by Paine and Carlezon (2009) upon acute treatment with haloperidol (0.25 mg/kg; s.c.) and clozapine (2.5 mg/kg; s.c.).

Several studies have also investigated the influence of long-term neurocognitive effects of APs in animal models. 14 days of treatment with clozapine (4 mg/kg/day; osmotic minipump delivery) ameliorated the scPCP induced deficits in response accuracy and reduced number of premature responses in the 5-CSRTT. However, in this study, the protocol for PCP dosing regimen is unusual as it treated rats with PCP (2mg/kg/day; s.c.) for two days after which rats underwent osmotic minipump surgery. Three days post-implant rats were treated with saline (s.c.) for 5 days followed by 5 days of PCP treatment, 30 minutes prior to each session. This brings the validity of these findings into question, since the acute effect of PCP interferes with task performance (Amitai et al., 2007). It could be suggested that this is not an appropriate model or study design (see **Table 1.2** for details). In another study, treatment with asenapine (0.075 mg/Kg twice daily; s.c.), risperidone (0.2 mg/kg/day; i.p.) and olanzapine (1.5 mg/kg/day; i.p.) significantly improved scPCP-induced deficits in reversal learning performance of an operant task on treatment days 3, 7, 17, 21 and 28. With olanzapine, asenapine and risperidone treatment, accuracy of performance showed a trend towards an improvement 24 h after the last olanzapine treatment. This suggests that the influence of these drugs on cognition might be long-lasting (McLean et al., 2010b). This compares to a study where a higher dose of olanzapine (3 mg/kg/bidaily; p.o.) failed to reverse an scPCP-induced deficit in the attentional set-shifting paradigm upon 21 days of treatment (Rodefer et al., 2008). In another experiment, socially-reared or isolation-reared rats were chronically treated with clozapine (5 and 10mg/kg/day; i.p.) for 6-8 weeks. Clozapine treatment significantly improved the performance of isolates in learning new strategies in an operant reversal learning paradigm (Li et al., 2007). 21 days of treatment with clozapine (5 mg/kg/day; i.p.) has also been reported to be effective in rescuing deficits of spatial working memory (measured by Morris water maze) in offspring of mice treated with poly (I:C) (Meyer et al., 2010).

Long-term treatment with clozapine but not haloperidol has also been consistently reported to rescue manipulation-induced deficit in NOR performance across models. For instance, treatment with haloperidol (1 mg/kg/day; p.o.) and clozapine (3 mg/kg/day; p.o.) was also reported to be ineffective in rescuing the

NOR deficit in DISC1 mutant mice treated with poly (I:C) following at least 7 days of treatment. In this study treatment with AP started 7 days prior to the behavioural testing and lasted for the duration of the study. However, the duration of study is unspecified in the report (Nagai et al., 2011). 14 days of treatment with clozapine (5mg/kg/day; i.p.) but not haloperidol (0.1 mg/kg/day; i.p.) also rescued the NOR deficit induced by 10 days of intermittent PCP treatment (10 mg/kg/day; 10 days; s.c.) (Hashimoto et al., 2005). Similar effects were observed when treating adult offspring of poly (I:C) treated mice with clozapine (5 mg/kg/day; i.p.) or haloperidol (0.1 mg/kg/day; i.p.) for 14 days (Ozawa et al., 2006). Contradictory evidence also exists. For instance, 14 days of treatment with haloperidol (1 mg/kg/day; i.p.) or clozapine (5 mg/kg/day; i.p.) was also reported to be ineffective in rescuing MK-801 (5 days; i.p.; no washout) induced deficit in NOR performance in mice (Ozdemir et al., 2012).

Studies of long-term treatment with APs in healthy rodents have yielded inconsistent results. For instance, 21 days of treatment with clozapine (5 mg/kg/day; i.p.) impaired performance of male mice in a spatial working memory paradigm (Meyer et al., 2010). Similarly, 6-8 weeks of treatment with clozapine (5 and 10mg/kg/day; i.p.) interfered with performance of healthy male Sprague-Dawley rats in an operant reversal learning paradigm (Li et al., 2007). In contrast, 14 days of treatment with haloperidol (1 mg/kg/day; i.p.) but not clozapine (5 mg/kg/day; i.p.) was found to impair NOR performance in healthy adult mice (Ozdemir et al., 2012). In contrast, In a study of healthy male Wistar rats, 14 days of treatment with olanzapine (2.5, 5 and 10 mg/kg/day; i.p.) and clozapine (0.3 mg/kg/day; i.p.) did not alter performance on the radial arm water maze task (Ortega-Alvaro et al., 2006). Physiologically normal rats treated with haloperidol (2 mg/kg/day; delivered via drinking water) and olanzapine (10 mg/kg/day; delivered via drinking water) show significant impairment on the Morris water maze task (measure of spatial working and memory) upon 90 but not 45 days of treatment. After 90 days, rats took significantly longer to find the hidden platform across several trials. Also, rats treated with haloperidol performed poorly on a greater number of trials in comparison to the olanzapine treated rats (Terry et al., 2002). In a similar experiment, 90 days of treatment with ziprasidone (12 mg/kg/day; delivered via drinking water) produced minor deficits in the water maze performance on day 90 and after 7 days of washout (Terry et al., 2006).

Details of these studies and their findings are summarised in **Table 1.2.** Collectively, these studies point towards the effectiveness of atypical APs in rescuing manipulation-induced deficits in a range of cognitive domains upon acute and long-term treatments, an effect that remains consistent regardless of the animal model used. In both acute and long-term experimental conditions, treatment with APs interferes with normal patterns of cognitive performance in physiologically healthy animals. Similar to clinical findings, evidence of reduced cortical volume has also been reported upon long-term treatment with APs in healthy rodents (Vernon et al., 2011; Vernon et al., 2012; Vernon et al., 2014). Whether these structural alterations are associated with cognitive impairments observed following long-term AP treatment in rodents is yet to be determined. Indeed, the effect of long-term treatment with APs on brain structure and its functional/cognitive consequences must also be studied in animal models of the disease. Collectively findings of the

neurocognitive effects of long-term treatment with APs in both disease models and healthy animals are confounded by methodological limitations hindering their translational power to the clinical setting. One common limitation is the use of inappropriate doses of APs and inadequate routes of drug delivery. The half-life of AP drugs are 4-6 times shorter in rodents compared to humans (Kapur et al., 2003). Therefore, even upon long-term dosing, once daily injections of APs in rodents fail to induce a stable drug brain and plasma concentration and are not representative of clinical practice (Kapur et al., 2003). Addressing these methodological limitations is essential in understanding the influence of long-term treatment with APs on cognition and will enable process of drug development for cognitive impairments associated with schizophrenia.

Authors	Animal Model	Treatment (mg/kg)	Behavioural Paradigm	Outcome	Limitations/Extra detail
Addy and Levin (2002)	Adult Female Sprague-Dawley **(n=47)**	Acute s.c. treatment with **Hal (0.04)** **Ris(0.05)** **Clz (1.25, 2.5)** **Vehicle**	Radial Arm maze (Spatial working memory)	Acute treatment with Clz but not Ris and Hal significantly impaired choice response accuracy in radial arm maze. All treatments significantly increased response latency	- This study also thoroughly investigated the influence of AP drug interaction with nicotine. Nicotine treatment with Hal and Ris improved performance compared to saline control. It also rescued Clz-induced spatial memory deficit.
Terry et al (2002)	Adult Male Albino Wistar Rats **(n=80)**	45 and 90 days of treatment with **Olz (10/day)** **Hal (2/day)** Administered through drinking water	Modified version of Morris Water Maze (Spatial learning and working memory)	At 90 but not 45 days, both Hal and Olz impaired spatial learning as evident in increase in the latency of swimming to the platform. Deficits were also apparent in platform hidden trials, manifested as the reduced number of cross overs to the location where platform used to be.	- Test conducted 4 days after last treatment at 45 and 90 days - Results suggest that the detrimental effects of long-term treatment with APs is persistent
Hashimoto et al (2005)	Male ICR Mice scPCP (10 mg/kg/day; s.c. Administered on days 1-5 and 8-12; followed by 3 days of WO) (Exact number of sample size not given)	Acute i.p. treatment with **Clz (5/day)** **Hal (0.1/day)** **Vehicle** 14 days of i.p. treatment with the same compounds at the same doses	NOR (1-day ITI)	Acute treatment with both APs was ineffective in rescuing scPCP-induced NOR deficit. 14 days of Clz but not Hal treatment rescued scPCP-induced deficit in mice.	- The total number of animals used was not reported - In acute and chronic studies NOR was performed 1h and 1 day after the acute/last dose of AP respectively. - In this study scPCP-induced deficit lasted for 6 weeks.
Abdul-Monim et al (2006)	Adult female LH rats ScPCP/Veh (2mg/kg; bidaily for 7 days; 7 days WO) **(n=6-8/group)**	Acute i.p. treatment with **Zip (2.5), Olz (1.5), Clz (5)** **Hal (0.05), CLP (2**) **Vehicle**	Operant Reversal Learning	Atypical but not typical agents rescued scPCP-induced deficit	- Studying a range of doses for each drug would have been more appropriate. - Selected dose for Clz, does not capture the therapeutic range of D2R occupancy

Ortega-Alvaro et al (2006)	Adult Male Wistar rats	Acute i.p. treatment with **Olz (2.5, 5, 10)** **Clz (5, 10, 20, 40)** **Vehicle** Long-term (14 days; dose/day i.p.) treatment with **Olz (2.5, 5, 10)** **Clz (2.5, 5, 10)** **Vehicle**	8-radial maze task (working memory)	Acute treatment with Olz and Clz induced a dose-dependent reduction in correct response, possibly due to sedative effects. Chronic treatment at lower doses did not impair performance, further supporting that deficits were due to acute sedative effect of treatment	- Authors have maintained the doses of Olz for the long-term study but have reduced the dose range of Clz - Selected doses for Clz (in both acute and long-term settings) fail to capture clinical range of D2R occupancy (Kapur et al., 2003)
Ozawa et al (2006)	Balb/C mice Pregnant dams treated with Poly (I:C) (5 mg/kg; i.p.) or vehicle every consecutive 6 days from gestational day 12-17. Offspring (male and female) separated from dam at 3 weeks old, **(n=47)**	14 days of i.p. treatment with **Clz (5 /day)** **Hal (0.1 /day)** Rats tested 1 day post last day of treatment	NOR (1 h or 1-day ITI)	Treatment with Clz but not Hal treatment rescued Poly (I:C) induced NOR deficit at both 1h and 1-day ITI	- Authors have only reported discrimination index as a measure of NOR performance. - Results of Vehicle treated offspring are not well specified. - There was no sex difference in the performance and treatment effect.
Terry et al (2006)	Adult Male Albino Wistar Rats **(n=36)**	45 and 90 days of treatment with **Zip (12/day)** **Hal (2/day)** Administered through drinking water	Modified version of Morris Water Maze	Significant increase in latency of locating hidden platform in both treatment groups at both time points. Reduced number of cross overs to the previous platform location in the probe trial. This was significant in the Hal and reached trend towards decrement in Zip group.	- Test conducted after 7 days of WO from 90 days of treatment - Results suggest that the detrimental effects of long-term treatment with APs is persistent
Amitai et al (2007)	Adult Male Wistar Rats **For acute study (n=32)** Within-subject cross-over design **For chronic study (n=22)** All rats received PCP (2 mg/kg/day; s.c.) for 2 day, then impanated with osmotic mini-pump to receive AP treatment. Three days post implant, rats received 5 days of vehicle followed by 5 days of PCP 30 mins prior to trial	Acute i.p. treatment with **Ris (0.25, 0.5, 1)** **Clz (1, 2, 3)** **QT (2.5, 5, 7.5)** **Hal (0.0125, 0.025, 0.05)** **Vehicle** 14 days of treatment via osmotic minipump **Clz (4 mg/kg/day)** **Vehicle**	5-CSRTT	Acute treatment with Ris and Clz at all doses and QT at high doses but not Hal (at any dose) significantly impaired attention (but not impulsivity) as measured by reduced percentage correct response. Longer treatment with Clz at 4 mg/kg ameliorated the scPCP induced deficits in response accuracy and reduced number of premature responses in the 5-CSRTT	- The PCP treatment regimen is questionable. Animals were treated for 2 consecutive days followed by a 8 day delay to be treated again for 5 consecutive days. - Animals were treated 30 mins pre-trial. Therefore, the acute effect of PCP treatment is inevitable. - Not an appropriate model for cognitive deficits in schizophrenia. - Doses of APs used do not reach clinically relevant D2R occupancy levels (Kapur et al., 2003).

Grayson et al (2007)	Adult Female LH rats ScPCP/Veh (2mg/kg; bidaily for 7 days; 7 days WO) **(7-12/group)**	Acute i.p. treatment with **Ris (0.05,0.1,0.2)** **Clz (1 and 5)** **Hal (0.05 and 0.075)** **Vehicle**	NOR (1 min ITI)	Clz at both doses and Ris at highest dose rescued scPCP-induced deficit, Hal was ineffective.	- Doses of AP used for this acute study do not fall within the therapeutic D2R occupancy levels (Kapur et al., 2003). - Unlike many other studies reported here, this study and other studies associated with the same group, perform all behavioural tests upon 7 days of WO from scPCP treatment to ensure drug residue does not interact with behaviour.
Li et al (2007)	Male Sprague-Dawley rats Isolation rearing (housed individually at weaning -3 weeks) or social rearing (group house at weaning) **(n=48)**	6-8 weeks of i.p. treatment with **Clz (5 and 10/day)**	Operant Reversal Learning	Isolates required significantly longer time to acquire a new strategy. They were also impaired on response inhibition. Clz treatment at both doses significantly ameliorated these effects in treated rats. In socially reared rats, Clz interfered with reversal learning but not acquisition of new strategies.	- The duration of the study is too long for daily i.p. injections as it might cause considerable pain and stress to the animal - Osmotic minipump implant would have been a better choice for long-term drug deliver.
Mclean et al (2008)	Adult Female LH rats ScPCP/Veh (2mg/kg; bidaily for 7 days; 7 days WO) **(n=50)**	Acute i.p. treatment with **Ris (0.2)** **Clz (2.5)** **Hal (0.05)** **Vehicle**	Attentional set shifting	Ris and Clz but not Hal rescued deficit in shifting attention between different sorting categories (extra-dimensional shift)	- This study has only used one dose of each drug. Since this was a validation study, using a range of treatment doses would have been more appropriate.
Rodefer et al (2008)	Adult Male Long Evans rats ScPCP/Veh (5mg/kg; bidaily for 7 days; 10 days WO) **(n=48) for each study**	Acute i.p. treatment with **Clz (0.1-5; i.p.),** **Olz (1.5-3; p.o.)** **Ris (0.01 and 0.3; i.p),** **Hal (0.01 and 0.1; i.p),** **Sertindole (0.63-2.5; p.o.), Vehicle (p.o.)** Long-term (21 days) treatment with **Olz (3/bidaily p.o),** **Sertindole (1.3/day ; p.o.), Vehicle (p.o.)**	Attentional set shifting	Upon acute treatment both typical and atypical agents but sertindole failed to rescue the scPCP-induced deficit in EDS. Effect of sertindole was observed with high doses. Long-term treatment revealed the same pattern of results.	- Created a dose response curve to identify the most effective dose for long-term treatment. - Route of drug delivery may not be appropriate due to the fast metabolism rate of APs in rodents. - Chosen doses of APs are within therapeutic range of D2R occupancy in the long-term but not acute investigations.

Paine and Carlezon (2009)	Adult Male Sprague-Dawley rats **(n=28)** Same group of rats were used for all treatments and doses in a cross-over design. Rats went through a WO period between each stage of study (5 stages)	Acute s.c. treatment with **Hal (0.25)** **Clz (2.50)** Rats were also treated with MK-801 acutely and acute MK-801 +Hal/Clz., sub-chronic MK-801 and scMK-801 +acute Hal/Clz For scMK-801 (0.5 mg/kg) rats were treated after test performance ever day for 12 days and were treated with Clz/Hal on days 6,8,10,12	5-CSRTT	Acute treatment with Hal and Clz significantly impaired performance. It significantly increased the number omissions, wrong responses, reward retrieval latency and correct response latencies. Both acute and scMK-801 induced deficits in task performance. Hal and Clz were more effective in treating acute MK-801 induced deficits than scMK-801.	- The study design is poor - Small sample size for the magnitude of experiments - For the scMK-801 studies, the NMDAR antagonist is injected post task performance. Therefore, the effect of residual drug on performance cannot be excluded. - Not an appropriate design for the study of cognitive impairments associated with schizophrenia. - Selected dose for Hal represents >80% D2R occupancy while selected dose for Clz offers <40%. For Hal this dose is higher than therapeutic D2R occupancy range (Kapur et al., 2003). For Clz this is below D2R occupancy range (Butnariu., 2017)
Snigdha et al (2010)	Adult Female Long Evans rats ScPCP/Veh (2mg/kg; bidaily for 7 days; 7 days WO) **(n=48)**	Acute i.p. treatment with **Olz (1 and 2)** **Ris (0.05 and 0.1)** **Melprone (1 and 3)** **Hal (0.05 and 0.1)** **Vehicle**	NOR (1 min ITI)	All APs but Hal were effective in rescuing scPCP-induced deficit	- The selected dose for Hal produces approximately 60-80% D2R occupancy which is within the clinical therapeutic occupancy range. Ris and Hal doses are associated with D2R occupancy levels which are lower than therapeutic range for each drug (Kapur et al., 2003; Butnariu., 2017
Mclean et al (2010b)	Adult Female LH rats ScPCP/Veh (2mg/kg; bidaily for 7 days; 7 days WO) **(n=100)**	28 days of treatment with **Asenapine (0.075; twice daily; s.c.)** **Ris (0.2/day; i.p.)** **Olz (1.5/day; i.p.)**	Operant Reversal Learning	All treatment groups rescued scPCP-induced deficit in reversal learning when tested on days 3, 7,17, 21 and 28 day of treatment. 24 h after last dose, there was a trend towards improved performance with all treatment groups	- Results suggest that treatment with these atypical agents might have beneficial long-lasting effects. - Route of drug administration is not appropriate for a chronic study. This is due to fast metabolism rate of APs in rodents.
Meyer et al (2010)	C57BL6/J Mice Poly (I:C) (5 mg/kg;i.v) on gestational day 17 Weaned at 3 weeks	21 days of i.p. treatment with **Clz (5/day)**	Morris water maze	Clz treatment improved working memory in offspring born to Poly (I:C) dams but significantly impaired performance in offspring born to vehicle treated dams.	- Route of drug administration is not appropriate for long-term study. - Sample size was not reported
Carli et al (2011)	Adult Male LH rats NMDAR antagonist (CPP) infused into the mPFC prior to each testing session **(n=30)**	Acute p.o. treatment with **Aripiprazole (1, 3)** **Olz (0.1, 0.3 and 1)** **Hal (0.1 mg/kg)** **Vehicle**	5-CSRTT	Aripiprazole at both doses and Olz (0.3 and 1 mg/kg) but not Hal rescued CPP induced deficit in response accuracy	- The animal model of choice is of limitations. - It resembles the acute NMDAR antagonist models rather than sub-chronic models - Important to determine whether cognition is restored if CPP is not administered.

Nagai et al (2011)	DISC-1 transgenic mice (C57BL/6N) Poly (I:C) (5 mg/kg/day from postnatal day 2 to 6; s.c.) Litter weaned at 3 weeks (n=11-14/group)	At least 7 days of p.o. treatment with **Hal (1/day)** **Clz (3/day)**	NOR (1h ITI)	Clz but not Hal rescued the manipulation induced deficit. Neither treatments compromised performance in healthy rats	- The sample size is unspecified - Duration of drug treatment is unspecified. Treatment started 7 days prior to the first behavioral test. There were total of 3 different tests (NOR, social interaction and LMA) performed.
Ozdemir et al (2012)	Adult Male Swiss Albino Mice scMK-801 (0.3 mg/kg/day for 5 days; i.p. No WO) **(n=47)**	14 days i.p. of treatment with **Clz (5/day)** **Hal (1/day)**	NOR (1h ITI) Test performed 24h after the last treatment	Both Clz and Hal failed to rescue MK-801 induced NOR deficit. In healthy rats Hal but not Clz impaired performance	- This study also investigated the influence long-term AP treatment on markers of synaptic functional integrity (SNAP-25) - Selected dose for Hal represents >80% D2R occupancy while selected dose for Clz offers <40%. For Hal this dose is higher than therapeutic D2R occupancy range (Kapur et al., 2003). For Clz this is below D2R occupancy range (Butnariu., 2017)
Neill et al (2016)	Adult Female LH rats ScPCP/Veh (2mg/kg; bidaily for 7 days; 7 days WO) **(n=240)**	Acute treatment with **Cariprazine (0.025, 0.1, 0.25; p.o.)** **Ris (0.1, 0.16; i.p.)**	NOR (1 min TI) Operant Reversal learning	Ris rescued scPCP-induced deficit in both measures. Cariprazine was effective at 0.025 and 0.1 mg in rescuing NOR deficit but at highest does it was associated with reduced LMA.	- The study only used female rats. It would have been better to also use males and explore possible sex differences in response to drug treatment

Table 1.2. Summary of pre-clinical studies investigating the influence of AP treatment on cognition. Hal: Haloperidol; Ris: Risperidone; Olz: Olanzapine; Clz: Clozapine; Zip: Ziprasidone; QT: Quetiapine; CLP: Chlorpromazine; WO: washout; i.p : intraperitoneal injections; p.o : Oral administration; LH : Lister Hooded rats

1.8. Synaptic plasticity

Neurons within local and widespread circuitries communicate through signals conducted by synapses. The incoming signal can dynamically alter the activity of the post-synaptic neuron and modulate the strength of synaptic connections. Collectively, these activity dependent modulations of synaptic strength are known as synaptic plasticity (Abbott and Regehr, 2004) which are thought to formulate the neural basis of learning, memory and cognition (Goto and Grace, 2008). Processes of synaptic plasticity are diverse and can be categorised as short-term and long-term plasticity. Short-term synaptic plasticity (STP) is defined as the activity-dependent alterations in synaptic weight which lasts from a few milliseconds to a few minutes (Regehr, 2012). STP can be investigated by paired-pulse stimulation (PPS). In most synapses, the field excitatory post-synaptic potential (fEPSP) in response to the second pulse of a stimulus pair is significantly enhanced compared to the first, if the second pulse is delivered shortly after the first (20-500 ms) (Citri and Malenka, 2008). This phenomenon known as the paired-pulse facilitation (PPF), lasts for a brief period of time ranging from a few milliseconds to seconds and is generally stronger for shorter interval between pulses (Zucker, 1989; Zucker and Regehr, 2002). Post-tetanic potentiation (PTP) is another form of short-term enhancement of synaptic activity, which is induced in response to tetanic high-frequency stimulation (HFS) (200 ms to 5 s in duration, 10-200 Hz) and lasts for a period of several minutes (Zucker and Regehr, 2002; Citri and Malenka, 2008).

Both PPF and PTP are of a presynaptic origin and are associated with an increase in the probability of neurotransmitter release as a result of an increase in calcium levels in the presynaptic terminals (Zucker, 1989; Zucker and Regehr, 2002; Abbott and Regehr, 2004). PPF is prominently explained by the residual calcium hypothesis, initially introduced by Katz and Miledi (1968). According to this hypothesis, once an action potential reaches the presynaptic terminal it triggers the opening of voltage-gated calcium channels located in axon terminals, allowing an influx of calcium. The increase in intracellular calcium concentration [Ca_{local}] triggers vesicle fusion, leading to neurotransmitter release into the synapse. After the voltage-gated calcium channels close, the Ca_{local} diffuses across the presynaptic boutons and binds to calcium-binding proteins while the residual calcium (Ca_{res}) is gradually expelled from the neuron. If the second pulse reaches the synaptic terminal while Ca_{res} is still available, it combines with the incoming calcium, signalling an increase in neurotransmitter release. PTP is also governed by similar mechanisms, whereby repeated action potentials increase [Ca_{local}] due to increased calcium influx. It is also suggested that in this case, [Ca_{res}] also remains elevated for a longer period of time and follows the time course of PTP (Regehr, 2012). PPF and PTP are predominately associated with neurons with an initially low probability of release. Paired-pulse depression (PPD) and post-tetanic depression can also occur in synapses with initially high release properties or in neurons where the vesicle pool is transiently depleted (Zucker and Regehr, 2002; Abbott and Regehr, 2004; Regehr, 2012).

Long-term potentiation (LTP) and its counterpart long-term depression (LTD) are forms of long-term synaptic plasticity that result in long-lasting increase or decrease in synaptic strength, respectively (Bliss

and Cooke, 2011). LTP, generally regarded as the most likely molecular model for learning, was initially reported in synapses between the medial perforant pathway and dentate gyrus in response to a brief train of high-frequency stimuli (Bliss and Lomo, 1973). LTP has since been widely demonstrated across the brain, including at all regions of the hippocampal formation (HF) and mPFC (Gemperle et al., 2003; Lynch, 2004; Xu and Yao, 2010). The process of LTP induction is often heavily dependent on the activation of the NMDAR. Although NMDAR-independent LTP also exists, NMDAR-dependent LTP is most prominent within all circuitries (Park et al., 2014). In a typical glutamatergic pathway (e.g. vHipp-mPFC), AMPA receptors (AMPAR) mediate the process of PPF (Jay et al., 1992; Gigg et al., 1994; Laroche et al., 2000) upon excitatory neurotransmitter release. Upon HFS, the summation of AMPAR-mediated currents strongly depolarises the membrane potential allowing for the Mg^{2+} to be removed from the NMDARs (Volianskis et al., 2015). Expression and maintenance of LTP relies on a complex chain of calcium-detection mechanisms which activates intracellular signalling cascades resulting in phosphorylation of the AMPARs to enhance their efficacy and de novo protein synthesis. Specifically, LTP is associated with phosphorylation of AMPAR GluR1 subunit at serine 831, a substrate for calcium-calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) (Lee et al., 1998; Lee et al., 2000) (**Figure 1.3**). These processes are skilfully reviewed by Bliss and Cooke (2011) and Citri and Malenka (2008).

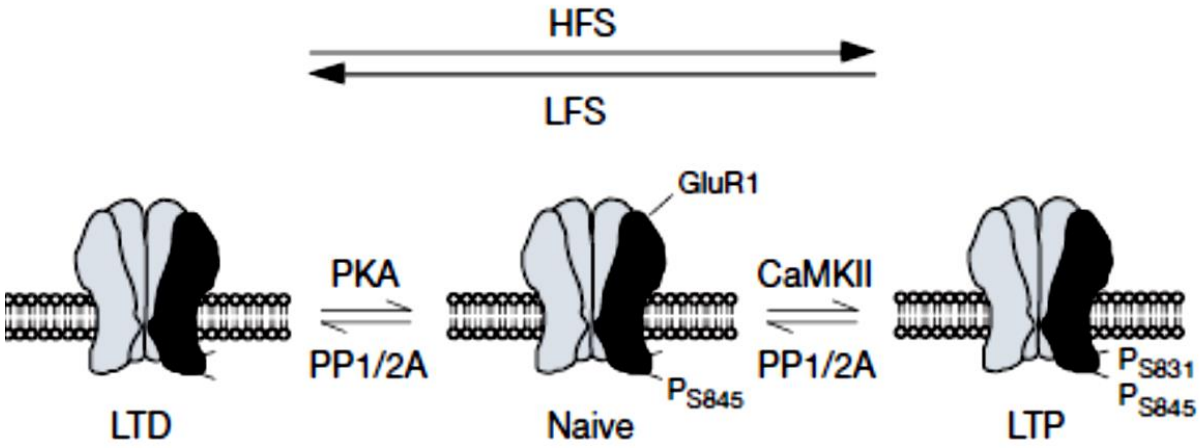

Figure 1.3. AMPA Receptor Alterations during long-term synaptic plasticity. HFS of a naïve synapse leads to activation of CaMKII and PKC which increases the phosphorylation of GluR1 at Serine 831 (S831), leading to LTP. LFS of a naïve synapse results in activation of protein phosphatases (PP), including (PP1/2A) and dephosphorylation of serine 845 (S845; PKA site), leading to LTD. LFS of a previously potentiated synapse also activates PP1/2A, leading to dephosphorylation of S831. Figure taken from Lee et al.,2000

LTD can also be established in excitatory synapses (Dudek and Bear, 1992). This phenomenon, generally regarded as a molecular model for forgetting (Foy, 2001) and removal of old memory (Dharani, 2015) suggests that activity and experience-dependent learning is encoded by a range of mechanisms that can be elicited within the same pathways (Citri and Malenka, 2008). In the hippocampus, LTD is typically induced by application of low-frequency stimulation (LFS) over a prolonged period of time (e.g. 900 stimuli at 1 Hz) (Dudek and Bear, 1992; Luscher and Malenka, 2012). Mechanisms of LTD are also NMDAR-dependent. Luscher and Malenka (2012) suggest that prolonged repeated LFS induces a modest depolarisation resulting in a modest and prolonged increase in calcium entry into the presynaptic neuron. In contrast to LTP, a modest elevation in the post-synaptic calcium levels is optimal for the induction of LTD (**Figure 1.4**). It is also suggested that by recruiting different signalling pathways, the processes of expression and maintenance of LTD represent the inverse mechanisms of LTP (Lee et al., 1998; Lee et al., 2000; Luscher and Malenka, 2012). Unlike LTP, LTD is associated with dephosphorylation of AMPAR GluR1 subunit at serine 845, which is associated with protein kinase A (PKA) (Lee et al., 1998; Lee et al., 2000) (**Figure 1.3**). These processes have been elegantly described by Luscher and Malenka (2012). While LTD reduces synaptic strength to below baseline levels, the activity-dependent process of depotentiation, reverses HFS-induced potentiation to baseline levels by dephosphorylating serine 831 site on the GluR1 subunit (Lee et al., 2000) (**Figure 1.3**). The process of depotentiation allows for neurons to retain their plasticity to further contribute to the memory processes. Examples of this phenomenon are seen in the Schaffer Collaterals (Lee et al., 1998; Lee et al., 2000; Sweatt, 2008) and also in the vHipp-mPFC pathway (see below; Burette et al., 1999). Whether LTP and LTD induction is governed by purely pre-synaptic and post-synaptic mechanisms remains a matter of debate among neuroscientists. Evidence for both of these mechanisms are supported in the literature (Lisman, 2009; Yang and Calakos, 2013).

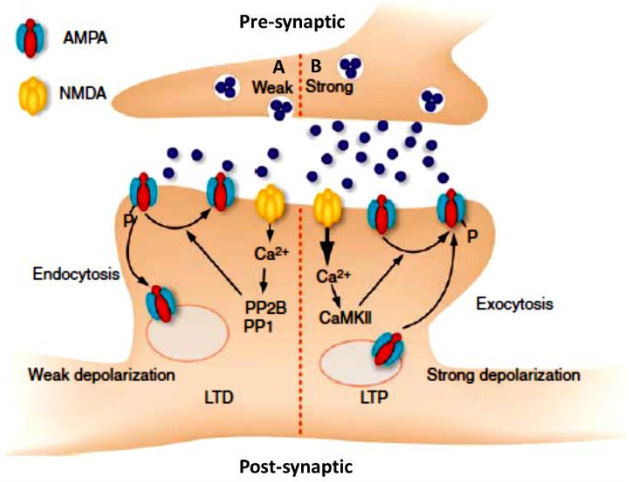

Figure 1.4. Concentration of post-synaptic Ca^{2+} influx determines the polarity of the response. (A) Weak activation of the pre-synaptic neuron, leads to a modest activation of NMDARs and Ca^{2+} influx. This triggers the signalling chain that leads to dephosphorylation and endocytosis (removal from post-synaptic membrane) of AMPARs, resulting in LTD (B) Strong stimulation of the pre-synaptic neuron, leads to a strong depolarisation of the post-synaptic neuron and high concentration of **Ca^{2+}** influx. This activates the signalling chain that leads to phosphorylation of AMPARs and their exocytosis (placement in post-synaptic membrane). Figure taken from Luscher and Malenka., 2012

1.9. Ventral Hippocampus (vHipp) – medial prefrontal cortex (mPFC) pathway: Anatomy and Function

Decades of research in both humans and animals has shown that the HF and PFC play separate but complementary roles in a range of cognitive and mnemonic processes, including episodic memory (Godsil et al., 2013; Eichenbaum, 2017). While the HF is implicated in processing spatial, contextual and temporal information of memory, the PFC is heavily involved in higher-order cognitive processes such as executive function and executive control of context-dependent memory retrieval (Kesner and Churchwell, 2011; Eichenbaum, 2017). Over the past several years, the significance of interaction between these two brain regions in mediating these functions has been highlighted. HF-PFC functional coupling is reported in humans and rodents during working memory tasks (Anderson et al., 2010; Benchenane et al., 2010; O'Neill et al., 2013), episodic memory (Gerlach et al., 2011; Veyrac et al., 2015) and context-dependent learning and memory retrieval (Milad et al., 2007; Barch and Ceaser, 2012; Navawongse and Eichenbaum, 2013; Brown et al., 2014). Their interaction is also essential for contextual emotional regulation and successful completion of goal-directed tasks (Godsil et al., 2013; Jin and Maren, 2015; Numan, 2015).

Anatomically, the HF and PFC are strongly connected through monosynaptic (direct) and multi-synaptic (indirect) pathways (Eichenbaum, 2017) (**Figure 1.5**). A single robust projection of neurons from the HF to the PFC forms the immediate direct anatomical connection between these structures which has been well characterised in both rats and monkeys (Hoover and Vertes, 2007). Studies injecting retrograde tracer into the mPFC of the rat have identified the vHipp and ventral subiculum as the origin of this robust direct projection (Jay et al., 1989; Hoover and Vertes, 2007). Injection of anterograde tracer in to the CA1 and subiculum revealed that these direct projections are most dense at the prelimbic (Prl) and infralimbic (IL) regions of the mPFC. These hippocampal projections are present across both superficial (II-III) and deep (V-VI) layers but are more densely distributed in deep layers of the Prl and superficial layers of the IL. Sparse projections can also be detected in the medial orbital cortex, a structure within rat PFC (Jay and Witter, 1991). Hereafter, this direct projection between the HF and PFC will be referred to as the **vHipp-mPFC** pathway.

Anterograde studies in primates have also identified projections form the CA1 regions that terminate in the orbital and mPFC (dLPFC, vLPFC), with high density in layers II and III (Zhong et al., 2006). A significant proportion of these connections originate from the rostral third of the hippocampus (Cavada et al., 2000) which is thought to be homologous to the vHipp in rats (Laroche et al., 2000). Due to the invasive nature of powerful trace-tracking techniques, detailed evidence of HF-PFC connections in humans are lacking in the literature (Godsil et al., 2013). However, evidence from diffusion tensor imaging measurements suggests that, in humans, the orbital and mPFC receives projections from the HF via the fornix (Croxson et al., 2005). A similar pattern is also observed in other primates, suggesting that the HF-PFC connection may be similar across primates (Croxson et al., 2005).

In rats (Sesack et al., 1989; Vertes, 2004; Hoover and Vertes, 2007) and monkeys (Carmichael and Price, 1995) there are no direct projections from the mPFC to the vHipp. Instead, the mPFC and HF communicate through various cortical and subcortical multi-synaptic pathways, which include amygdala, ventral tegmental area (VTA), parahippocampal cortices (Agster and Burwell, 2009) and thalamus (Cassel et al., 2013; Jin and Maren, 2015). For instance, the nucleus reuniens (RE) of the thalamus receives bidirectional projections from all regions of the mPFC and the HF (Vertes, 2002; Vertes et al., 2006). Therefore, RE is in the key position to relay information between these two regions and to synchronise their activity (Cassel et al., 2013; Eichenbaum, 2017). Similarly, the nucleus accumbens (NAcc; both core and shell) , a central component of the limbic system also receives converging input from mPFC, HF and the amygdala, suggesting that this structure integrates emotional information with mPFC-HF cognitive information (e.g. spatial context and behavioural strategies) to guide goal-directed behaviour (Vertes, 2004; Hoover and Vertes, 2007; Goto and Grace, 2008; Jin and Maren, 2015). The mPFC is also indirectly connected to the HF though its connections to the entorhinal cortex and the perirhinal cortex, which have bidirectional connections with each other and the HF (Agster and Burwell, 2009; Preston and Eichenbaum, 2013; Eichenbaum, 2017) (**Figure 1.5**)

While the pattern of connections from the HF to the PFC appears preserved across species, cross-species differences in mPFC homology does not allow for definitive comparisons of the function of the HF-PFC connections (Godsil et al., 2013). Functional homology of the mPFC across species is extensively debated in the literature (Uylings et al., 2003; Kesner and Churchwell, 2011). While a universally accepted cross-species comparative model does not exist, substantial evidence suggests that the mPFC in rodents supports cognitive functions mediated by the dLPFC and vLPFC in primates and humans. It can be argued that unlike humans and monkeys, the dLPFC and vLPFC are not distinctly differentiated (Kesner and Churchwell, 2011; Eichenbaum, 2017). Nevertheless, it has been suggested that Prl and IL are functionally homologous to primate dLPFC and vLPFC respectively (Vertes, 2004; Hoover and Vertes, 2007; Kesner and Churchwell, 2011). Therefore, rodents can be beneficial models for studying the cognitive and mnemonic processes mediated by the PFC and HF in primates and humans (**Figure 1.6**).

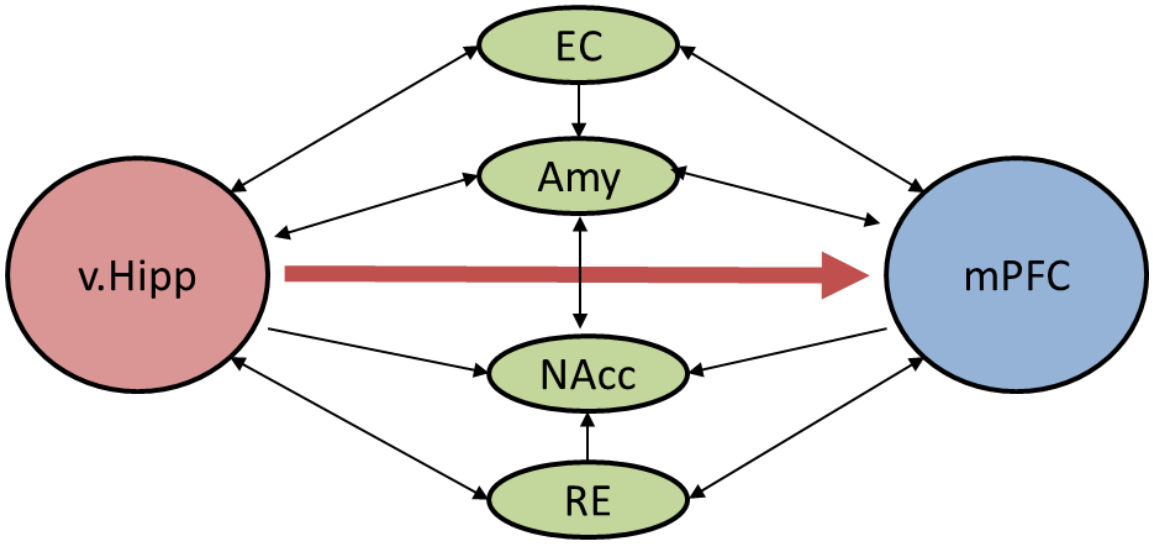

Figure 1.5. **Representing the direct and indirect connections between the vHipp and the mPFC.** The vHipp and mPFC have a robust direct connection (Pink arrow) but there are no direct return connections from the mPFC to the HF. Instead the mPFC and vHipp each form bidirectional connections with the entorhinal cortex (EC), amygdala (Amy) and the Nucleus Reuniens (RE) where various information from vHipp and mPFC can converge and be integrated with other sources of input. RE is important for synchronising the functional interaction between the vHipp-mPFC. Nucleus accumbens (NAcc, both core and shell) receives unidirectional input from RE, vHipp and mPFC and bidirectional connections from the Amy, hence is important for integrating vHipp-mPFC interaction with emotional information guiding goal-directed behaviour. Figure adapted from Jin and Maren (2015)

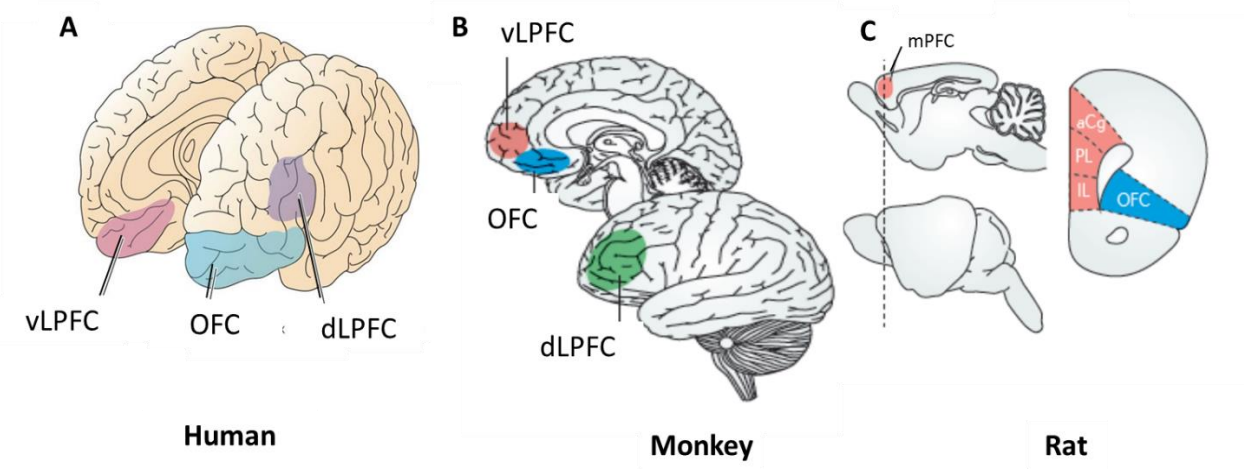

Figure 1.6. Conservation of the mPFC in (A) human, (B) monkey and (C) rat. The mPFC in rat supports the function of dLPFC and vLPFC in monkeys and humans. However, these two regions are not clearly differentiated in the rat. It is nevertheless suggested that the Prl region of the mPFC in the rat resembles the dLPFC in monkeys and humans while the IL resembles the vLPFC. Figure adapted from Eichenbaum (2017)

Thus far, several hypotheses have been proposed to explain the functional interaction between the HF and PFC. Numan (2015) suggests that the dorsal hippocampus (dHipp) can modulate the ongoing PFC-mediated context-dependent cognitive task performance (Colgin, 2011). In this hypothesis, the dHipp assumes the role of a comparator system (Kumaran and Maguire, 2006; Duncan et al., 2012). Since the dHipp generates specific contextual and spatial representations of events (Duncan et al., 2012; Komorowski et al., 2013), it can formulate a match/mis-match signal by comparing the PFC-mediated context-dependent cognitive strategy in response to an event with the representations of previous experiences (Kumaran and Maguire, 2006; Numan, 2015). The result match/mis-match signal is then communicated to the mPFC via vHipp, whereby the mPFC consolidates or modifies the action plan. While this model highlights the role of the dHipp and its influence on context-dependent memory retrieval, recent emerging evidence suggests that the mPFC also exerts a strong cognitive control by directing context-specific memory retrieval and points towards a bi-directional flow of information between these structures (Navawongse and Eichenbaum, 2013).

Evidence suggests that unlike the dHipp, vHipp formulates more generalised representations across events associated with spatial context (Komorowski et al., 2013). Substantial evidence also suggests that the mPFC formulates abstract representations of strategies and context-specific behavioural plans (Hyman et al., 2005; Rich and Shapiro, 2009; Preston and Eichenbaum, 2013). These neural representations in the mPFC appear to hold general information about relevant experiences associated with certain contexts (Hyman et al., 2012) and can switch between strategies when contingencies change (Durstewitz et al., 2010). It is suggested that when cued to a specific experience or context, the vHipp provides context-defining information to the mPFC through the direct vHipp-mPFC connection. The mPFC then chooses context-appropriate behavioural strategy and exerts its top-down control to the dHipp to retrieve context-specific details while repressing context inappropriate representations. Based on this hypothesis, the conflict between representations are also detected and resolved at the level of mPFC (Preston and Eichenbaum, 2013; Eichenbaum, 2017).

It is suggested that disruption in activation of context-relevant information and formulation of correct association between relevant stimuli, fundamental function of HF-PFC interaction, is a core cognitive deficit associated with schizophrenia (Holmes et al., 2005; Eichenbaum, 2017). This is supported by a large body of evidence supporting the disruption in HF-PFC interaction in patients with schizophrenia (Godsil et al., 2013; Meyer-Lindenberg et al., 2005; Bahner and Meyer-Lindenberg, 2017) and in animal models of the disease (Sigurdsson et al., 2010; Sigurdsson, 2015). These will be further discussed in more detail in **Chapter 5**. With specific relavance to this project is the finding of reduced connectivity between the HF-PFC in the scPCP model for cognitive impaiments with schizophrenia (Dawson et al., 2014). However, synaptic properties of the direct vHipp-mPFC connections are yet to be elucidated in the scPCP model for cognitive impairments with schizophrenia. Examining this pathway is highlighted as one of the objectives of the work presented in this thesis.

1.10. Synaptic plasticity in the vHipp-mPFC pathway

Stimulation of the vHipp (ventral CA1/subiculum) evokes a characteristic monosynaptic response in the mPFC (**Figure 1.7 A**), which is composed of a slow-rising positive wave followed by an early large EPSP (negative deflection) and an early positive-going inhibitory component (inhibitory post-synaptic potential; IPSP) (**Figure 1.7 B**) (Laroche et al., 1990; Degenetais et al., 2003). *In vivo* extracellular field potential recordings show that STP is predominantly observed as facilitation in the mPFC following PPS of vHipp afferents (Laroche et al., 1990). Intracellular recordings from the subset of pyramidal neurons suggest that in addition to PPF, PPD is also observed in some neurons, independent of cell subtype (Degenetais et al., 2003). This suggests that factors such as the properties of the dendritic spines, location of the synapses and the previous activity history of the cells give rise to neurons with high and low neurotransmitter release probabilities in the mPFC, leading to PPD and PPF, respectively (Abbott and Regehr, 2004).

Evidence for long-lasting synaptic plasticity in the vHipp-mPFC pathway came from studies applying HFS to the vHipp. Laroche et al (1990) reported a rapidly induced LTP in the mPFC upon application of HFS to the vHipp (2 series of 10 trains at 250 Hz, applied 10 minutes apart) of anaesthetised rats which remained stable for 4 hours. The same HFS protocol also induces LTP in vHipp-mPFC pathway in awake rats, which persists for up to 3 days (Jay et al., 1996). In a recent study of awake, freely moving rats, similar patterns and a more prolonged duration of activity (3-7 days) were observed by a different HFS protocol (10 series of 5 trains at 400 Hz) (Taylor et al., 2016). In the same study, consecutive administration of HFS over 3 days, induced a robust LTP which lasted for an average of 21 days in the mPFC of rats. This has implications in mPFC-dependent learning and memory consolidation (Taylor et al., 2016). The mechanisms of PPF and LTP induction in the vHipp-mPFC pathway are similar to those described in **Section 1.8**. In the vHipp-mPFC pathway, PPF is mediated by the AMPAR, since these responses were inhibited in the presence of cyanquixaline (CNQX; AMPAR antagonist) (Jay et al., 1992). In the vHipp-mPFC pathway, LTP induction is also dependent on the activation of the NMDARs (Jay et al., 1995) (**Figure 1.7.C**). Similar to hippocampus (Citri and Malenka, 2008; Luscher and Malenka, 2012), expression and maintenance of LTP in the mPFC involves activation of a range of protein complexes including PKC and CaMKII and PKA (Jay et al., 1998; Laroche et al., 2000).

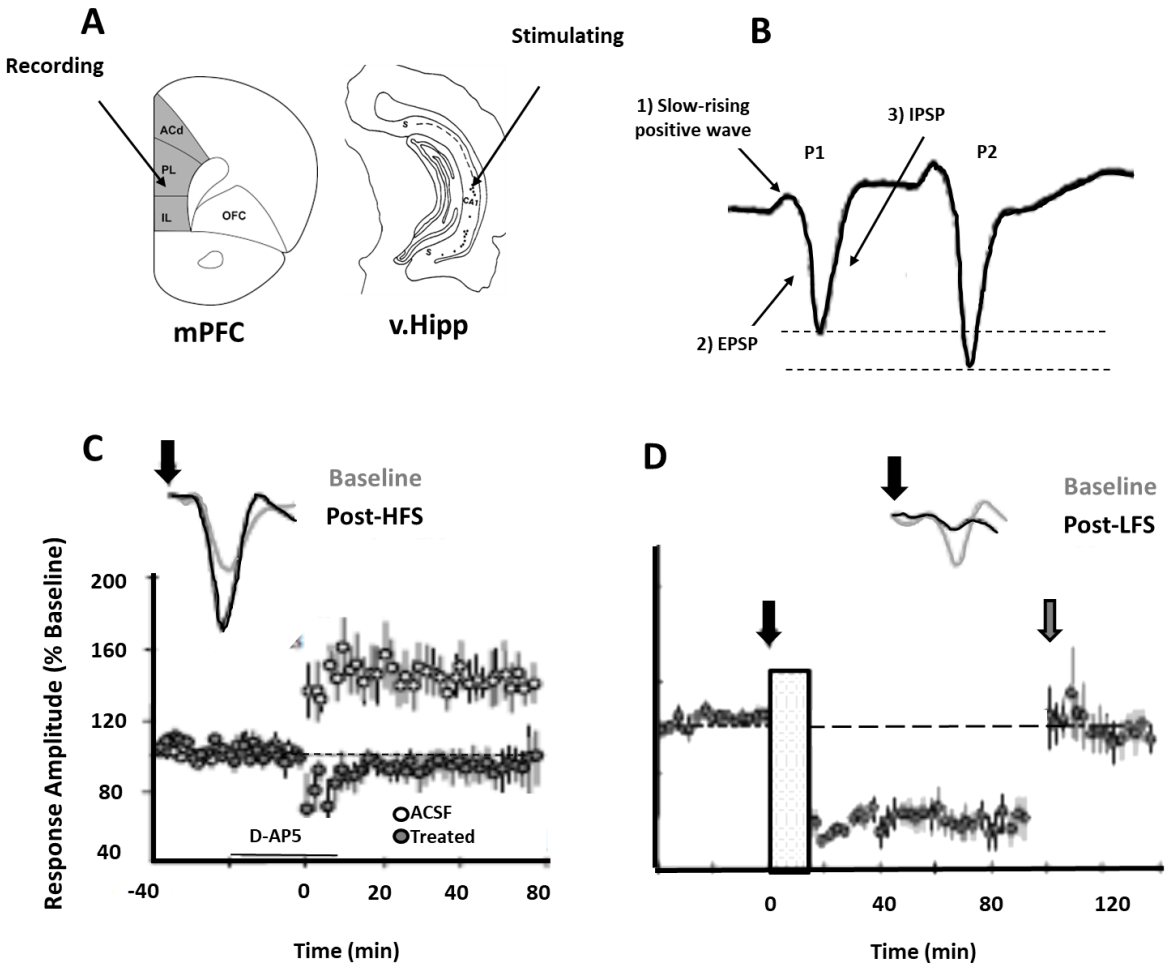

Figure 1.7. Typical PPF, LTP and LTD patterns in vHipp-mPFC pathway. (A) Represents typical stimulation (vHipp) and recording sites (Prl). (B) A typical response recorded from the mPFC upon vHipp stimulation. Response consists of 3 distinct phases. This also shows that the system supports PPF as a form of STP as represented by an increase in the amplitude of the response to the second pulse (P2) compared to the first (P1) (dotted line) (Laroche et al., 1990). (C) Induction of LTP in the mPFC upon HFS of vHipp. Infusion of D-AP5 into the mPFC under anesthetised conditions inhibits induction of LTP. Arrows show HFS (250 Hz, 200 ms, 2 series) (Jay et al., 1995). (D) Induction of LTD in the mPFC upon stimulation of vHipp with 900 stimulus trains (5 pulses at 250 Hz) at 1 Hz (Black arrow; grey block shows duration of stimulation) which was readily reversible by application of 12 stimulus trains (50 pulses at 250 Hz) at 0.1 Hz (Grey arrow) (Takita et al., 1999). Each dot in C and D represents average of 4 consecutive responses. Figure adapted from mentioned references and Jay et al (2004). All recordings presented here were obtained in-vivo from rats under anaesthetised conditions.

While LTP is readily inducible in the vHipp-mPFC pathway *in vivo*, induction of LTD and depotentiation of previously potentiated synapses have been challenging. Prolonged 1Hz LFS (900 single pulses), which typically induces robust LTD in rat hippocampal slices (Dudek and Bear, 1992), was also found to induce LTD in mPFC slices under *in vitro* conditions in young animals (Kirkwood et al., 1993). However, LFS of the vHipp with the 900 single pulses at 1Hz or a similar protocol (450 pairs of pulses at 1Hz, 35 ms inter-pulse interval) fails to induce LTD in the mPFC of anaesthetised rat. Instead both protocols induced a transient depression in response amplitude in the mPFC, which recovered to baseline within 5 minutes

post-LFS, followed by a gradual, late-onset potentiation. A similar effect was observed when LFS was applied to previously potentiated synapses (in presence of LTP). In this case also, both LFS protocols induced a significant depression in the response. This effect was transient, so that response values reached potentiated (LTP) levels 10-30 minutes after LFS (Burette et al., 1997). In a related study, application of trains of HFS (5 pulses at 250 Hz) applied at 1Hz to the vHipp induced a prolonged LTD in the mPFC of anaesthetised rats which was maintained for 13 hours. This LTD was found to be readily reversible in response to 50 pulses (250 Hz) applied at 0.1Hz to the vHipp (Takita et al., 1999) (**Figure 1.7 D**).

Furthermore, the typical LFS protocol failed to induce depotentiation in previously potentiated synapses. However, depotentiation was observed following PPS (900 bursts at 1Hz, 5ms inter-pulse interval; IPI) in previously potentiated synapses. This induced response sizes that were significantly below potentiated responses but not significantly different from baseline, showing a pattern of depotentiation in the mPFC. It is noteworthy that 20-30 mins post-LFS, amplitude of the depotentiated response started to increase, reaching a stable state that was significantly above baseline (Burette et al., 1997). Similar to observations in the hippocampus, LFS of the mPFC through pairs of pulses with very short IPI could induce a moderate activation of the NMDARs, leading to expression of depotentiation and LTD (Laroche et al., 2000). Collectively, these findings suggest that the synaptic weight in vHipp-mPFC pathway can be modulated to express LTP, LTD and depotentiation which supports its role in mediating learning and mnemonic process. In the vHipp-mPFC pathway, processes of synaptic plasticity are also modulated by dopamine and noradrenaline both of which are important for higher-order cognitive processes.

LTP induction and maintenance in the vHipp-mPFC pathway is regulated by modulatory neurotransmitters such as dopamine (Gurden et al., 1999; Gurden et al., 2000). Ventral tegmental area (VTA) provides the primary dopaminergic input to the mPFC. These afferents innervate dendritic spines of pyramidal neurons and GABAergic interneurons in layer 5-6 of the mPFC and are located in close proximity to the excitatory input from the vHipp (Carr and Sesack, 1996). HFS stimulation of the VTA afferents (50 Hz, 2 s) prior to HFS (10 trains of stimuli, 250 Hz, 200 ms applied at 0.1 Hz) of the vHipp induces a strong long-lasting enhancement in mPFC LTP while its electrolytic lesion dramatically decreases LTP in this pathway (Gurden et al., 1999) (**Figure 1.8 A and B**). The findings of transient elevation in dopamine release in the mPFC upon HFS of the vHipp further highlights the significance of dopamine in LTP maintenance and induction in this pathway (Gurden et al., 2000; Jay et al., 2004; Matsumoto et al., 2008).

Evidence suggests that the dopamine control of the NMDAR-dependent LTP is mediated through the action of dopamine D1 receptors (D1R) (Gurden et al., 2000). The synergistic effect of D1R and NMDAR interaction is thought to be mediated through PKA, which is activated through the action of dopamine on D1R (Gurden et al., 2000) and upon activation of NMDAR (Jay et al., 1998). In the rat mPFC, D1R and D2R are expressed in both pyramidal neurons and GABAergic interneurons, with concentrated expression in layer V (Santana et al., 2009). At the microcircuit level, activation of the D2R located on inhibitory interneurons supresses inhibition and facilitates D1R-NMDAR interaction in induction of LTP (Xu and Yao,

2010). Noradrenaline also regulates synaptic plasticity in the vHipp-mPFC pathway (Lim et al., 2010). Stimulation of locus coeruleus (LC) (12 Hz, 200 ms; this protocol promotes noradrenaline release), which is the primary source of adrenergic input to the mPFC facilitates vHipp-mPFC LTP (**Figure 1.8. C**). This is while inactivation of LC impairs LTP in this pathway, an effect which is also present upon blockade of the α_2-adrenoceptor in the mPFC (Lim et al., 2010).

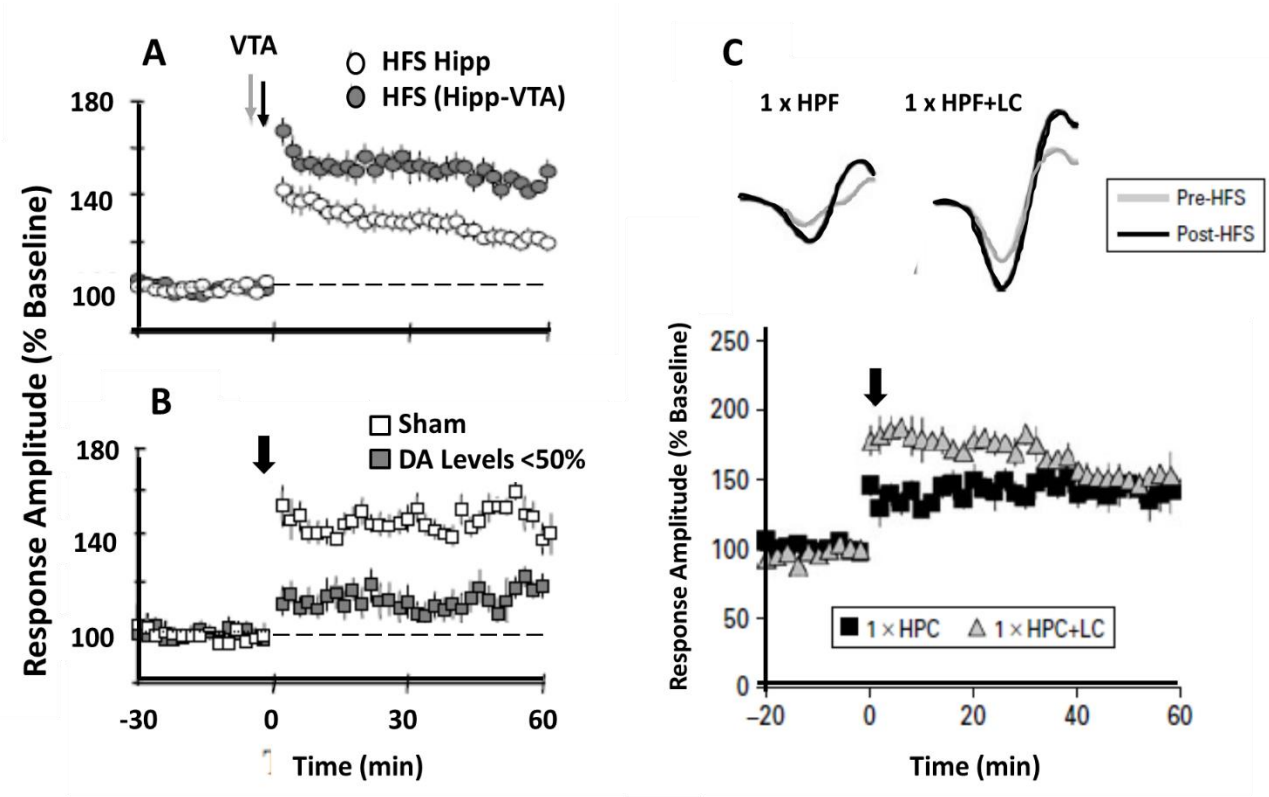

Figure 1. 8. The effect of modulating neurotransmitters on LTP in the vHipp-mPFC pathway. (A) HFS (50 Hz, 2 s) of the VTA prior to HFS (10 trains of stimuli,250 Hz, 200 ms applied at 0.1 Hz) to the vHipp, significantly potentiated the response amplitude in the mPFC (Gurden et al., 1999). (B) Lesion to the VTA (associated with 50% reduction in cortical DA levels) significantly impaired LTP in the mPFC compared to the sham controls (Gurden et al., 1999). (C) 1 time application of HFS (10 trains of stimuli,250 Hz, 200 ms applied at 0.1 Hz) to the vHipp was associated with expected LTP. This was significantly higher when accompanied by stimulation of the LC (12 Hz, 200 ms). This stimulation pattern is associated with an increase in noradrenaline release in the mPFC (Lim et al., 2010).

1.11. Synaptic plasticity in schizophrenia

As discussed in **Section 1.4** , schizophrenia is associated with imbalances in both glutamatergic and dopaminergic transmission. Genes coding for the receptors associated with these neurotransmitter systems (Harrison, 2015) as well as genes coding for the molecular structures involved in the transmission cascades associated with receptor activation (such as neuregulin 1 and its receptor erbB4) (Stefansson et al., 2002; Shen and Chen, 2012) are identified as risk factors associated with schizophrenia (Stefansson et al., 2002; Harrison and Weinberger, 2005). Given the key role of NMDAR in mediating LTP and LTD, these findings point towards disturbed plasticity processes associated with the disease (Goto et al., 2010).

In humans, LTP-like plasticity can be induced by paired-associative stimulation (PAS) using the transcranial magnetic stimulation technique in the motor cortex (Frantseva et al., 2008) and the PFC (Salavati et al., 2018). Studies in patients with schizophrenia, show a significant impairment in the induction of PAS-induced LTP-like activity in the motor cortex, which is associated with poor motor learning and performance (Frantseva et al., 2008). The same group also reported deficits in activity-dependent plasticity manifested as failure in motor re-organisation in medicated and un-medicated patients with schizophrenia (Daskalakis et al., 2008). More recent studies using transcranial stimulation, suggest that, in comparison to FEP patients, those in the chronic stage of the disease show a significant reduction in LTP, while both groups show significant cortical disinhibition to the same extent (Hasan et al., 2011). This group also reported deficits in LTD induction in patients with schizophrenia, which the authors attribute to hypofunctional NMDARs and an increase in $GABA_B$ receptor activity (Hasan et al., 2012).

Disrupted synaptic plasticity is also observed in animal models for schizophrenia. In these models, disturbances are manifested as an enhancement in LTP-like activity. For instance, in the MAM-17 model, HFS of the vHipp induced robust LTP in the mPFC that was significantly stronger in comparison to saline treated rats (Goto and Grace, 2006). Similarly, acute systemic injection of MK-801 (0.1 mg/kg) induced a gradual and significant potentiation in the vHipp-mPFC response. This effect was induced in the absence of exogenous HFS to the vHipp and was significantly stronger than HFS-induced LTP in vehicle treated rats. In the presence of MK-801, subsequent application of HFS failed to further potentiate the synapses, suggesting that prior MK-801 treatment masks HFS-induced LTP (Blot et al., 2015). This observation agrees with previous findings where acute systemic administration of PCP (Jodo et al., 2005; Kargieman et al., 2007) and MK-801 (Homayoun and Moghaddam, 2007) significantly increases the firing rate of mPFC pyramidal neurons. Nevertheless, given that LTP induction in the vHipp-mPFC pathway is NMDAR-dependent (Jay et al., 1995), the observation of LTP-like activity in presence of MK-801 is intriguing. This could partly be explained by the phenomenon of cortical disinhibition, which occurs due to the preferential action of the NMDAR antagonists on the NMDARs located on GABAergic inhibitory neurons (Homayoun and Moghaddam, 2007).

Several lines of evidence suggest that disinhibition in the mPFC is unlikely to solely contribute to these observed effects. Unlike its systemic treatment, local infusion of MK-801 or PCP to the mPFC does not alter the activity of the neurons in this region. However, local infusion of the same compound in the vHipp (Jodo et al., 2005) or mediodorsal thalamic nucleus (Kiss et al., 2011a; Kiss et al., 2011b) induces similar effects as its systemic administration. This is further supported by findings that suggest minimal involvement of NMDAR in the activity of the fast-spiking parvalbumin interneurons in the mPFC (Rotaru et al., 2011). In a series of experiments, Blot and colleagues (2015) revealed that MK-801-induced LTP shares the same expression mechanism as HFS-induced LTP. These authors further suggested that, similar to HFS (Gurden et al., 1999; Matsumoto et al., 2008), acute application of MK-801 induces an efflux of dopamine (Adams and Moghaddam, 1998; Lopez-Gil et al., 2007) and glutamate (Homayoun and Moghaddam, 2007), possibly due to its disinhibitory effect, the interaction of which is essential for induction and maintenance of LTP in the vHipp-mPFC pathway (Gurden et al., 1999; Gurden et al., 2000). NMDARs act as coincident detectors. The abnormal augmented synaptic plasticity could reflect reduced signal-to-noise ratio in the mPFC, resulting in the aberrant association between stimuli, a prominent feature of psychosis and cognitive impairments associated with schizophrenia (Goto et al., 2010; Blot et al., 2015).

Differences between human and animal *in vivo* studies could be due to inter-species variations and differences in the brain regions studied. While animal studies have primarily focused on the synaptic properties of the vHipp-mPFC pathway, human studies have investigated these processes in motor cortex due to ease of access. To the best of my knowledge, there are no reports of synaptic plasticity in the vHipp-mPFC pathway in patients with schizophrenia. As discussed in **Section 1.5.3**, acute administration of NMDAR antagonists may not be as representative of the chronic nature of the disease as a more chronic treatment regimen. It is, therefore, essential to investigate these effects in animal models, such as the scPCP rat model, that better represent disease chronicity and long-lasting behavioural and pathophysiological symptoms.

1.12. Influence of antipsychotics on synaptic plasticity

Substantial evidence suggests that APs modulate synaptic plasticity. However, these effects are found to vary across brain regions and between typical and atypical agents. Variations in experimental set up (*in vivo* vs. *in vitro* and stimulation protocols) could account for differences in the effects of APs on synaptic plasticity (Goto et al., 2010; Price et al., 2014). For instance, long-term (21 days) but not acute treatment with haloperidol (1 mg/kg/day; i.p.) promoted LTP in striatum slices upon HFS of the corticostratal pathway (three trains, 3s in duration, 100Hz; applied at 20s intervals) (Centonze et al., 2004). In contrast, acute treatment with haloperidol (0.8 mg/kg; i.p.) inhibited the *in vivo* induction of LTP in the dentate gyrus upon HFS of the perforant pathway (1 train of 60Hz in 1s, repeated 3 times with 3min intervals) (Jibiki et al., 1993). In a similar preparation of the same pathway, acute systemic treatment with clozapine both at low (10 mg/kg; i.p.) and high (20 mg/kg; i.p.) doses significantly potentiated the magnitude of LTP in the dentate gyrus upon HFS of the perforant pathway with the same induction protocol (Kubota et al., 1996).

Bath application of clozapine (10 µM) significantly facilitated LTP in layer V of mPFC slice preparations upon HFS of layer II (1 train, 1s, 50Hz followed by 4 trains of the same frequency every 10s applied 15 minutes after the first HFS-train set) (Gemperle et al., 2003). The same group reported significantly lower LTP levels in slice preparation of the same circuitry, after 21 days of clozapine treatment (30 mg/kg/day; via drinking water) (Gemperle and Olpe, 2004). In the same circuitry, bath application of haloperidol (0.2 µM) and iloperidone (2.5 µM) did not have an effect on LTP induction (Gemperle et al., 2003). Similarly, Xu et al (2009) also found no change in slice LTP induction in layer V of the Prl/IL upon stimulation of layer II-III in mice treated with haloperidol. This was the case for both acutely (1 mg/kg; i.p.) and chronically (14 days, 0.5 mg/kg/day; i.p.) treated animals (Xu et al., 2009). Similarly, acute treatment with haloperidol (1 mg/kg; i.p.) had no effect on mPFC LTP upon stimulation of vHipp under anaesthetised conditions. However, acute treatment with clozapine (20 mg/kg; i.p.) facilitated LTP induction in the same pathway (Matsumoto et al., 2008). Consistent with these findings, local infusion of other D2R antagonists such as sulpiride (5-10 mM) into the mPFC had no effect on the induction of LTP after HFS of the vHipp (Gurden et al., 2000). Contrary evidence has also been reported, whereby, bath application of haloperidol (2 µM) to mPFC slices of physiologically healthy mice completely abolished the induction of spike-timing dependent LTP in layer V upon stimulation of layer II-III under intact GABAergic transmission (Xu and Yao, 2010).

The significance of synaptic plasticity as the cellular foundations of cognition was highlighted in previous sections. Therefore, findings of electrophysiological studies reported here are of some relevance when considering the neurocognitive effects of AP treatments. Based on these studies, the influence of haloperidol on LTP in the mPFC and vHipp-mPFC pathway appears minimal. This may highlight the relative ineffectiveness of this AP in improving cognitive impairments both in humans and in disease models. This contrasts with the modulation of synaptic plasticity by clozapine, which might point towards its beneficial neurocognitive properties. With the limited information at hand, however, the interaction between the influence of AP treatments on synaptic plasticity and its potential neurocognitive effects are merely speculative. While these studies may elucidate the mechanism of action of APs in local circuitries that mediate cognitive function, they are substantially limited in their translational validity both to disease and *in vivo* state. This is because the majority of these studies report the effects of AP treatment in slice or *in vivo* preparations in physiologically healthy animals. Based on the presented findings, there is a clear gap in current understanding with regards to the influence of long-term treatment with APs. Investigating these effects in a well validated model such as the previously described scPCP model, could contribute to the understanding of the neurophysiological effects of APs on neural substrates of cognition.

1.13. General Aims and Objectives

Our limited understanding of the long-term effects of APs on cognition hinders advances in drug development and negatively impacts the quality of life and functional outcome of patients with schizophrenia. Studying the neurocognitive effects of APs in the clinical setting is made complex by various confounding variables including polypharmacy. It is therefore most essential that these effects are studied in the pre-clinical setting, using well-validated models of relevance to the disease. To date, results of the few studies investigating the influence of long-term treatment with APs on cognition, are of limited translational validity due to poor choice of treatment dose and method of drug delivery. Even fewer studies have examined the influence long-term treatment with APs on the synaptic properties of pathways implicated in mediating higher-order cognitive functions.

The studies presented in this thesis aim to address the highlighted methodological issues associated with pre-clinical studies and to provide an in-depth examination of the neurocognitive effects of these compounds upon long-term treatment. Since the mechanisms of synaptic plasticity are fundamental to cognition, this project also aims to investigate the influence of long-term AP treatment on synaptic properties of the vHipp-mPFC pathway. Investigations will be conducted in the scPCP model for cognitive impairments associated with the disease, using a range of behavioural and electrophysiological techniques. It is noteworthy that the synaptic properties of the vHipp-mPFC pathway have not been previously examined in the scPCP model. Therefore, the work provided in this thesis will expand the boundaries of face validity associated with this model.

The specific objectives of the project are outlined below:

1. To examine the influence of long-term treatment with haloperidol and olanzapine on cognitive performance using variations of the NOR paradigm (dNOR and cNOR). This objective is addressed in **Chapter 3** and **Chapter 4** presented in this thesis.

2. To characterise the synaptic properties of the vHipp-mPFC pathway in the scPCP model. This is an essential step towards fulfilment of the final objective of this project. In addition, this allows for validation of the scPCP model from an electrophysiological perspective. This objective is addressed in **Chapter 5**.

3. Investigate the influence of long-term treatment with haloperidol on the short- and long-term synaptic properties of the vHipp-mPFC pathway. This objective is addressed in **Chapter 6**.

Chapter 2

Materials and Methods

2.1. Animals

All animals used for studies in this thesis were adult female Lister Hooded rats (Charles River, UK). Rats were housed in groups of 5 in individually ventilated cages with two levels (GR1800 Double-Decker Cage, Techniplast, UK) maintained at controlled temperature (21 ± 2˚C), humidity (45 ±10%) and illumination (12:12h light/dark cycle, lights on at 7am) throughout all studies. Food (Special Diet Services, Sussex, UK) and water were available *ad libitum* for all animals in all studies reported in this thesis. All experimental procedures were conducted in the light phase and commenced 7 days after the arrival of the animals to the Biological Service Facility (BSF) of the University of Manchester. All procedures were undertaken under UK Home Office licence in accordance with the Animals Scientific Procedures Act (1986) and were approved by the University of Manchester Animal Welfare and Ethical Review Body. The number of animals used in each study is reported in each experimental chapter.

2.2. Drugs and compounds Preparation and Administration

A list of all drugs and compounds used in studies reported in this thesis is provided in **Table 2.1**. In addition, details of dose, volume and route of administration are also reported. Briefly, drugs were administered via intraperitoneal (i.p.), subcutaneous (s.c.) and oral (p.o.) routes and osmotic minipumps which were implanted subcutaneously (see **Section 2.5**). Based on standard practice in our laboratory, all drugs were delivered in a volume of 1 ml/kg. This is within the dose volume range (maximum 5 ml/kg) specified in the project licences under which these studies were conducted and are in agreement with the guidelines of substance administration to laboratory animals (Diehl et al., 2001; Morton et al., 2001). Drugs requiring an oral route of administration were delivered via a 16G, 3 inch long gavage needle with a 3 mm ball (Sigma-Aldrich, UK).

2.3. Sub-Chronic PCP/Vehicle Treatment

Rats were randomised to receive vehicle (0.9% saline; i.p.) or phencyclidine hydrochloride (PCP, 2 mg/kg; i.p.) twice daily for 7 days. This was followed by a 7- day washout (WO) period. The WO period is necessary to eliminate any behavioural and electrophysiological changes caused by acute drug withdrawal or residual PCP present in the system. Adapted from previous studies conducted by Jentsch and colleagues (Jentsch et al., 1997; Jentsch et al., 1998), this is now the standard dosing regimen routinely used in our laboratory. Data from our laboratory (Neill et al., 2010; Neill et al., 2014; Cadinu et al., 2017) and others (Jenkins et al., 2008; Jenkins et al., 2010; McKibben et al., 2010) suggest that this regimen induces a wide range of cognitive and negative symptoms as well as pathophysiology associated with schizophrenia. For a review of previous work with this model please refer to **Chapter 1.5.3 and 1.7.2.** It is important to note that while the described sub-chronic PCP (scPCP) dosing regimen or similar protocols (see **Chapter 1.5.3**) are most commonly applied to rats, the scPCP regimen used in mice generally requires a higher dose (5-10 mg/kg)

and the dose and duration of treatment appears to be varied across the literature (Hashimoto et al., 2005; Brigman et al., 2009; Nomura et al., 2016).

Drug	Formulation	Source	Dose (mg/kg)	Route	Preparation	Pre-treatment time
Phencyclidine Hydrochloride	Powder	Sigma Aldrich (UK)	2	i.p.	Dissolved in 0.9% saline	-
Haloperidol	Liquid	Janssen-Cilag Ltd.	0.1	p.o.	Diluted in 0.9% saline	90 mins
Haloperidol	Powder	Alfa Aesar	0.5	Minipump	Dissolved in ascorbic acid and diluted in the PH adjusted β-hydroxypropylcyclodextrin. Brought to PH 6 by ascorbic acid and NaOH (1M)	-
Olanzapine	Powder	Kemprotec Ltd.	1.5	p.o.	Dissolved in a small volume of Acetic acid (15 µl/10 mg of olanzapine), adjusted to pH 6-6.5 by NaOH (1M) and brought to volume with 0.9% saline	90 mins
d-Amphetamine	Powder	Sigma Aldrich (UK)	1	i.p.	Dissolved in 0.9% saline	30 mins
Propranolol	Powder	Sigma Aldrich (UK)	10	i.p.	Dissolved in 0.9% saline	30 mins
β-hydroxypropylcyclodextrin	Powder	Cavasol W7 HP Pharma	-	Minipump	20% w/v, dissolved in dH_2O. Adjusted to pH 6 by ascorbic acid and NaOH (1M)	-
Buprenorphine	Liquid	Vetergesic (UK)	0.03	s.c.	Diluted in 0.9% saline	-
Urethane	Powder	Sigma Aldrich (UK)	1.5 (g/kg)*	i.p.	30% w/v Dissolved in distilled water	-
Sodium Citrate	Powder	Melford	-	-	3.8% w/v Dissolved in distilled water	-
Dulbecco's Phosphate-buffered Saline 1x (without $CaCl_2$ and $MgCl_2$)	Liquid	Sigma Aldrich (UK)	-	-	200 of 10x PBS in 1800 ml of Distilled water	-
Paraformaldehyde (4% v/v)	Liquid	Genta Medical	-	-	160 ml of PFA (50% v/v) in 1840 ml Distilled water	-
Cryoprotectant	Liquid	Sigma Aldrich (UK)	-	-	30ml of glycerol,30ml of glycoethylene and 40 ml of 1xDPBS	-

Table 2.1. A summary of all compounds used in the experimental chapters including the dose of treatment, methods of drug preparation and route of delivery. * For urethane only dose was based on g/kg.

2.4. Behavioural tasks

2.4.1. Novel Object Recognition task

The Novel Object Recognition task (NOR) forms a major part of the studies reported in this thesis. According to the aims and objectives of each study, either the disrupted NOR (dNOR) or the continuous NOR (cNOR) were employed. A detailed account of NOR protocols is provided below.

2.4.1.1. NOR Apparatus

NOR was conducted in open Plexiglas boxes (52 cm w, 52 cm L, 30 cm h). Each box consisted of black walls and a white floor which was divided into 9 square sectors (**Figure 2.1**). The object pairs used in NOR consisted of Coca-Cola ® cans and brown medicine bottles (1 and 2), black clay flower pots and white Frijj ® milkshake bottles filled with water (3 and 4), empty blue pepper dispenser and small transparent glasses (5 and 6), transparent square candle holders and small blue clay bowls (7 ad 8) (**Figure 2.2**). Lighter objects were set in place using Blu tack. All objects had been previously validated in our laboratory to ensure that rats do not have a preference for either object in object pairs. Object placement was pseudorandomised between rats and NOR test boxes (**Figure 2.3**) to prevent potential effects of spatial bias on performance. In studies reported in this thesis (particularly in **Chapters 3** and **4**), NOR tests were conducted at several time points throughout treatment duration. To prevent over-exposure to one object pair, different sets of objects were employed at each testing session. This was particularly important if several NOR testing sessions were planned to be conducted within short intervals of each other. **Table 2.2** represents an example of object randomisation used (for one rat) in **Chapter 4**, which employed the use of all mentioned object pairings due to frequency and the short interval between NOR testing sessions.

2.4.1.2. Habituation

To reduce stress during testing, all rats were habituated to the empty NOR box and testing environment two days prior to the first NOR. Habituation protocol was as follows:

- Group Habituation: Two days prior to the first NOR, rats were placed in the testing box with their cage mates for 15 minutes.
- Individual Habituation: One day prior to the first NOR, rats were individually placed in the NOR box for 10 minutes.

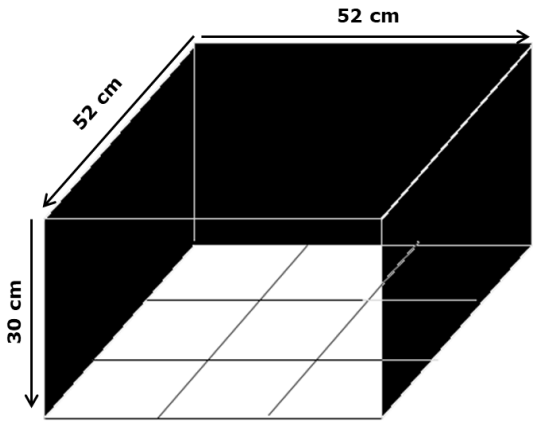

Figure 2.1. Schematic representation of the NOR test box

Figure 2.2. Object pairs used in the NOR test

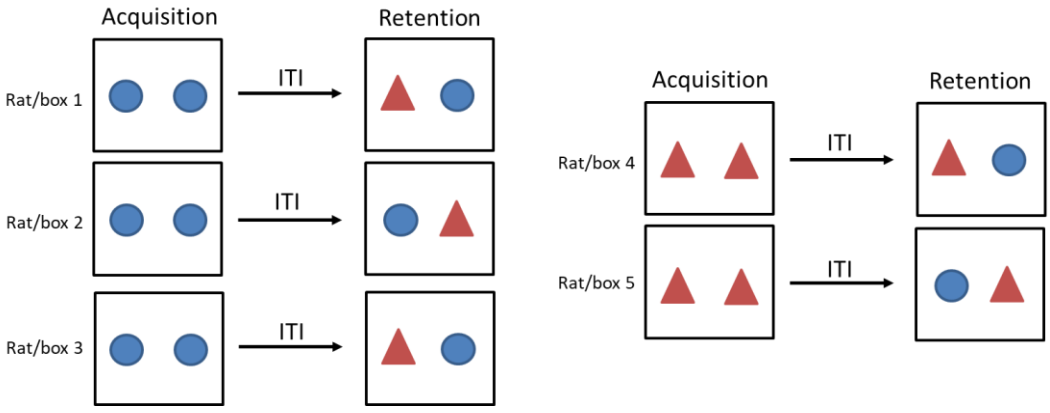

Figure 2.3. NOR object-box/rat counterbalancing. Objects were counterbalanced based on their left/right position and novelty/familiarity

NOR session	Acquisition		Retention	
dNOR-1	2	2	2	1
cNOR-1	4	4	4	3
dNOR-2	5	5	5	6
cNOR-2	7	7	8	7
dNOR-3	4	4	3	4
cNOR-3	1	1	2	1

Table 2. 2. Example of object counterbalancing over several NOR testing sessions for one rat. This format of counterbalancing was employed in chapter 4 whereby each dNOR and cNOR testing session was separated by 1 day. Refer to chapter 4 for details of NOR testing sessions and experimental design. Numbers refer to the object pairs used in each trial. Red numbers represent the novel objects.

2.4.1.3. NOR testing protocol

2.4.1.3.1. Acquisition Phase

Two identical objects were placed equidistant from each other and the walls of the testing arena. Rats were placed into the box (facing the corner) and left to explore the objects for 3 minutes (**Figure 2.4 A**). Objects were removed from the box at the end of this phase.

2.4.1.3.2. Inter-trial-Interval

The acquisition phase was followed by an inter-trial-interval (ITI) (**Figure 2.4 B**). The ITI was kept at 1 minute in all studies reported in this thesis. scPCP treatment induces a reproducible deficit in NOR performance which is apparent at short ITIs (i.e. 1 minute). This deficit, which could partly be explained by the susceptibility of scPCP treated animals to distraction at short ITIs (see dNOR below), has been well validated in our laboratory (Grayson et al., 2007; Snigdha et al., 2010; Grayson et al., 2014; Cadinu et al., 2017). The location of the rat during the ITI determined the main and only difference between the dNOR and cNOR.

- dNOR: At the end of the acquisition phase, the rat was immediately removed from the NOR box and placed into an open unfamiliar Plexiglas box (24 cm w, 44 cm L, 19 cm h) where it remained for 1 minute (element of distraction). During this time, the NOR box was cleaned using water-soaked cloth and objects for the next phase were placed in the box (**Figure 2.4 B_1**).

- cNOR: At the end of the acquisition phase, objects were immediately removed from the NOR box while the rat remained in the NOR box for 1 minute. In the cNOR, the NOR box was not cleaned in the ITI. Objects for the next phase were introduced to the rat at the end of the 1-minute ITI (**Figure 2.4 B_2**).

2.4.1.3.3. Retention Phase

A novel object and a replica of the familiar object (to remove any potential odour cues) were placed in the box. The location and the orientation of the objects were the same as the acquisition phase. In the retention phase, rats were left to explore the objects for another 3 minutes (**Figure 2.4 C**). At the end of this phase, rats were returned to home cage and the NOR box and objects were thoroughly cleaned with 70% ethanol.

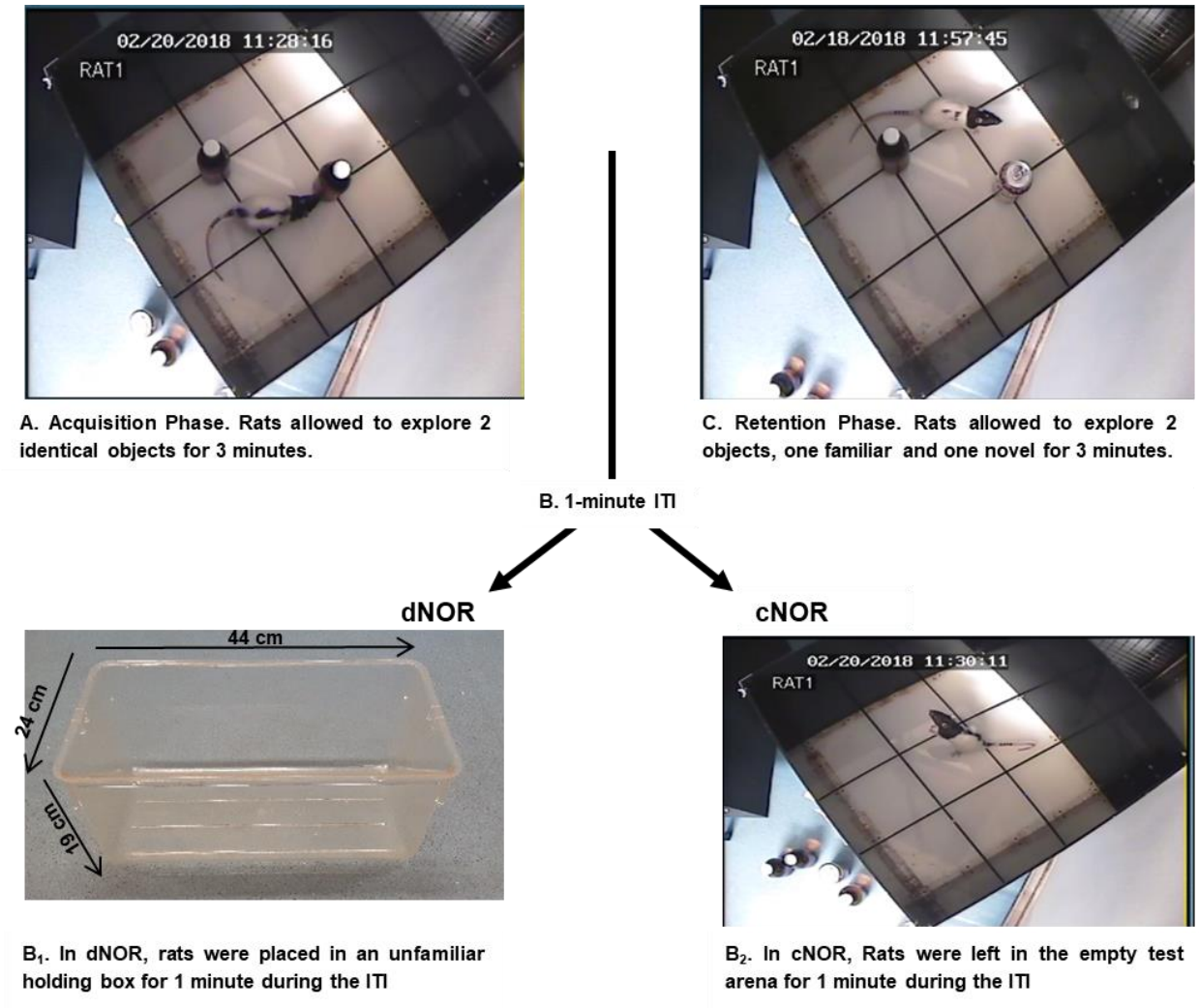

Figure 2.4. Representation of the dNOR and cNOR tests. (A) Represents the Acquisition phase of the trial (B) Represents the Inter-trial Interval (ITI). Location of the rat during the ITI is different for B_1 (dNOR) and B_2 (cNOR) (C) Represents the Retention phase of the trial.

2.4.1.4. Behavioural Analysis

All NOR testing sessions were filmed and video recorded for subsequent blind analysis. It is important to note that I was not blind to the treatment at the time of testing since drug preparation and its administration was carried out by myself. However, the videos recorded at each NOR testing session were devoid of any labels that could reveal the animal ID and their treatment history. Therefore, the videos were essentially analysed blind to treatment and rat ID and were decoded only when all necessary information were extracted from recordings. From each video, object exploration time in each phase of the NOR task was recorded using the "Jack River-Auty" online stopwatch (http://jackrrivers.com/program/). Object exploration was defined as licking and sniffing whilst leaning on or touching the object. Turning towards and sitting on or next to the object without sniffing was not considered exploration. This definition of object exploration is consistent across literature (Ennaceur et al., 1997; Antunes and Biala, 2012) and is commonly used in our laboratory (Grayson et al., 2007). Behavioural trials were excluded from analysis if animals either explored objects for one or less than one second or jumped onto the edge of the NOR test box. From the recorded object exploration times the following factors were calculated:

- Total Exploration Time (s): In each phase of the task total exploration time was calculated. This could be regarded as a measure of general interest and interaction with the object.

Total Exploration Time = object 1 exploration (s) + object 2 exploration (s)

- Discrimination Index (DI): DI is expressed as the ratio of novel to total object exploration time in the retention phase.

$$\textbf{DI} = \frac{(\boldsymbol{Novel\ object\ exploration\ time - Familiar\ object\ exploration\ time})}{\boldsymbol{Total\ object\ exploration\ time}}$$

Locomotor activity (LMA) was also evaluated by counting the total number of lines crossed by each rat in both the **acquisition** and the retention phase.

2.4.1.5. Statistical Analysis of NOR

All data were tested for normality (Shapiro-Wilk analysis) and transformed using Log_{10} transformation when appropriate. Object exploration data were analysed by mixed-designed two-way (task and treatment) ANOVAs to determine the main effect of task (Left vs. Right in acquisition phase and Novel vs. Familiar in the retention phase; within-subjects variable) and drug treatment (between-subjects variable) on object exploration time. This was followed by two-tailed paired Student's t-tests (employed to compare between novel vs. familiar object exploration time within each treatment group) and independent samples Students' t-test or planned Bonferroni pair-wise comparisons (to compare total object exploration times between the treatment groups). Discrimination index (DI) and locomotor activity (LMA) data were analysed by independent Student's t-tests or one-way ANOVAs. The latter was followed by planned Bonferroni pair-wise comparisons. Furthermore, DI scores were compared against zero using Mann-Whitney U test (nonparametric independent Samples t-test).

Planned Bonferroni pair-wise comparisons are defined separately in each experimental chapter. For all Bonferroni comparisons, the significance threshold (i.e. α-value) was set at **0.05**. The *p*-values were adjusted by multiplying the unadjusted *p*-value (obtained from planned LSD comparisons) by the number of comparisons made. This is the method recommended by IBM SPSS Statistics Support (2016a) to adjust for multiple comparisons using Bonferroni corrections. Number of comparisons is defined separately in each experimental chapter. All data were analysed using SPSS IBM (Version 23). For the two-way ANOVAs, tests of homogeneity of variance were conducted when necessary. Since the within-subject variables had 2 conditions only (i.e. left vs. right; novel vs. familiar), no corrections for equality of variance were required when reporting the within-subjects effects. Between-subjects variables were tested for homogeneity of variance using Levene's test. Unless specified, results of between-subjects comparisons were reported uncorrected when the assumption of equal variances was not rejected. Similarly, Levene's test was conducted when using a one-way ANOVA. No corrections for equality of variance were required when reporting the results of the one-way ANOVA. All analyses were performed by IBM SPSS (Version 23).

2.4.2. Locomotor Activity (LMA) test

The purpose of this test was to assess the influence of drug treatment on the activity levels of the animals. Given the automated setting of the testing chambers, the subjective influence of the experimenter was removed from the analysis.

2.4.2.1. LMA Apparatus

Locomotor Activity (LMA) was tested using the automated testing chambers (Photobeam Activity system, San Diego instruments). The testing chambers were translucent Plexiglas boxes (24 cm w, 44 cm L, 19 cm h) with a perforated translucent Plexiglas lid (to allow air flow). Each box contained a small amount of saw-dust to provide insulation for the animals. All boxes were positioned in a locomotor and rearing frame (**Figure 2.5 A**). The locomotor frame contained 2x4 beams on the X-axes and 2x8 beams on the Y-axes. The rearing frame contained 2x8 beams on the X-axes only (**Figure 2.5 B**). All boxes were placed in a three-tier shelf against the wall, in a total bank of 12 boxes (**Figure 2.5 C**). All testing chambers were connected to a control box which was connected to a computer. The system was controlled using Pas764 LMA software to record the number of interruptions to the beam along the X and Y axes.

2.4.2.2. Habituation

Rats were habituated to the LMA testing boxes one day prior to testing. This involved leaving individual rats in the LMA boxes for an hour.

2.4.2.3. LMA Testing protocol

On the day of testing, rats were placed in the testing chambers and a baseline measure of their activity was recorded every 5 minutes for 30 minutes. Rats were then treated with an acute dose (i.p.) of the experimental compound and were immediately put back in the LMA box. Following treatment, LMA activity was recorded every 5 minutes for 60 minutes. Boxes were thoroughly cleaned with 70% ethanol and saw dust was replaced between rats.

2.4.2.4. LMA Analysis

The software automatically calculated the total number of beam breaks for central and peripheral activity in 5-minute bins. From these measures the following factors were calculated:

- Total LMA (Central + Peripheral)
- Area under the curve (AUC) for total LMA: AUC represents the accumulation of activity over time in each group. This was calculated using GraphPad Prism (Version 8.0.2).

2.4.2.5. Statistical Analysis

Data were examined for normality using the Shapiro-Wilk analysis test and transformed when necessary using Log_{10} transformation. AUC for the total LMA during the test phase (not baseline) was analysed using an independent one-way ANOVA followed by planned LSD *post-hoc* analysis. Homogeneity of variance was assessed by the Levene's test. Assumption of equal variance was maintained unless otherwise stated. Therefore, the results of the ANOVA were reported uncorrected. All statistical analyses were conducted using IBM SPSS (Version 23).

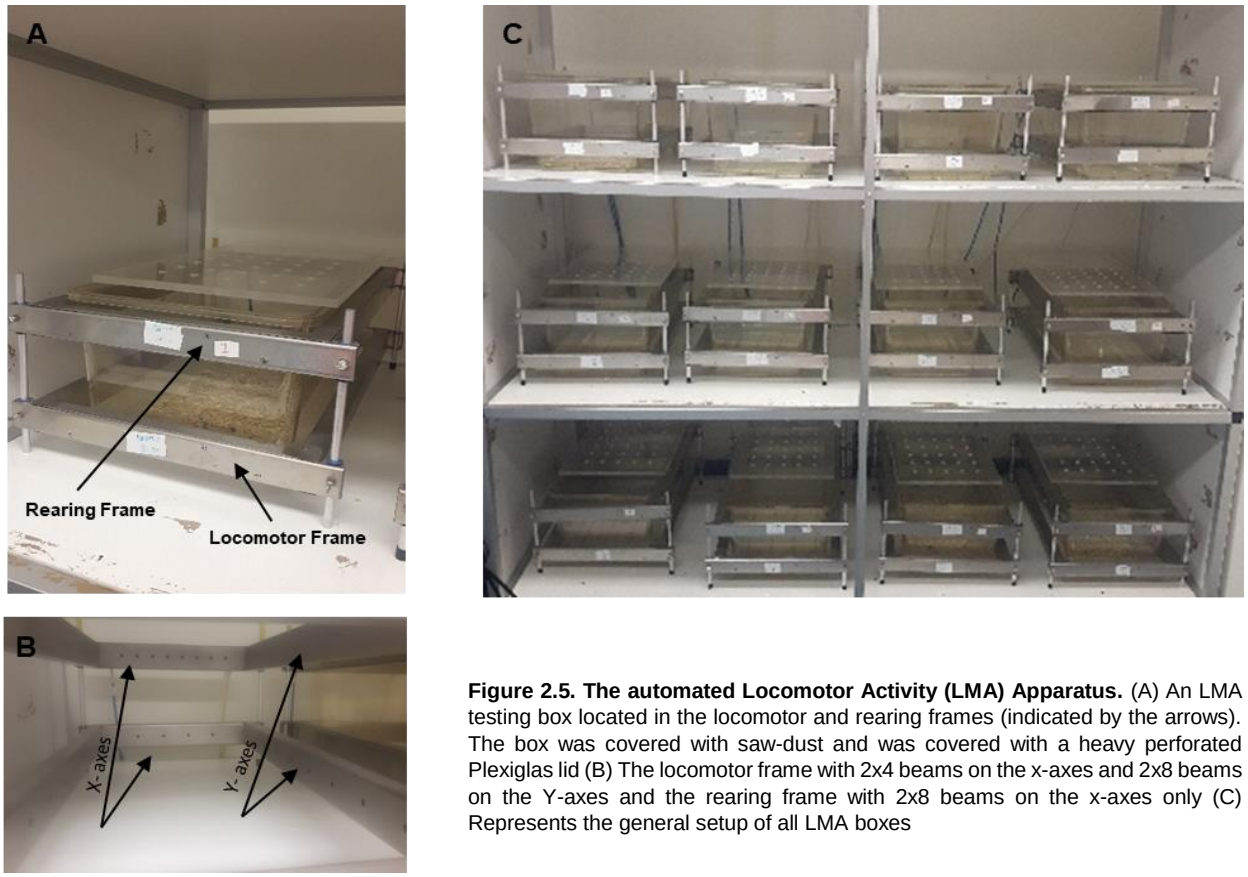

Figure 2.5. The automated Locomotor Activity (LMA) Apparatus. (A) An LMA testing box located in the locomotor and rearing frames (indicated by the arrows). The box was covered with saw-dust and was covered with a heavy perforated Plexiglas lid (B) The locomotor frame with 2x4 beams on the x-axes and 2x8 beams on the Y-axes and the rearing frame with 2x8 beams on the x-axes only (C) Represents the general setup of all LMA boxes

2.5. Osmotic minipumps

The half-life of APs is, on average, 4 to 6 times shorter in rodents (2.5 h for olanzapine and 1.5h for haloperidol) than in humans (21-54 hours for olanzapine and 14.5-36.7 hours), hence, single daily treatments in rodents is not a clinically translatable method of drug administration (Kapur et al., 2003). This can be overcome by dosing animals multiple times in a day. This however might not be sustainable for long dosing schedules as dosing is stressful to the animals (Stuart and Robinson, 2015).Osmotic minipumps are a reliable method of drug delivery (Vernon et al., 2011; Vernon et al., 2012; Vernon et al., 2014). Given the set reservoir capacity and flowrate, the pump releases a set volume of drug into the system to provide a sustained drug plasma level (Kapur et al., 2003). Osmotic minipumps are advantageous in comparison to other methods of drug delivery as they minimise the stress of daily dosing for the animal, overcome the fast half-life of the drug by their sustained release mechanism and allow for long-term drug treatment in rodents.

2.5.1. Osmotic Minipump Mechanism

As shown in **Figure 2.6,** the pump is comprised of an impermeable reservoir where the drug is loaded. This is surrounded by the osmotic layer (the salt sleeve) which plays a key role in the mechanism of the pump. The outer layer of the pump is made of a semipermeable membrane. The construction of this membrane and its permeability to water determines the rate of drug delivery. The tube at the centre of the reservoir is the space through which the pump is filled. The osmotic minipump operates due to the difference in osmotic pressure between the osmotic layer within the pump and the environment where the pump is implanted. The difference in the pressure allows water to flow in through the semipermeable membrane into the pump. This causes the osmotic layer to compress the flexible reservoir and to flux the drug out through the flow moderator at a pre-determined rate (Theeuwes and Yum, 1976).

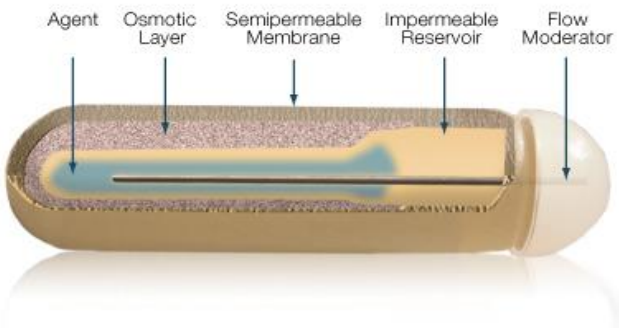

Figure 2.6. Structure of an osmotic mini-pump. Image taken-from-the-following-website: http://www.alzet.com/products/ALZET_Pumps/howdoesitwork.html

2.5.2. Technical Description of Minipumps used

Table 2.3 provides a brief description of the Alzet Osmotic minipumps (2ML4 model) used in experiments reported here. Most of the Minipumps were kindly provided by Dr. Anthony Vernon (King's College London).

Pump Model	2ML4 (Alzet)	**Flow rate**	2.5 µ/h (± 0.05 µ/h)
Duration	28 days	**Reservoir Volume**	2 ml
Length	5.1 cm	**Diameter**	1.4 cm
Weight (empty)	5.1 g	**Weight Full**	7.6 g
Average Weight at 28 days *	7.6 g	**Outer later Material**	Cellulose ester blend
Drug Reservoir Material	Thermoplastic hydrocarbon elastomer	**Flow moderator Material**	Stainless Steel

Table 2.3. A brief description of the osmotic minipumps. * The weight of the pump was measured at the end of the study presented in chapter 6 only for each animal and the average pump weight is reported here.

2.5.3. Filling the Minipump

Minipumps were filled under sterile conditions using a 2.5 ml syringe and a 25-gauge filling tube provided by the manufacturer. With the flow moderator removed, the pump was held in an upright position and the filling tube was inserted through the opening at the top of the pump until it could go no further (this placed the filling tube at the bottom of the reservoir). Drug was then displaced from the syringe into the pump very slowly to prevent the formation of air bubbles in the pump. When the solution appeared at the outlet of the pump, the filling tube was slowly removed and the flow moderator was replaced. Correct pump filling was achieved when a small amount of fluid came out of the flow moderator after its placement in the pump. Following on the protocol used by Vernon et al (2011; 2012; 2014), the minipumps used in the studies presented in this thesis were not primed prior to implant.

The volume of solution inserted into each pump was 2ml. The concentration of the drug was determined using the online interactive calculator provided by Alzet taking into account factors including the pump flow rate and reservoir volume http://www.alzet.com/products/guide_to_use/AZLET_update06.swf .

2.5.4. Osmotic Minipump Implant Protocol

Anaesthesia was induced using isoflurane (5% flow rate in 2 l/min O_2) in an induction chamber. A 3x5 cm surface area between the shoulders of the rat was then shaved before the rat was transferred to a sterile surface for the surgical procedure. Throughout the procedure anaesthesia was maintained at 3.5% isoflurane in 2 l/min O_2. The incision site was cleaned with ionated povidone cutaneous solution (10% w/v, EcoLab Ltd. Leeds) using sterile cotton buds. A 2-2.5 cm incision was made in the skin using a scalpel. The opened cavity was irrigated with sterile 0.9% saline solution. Using a pair of blunt end scissors, the

skin was slowly separated from the connective tissue to form a pocket underneath the skin. It was ensured that the pocket was large enough to enclose the pump as far away from the incision site as possible. The pre-filled pump was wiped with 70% alcohol wipes (Sani-Cloth®, PDI, UK) and inserted into the pocket with the flow moderator cap facing towards the head of the animal. The incision site was then closed using number 3-0 silk non-absorbable suture (Mersilk, Johnson and Johnson Medical Ltd. UK). The interrupted stitching technique was employed as this allowed for the knots to be hidden, hence, reducing the possibility of wound opening when rats start grooming. The incision site was cleaned again with iodine solution. An acute dose of Buprenorphine (0.03 mg/kg) was injected subcutaneously. The animal was then placed in a clean recovery box covered with saw-dust and kept at 27-30 ˚C until it gained consciousness. Animals were returned to home-cage when they started to groom, eat, and drink water in the recovery cage.

2.5.5. Peri and Post-Operative care

In studies using osmotic minipumps for drug delivery (**Chapters 4** and **6**), rats were weighed daily for 7 days prior to the operation. The post-operative care varied slightly for each study. In chapter 4, rats were weighed daily until the end of the study (28 days post operation). In **Chapter 6**, rats were weighed daily for 7 days post-implant and then on a weekly basis until the end of the study. For all minipump implanted animals, food intake was monitored for 7 days prior to the surgery. For a week post-operation, a portion of food soaked in water was placed on the cage floor for ease of access and consumption.

2.6. Acute Electrophysiology Technique

Acute (non-recovery) electrophysiological techniques were used to study the synaptic properties of the ventral hippocampus (vHipp) - medial prefrontal cortex (mPFC) pathway in **Chapters 5 and 6.** All electrophysiological recordings were obtained under anaesthetised conditions.

2.6.1. Anaesthesia

To achieve surgical-plane anaesthesia, rats were treated with an i.p. injection of urethane (30% w/v in distilled water, 1.5 g/kg). Pedal-withdraw and corneal reflex were monitored to assess the depth of anaesthesia. Supplementary doses of urethane (0.1-0.2 ml, 30% w/v, 1.5 g/kg) were provided when strong-moderate pedal-withdraw reflex was still present 20 minutes after the first injection. Surgical procedure proceeded upon complete areflexia. Rats remained anaesthetized for an average of 9 hours. Urethane was chosen due to its long duration of action, minimal effect on cardiovascular and respiratory systems and its relatively minimal interference with major neurotransmitter systems (Hara and Harris, 2002). Furthermore, it has been shown that urethane induces a state of behavioural unconsciousness mimicking that of natural sleep during which different states of network oscillations can be monitored. This suggests minimal interference of this anaesthetic agent to brain dynamics (Clement et al., 2008).

2.6.2. Surgical Procedure

Once in surgical plane, rats were mounted on to a stereotaxic frame (Kopf 1430, USA) whereby the position of the head was stabilised by ear and incisor bars. A rectal probe and a homoeothermic heat-pad (Harvard Apparatus, USA) were used to monitor and maintain body temperature at approximately 37 ˚C. The animal's head was cleaned using 70% alcohol wipes (Sani-Cloth®, PDI, UK). Then using a scalpel an incision was made to expose the skull along the midline of the head. Any connective tissue was removed from the skull to expose and identify the brain landmarks (Bregma, and Lambda). Thereafter, the coordinates for craniotomies were marked on the skull. To target the prelimbic (Prl) region of mPFC, craniotomies were made at 3.2 mm anterior to Bregma and 1.5 mm lateral to the midline (B+3.2 mm, ML= 1.5 mm) (**Figure 2.7 A**). To target the CA1 region of vHipp, a craniotomy was made at 6.5 mm posterior to Bregma and 5.5 mm lateral to the midline (B-6.5 mm, ML=5.5 mm) (**Figure 2.7 B**). These co-ordinates were based on the study by Laroche et al (1990) with electrode placements confirmed *post-hoc* using Paxinos and Watson (2007) rat brain atlas (atlas **Figures 10 and 87**) (**Figure 2.7 A and B**).

Craniotomies were drilled over the left hemisphere with a 0.7 mm drill bit (Fine Science Tools, Germany) using a high-speed hand-held drill (Foredom, USA). Care was taken to ensure the overlaying dural blood vessels remained intact during the process. Once craniotomies were completed, the dura covering cranial surface was removed (using a pair of angled tweezers) to expose brain surface and allow smooth electrode insertion. The exposed brain tissue was kept moist prior to electrode insertion using sterile saline-soaked cotton wool.

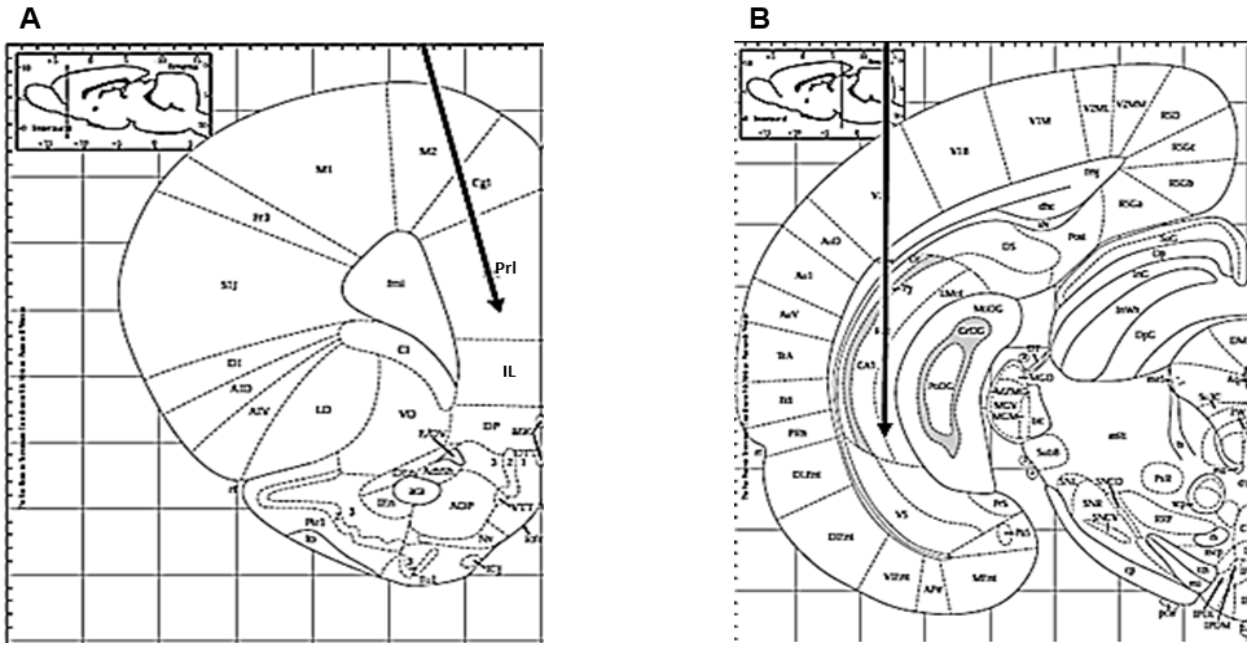

Figure 2.7. Target recording and stimulation sites. (A) mPFC recording site in left hemisphere. The arrow tip shows the ideal final position of the electrode in the Prl cortex. The coordinates were: B+3.2mm, ML= 1.5mm. Insertions were made at a 10˚ angle from midline to avoid the medial venous sinus, depth= 4mm (B) vHipp stimulation site in left hemisphere. The arrow tip shows the ideal final position of the electrode in ventral CA1. The coordinates were: B-6.5mm, ML= 5.5mm, depth= 4.4-5.5mm. Figures adapted from Paxinos and Watson (2007)

2.6.3. Electrode Configuration and Placement

Two different recording electrode configurations were used in the electrophysiological studies. In chapter 5, the recording electrode used (NeuroNexus Tech, USA) consisted of two silicon shanks, each with 16 recording channels (16 x 2 = total 32 recording sites). On each shank, channels were arranged linearly and separated by 100 µm centre-to-centre spacing with the two shanks 500 µm apart (**Figure 2.8 a**). In chapter 6, the E-2 series 2x16 recording electrode (Cambridge NeuroTech) was used. On each shank, channels were arranged in a polytrode configuration. The first two channels were separated by 25 µm while the rest were separated by 40 µm. The two electrode shanks were 250 µm apart (**Figure 2.8 b**). In both experiments, the recording electrode was placed at the surface of the mPFC and 10° angle and slowly lowered by 4 mm to target the Prl region of the mPFC (**Figure 2.7 A**). Before insertion, recording electrodes were coated in fluorescent dye, DiI (1, 1′-Dioctadecyl-3, 3, 3′, 3′ tetramethylindocarbocyanine perchlorate, Sigma-Aldrich, UK) to reveal the electrode track *post-hoc.*

The stimulating electrode consisted of a pair of twisted Teflon-insulated stainless-steel wires (CA151316, Advent Research Material Ltd) (**Figure 2.8 c**). The wires were twisted 30-40 times and the electrode was approximately 15000 µm in length. The ends of the electrodes were trimmed with a sharp pair of scissors and checked for lack of electrical short using voltmeter. The stimulating electrode was placed at the surface of the cortex and lowered to 4.4-5.5 mm to target the CA1 region of the vHipp (**Figure 2.7 B**). The depth of the electrode was adjusted during recording from Prl to achieve optimum response shape (**Figure 2.9**) as previously shown by Laroche et al (1990).

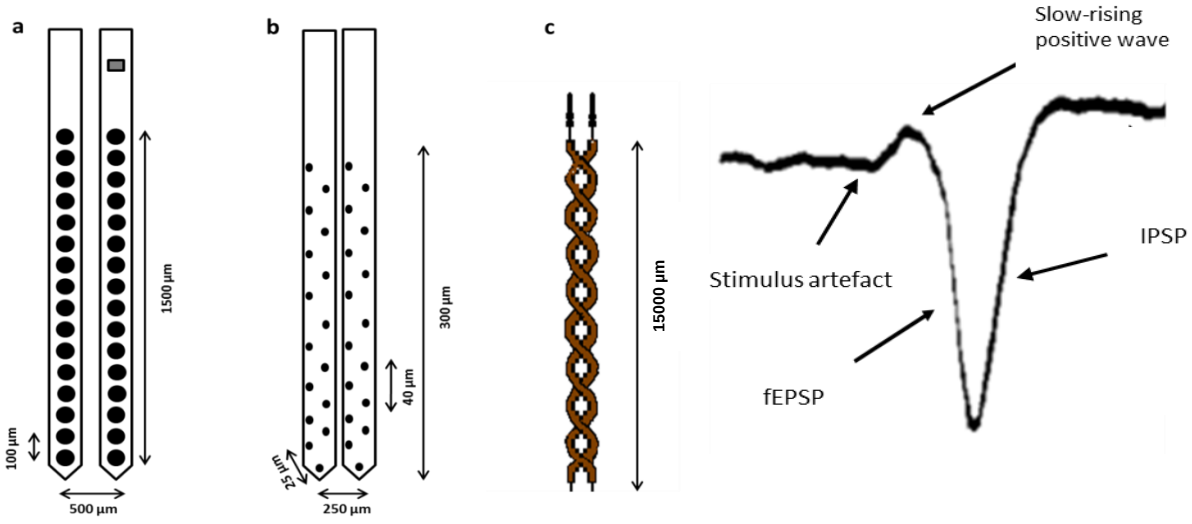

Figure 2.8. Schematic representation of recording and stimulating electrodes. (a) 2x16 electrode, linear channel configuration, used in chapter 5. (b) 2x16 electrode, polytrode channel configuration used in chapter 6. (c) Stimulating electrode, made of two strands of stainless steel (twisted together 30-40 times), made to 15000 µm.

Figure 2.9. Typical shape of the field EPSP response recorded from the pre-limbic region of the mPFC following stimulation of the CA1 region of the vHipp. The depth of the stimulating electrode in the present studies was adjusted to achieve a clear response as represented here. Figure taken from (Laroche et al., 1990).

2.6.4. Recording Protocol

Two recording protocols (**A and B**) were used in the electrophysiological studies reported in this thesis. Each protocol was of 2 main phases which are outlined below. In recording protocol (**A**), phase one consisted of 2 steps and phase 2 of 4 steps. In recording protocol (**B**), phase one consisted of one step and phase 2 of 4 steps. For a schematic summary of the recording protocol, see **Figure 2.10.**

In general, the recording electrodes were connected to a 32-channel electrode interface board (EAG0517/18783, Plexon, USA) to which a head stage amplifier was connected (x20 fixed gain, 09-26-A-01 Plexon, USA). Data were referenced to ground and signals recorded using a Recorder64 system (12-bit A/D; Plexon Inc, USA). Final gain was adjusted to x2000-2500 for all recordings. Local Field Potentials were sampled at 5 KHz with a low-pass filter of 100Hz. Spiking activity was recorded at 40 KHz sampling frequency, with a high-pass filter of 300 Hz. A voltage threshold to capture spiking activity was set manually for individual channels. All data were recorded to a PLX format file. Stimulus triggers were recorded to an event channel in the PLX file. These specifications remained consistent throughout the recording protocol.

Phase One – Spontaneous and Toe-pinch Recording Protocol

Step 1. Upon insertion of the recording electrodes into the Prl, the spontaneous activity was recorded for 20 minutes.

Step 2. The activity of the system was recorded in response to sensory stimulations. Toe-pinches were delivered every 30 seconds for 20 minutes. In recording protocol (**B**) this step was eliminated.

Data obtained from this phase were for another experimenter and will not be presented here.

Phase Two: Evoked (stimulation) Recording Protocol

To evoke responses in the mPFC, electrical stimuli were delivered to the vHipp through the stimulating electrode. The stimulating electrode was connected to an isolated constant current stimulator (DS3, Digitimer, UK) which allowed for precise control of the current amplitude, polarity and duration. The DS3 was triggered by 1ms 5V square wave pulses in various patterns controlled by LabVIEW software (Version 8 National Instruments, USA), using a national instruments PCI card (PCI-6071E). Stimulus duration was set on the DS3 at 200µs throughout the recording protocol. The output of each channel on the recording electrode could be visualised (four at any one time) on an oscilloscope (HAMEG instruments, GmbH, US) to allow the response shapes to be monitored in more detail. This helped to determine whether the electrodes were correctly positioned at their target. A typical extracellular vHipp-evoked local field potential in the mPFC consisted of a slow-rising positive wave, followed by a large negative deflection (fast excitatory post-synaptic potential; fEPSP) followed by an inhibitory post-synaptic potential (**Figure 2.9**). The following describes each step into the evoked recording protocol. The order of the recording steps in each protocol is indicated by assigning **A** or **B** to step numbers.

Step 3A/2B. Assessment of Synaptic Connectivity

Once a stable characteristic response was obtained, an input-output (I/O) curve was plotted by application of 20 pairs of pulses (PP, first pulse, P1; second pulse P2) with 50ms inter-pulse interval (IPI) and 3s interval between pairs of pulses (IBP). The sets of 20 pairs of pulses were applied at different 8 current intensities (100, 200, 300, 400, 500, 600, 700, 800 μA) in a random order. Response amplitude at each current intensity was monitored and stimulating intensity was then set at the current eliciting half maximum response for other steps in the protocol. This was typically between 300-350 μA.

Step 4A/3B. Assessment of Short-term synaptic plasticity using Paired-Pulse Stimulation

Paired-pulse stimulation (PPS) protocol was also utilised to investigate ability of the vHipp-mPFC synaptic connections to support short-term synaptic plasticity. 20 PP (first pulse, P1; second pulse P2) were applied at 7 varying IPI (25, 50, 100, 150, 200, 250, 500, 1000 ms) and 3s IBP. The IPI to be applied was chosen randomly.

Step 5A/5B. Long-term Potentiation (LTP)

The ability of the vHipp-mPFC synaptic connections to support long-term synaptic plasticity (LTP) was assessed using a High-Frequency Stimulation (HFS) protocol. A baseline measure was recorded prior to the HFS, by applying a series of PPS (30 PP) at 50ms IPI (30s IBP) for 15min. A HFS (bursts of 20 pulses at 200 Hz, repeated 5 times with bursts separated by 2s) was then applied to the vHipp. This was followed by a series of PPS (60 PP for 30 mins in recording **protocol A** and 30 PP for 15 mins in recording **protocol B**) applied at 50 ms IPI (30s IBP) post-HFS, to assess the effects of HFS. Furthermore, step 4A/3B of the protocol was repeated to assess the short-term plasticity of the synapses post-HFS. In recording protocol (B), this step (5B) was recorded last.

Step 6A/4B. Low-Frequency Stimulation (LFS)

The ability of the synapses to reverse the LTP was assessed using a LFS protocol. The LFS consisted of a train of 900 PPS (IPI = 5 ms, IBP = 1s) delivered at 1Hz to the system. In recording protocol (**A**) the effect of repeated LFS was examined on previously potentiated vHipp-mPFC synapses. This stimulation protocol has been shown to depotentiate responses in previously potentiated synapses (Burette et al., 1997; **Chapter 1.10**). This was followed by a series of PPS (30 PP) at 50ms IPI (30s IBP) for 15min (Post-LFS) to assess the effects of LFS. Furthermore, step 4A/B3 of the protocol was repeated to assess the short-term plasticity of the synapses post-LFS. In recording protocol (**B**), this step was recorded prior to the LTP induction protocol. Therefore, in recording protocol (**B**), the effect of LFS was examined on previously non-potentiated synapses followed by HFS.

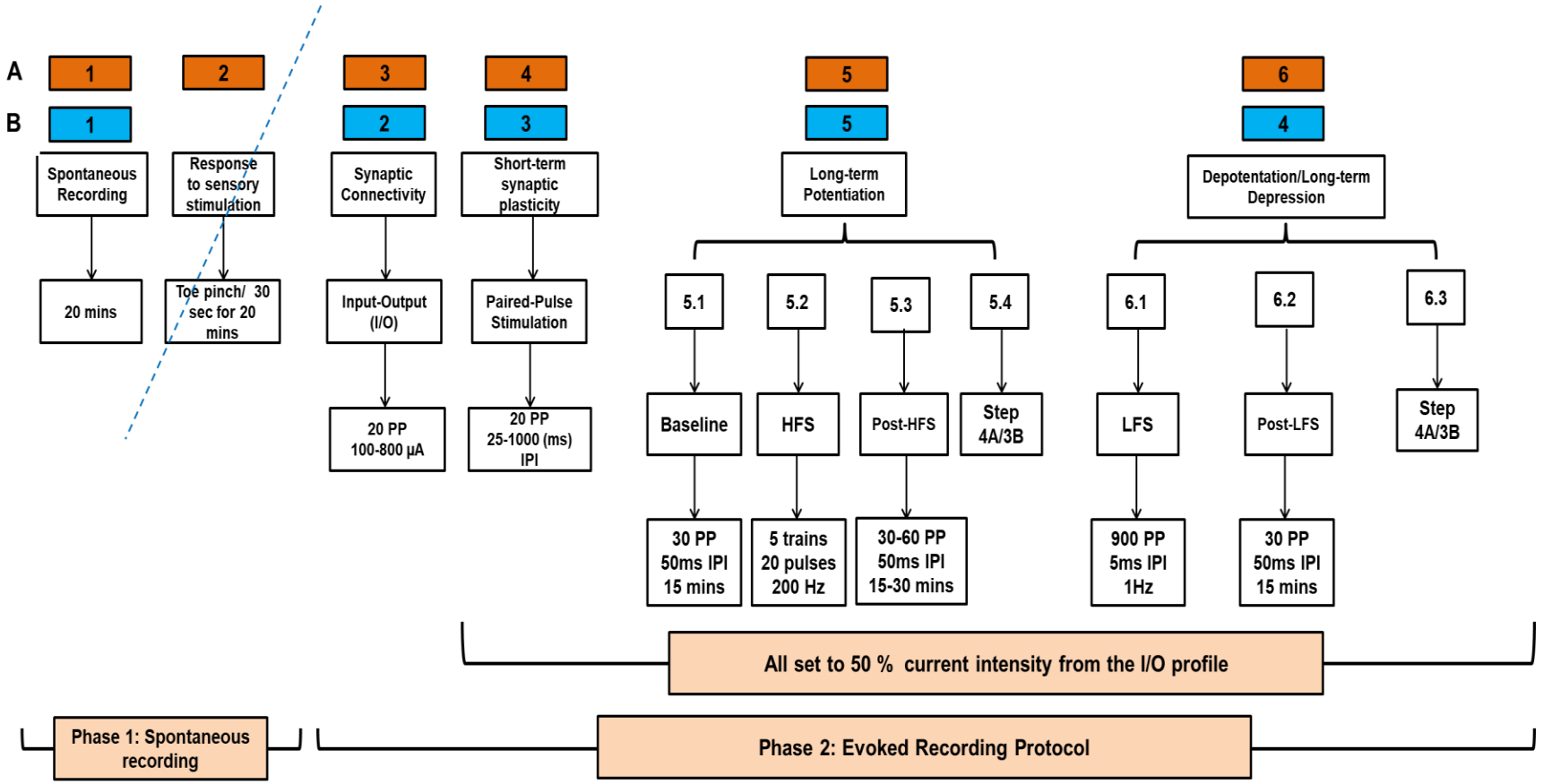

Figure 2.10. A schematic summary of the recording protocols. The orange numbered boxes represent the order of the recording steps in Recording Protocol (A). The Blue numbered boxes represent the order of recording steps in Recording Protocol (B). In protocol (B) activity in response to sensory stimulation (toe-pinch) was not recorded (indicated by the blue dotted line) and LFS was administered first followed by long-term potentiation protocol. PP: Paired-Pulses; IPI: Inter-pulse Interval; HFS: High Frequency Stimulation; LFS: Low Frequency Stimulation.

2.6.5. Statistical Analysis of Electrophysiology Data

For the purposes of this thesis, only the Stimulation Recording Data were analysed and reported. Data from all stimulation recording protocols were imported to data analysis software Spike 2 (Version 6.08, Cambridge Electronic Design LTD, UK). Using this software, the slope and the amplitude of the evoked response recorded from the mPFC were measured. For each animal, the channel with the highest slope facilitation (%) in the baseline PPS recording (at 50 ms IPI) (step 4A/3B) was selected and analysed for all the other recording steps. Slope was measured from the steepest segment of the fEPSP response. Amplitude of the response was measured as the distance between the peak (top of the upward segment of response) to the trough (**Figure 2.11**). All graphs were plotted in GraphPad Prism (ersion 8.0.2).

2.6.5.1. Analysis of I/O and PPS Data

Using the inbuilt *waveform average* function in Spike2, the 20 PP recorded during the I/O protocol were analysed to obtain an average response at each current intensity. Then the slope and the amplitude of the first pulse (P1) in the pair of pulses was measured from the average waveform. To analyse the I/O data, the amplitude and slope of P1 was then plotted against current intensity to obtain the I/O curve profile. Stronger current intensities were expected to evoke larger responses.

Similarly, 20 PP recorded at each IPI in the PPS protocol were analysed to obtain an average waveform shape. Then the slope of both P1 and Pulse 2 (P2) within the averaged waveform was measured (**Figure 2.11**). % Paired-Pulse index (PPI) was calculated using the formula below:

$$\%\ PPI = \left(\frac{(Slope\ 2 - Slope1)}{(Slope\ 1)} \right) x\ 100$$

A positive % PPI signified a steeper slope in P2 suggesting facilitation in the response while a negative % PPI signified a shallower slope in P2 and depression in the response in the paired-pulse protocol. The same formula was also used to calculate %PPI for amplitude measures. Prior to statistical analysis data were tested for normality. No transformations were required. Mixed-design two-way ANOVAs were used to analyse data obtained from the I/O (treatment and current intensity) and the PPS protocols (step 4A/3B) (treatment and IPI). Since within-subjects variables had more than 2 conditions, data were tested for equal variances using Mauchly's test of sphericity. Upon violation of this assumption, results were reported using the Greenhouse-Geisser corrections. In these studies, all within-subjects variables were reported using this correction. Between-subjects variables were tested for homogeneity of variance using Levene's test. Unless specified, results of between-subjects comparisons were reported uncorrected when the assumption of equal variances was not rejected. This was followed by planned Bonferroni paired-wise comparisons. Repeated measure two-way ANOVAs were also used to compare short-term synaptic plasticity across the three recorded conditions (baseline, post-HFS and post-LFS) within individual

treatment groups. Bonferroni pair-wise comparisons were used when appropriate. For all Bonferroni pair-wise comparisons, the significance threshold was kept at **α=0.05**. The *p*-values were adjusted by multiplying the unadjusted *p*-value (obtained from LSD comparisons) by the number of comparisons made. This is the method recommended by IBM SPSS Statistics Support (2016a) to adjust for multiple comparisons using Bonferroni corrections. Number of comparisons is defined in appropriate sections separately for each experimental chapter. All data were analysed using SPSS IBM (Version 23).

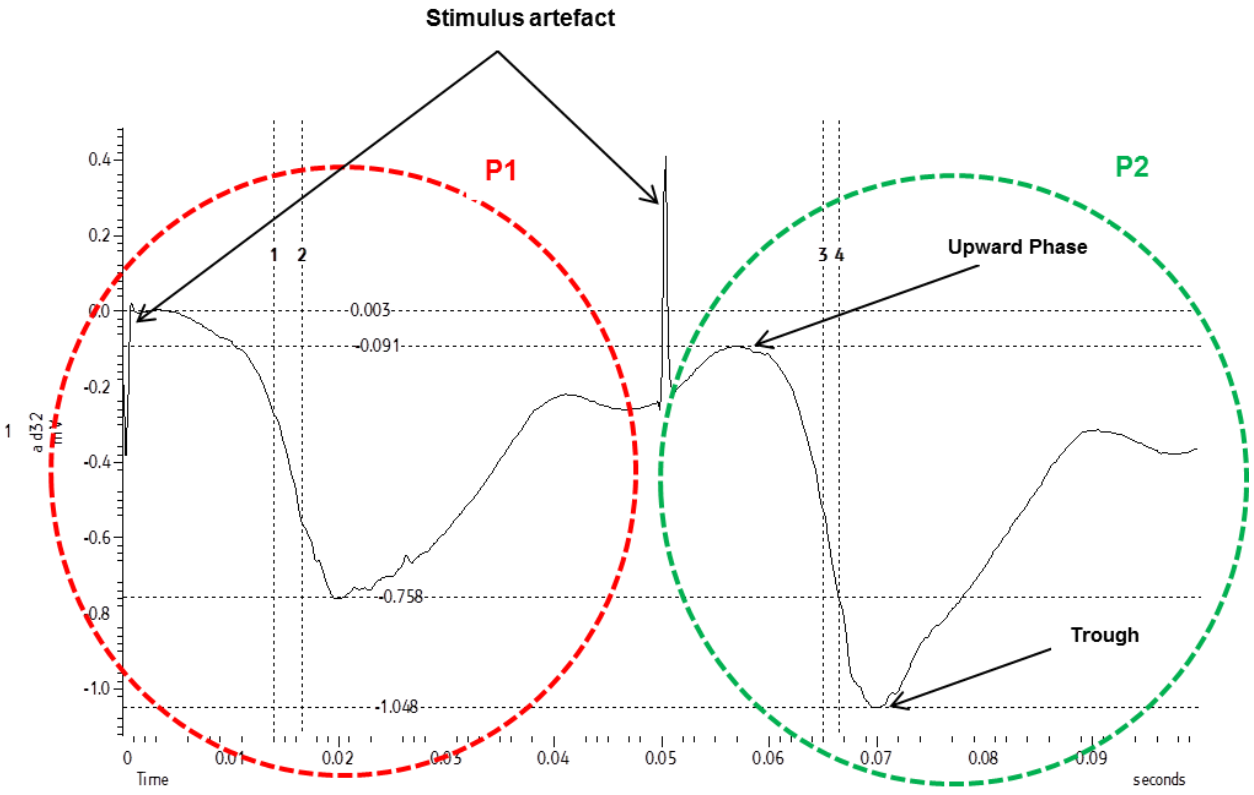

Figure 2.11. Analysis of the paired-pulse responses. Vertical cursors were placed on the steepest and most straight downward segment of the averaged response. The amplitude was measured by placing one horizontal cursor at the top of the upward phase of the response and another on the trough. For the I/O and baseline responses, only the slope and the amplitude of the response to the first pulse (P1, red circle) were measured. For the Paired-Pulses responses, slope of both P1 and P2 (green circle) were measured. Depicted trace is representative of traces obtained from the electrophysiological studies presented in this thesis.

2.6.5.2. Assessment of HFS and LFS effects

In order to assess the effects of HFS and LFS on fEPSP, the P1 slope and amplitude of responses recorded during the baseline measure (30 PP) were normalised to their average response value. Same parameters were measured for post-HFS and post-LFS responses. These responses were then expressed as a percentage change to the baseline. For these recordings, response slope was measured using the *W-FP* inbuilt script in spike2 while response amplitude was measured manually as described above (**Figure 2.11**). Prior to statistical analysis data were tested for normality. No transformations were required. Data were analysed using mixed-design two-way (treatment and stimulation pattern) ANOVAs to examine the main effects of treatment (between-subjects variable) and stimulation pattern (within-subjects variable) on response slope and amplitude. This was followed by *post-hoc* Bonferroni corrections. For all Bonferroni pair-wise comparisons, the significance threshold was kept at **α=0.05**. The *p*-values were adjusted by multiplying the unadjusted *p*-value (obtained from LSD comparison) by the number of comparisons made. This is the method recommended by IBM SPSS Statistics Support (2016a) to adjust for multiple comparisons using Bonferroni corrections. Number of comparisons is defined in appropriate sections separately for each experimental chapter. Since within-subjects variables had more than 2 conditions, data were tested for equal variances using Mauchly's test of sphericity. Upon violation of this assumption, results were reported using the Greenhouse-Geisser corrections. In these studies, all within-subjects variables were reported without this correction. Between-subjects variables were tested for homogeneity of variance using Levene's test. Results of between-subjects comparisons were also reported uncorrected for these measures. Tests of normality and homogeneity of variance were conducted in SPSS IBM (Version 23). However, the statistical analysis (mixed-design two-way ANOVA) was conducted in GraphPad Prism (Version 8.0.2). Unlike SPSS which processes missing values by list-wise deletion (resulting in reduced sample size) (IBM SPSS Statistics Support., 2016b), GraphPad Prism is better equipped to incorporate missing values into the analysis (GraphPad., 2009).

2.7. Transcardial Perfusion Technique

Animals in all studies reported here were subjected to transcardial perfusion as a final step. In the electrophysiology studies, rats were already anaesthetised with the non-recovery agent urethane for the purpose of the electrophysiology procedures, therefore, further anaesthetic was not required. In the other studies, anaesthesia was induced using isoflurane (5% flow rate in 2 l/min O_2) in an induction chamber. Concentration of the inhalation anaesthetic agent remained constant during the entire procedure.

Anaesthetised rats were transferred onto a down flow table. Blinking and withdrawal reflexes were tested to ensure sufficient depth of anaesthesia. First, the sternum was identified and an incision was made with sharp scissors to reveal the xiphoid cartilage. The incision was then extended laterally to expose the diaphragm. Next, the diaphragm was cut followed by cuts through the ribs on either side of the sternum to allow the ribcage to be retracted and clamped (using Kelly clamps) to expose the heart. Care was taken to

avoid damage to the lungs and other internal organs. The heart was secured with a pair of artery clamps. A small cut was then made in the left ventricle and a small gavage needle connected to a perfusion pump was inserted into the cut and advanced into the aorta. Once the needle was visualised from the aorta, it was pulled back and secured with the same pair of artery clamps. A small cut was then made in the right atrium to allow blood to flow out. The Pump (Watson Marlow, 3135) was set at 10-15 ml/min flow rate. In all studies, the animals were perfused with BPS 1x for 6 minutes or until the solution flowing out of the heart became clear and the liver became pale in colour. In electrophysiology studies only, the last step was followed by perfusion with paraformaldehyde (PFA 4%) for another 4-5 minutes in order to fix the brain tissue in the skull. After the end of perfusion, clamps and needles were removed, head separated from the body and brain extracted from the skull. **Figure 2.12** shows the difference between a perfused and non-perfused brain extracted from the skull. **Table 2.4** provides a summary of the culling procedure for each study.

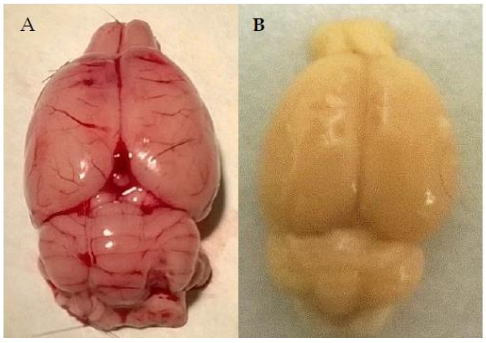

Figure 2.12. An example of a (A) Non-perfused and a (B) Perfused Brain. Image taken from William Watremez PhD Thesis (2016).

In studies using osmotic minipumps for drug delivery, blood samples (approximately 2 ml) were also collected from the heart prior to perfusion. Blood was collected using a 26 ¾ gauge needle and a 2.5 ml syringe pre-treated with sodium citrate (3.8% w/v) (0.1 ml of Sodium citrate for 1 ml of blood). Blood was then immediately stored in EDTA treated tubes. For blood plasma preparations, refer to **Section 2.8.2**.

2.8. Tissue Storage and Preparation

2.8.1. Brain Tissue Storage

After the end of perfusion, rat brains were processed and stored in a way most appropriate for the intended post-mortem analysis. In the electrophysiological studies, whole extracted brain was stored in PFA (4%) which was replaced by sucrose (30%) after 72h. The tissue remained in sucrose until it sank to the bottom of the container. The tissue was then sliced using freezing sledge microtome. In other studies, the brain was cut in half to separate the right and left hemispheres upon extraction. The right and left hemispheres were then prepared as follows:

- One hemisphere was rapidly frozen in chilled isopentane. The tissue was then stored at -80˚C.
- The other hemisphere was stored in PFA (4%) which was replaced by sucrose (30%) after 72h. The tissue remained in sucrose until it sank to the bottom of the container. Then the brain was rapidly frozen in chilled isopentane and stored in -80˚C.

A summary of this information for each study is provided in **Table 2.4**.

Study/ Chapter	Perfusion	Cardiac Blood	Brain Tissue/Blood Tissue
1/3	BPS (1x) Only	-	Right and Left hemispheres randomly assigned to: 1. Rapidly frozen in cilled Isopentane, stored in -80˚C 2. Post-Fixed in PFA (4%) which was replaced by sucrose (30%) after 72h. When sunk in sucrose, tissue was rapidly frozen in isopentane, stored in -80˚C
2/4	BPS (1x) Only	2 ml	Same as 1. Blood was centrifuged at x10^4 /min, for 10 minutes to collect plasma.
3/5	BPS (1x) and PFA (4%)	-	Whole brain stored in PFA (4%) which was replaced by sucrose (30%) after 72h. When sunk in sucrose, tissue was sliced (60 µm) and stained for electrode placement confirmation
4/6	BPS (1x) and PFA (4%)	2 ml	Same as 3. Prior to slicing, tissue was cut to separate right and left hemispheres. Left hemisphere was processed as 3. From the right hemisphere 1 in 4 serial sections (30 µm) were collected from mPFC, dHipp and vHipp for parvalbumin-immunohistochemistry. Blood samples were processed as defined in 2.

Table 2.4. A Summary of culling procedure and tissue preparation and storage for each study

2.8.2. Blood sample storage/Analysis

Collected Blood samples were centrifuged at x10 ^{4} /min for 10 minutes (Eppendorf centrifuge 5415c). This is the standard operating procedure in our laboratory to collect plasma. The plasma samples were then stored at -80˚C. Samples from study 2 (chapter 4) were processed to determine plasma drug concentration. This analysis was performed by Cyprotex Discovery Limited, an external company, using a standardised HPLC protocol. Based on the protocol provided by the company, plasma samples were treated with organic solvent (Methanol or acetonitrile) and following protein precipitation samples were either centrifuged (30 mins, 4˚C, 5x10^3) or filtered through a 96-well precipitation plate under positive pressure. The resulting supernatant/filtrate was further diluted in HPLC-grade water and analysed by LC-MS/MS. The concentration of the drug was measured from a standardised calibration curve.

2.8.3. Histology

When sunk in sucrose, the brain tissues collected from the electrophysiological studies were sliced using a frozen-sledge microtome (SM24000 Leica microsystems, Germany) (**Figure 2.13**). Coronal sections (60 µm thick) were obtained from the mPFC and vHipp, where recording and stimulating electrode were inserted respectively. Slices were first immersed in PBS, then mounted on Superfrost Plus slides (Thermo Fischer Scientific, Menzel-Gläzer Superfrost Plus, Germany) and then covered to protect from light and left to dry overnight. Then the tissue was stained with nuclear dye 4′,6-Diamidine-2′-phenylindole dihydrochloride (DAPI) (Invitrogen, Life technologies corporation, USA) and left to dry overnight in the dark. DAPI was used to allow for rough identification of the tissue cytoarchitecture. Furthermore, the specific

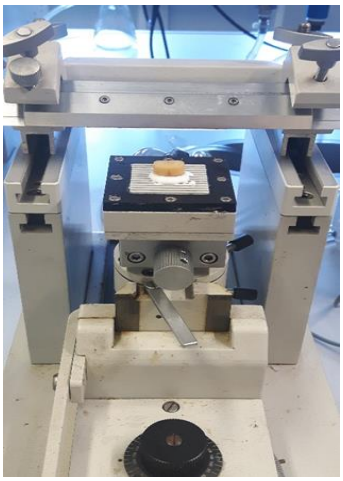

Figure 2.13. Typical tissue set- up on microtome

formulation of this dye (Gold antifade reagent) prolonged the life of the florescent dye present in the mPFC sections. The slides were processed through a slide scanner (Laser 2000, UK) and the images were processed in Case Viewer (3DHISTECH, Hungary). The location of the recording electrode was identified by traces of the Dil-fluorescent dye. The location of the stimulating electrode site could be identified by the tissue damage caused by the electrode's thickness. Processed images were then compared against the Paxinos and Watson (2007) rat brain atlas to confirm whether the electrodes had reached their targets (**Figure 2.14 A1 and B1**). Animals were excluded if either electrode failed to hit the target region (**Figure 2.14 A2 and B2**) In the study presented in **Chapter 6**, the sucrose infused tissue was cut and the hemispheres were separated prior to slicing. The left hemisphere was processed as explained above for electrode placement confirmation. 1 in 4 serial coronal sections (30 µm thick) were obtained from the mPFC, dorsal hippocampus (dHipp) and vHipp from the right hemisphere. The sections were stored in cryoprotectant at -20˚C to be processed for parvalbumin immunostaining. Due to time constraints, parvalbumin immunostaining was not completed as part of this thesis.

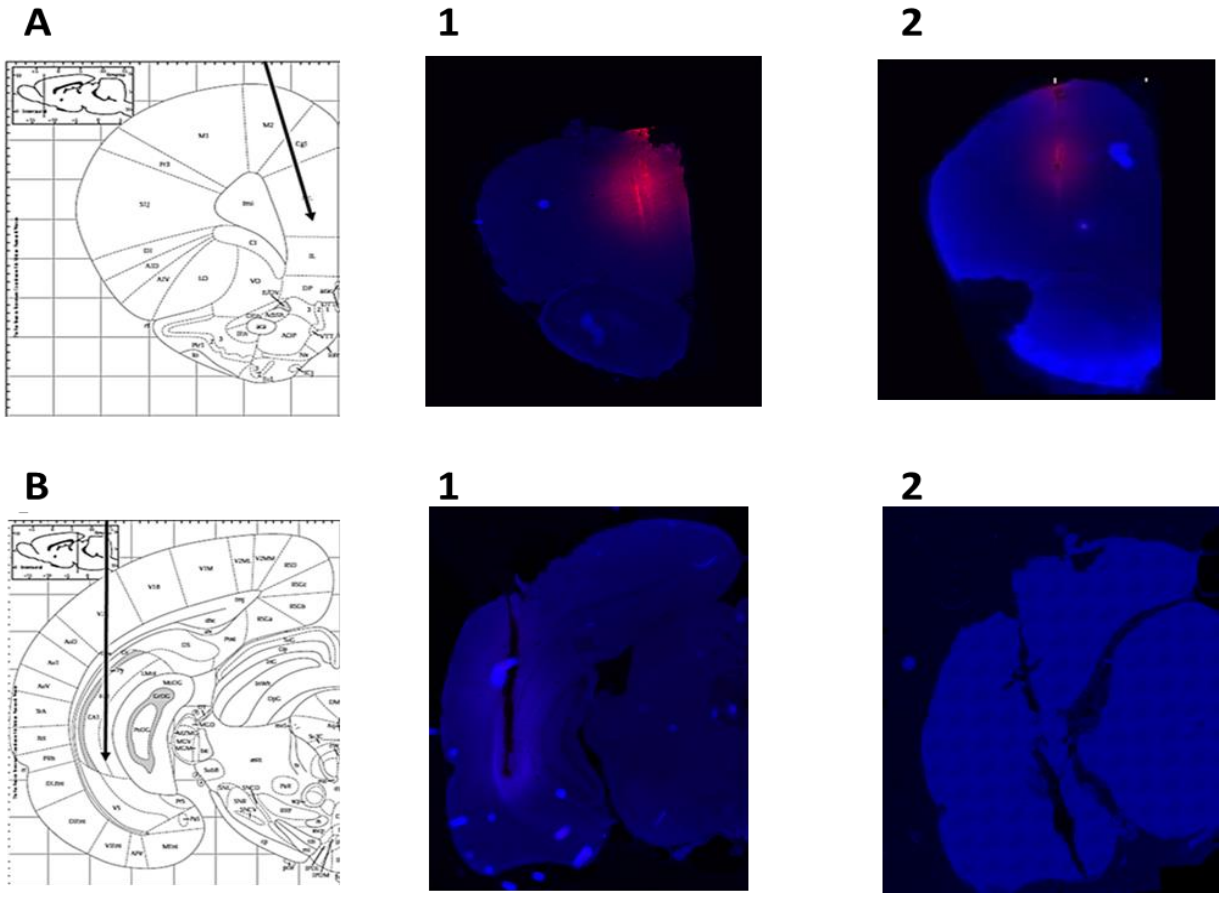

Figure 2.14. Histology for electrode placement confirmation. (A) mPFC recording site in left hemisphere. The arrow tip shows the ideal final position of the electrode in the Prl cortex (A1) Tissue analysis confirming the ideal recording electrode placement in the mPFC (A2) Electrode target missed in the mPFC (B) vHipp stimulation site in left hemisphere. The arrow tip shows the ideal final position of the electrode in ventral CA1. (B1) tissue analysis confirming the ideal stimulating electrode placement in the vHipp (B2) Electrode target missed in the vHipp.

Chapter 3

Study 1-An investigation into the neurocognitive effect of haloperidol and olanzapine upon 22 days of treatment

3.1. Introduction

In patients with schizophrenia, impairments in cognitive function have been established as an important symptom domain (Kalkstein et al., 2010; Millan et al., 2012). Moderate to severe impairments across higher-order cognitive processes including executive function, episodic memory and visual learning and memory have been well-documented in patients with schizophrenia (Keefe et al., 2007a; Keefe and Fenton, 2007; Keefe and Harvey, 2012; Han et al., 2015). These impairments which are present before disease onset (Bora, 2015) and in first episode psychosis (FEP) (Bora and Murray, 2014), persist at chronic stages of the disease and throughout the life of patients (Addington et al., 2005; Hoff et al., 2005; Bozikas and Andreou, 2011; Irani et al., 2011; Hill et al., 2010; Bora, 2015). Symptoms of cognitive impairments have long-term negative effects on patients' functional outcome (discussed in **Chapter 1.2.1**) and quality of life (Green et al., 2000; Green et al., 2004; Green et al., 2015; Schmidt et al., 2011).

Antipsychotic (AP) drugs are the first line of treatment for management of schizophrenia. Current guidelines recommend patients to remain on AP treatment for long-term (Moncrieff, 2015; NICE Guidelines), however, the long-term neurocognitive effects of these treatments remains controversial (Keefe and Harvey, 2012). Short-term clinical trials in FEP patients show a significant but small improvement in cognitive performance upon treatment with both typical and atypical APs (Keefe et al., 2004; Harvey et al., 2005; Keefe et al., 2006; Keefe et al., 2007b; Crespo-Facorro et al., 2009). Keefe and colleagues (2004), showed a significant improvement in cognitive performance on tests of vigilance, attention, verbal fluency and working memory upon 3 months of treatment with low-dose haloperidol (5.54 mg/day) and olanzapine (10 mg/day). Performance in these cognitive domains and the weighted composite scores (calculated from scores of each test) was significantly higher in olanzapine treatment group, suggesting that overall, olanzapine may have been more beneficial that haloperidol treatment. In the same sample, similar results emerged at the 6 month follow-up when comparing the weighted composite scores. Interestingly, there was no significant difference in performance in individual cognitive domains or in weighted composite scores between the treatment groups at 1 year and 2 year follow-up (Keefe et al., 2006). These findings were confirmed in an independent cohort at 1 year (Crespo-Facorro et al., 2009) and 3 year (Ayesa-Arriola et al., 2013) follow-up time points.

Cognitive effects of treatment with risperidone and haloperidol follow a similar pattern. Three months of treatment with risperidone (3.3 mg/day) and haloperidol (2.9 mg/day) significantly improved cognitive performance in tasks of episodic memory, vigilance, attention and visuospatial processing. However, risperidone was significantly more effective in improving performance on tasks of executive function and verbal fluency (Harvey et al., 2005). While maintaining the finding of significant improvement in cognitive performance, longer-term studies do not show a difference between these treatment groups at the 1 year (Crespo-Facorro et al., 2009) and 3 year (Ayesa-Arriola et al., 2013) follow-up time points.

Early clinical trials in chronically ill patients provided reports of the ineffectiveness and in some cases deleterious effects of typical antipsychotics (APs) on cognitive performance while pointing to the superiority of atypical agents in improving cognition (Buchanan et al., 1994; Gallhofer et al., 1996; Keefe et al., 1999; Purdon et al., 2001; Bilder et al., 2002). In most of these studies (described in detail **in Chapter 1.7.1 and Table 1.1**), high doses of haloperidol were used as a comparator to other atypical APs. It is well-known that at high doses, haloperidol can interfere with cognitive performance due to its deleterious side-effects (Keefe et al., 2004; Woodward et al., 2007). Methodologically modified clinical trials in chronically ill patients point towards findings similar to FEP (Green et al., 2002; Han et al., 2015). Perhaps the best example of such studies is the US Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE). These randomised trials included chronically ill patients with an average of 14 years previous AP treatment. Findings of these trials also suggested a small but significant improvement in the cognitive performance of patients treated with typical and atypical APs, with no significant difference between treatment groups (Keefe et al., 2007a; Keefe et al., 2007b). Generally, findings of these studies have been supported by meta-analyses suggesting an improvement in cognitive performance with both typical and atypical APs (Woodward et al., 2005; Desamericq et al., 2014).

Results of these studies should, however, be interpreted with caution. The neurocognitive improvements reported in these studies are associated with small effect sizes and can, to some extent, be explained by other factors. For instance, evidence suggests that the neurocognitive improvements observed with AP treatments are partly explained by changes in clinical symptoms in studies of both FEP and chronic patients (Keefe et al., 2004; Harvey et al., 2005; Keefe et al., 2006; Keefe et al., 2007b; Keefe and Harvey, 2012). Furthermore, in studies including healthy participants as controls, the magnitude of improvement in cognitive performance of patients on atypical AP are comparable to that of healthy participants, highlighting the influence of practice effect on the outcome (Crespo-Facorro et al., 2009; Ayesa-Arriola et al., 2013). It has also been suggested that haloperidol (independent of dose) might impede practice effect (Woodward et al., 2007), hence appear less effective in improving cognitive performance. This might explain the apparent superiority of atypical AP in studies with short follow-up, which is eliminated when the test-retest interval is prolonged. In addition, factors such as duration of un-treated psychosis (Chang et al., 2013), hospitalisation period, poly-pharmacy (Hori et al., 2006), adjunct treatment with anticholinergic drugs (Eum et al., 2017) and treatment discontinuation confound interpretation of these findings, leaving the effect of long-term AP treatment on cognition controversial (Keefe and Harvey, 2012; Han et al., 2015). Most importantly, it remains to be elucidated whether these small neurocognitive improvements confer to improve functional outcome.

Given that patients tend to remain on APs for life, the duration of AP treatment in studies reported above are relatively short and may not be representative of chronic treatment. While randomised clinical trials are well equipped for examining short-term treatment efficacy and its side effects, they are not feasible for studies of long-term treatment effects (Young et al., 2015). Naturalistic studies provide a more realistic

setting for studying the influence of long-term AP treatment on neurocognitive functioning (Young et al., 2015). Recent naturalistic studies have shown that higher life-time accumulative dose of AP was significantly negatively associated with scores of global cognitive performance in chronically ill patients (mean illness duration of 16.5 years) (Husa et al., 2017). Specifically, higher life-time accumulative dose of AP was associated with poorer baseline performance in tasks of verbal learning and memory. Performance in this domain of cognition had significantly declined at the 9-year follow-up as a function of higher accumulative AP exposure (Husa et al., 2014). Furthermore, an association between AP treatment and brain volume loss has also been established (Ho et al., 2011; Fusar-Poli et al., 2013). It is thought that this volume loss may be associated with cognitive decline in patients with schizophrenia (Pol and Kahn, 2008; Andreasen et al., 2011; Dempster et al., 2017). Given the importance of cognitive function in day to day life and functional outcome, understanding the influence of long-term treatment with APs is essential.

In pre-clinical settings, studies of acute AP treatment outnumber those investigating the long-term neurocognitive effects of these compounds. Collectively, studies of acute AP treatment in animal models of the disease support the superiority of atypical over typical APs (Grayson et al., 2007; Idris et al., 2010; Snigdha et al., 2010; Snigdha et al., 2011a; Cadinu et al., 2017). Studies with longer treatment duration consistently report the ineffectiveness of haloperidol in rescuing manipulation-induced cognitive deficits in animal models of the disease. For instance, 14 days of treatment with haloperidol (0.1 mg/kg/day; intraperitoneal injections; i.p.) failed to rescue deficit in the disrupted novel object recognition (dNOR) task induced by 10 days of intermittent PCP treatment (10 mg/kg/day; 10 days; subcutaneous injections (s.c.), 3 days of washout) in mice (Hashimoto et al., 2005). A similar dose and treatment duration with haloperidol was also found to be ineffective in rescuing NOR deficit in adult offspring of mice treated with poly (I:C) (Ozawa et al., 2006). Similarly, 14 days of treatment with a higher dose of haloperidol (1 mg/kg/day; i.p.) also failed to rescue the sub-chronic MK-801-induced (0.3 mg/kg/day for 5 days; i.p., no washout) deficit in the NOR task while significantly impairing performance in healthy mice (Ozdemir et al., 2012). A similar dose and treatment duration with haloperidol was reported to be ineffective in rescuing NOR deficit in DISC1 mice (mutation in Disrupted In Schizophrenia 1 gene) treated with poly (I:C) (dual hit model) (Nagai et al., 2011). Only a few studies have investigated the long-term effects of olanzapine on cognition. In one study, 21 days of treatment with olanzapine (3 mg/kg/bidaily; oral administration; p.o.) in adult male Long Evans rats was also ineffective in rescuing sub-chronic pencyclidine (scPCP; 5mg/kg/bidaily for 7 days; i.p.; 10 days WO) induced deficit in attentional set-shifting paradigm (Rodefer et al., 2008). In contrast 28 days of treatment with a lower dose of olanzapine (1.5 mg/Kg/day; i.p.) rescued the scPCP-induced (2 mg/kg/ bidaily for 7 days; i.p.; 7 days of WO) deficit in a reversal learning operant task in adult female Lister Hooded rats (McLean et al., 2010b). Differences in these findings could be due to variations in scPCP dosing protocol, sex and strain differences as well as differences in tested behavioural paradigms.

A number of studies have also investigated the neurocognitive effects of long-term treatment with APs in physiologically healthy animal. For instance,14 days of treatment with olanzapine (2.5, 5, 10 mg/kg/day; i.p.) was found not to impair performance on the radial arm maze, a working memory test, in healthy adult male Wistar rat (Ortega-Alvaro et al., 2006). Similarly, 45 days of treatment with haloperidol (2 mg/kg/day; through drinking water), olanzapine (10 mg/kg/day; administered via drinking water) and ziprasidone (12 mg/kg/day; administered via drinking water) did not have a deleterious effect on performance in the Morris water maze, a test of spatial learning and working memory, in healthy adult male Wistar rats when tested after 7 days of washout (Terry et al., 2002; Terry et al., 2006). This is while 90 days of treatment with the same compounds significantly impaired spatial learning and memory (Terry et al., 2002; Terry et al., 2006). It is noteworthy that the studies reported here are not exhaustive. Most of these studies have also reported effects of other APs. However, given the focus of this chapter on haloperidol and olanzapine, only relevant results were reported. A detailed summary of these studies is reported in **Chapter 1.7.2** and **Table 1.2.**

Assuming that each rat month equates to 3 human years (Sengupta, 2013), findings of the studies reported above (ranging from approximately 1.5 to 3 human years) are comparable to those of clinical trials with similar length and point towards poor treatment efficacy. Only two studies reported by Terry and colleagues (2002; 2003) have investigated the neurocognitive effects of APs beyond 3 years (45 to 90 rat days equating to approximately 4.5 to 9 human years), the results of which are comparable to the naturalistic studies reported above. However, their translational validity of the findings of Terry and colleagues (2002; 2006) are limited since studies were conducted in physiologically healthy animals. Using clinically incomparable doses of AP medication and lack of appropriate control groups are common limitations of the methodology of pre-clinical studies reported here. Therefore, findings of studies conducted in animal models are also poorly translated into clinical practice.

This study aims to bridge this translational gap by addressing some of these methodological issues. To that effect, the influence of 22 days (22 rodent days equates to approximately 2.5 human years) of treatment with haloperidol and olanzapine on cognition was investigated in the scPCP model of cognitive impairments in schizophrenia. The choice of treatment duration (22 days) in the study reported here was based on the lack of evidence on the neurocognitive effects of prolonged treatment with haloperidol and its comparison to olanzapine in a well-validated model. Previous research in our laboratory (Abdul-Monim et al., 2006; Abdul-Monim et al., 2007; Grayson et al., 2007; Neill et al., 2010; McLean et al., 2011; Snigdha et al., 2011a; Neill et al., 2014; Cadinu et al., 2017) and others (Egerton et al., 2008; Rodefer et al., 2008; McKibben et al., 2010; McAllister et al., 2015) has shown that repeated treatment with PCP induces a range of cognitive deficits and neurobiological abnormalities in rats (Cochran et al., 2003; Abdul-Monim et al., 2007; Jenkins et al., 2010) which are comparable to those seen in patients with schizophrenia (Lewis et al., 2012) (see **Chapter 1.5** for review). Therefore, this model is increasingly used as a valuable tool to investigate disease mechanisms and drug development. Here, haloperidol was chosen as it is the drug against which most other APs are compared in clinical trials (Keefe et al., 2004; Keefe et al., 2006).

Olanzapine was chosen as it has the lowest discontinuation rate in clinical practice (Lieberman et al., 2005; Lewis and Lieberman, 2008; Zhang et al., 2013).

In this study, performance of the animals was tested using the dNOR paradigm. The dNOR paradigm is amongst the behavioural tests most commonly used in pre-clinical research (Grayson et al., 2015b) and is identified as a standardised test of visual learning and memory by the MATRICS consortium (Measurements and Treatment Research to Improve Cognition in Schizophrenia) (Young et al., 2009). By extension, this test can provide information about elements of episodic memory as it relies on the ability to identify previously encountered elements or episodes (Morici et al., 2015). These cognitive domains are amongst those severely disrupted in patients with schizophrenia, hence the use of the NOR task in this study is justified.

Throughout this study, the inter-trial interval (ITI; the delay between the acquisition and the retention phase of the dNOR paradigm) was kept at 1 minute. Findings from our laboratory (Grayson et al., 2007; Snigdha et al., 2011a; Grayson et al., 2014) and others (Mclean et al., 2017) have consistently reported a significant deficit in dNOR performance in scPCP (same dosing regimen used in the studies presented in this thesis. See **Section 3.2.2** and **Chapter 2.3**) treated rats at short ITIs (10s, 1 minute and 10 minute). This deficit in dNOR performance can in part be explained by the susceptibility of the scPCP treated rats to distraction at short ITI (Grayson et al., 2014; Mclean et al., 2017), which is in line with clinical observations of effects of distraction on cognitive performance of patients with schizophrenia (Cellard et al., 2007; Anticevic et al., 2011)

The critical role of the perirhinal cortex in NOR performance was discussed in depth in **Section 1.3.1.** Several lines of evidence suggest that the medial prefrontal cortex (mPFC) may also be involved in object recognition memory (Asif-Malik et al., 2017; Mclean et al., 2017) (see **Section 1.3.1** for a detailed discussion). Indeed, PFC dysfunction, including reduced dopamine transmission in this region, is thought to underlie the cognitive impairments associated with schizophrenia (Goldman-Rakic et al., 2004; Rao et al., 2018). In particular, dopamine release during the delay period of tasks of working memory is essential for task-goal maintenance and sustained attention (Goldman-Rakic et al., 2004). It is thought that reduced dopamine release might be associated with increased susceptibility to distraction (Durstewitz et al., 2000). Reduced dopamine transmission is a hallmark of scPCP treatment (Jentsch et al., 1997). A recent microdialysis study revealed a significant increase in mPFC dopamine concentration in control rats during the retention phase of the dNOR task following a short (10 min) ITI. This effect, which was absent in the scPCP treated animals, supports the role of mPFC dysfunction, dopamine dysregulation and susceptibility to distraction (key aspects of schizophrenia symptomatology) at short ITIs (Mclean et al., 2017). As such, the decision to select a short ITI (1 minute) is justified.

3.2. Materials and Methods

3.2.1. Animals

A total of 60 adult female Lister Hooded rats (Charles Rivers, UK) with starting weight of 190-230 g were used in this experiment. For details on housing conditions and food and water availability, refer to **Chapter 2.1.**

3.2.2. Drug Administration

3.2.2.1. Sub-Chronic PCP/Vehicle Treatment

Rats were randomised to receive vehicle (0.9% Saline; i.p., n=15) or PCP (2 mg/kg; i.p., n=45) twice a day for 7 days. This was followed by a 7-day washout (WO) period. For more detail on this treatment protocol, refer to **Chapter 2.3.**

3.2.2.2. Chronic Treatment with Antipsychotic Drugs

Treatment with AP agents started on the last day of WO from scPCP/scVeh treatment. Accordingly, scPCP treated rats were further randomised to receive vehicle (Veh, 0.9% Saline; p.o., scPCP-control n=15), haloperidol (Hal, 0.1 mg/kg; p.o., scPCP-Hal n=15) or olanzapine (Olz, 1.5 mg/kg; p.o., scPCP-Olz n=15) for 22 days. Sub-chronic Vehicle (scVeh) treated rats further received vehicle treatment for 22 days (p.o., scVeh-control n=15). Please note that vehicle solution was administered orally to keep treatment conditions consistent for rats in all treatment groups. For details of drug preparation, refer to **Chapter 2.2.**

3.3. Experimental design

Throughout this study, cognitive performance of the animals was examined at different time points using the dNOR test. This test was carried out as described in detail in **Chapter 2.4.1** and formed the main experimental outcome of this study. Throughout this study, the ITI (the delay between the acquisition phase and the retention phase of the dNOR) was kept at 1 minute (refer to the introduction of this chapter for more detail on the choice of this ITI). The first dNOR test was conducted on the first day of treatment with Hal and Olz (dNOR-1). The dNOR was then repeated every week on day 8 (dNOR-8), 15 (dNOR-15) and 22 (last day, dNOR-22) of treatment. These dNOR testing sessions were conducted 90 minutes after treatment administration. At chosen doses, this pre-treatment time is associated with no sedation and falls within the boundaries of drug half-life (1.5h for Hal and 2.5h for Olz). On the last day of treatment with APs, 5 animals per treatment group were randomly sacrificed (endpoint 1) while the remaining animals (10 per treatment group) underwent a 7-day WO period. The performance of the remaining animals was then tested in the dNOR on day 1 (dNOR-WO-1) and day 7 (dNOR-WO-1) of the WO period. On the last day of the WO, a second cohort of rats (5 animals per treatment group) was sacrificed (endpoint 2). At both study endpoints,

animals were sacrificed by transcardial perfusion as described in **Chapter 2.7.** Collected brain tissue was prepared and stored as described in **Chapter 2.8.** The choice of sample size (n=5 from each treatment group at each designated endpoint) for post-mortem tissue analysis was based on previous pilot investigations in our laboratory, indicating that a sample size of 4-5 per treatment group was sufficient for achieving a high statistical power (80%). The remaining 20 animals (tissue not needed), were sacrificed by an overdose of CO_2. **Figure 3.1** depicts a summary of the experimental design.

3.3.1 Exclusion Criteria

At all tested time points, behavioural trials were excluded from analysis if animals explored objects for one or less than one second or jumped onto the edge of the NOR box. Based on these criteria, 7 rats were excluded from dNOR-1, 8 were excluded from dNOR-8 and 7 excluded from dNOR-22. On day 15 of treatment, 7 animals from scPCP-control, scPCP-Hal and scPCP-Olz treatment groups were used in a different study for another experimenter. From the total number of animals remained at this time point (n=39), another 11 rats were excluded based on the described exclusion criteria. In total, therefore, 32 rats were excluded from analysis at dNOR-15. As stated in the previous section, 5 rats per treatment group were culled prior to dNOR-WO-1 (endpoint 1). From remaining animals (n=10/treatment group), 7 rats were excluded from dNOR-WO-1 and 9 from dNOR-WO-2. **Table 3.1** provides a detailed summary of the number of rats excluded per NOR testing session throughout this study.

NOR testing session	Treatment				Total Excluded
	scVeh-control (n=15)	scPCP-control (n=15)	scPCP-Hal (n=15)	scPCP-Olz (n=15)	
dNOR-1	2	1	1	3	7
dNOR-8	2	2	2	2	8
dNOR-15	5	8	8	11	32
dNOR-22	2	1	2	2	7
	scVeh-control (n=10)	scPCP-control (n=10)	scPCP-Hal (n=10)	scPCP-Olz (n=10)	
dNOR-WO-1	2	2	2	1	7
dNOR-WO-7	2	2	3	2	9

Table 3.1. Summary of the number of rats excluded per NOR testing session in Chapter 3. In the first 4 NOR testing sessions (throughout long-term AP treatment), each treatment group included 15 rats. Therefore, the number of rats excluded and presented here are out of 15/treatment group. After dNOR-22, 5 rats per treatment group were sacrificed (end point 1). Therefore the number of rats excluded and presented here are out of 10/treatment group.

3.4. Statistical Analyses

Object exploration times in the acquisition and the retention phase were analysed using mixed-design two-way (task and treatment) ANOVAs to determine the main effect of task (exploring left/right identical object in acquisition phase and novel/familiar in retention phase; within-subjects variable) and treatment (between-subjects variable). Paired Student's t-tests (comparison between novel vs. familiar object exploration time within each treatment group) and planned Bonferroni pair-wise comparisons (scPCP-control vs. scVeh-control; scPCP-control vs. scPCP-Hal; scPCP-control vs. scPCP-Olz; scPCP-Hal vs. scPCP-Olz; to compare total object exploration times between defined treatment groups in each phase) tests were conducted when appropriate. Discrimination index (DI) and locomotor activity (LMA) data were analysed using a one-way ANOVA, followed by planned Bonferroni pair-wise comparisons (scPCP-control vs. scVeh-control; scPCP-control vs. scPCP-Hal; scPCP-control vs. scPCP-Olz; scPCP-Hal vs. scPCP-Olz). DI data were further compared against zero using Mann-Whitney U test. For Bonferroni pair-wise comparisons, the significance threshold (α value) was set at **0.05**. Adjusted *p*-values were calculated by multiplying the uncorrected *p*-values (obtained from LSD planned comparisons) by the number of comparisons made. In case of the study presented in this chapter the unadjusted *p*-values were multiplied by 4, which is the number of defined planned comparisons. This is the method recommended by IBM SPSS Statistics Support (2016a) to adjust for multiple comparisons using Bonferroni corrections. The same format of analysis was repeated for all tested time points. All Data are presented as Mean ± SEM. All statistical analyses were conducted using IBM SPSS (Version 23). For details on tests of normality and homogeneity of variance, refer to **Chapter 2.4.1.5**.

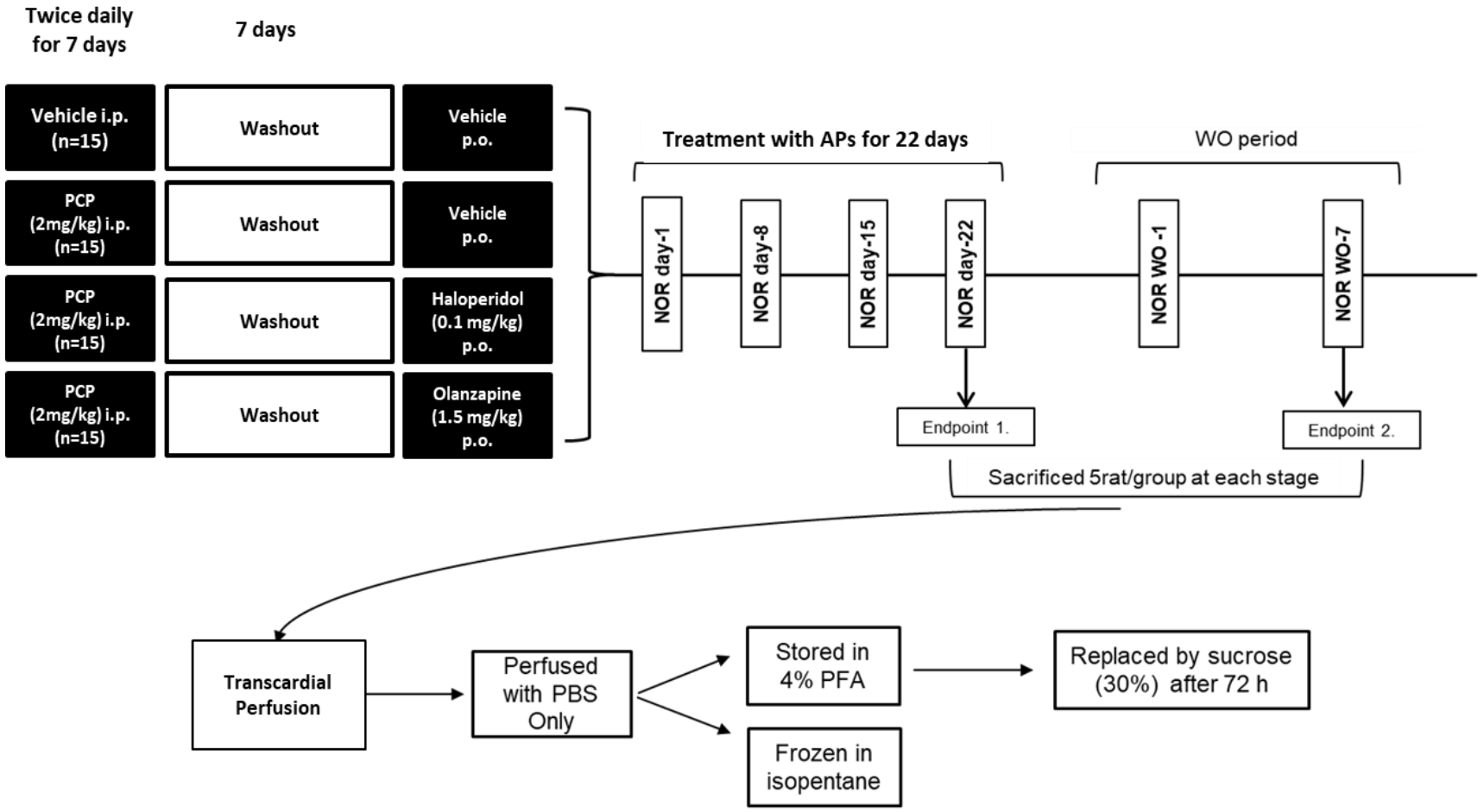

Figure 3.1. A Schematic summary of the experimental design, tissue preparation and storage for chapter 3. scPCP/scVeh treated animals were treated with Hal (0.1 mg/kg), Olz (1.5 mg/kg) or vehicle for 22 days followed by a 7-day WO period. Cognitive performance was tested at 6 times points using the dNOR test. 5 rats per treatment group were sacrificed at the two designated experimental endpoints and tissue was prepared as previously described. Remaining 20 animals (no tissue needed), were sacrificed by an overdose of CO_2.

3.5. Results

3.5.1. NOR performance on first day of treatment (dNOR-1)

3.5.1.1. Acquisition Phase

Rats in all treatment groups spent similar time exploring the left/right identical objects ($F_{1, 49}$= 0.002, p=0.96) (**Figure 3.2 a**). Treatment had no significant effect on the total object exploration time ($F_{3,49}$ =1.95, p=0.13). Planned Bonferroni analysis also did not detect any significant pair-wise differences in total object exploration times between the treatment groups (**Table 3.2**). There was also no significant task x treatment interaction ($F_{3,49}$ =0.75, p=0.52) on total object exploration time.

3.5.1.2. Retention Phase

Across all treatment groups, rats explored the novel object significantly more than the familiar object ($F_{1,49}$= 95.50, p<0.0001). Paired Student's t-test *post-hoc* analysis within individual treatment groups showed that rats in the scVeh-control (t_{12} = 4.71, p<0.001), scPCP-control (t_{13} = 10.30, p<0.0001), scPCP-Hal (t_{13} = 5.02, p<0.0001) and scPCP-Olz (t_{11} = 2.67, p<0.05) explored the novel object significantly more than the familiar object (**Figure 3.2 b**). There was no significant main effect of treatment ($F_{3,49}$ = 1.55, p=0.21) on total object exploration time. Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total object exploration time between the treatment groups in the retention phase (**Table 3.2**). There was also no significant task x treatment interaction ($F_{3,49}$ = 2.32, p=0.08) on total object exploration time.

3.5.1.3. Discrimination Index

Results of a one-way ANOVA revealed no significant effect of treatment on the DI ($F_{3,49}$ =2.37, p=0.08). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the DI between the treatment groups. Results of the Mann-Whitney U test revealed that the DI for the scVeh-control (U = 0, p<0.0001), scPCP-control (U = 0, p<0.0001), scPCP-Hal (U = 15, p<0.0001) and scPCP-Olz (U = 30, p<0.01) treatment groups was significantly superior to zero (**Figure 3.2 c**). The results of the Mann-Whitney U test reflect the findings of object exploration time in the retention phase.

3.5.1.4. Locomotor Activity

Results of the one-way ANOVA showed a significant effect of treatment on the number of line crossings ($F_{3,49}$ = 3.76, p<0.05). Planned Bonferroni comparisons showed that rats in the scPCP-control treatment group were significantly less active than rats in the scVeh-control treatment group (p=0.028). There were no other significant pair-wise differences in the LMA between treatment groups (**Figure 3.2 d**).

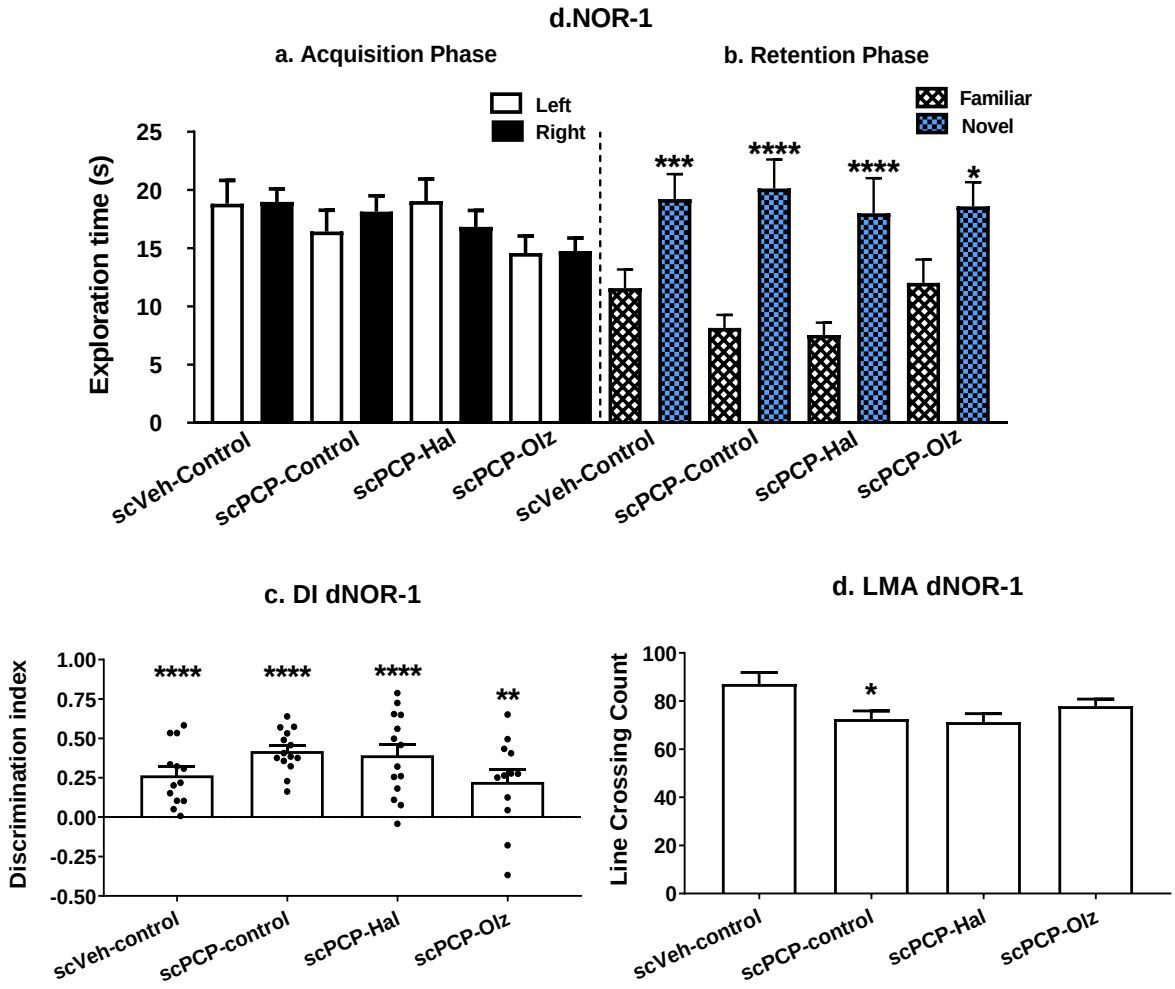

Figure 3.2. NOR performance on the first day of treatment (dNOR-1) (a) Rats in all treatment groups spent similar time exploring the right and the left object in the acquisition phase, while (b) exploring the novel object significantly more than familiar in the retention phase, **p*<0.05, ****p*<0.001, *****p*<0.0001 (c) Ratio of novel to total object exploration time. No significant pair-wise differences were detected in the DI between the treatment groups. ***p*<0.001, *****p*<0.0001 vs. Zero. Each dot represents the DI score for 1 rat (d) Number of line crossings representing the locomotor activity during both the acquisition and the retention phase of the NOR, **p*<0.05, vs. scVeh-control. No other significant pair-wise differences were detected in the LMA between the treatment groups. Data are presented as Mean ± SEM, (n=12-14/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVeh-control (n=13)	37.79 ± 2.2	30.79 ± 3.3
scPCP-control (n=14)	34.58 ± 2.4	28.26 ± 3.3
scPCP-Hal (n=14)	35.88 ± 3.0	25.53 ± 3.4
scPCP-Olz (n=12)	29.33 ± 2.0	30.60 ± 2.7

Table 3.2. Total object exploration times in each phase of dNOR test on first day of treatment. Total object exploration times were similar between the treatment groups in both the acquisition and the retention phase. Data are presented as Mean ± S.E.M, (n=12-14 per group).

3.5.2. NOR performance on day 8 of treatment (dNOR-8)

3.5.2.1. Acquisition Phase

Rats in all treatment groups spent similar time exploring the left/right identical objects ($F_{1, 48}$= 0.16, p=0.69) (**Figure 3.3 a**). Treatment had a significant effect on total object exploration time ($F_{3,48}$ =3.12, p<0.05). Planned Bonferroni comparisons showed that the total object exploration time was significantly lower in the scPCP-control group in comparison to the scVeh-control group (p=0.02). No other significant pair-wise differences were detected in total object exploration time between the treatment groups in this phase of the task (**Table 3.3**). There was also no significant task x treatment interaction ($F_{3,49}$ =0.39, p=0.52) on total object exploration time.

3.5.2.2. Retention Phase

Across all treatment groups, there was a significant difference in the novel/familiar object exploration times ($F_{3,48}$ = 36.03, p<0.0001). Paired Student's t-test *post-hoc* analysis within individual treatment groups showed that rats in the scVeh-control (t_{12} = 4.42, p<0.001), scPCP-Hal (t_{12} = 3.60, p<0.01) and scPCP-Olz (t_{12} = 2.6, p<0.05) but not in the scPCP-control (t_{12} = 1.64, p=0.13), explored the novel object significantly more than the familiar object (**Figure 3.3 b**). There was no significant main effect of treatment ($F_{3,48}$ = 1.16, p= 0.33) on total object exploration time. Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total object exploration time between the treatment groups in the retention phase (**Table 3.3**). There was also no significant task x treatment interaction ($F_{3,48}$ = 1.30, p=0.28) on total object exploration time.

3.5.2.3. Discrimination Index

Results of the one-way ANOVA revealed no significant effect of treatment on the DI ($F_{3, 48}$=1.21, p=0.31). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the DI between treatment groups. Results of the Mann-Whitney U test revealed that the mean DI value for the scVeh-control (U=26, p<0.01), scPCP-Hal (U=26, p<0.01) and scPCP-Olz (U=26, p<0.01) treatment groups was significantly superior to zero. Consistent with the exploration time data, DI of the scPCP-control (U=52, p=0.07) treatment group was not significantly higher when compared against zero (**Figure 3.3 c**). The results of the Mann-Whitney U test reflect the findings of object exploration times in the retention phase.

3.5.2.4. Locomotor Activity

The one-way ANOVA revealed a significant effect of treatment on the number of line crossings ($F_{3, 48}$ = 10.42, p<0.0001). Planned Bonferroni comparisons detected that the LMA was significantly lower in the scPCP-control treatment group in comparison to the scVeh-control group (p=0.0003). No other significant pair-wise differences were detected between the treatment groups (**Figure 3.3 d**).

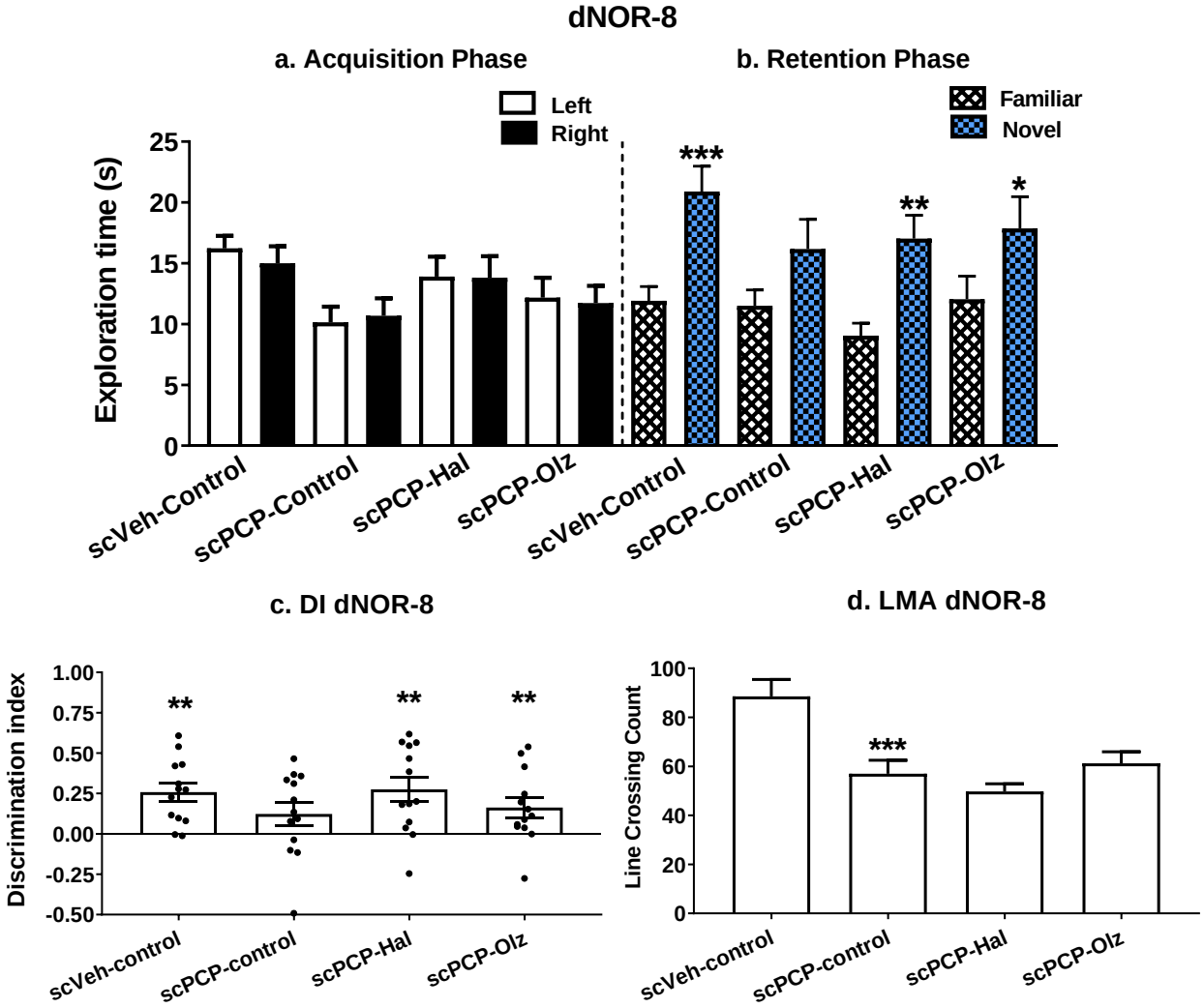

Figure 3.3. NOR performance on day 8 of treatment (dNOR-8) (a) Rats in all treatment groups spent similar time exploring the right and the left object in the acquisition phase (b) Rats in all groups apart from the scPCP-control group explored the novel object significantly more than familiar in the retention phase, **p*<0.05, ***p*<0.01, ****p*<0.001 (c) Ratio of novel to total object exploration time was similar between the treatment groups. ***p*<0.01 vs. Zero. Each dot represents the DI score for 1 rat. (d) Number of line crossings representing the locomotor activity during both the acquisition and the retention phase of the NOR. ****p*<0.001 vs. scVeh-control. No other significant pair-wise differences were detected in the LMA between the treatment groups. Data are presented as Mean ± SEM, (n=13/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVeh-control (n=13)	31.23 ± 1.9	32.81 ± 2.5
scPCP-control (n=13)	20.83 ± 2.5 *****	27.69 ± 3.0
scPCP-Hal (n=13)	27.71 ± 2.3	26.08 ± 2.1
scPCP-Olz (n=13)	23.89 ± 2.7	29.91 ± 3.9

Table 3.3. Total object exploration times in each phase of dNOR test on day 8 of treatment. Total exploration time in the acquisition phase was significantly lower in the scPCP-control group when compared to the scVeh-control group **p*<0.05. No other significant pair-wise differences were detected between the treatment groups in the acquisition and the retention phase. Data are presented as Mean ± S.E.M, (n=13/ group).

3.5.3. NOR performance on day 15 of treatment (dNOR-15)

3.5.3.1. Acquisition Phase

Rats in all treatment groups spent similar time exploring the left/right identical objects ($F_{1, 25}$= 0.04, p=0.84) (**Figure 3.4 a**). Treatment had no significant effect on the total object exploration time ($F_{3,25}$ = 0.60, p=0.61). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total exploration time between the groups (**Table 3.4**). There was no significant task x treatment interaction ($F_{3,25}$ = 1.05, p=0.38).

3.5.3.2. Retention Phase

Across all treatment groups, there was a significant difference in novel/familiar object exploration times ($F_{1,25}$ = 10.58, p<0.01). Paired Student's t-test *post-hoc* analysis within individual treatment groups showed that rats in the scVeh-control (t_9 = 2.32, p<0.05) only, explored the novel object significantly more than the familiar object (**Figure 3.4 b**). Treatment had no significant effect on total object exploration time ($F_{3,25}$ = 0.61, p=0.61). Planned Bonferroni comparisons did not detect any significant pair-wise differences in total exploration time between the groups in this phase (**Table 3.4**). There was also no significant task x treatment interaction ($F_{3,25}$ = 0.80, p=0.50) on total object exploration time.

3.5.3.3. Discrimination Index

Results of the one-way ANOVA showed no significant effect of treatment on the DI ($F_{3,25}$ =1.12, p=0.35). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the DI between the treatment groups. Results of the Mann-Whitney U test revealed that the DI for the scVeh-control (U=25, p<0.05), scPCP-control (U=10, p<0.01) and the scPCP-Olz (U=0, p<0.001) treatment groups but not the scPCP-Hal (U=40, p=1.00) treatment group was significantly superior to zero (**Figure 3.4 c**).

3.5.3.4. Locomotor Activity

Results of the one-way ANOVA showed a significant effect of treatment on the locomotor activity of the rats ($F_{3,25}$ =3.95, p<0.05). However, planned Bonferroni comparisons did not detect any significant pair-wise differences in LMA between the treatment groups. No other significant pair-wise differences were detected in the LMA between the treatment groups (**Figure 3.4 d**)

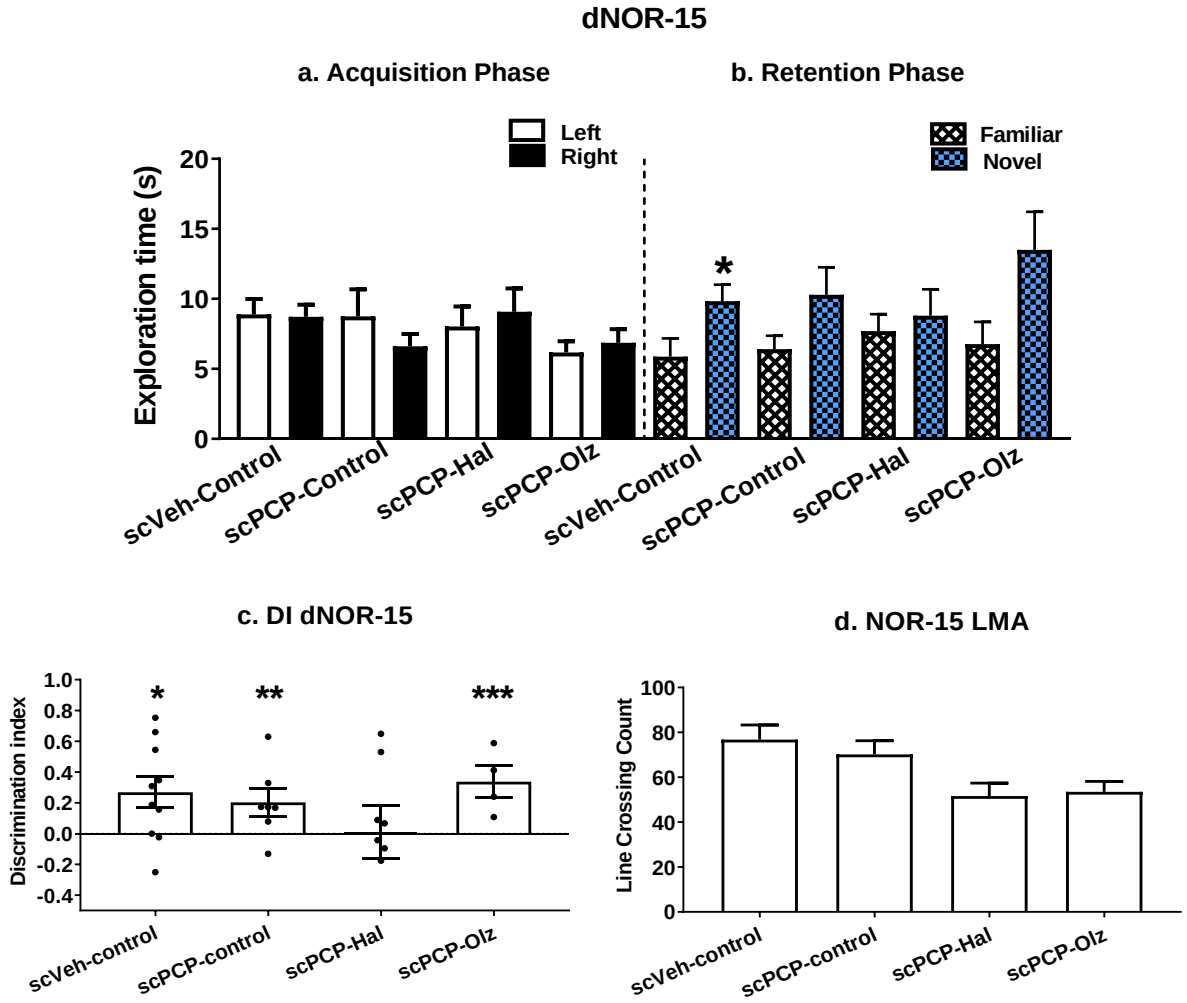

Figure 3.4. NOR performance on day 15 of treatment (dNOR-15) (a) Rats in all treatment groups spent similar time exploring the right and the left object in the acquisition phase (b) Only rats in the scVeh-control treatment group explored the novel object significantly more than familiar in the retention phase, *p<0.05 (c) Ratio of novel to total object exploration time was similar between the treatment groups. *p<0.05, **p<0.01, ***p<0.001 vs Zero. Each dot represents the DI score for 1 rat. (d) Number of line crossings representing the locomotor activity during the acquisition and the retention phase of the NOR. No significant pair-wise differences were detected in the LMA between the treatment groups. Data are presented as Mean ± SEM, (n=4-10/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVeh-control (n=10)	17.61 ± 1.6	15.71 ± 1.8
scPCP-control (n=7)	15.36 ± 2.52	16.68 ± 2.2
scPCP-Hal (n=7)	17.10 ± 2.7	16.5 ± 1.6
scPCP-Olz (n=4)	23.61 ± 10.48	7.1 ± 1.5

Table 3.4. Total object exploration time in each phase of dNOR test on day 15 of treatment. Total object exploration times were similar between the treatment groups in the acquisition and the retention phase. Data are presented as Mean ± S.E.M, (n=4-10/ group).

3.5.4. NOR performance on day 22 of treatment (dNOR-22)

3.5.4.1. Acquisition Phase

Rats in all treatment groups spent similar time exploring the left/right identical objects ($F_{1,49}$=0.06, p=0.80) (**Figure 3.5 a**). Treatment had no significant effect on the total object exploration time ($F_{3,49}$=0.016, p=0.99). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total exploration time between the treatment groups (**Table 3.5**). Similarly, there was no significant task x treatment interaction ($F_{3,49}$ = 0.21, p=0.89).

3.5.4.2. Retention Phase

Across all treatment groups, there was a significant difference in novel/familiar object exploration times ($F_{1,49}$ =30.11, p<0.001). Paired Student's t-test *post-hoc* analysis within individual treatment groups showed that rats in the scVeh-control (t_{12} = 2.89, p<0.05), scPCP-control (t_{13} = 2.91, p<0.05) and the scPCP-Hal (t_{12} = 3.53, p<0.01) explored the novel object significantly more than the familiar object. This effect approached significance in the scPCP-Olz (t_{12} = 2.08, p=0.06) treatment group (**Figure 3.5 b**). Treatment had no significant effect on total object exploration time ($F_{3,49}$=1.81, p=0.15). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the total object exploration time between the treatment groups (**Table 3.5**). There was no significant task x treatment interaction ($F_{3,49}$=0.02, p=0.89) on object exploration time.

3.5.4.3. Discrimination Index

The one-way ANOVA showed no significant effect of treatment on the DI ($F_{3,49}$=0.20, p=0.89). Results of the planned Bonferroni comparisons also did not detect any significant pair-wise differences in the DI between treatment groups. Results of the Mann-Whitney U test revealed that the DI for the scVeh-control (U=28, p<0.001), scPCP-control (U=42, p<0.01), scPCP-Hal (U=42, p<0.05) and the scPCP-Olz (U=42, p<0.05) treatment groups was significantly superior to zero (**Figure 3.5 c**). Results of the Mann-Whitney U test reflect the findings of the object exploration times in the retention phase.

3.5.4.4. Locomotor Activity

The one-way ANOVA showed no significant effect of treatment on the locomotor activity ($F_{3,49}$=1.00, p=0.39). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the LMA between the treatment groups (**Figure 3.5 d**).

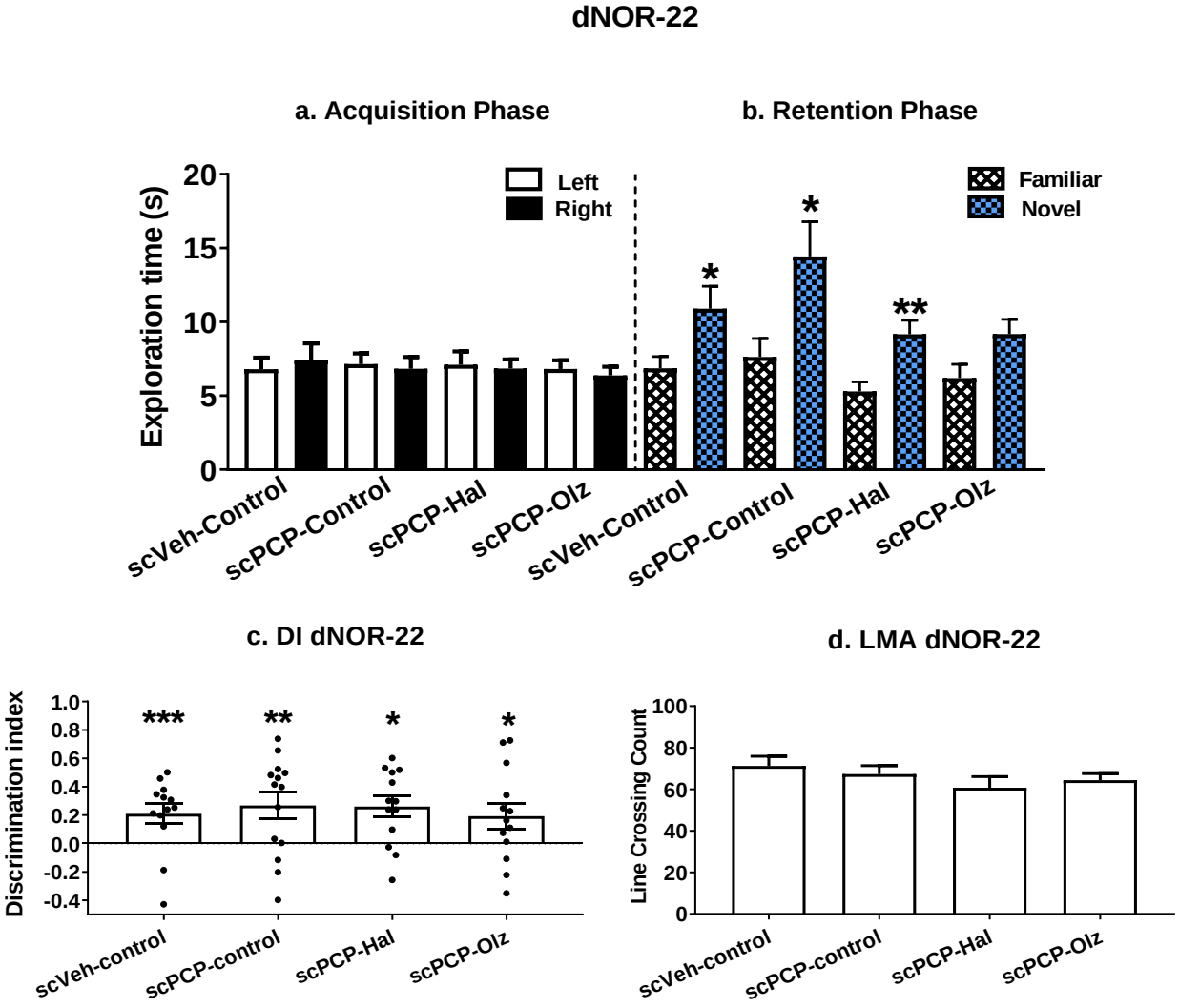

Figure 3.5. NOR performance on day 22 (last-day) of treatment (dNOR-22) (a) Rats in all treatment groups spent similar time exploring the right and the left object in the acquisition phase while (b) rats in the scVeh-control, scPCP-control and scPCP-Hal, but not the scPCP-Olz treatment group explored the novel object significantly more than familiar in the retention phase, **p*<0.05,***p*<0.01 (c) Ratio of novel to total object exploration time was similar between the treatment groups. **p*<0.05, ***p*<0.01, ****p*<0.001 vs Zero. Each dot represents the DI score for 1 rat. (d) locomotor activity levels were similar between the treatment groups. Data are presented as Mean ± SEM, (n=13-14/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVeh-control (n=13)	14.23 ± 1.5	17.75 ± 2.1
scPCP-control (n=14)	13.98 ± 1.14	22.06 ± 2.7
scPCP-Hal (n=13)	13.94 ± 1.3	14.45 ± 1.2
scPCP-Olz (n=13)	13.19 ± 0.7	15.37 ± 1.4

Table 3.5. Total object exploration time in each phase of dNOR test on day 22 of treatment. Total object exploration times were similar between the treatment groups in the acquisition and the retention phase. Data are presented as Mean ± S.E.M, (n=13-14/ group).

3.5.5. NOR performance on first day of washout period (dNOR-WO-1)

3.5.5.1. Acquisition Phase

Rats in all treatment groups spent similar time exploring the left/right identical objects ($F_{1,29}$=0.25, p=0.61) (**Figure 3.6 a**). There was no significant main effect of treatment on total object exploration time ($F_{3,29}$=2.14, p=0.11). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total object exploration times between the treatment groups (**Table 3.6**). There was no significant task x treatment interaction ($F_{3,29}$=0.46, p=0.70) on total object exploration time.

3.5.5.2. Retention Phase

Across all treatment groups, there was a significant difference in novel/familiar object exploration times ($F_{1,29}$ = 14.341, p<0.001). Paired Student's t-test *post-hoc* analysis within individual treatment groups showed that only rats in the scVeh-control (t_7 = 2.98, p<0.05) explored the novel object significantly more than the familiar object. This effect was absent in all other treatment groups (**Figure 3.6 b**). Treatment had no significant effect on total object exploration time ($F_{3,29}$ =0.19, p=0.90). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the total object exploration time between the treatment groups (**Table 3.6**). There was no significant task x treatment interaction ($F_{3,29}$=1.34, p=0.28) on total object exploration time.

3.5.5.3. Discrimination Index

The one-way ANOVA revealed no significant effect of treatment on the DI data ($F_{3,29}$=1.54, p=0.22). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the DI between the treatment groups. No other significant pair-wise differences were detected in the DI between the treatment groups. However, results of the Mann-Whitney U test revealed that the DI for the scVeh-control (U=0, p<0.001) but not scPCP-control (U=18, p=0.06), scPCP-Hal (U=27, p=0.34) and the scPCP-Olz (U=27, p=0.20) treatment groups was significantly superior to zero (**Figure 3.6 c**). Results of the Mann-Whitney U test reflect the findings of the object exploration times in the retention phase.

3.5.5.4. Locomotor Activity

The one-way ANOVA revealed no significant effect of treatment on locomotor activity of the rats across all treatment groups ($F_{3,29}$= 1.05, p=0.38). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in LMA between treatment groups (**Figure 3.6 d**).

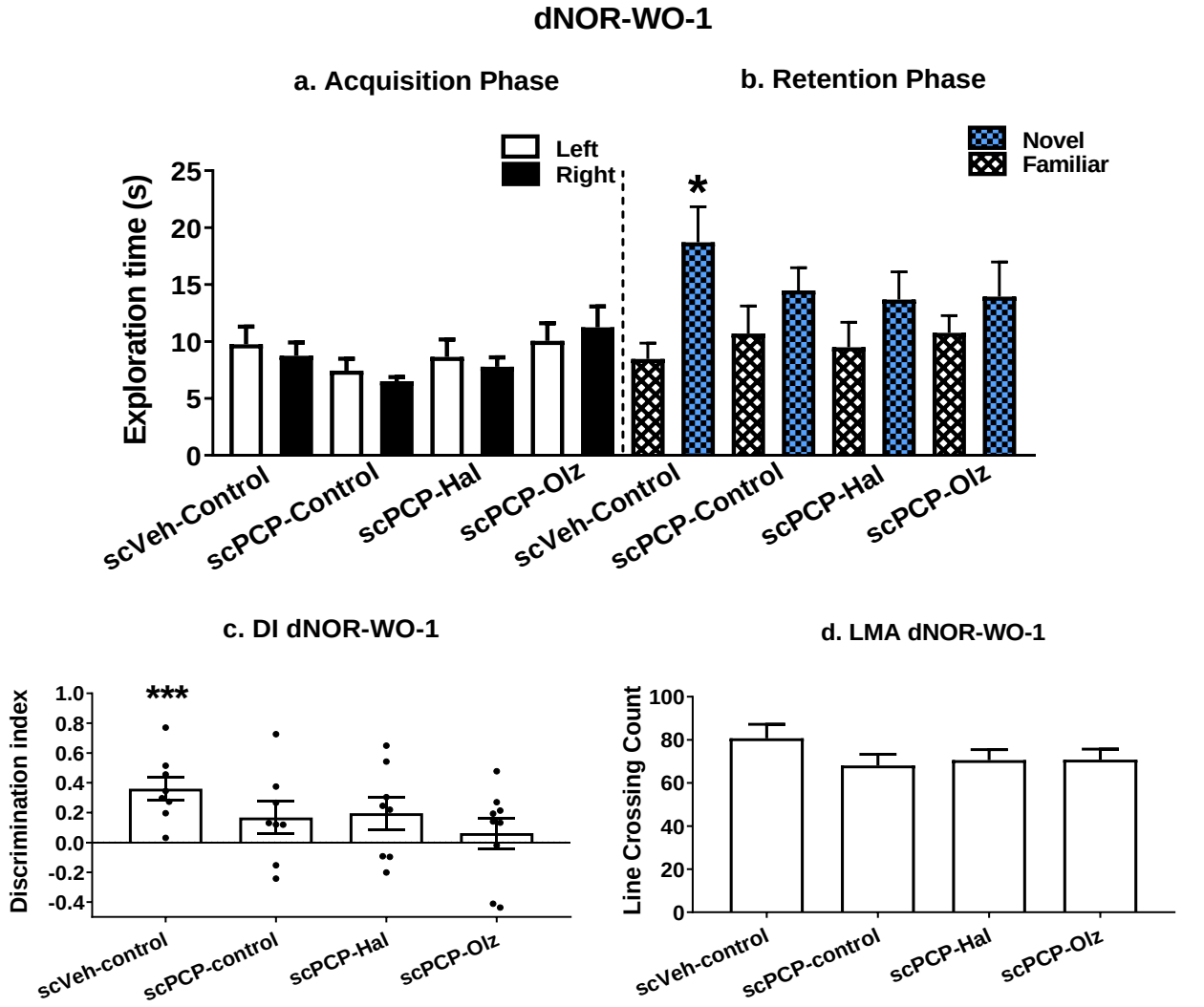

Figure 3.6. NOR performance on first day of washout (dNOR-WO-1) (a) Rats in all treatment groups spent similar time exploring the right and the left object in the acquisition phase (b) Only rats in the scVeh-control treatment group explored the novel object significantly more than familiar in the retention phase, *p<0.05 (c) Ratio of novel to total object exploration time was similar between the treatment groups. ***p<0.001 vs Zero. Each dot represents the DI score for 1 rat (d) locomotor activity levels were similar between the treatment groups. Data are presented as Mean ± SEM, (n=8-9/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVeh-control (n=8)	18.49 ± 1.9	27.17 ± 3.3
PCP-control (n=8)	13.95 ± 1.2	25.19 ± 3.1
scPCP-Hal (n=8)	16.43 ± 1.5	23.17 ± 3.9
scPCP-Olz (n=9)	21.29 ± 2.9	24.70 ± 4.2

Table 3.6. Total object exploration time in each phase of dNOR test on first day of WO (dNOR-WO-1). Total object exploration times were similar between treatment groups at both the acquisition and the retention phase. Data are presented as Mean ± S.E.M, (n=8-9 / group).

3.5.6. NOR performance on day 7 of washout period (NOR-WO-7)

3.5.6.1. Acquisition Phase

Rats in all treatment groups spent similar time exploring the left/right identical objects ($F_{1,27}$=0.20, p=0.65) (**Figure 3.7 a**). Treatment had no significant effect on total object exploration time ($F_{3,27}$=0.59, p=0.62). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total object exploration time between the treatment groups (**Table 3.7**). There was no significant task-treatment interaction ($F_{3,27}$= 1.02, p=0.40) on total object exploration time.

3.5.6.2. Retention Phase

Across all treatment groups, there was a significant difference in novel/familiar object exploration times ($F_{1,27}$=26.92, p<0.001). Paired Student's t-test *post-hoc* analysis within individual treatment groups showed that rats in the scVeh-control (t_7 = 3.002, p<0.05) and scPCP-Hal (t_6 = 3.50, p<0.05) treatment groups explored the novel object significantly more than the familiar objects. This effect was absent in the scPCP-control (t_7 = 1.85, p=0.10) but approached significance in the scPCP-Olz (t_7 = 2.33, p=0.052) treatment groups (**Figure 3.7 b**). There was no significant main effect of treatment ($F_{3,27}$=1.18, p=0.33) on total object exploration time. Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the total object exploration time between treatment groups (**Table 3.7**). There was no significant task x treatment interaction ($F_{3,27}$=0.51, p=0.68) on total object exploration time.

3.5.6.3. Discrimination Index

Results of the one-way ANOVA revealed no significant effect of treatment on the DI data across all treatment groups ($F_{3,27}$ =0.45, p=0.72). The planned Bonferroni comparisons also did not detect any significant pair-wise differences in the DI between the treatment groups. Results of the Mann-Whitney U test revealed that the mean DI value for the scVeh-control (U=8, p<0.01), scPCP-Hal (U=8, p<0.05) and the scPCP-Olz (U=8, p<0.01) treatment groups but not the scPCP-control (t_7 = 1.86, p=0.10) treatment group was significantly superior to zero (**Figure 3.7 c**). Results of the Mann-Whitney U test support the findings of the object exploration times in the retention phase.

3.5.6.4. Locomotor Activity

Results of the one-way ANOVA revealed no significant effect of treatment on the number of line crossings ($F_{3,27}$ = 2.57, p=0.07). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the LMA between the treatment groups (**Figure 3.7 d**).

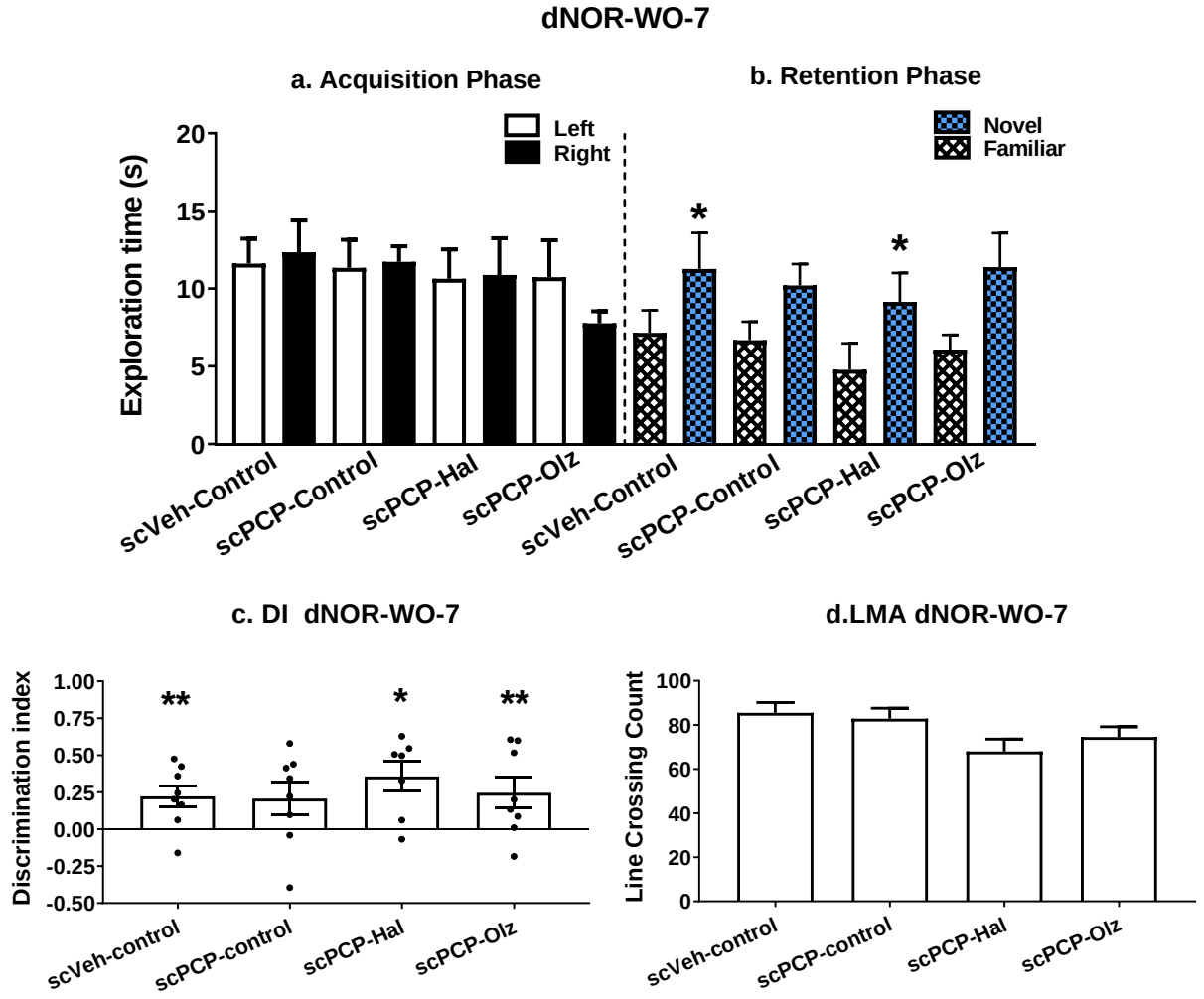

Figure 3.7. dNOR performance on day 7 of washout (dNOR-WO-7). (a) Rats in all treatment groups spent similar time exploring the right and the left object in the acquisition phase (b) Only rats in the scVeh-control and scPCP-Hal group explored the novel object significantly more than familiar in the retention phase, *p<0.05 (c) Ratio of novel to total object exploration time was similar between the treatment groups. *p<0.05, **p<0.01 vs Zero. Each dot represents the DI score for 1 rat. (d) Number of line crossings representing the locomotor activity during the acquisition and retention phase of the dNOR were similar between the treatment groups. Data are presented as Mean ± SEM, (n=7-8/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVeh-control (n=8)	23.94 ± 3.5	18.41 ± 3.5
PCP-control (n=8)	23.07 ± 2.4	16.88 ± 1.5
scPCP-Hal (n=7)	21.51 ± 3.8	13.93 ± 3.3
scPCP-Olz (n=8)	18.50 ± 2.7	17.46 ± 2.3

Table 3.7. Total object exploration time in each phase of dNOR test on day 7 of WO (dNOR-Wo-7). Total object exploration times were similar between treatment groups at both the acquisition and the retention phase. Data are presented as Mean ± S.E.M, (n=7-8 / group).

NOR-day	Treatment group	Acquisition (Did rats have a preference for the left or the right object?)	Retention phase (Did rats explore the novel object significantly more than the familiar?)	Discrimination Index (compared vs. zero and compared between groups)	Locomotor activity
NOR-1	1: scVeh-Control	NO	Yes: $p<0.001$	DI >0: $p<0.0001$	-
	2: scPCP-Control		Yes: $p<0.0001$	DI >0: $p<0.0001$	Sig lower than 1 only, $p=0.028$
	3: scPCP-Hal		Yes: $p<0.0001$	DI >0: $p<0.0001$	N.S. compared to 2
	4: scPCP-Olz		Yes: $p<0.05$	DI >0: $p<0.01$	N.S. compared to 2
NOR-8	1: scVeh-Control	NO	Yes: $p<0.001$	DI >0: $p<0.01$	-
	2: scPCP-Control		NO	DI >0, N.S. against Zero, $p=0.07$	Sig lower than 1 only, $p=0.0003$
	3: scPCP-Hal		Yes: $p<0.01$	DI >0: $p<0.01$	N.S. compared to 2
	4: scPCP-Olz		Yes: $p<0.05$	DI >0: $p<0.01$	N.S. compared to 2
NOR-15	1: scVeh-Control	NO	Yes: $p<0.05$	DI >0: $p<0.05$	-
	2: scPCP-Control		NO	DI >0, $p<0.01$	N.S. compared to any group
	3: scPCP-Hal		NO	DI >0, N.S. against Zero, $p=1.00$	N.S. compared to 2
	4: scPCP-Olz		NO	DI >0: $p<0.001$	N.S. compared to 2
NOR-22	1: scVeh-Control	NO	Yes: $p<0.05$	DI >0: $p<0.001$	-
	2: scPCP-Control		Yes: $p<0.05$	DI >0: $p<0.01$	N.S. compared to any group
	3: scPCP-Hal		Yes: $p<0.01$	DI >0: $p<0.05$	N.S. compared to 2
	4: scPCP-Olz		NO	DI >0, $p<0.05$	N.S. compared to 2
NOR-WO-1	1: scVeh-Control	NO	Yes: $p<0.05$	DI >0: $p<0.001$	-
	2: scPCP-Control		NO	DI >0, N.S. against Zero, $p=0.06$	N.S. compared to any group
	3: scPCP-Hal		NO	DI >0, N.S. against Zero, $p=0.34$	N.S. compared to 2
	4: scPCP-Olz		NO	DI >0, N.S. against Zero, $p=0.20$	N.S. compared to 2
NOR-WO-7	1: scVeh-Control	NO	Yes: $p<0.05$	DI >0: $p<0.01$	-
	2: scPCP-Control		NO	DI >0, N.S. against zero, $p=0.08$	N.S. compared to any group
	3: scPCP-Hal		Yes: $p<0.05$	DI >0: $p<0.05$	N.S. compared to 2
	4: scPCP-Olz		NO: $p=0.052$	DI >0: $p<0.01$	N.S. compared to 2

Table 3.8. Summary of the NOR results for Chapter 3. For Discrimination Index, p values represent comparisons against zero There were no significant pair-wise differences in the DI between the treatment groups at any of the tested time points. **N.S.**: Not Significant

3.6. Discussion

Determining the neurocognitive effects of long-term treatment with APs remains a highly unmet clinical need. Specifically, the neurocognitive effects of long-term treatment with haloperidol and olanzapine remain to be elucidated. In clinical trials, neurocognitive effects of haloperidol and olanzapine are frequently compared against each other. As described in detail in the introduction of the present chapter, the neurocognitive effects of long-term treatment with these APs remains controversial (Keefe et al., 2004; Keefe et al., 2006; Crespo-Facorro et al., 2009; Ayesa-Arriola et al., 2013). Thus far, pre-clinical studies have also been unable to fully address these controversies due to methodological limitations such as using clinically incomparable doses of APs and poor experimental design which limits the translational validity of these findings to clinical practice. Furthermore, evidence of direct comparison between the neurocognitive effects of haloperidol and olanzapine upon short and long-term is lacking in the literature of pre-clinical studies. By addressing some of these limitations, this study aimed to investigate the influence of 22 days (approximately 2.5 human years) of treatment with haloperidol and olanzapine on cognition in the well-validated scPCP model for cognitive impairments associated with schizophrenia. Additionally, by including a week-long washout period at the end of AP treatment schedule, this study investigated whether the neurocognitive effects of tested compounds last upon treatment cessation.

The scPCP-induced deficit in the NOR, manifested as the absence of novelty preference, is frequently and reliably replicated in the literature (Grayson et al., 2007; McKibben et al., 2010; Snigdha et al., 2010; Snigdha et al., 2011a; McLean et al., 2011; Horiguchi et al., 2012; McAllister et al., 2015) hence can be considered as a valid test of cognitive dysfunction in this model. Furthermore, this test provides information regarding basic perceptual stages of visual recognition and memory as well as episodic memory, deficits of which are reported in patients with schizophrenia (Libby et al., 2013; Wang et al., 2014; Ragland et al., 2015). Based on the current evidence and its routine use in our laboratory the scPCP model and the NOR paradigm were chosen to investigate the objectives of this experiment.

A summary of the main results is provided in **Table 3.8.** In this study, rats across all treatment groups and at individual time points spent similar time exploring the identical objects in the acquisition phase, suggesting no preference for the object location in the NOR test box (right vs. left). In agreement with previous findings, rats in the scVeh-control group explored the novel object significantly more than the familiar object across all tested time points. The expected scPCP-induced deficit in the retention phase of the NOR task was absent in the scPCP-control rats when tested on the first and last day (day 22) of the AP treatment schedule. This is while the same group of rats failed to perform the task when tested on days 8 and 15 of treatment and on days 1 and 7 of WO from AP treatment. Rats in the scPCP-Hal and scPCP-Olz treatment groups also explored the novel object significantly more than the familiar object when tested on days 1 and 8 of treatment. The scPCP-Hal but not the scPCP-Olz treated group was also successful in performing the NOR task on day 22 of dosing and on the last day of WO from AP treatment. On other time

points, rats in the scPCP-Hal and scPCP-Olz treatment groups spent similar time exploring the novel and the familiar object.

As reflected in the results of the one-way ANOVA, DI remained unaffected by treatment across all tested time points. Generally, the results of DI can vary between +1 and -1 with positive values representing a preference towards the novel object (Antunes and Biala, 2012). Across all tested time points, the DI of all treatment groups remained above zero, pointing towards the tendency of rats to explore novel object more than the familiar object (**Table 3.8**). Reflecting the findings of exploration time in the retention phase, the DI of the scVeh-control group was significantly superior to zero at all tested time points indicating a strong novelty preference in this treatment group. At time points where rats in the scPCP-control explored the novel object significantly more than the familiar object, the mean DI value was also significantly superior to zero (days 1 and 22 of treatment). Similarly, the mean DI value (DI>0) in the scPCP-control treatment group was not significantly different from zero at time points where rats spent similar time exploring the novel/familiar objects (days 8 of treatment and day 1 and 7 of WO from AP treatment), except at dNOR-15, where DI was significantly superior to zero. There was no significant difference in the DI between the scVeh-control and the scPCP-control treatment groups at any of the tested time points. Collectively, these results suggest that the scPCP-control treatment group had a tendency towards exploring the novel object more than the familiar, even on time points where the DI was not significantly superior to zero. This is also evident from (**Figures 3.2 c, 3.3 c, 3.4 c, 3.5 c, 3.6 c and 3.7 c**), depicting the DI score of individual animals tested in relevant time points. When comparing the DI of scPCP-Hal treatment group to zero a similar pattern emerges. Accordingly, the mean DI value was significantly superior to zero at time points where the rats spend more time exploring the novel rather than the familiar object. This is while the DI value for the scPCP-Olz treatment group was significantly superior to zero even at time points where rats spent similar time exploring novel/familiar objects (except at day 1 of WO from AP). In addition to these findings, planned comparisons did not detect any significant pair-wise differences between the scPCP-Hal and scPCP-Olz and their control (scPCP-control) at any of the tested time points. Collectively, these result point towards the strong tendency of rats in these treatment groups to explore novelty.

Based on these variable findings, it is difficult to ascertain the presence of a robust scPCP induced deficit in the scPCP-control rats, hence it is challenging to meaningfully interpret the findings of the AP treated cohorts. Nonetheless, the finding of similar DI across all four treatment groups at tested time points during AP treatment, might suggest that long-term treatment with haloperidol and olanzapine are not detrimental to performance in the NOR task. This is supported by the findings of similar DI between treatment groups in the WO period from AP treatment. Other studies have also shown that treatment with higher doses of haloperidol (2 mg/kg/day) (Terry et al., 2002; Terry et al., 2006) and olanzapine (2, 5, 10 mg/kg/day) do not impair performance on tasks of spatial learning and memory for up to 45 days of treatment in healthy rats (Terry et al., 2002; Terry et al., 2006; Ortega-Alvaro et al., 2006). In fact, 28 days of treatment with olanzapine (1.5 mg/kg/day; i.p.) improved performance in a reversal learning paradigm in scPCP treated

rats, which also lasted 24h after treatment withdrawal (Mclean et al.,2010b; but also see Rodefer et al., 2009). This is while treatment with haloperidol has been reported to be ineffective in rescuing DISC1-poly (I:C) (Nagai et al., 2011) and MK-801-induced (Ozdemir et al., 2012) NOR deficit upon 7 and 14 days of treatment, respectively.

The doses of APs used in this study were relatively low. According to Kapur et al (2003), haloperidol (0.1 mg/kg) and olanzapine (1.5 mg/kg) occupies approximately 80% of the dopamine D2 receptors upon a single subcutaneous dose in adult male Sprague-Dawley rats. These doses and route of administration are associated with low or no instances of catalepsy in treated animals. This is while the oral route administration of same doses of these drugs is associated with 40-45% of dopamine D2 receptor (D2R) occupancy in adult male Sprague-Dawley rats (Barth et al., 2006). In this study, the oral route of administration was chosen to mimic the human situation.

Currently, there are no direct comparisons in the pharmacokinetic properties of olanzapine and haloperidol treatments in male and female rodents. However, observations from clinical studies suggest higher AP plasma levels in females in comparison to males after receiving the same dose of the drug (Seeman, 2004; Weston-Green et al., 2010). Higher AP plasma levels in females may suggest that lower doses of AP are sufficient in maintaining clinically effective D2 receptor occupancy levels (Seeman, 2004). Accordingly, it is plausible that the doses of AP used in this study, provide D2R occupancy at clinically relevant levels. It is noteworthy that the AP doses chosen in this study did not induce sedation as the locomotor activity in the scPCP-Hal and scPCP-Olz treated groups were similar to the scPCP-control group across all tested time points. This is also reflected in the total object exploration times. There were no significant differences in total object exploration time in either phase of the NOR between the scPCP-Hal and scPCP-Olz and their control (scPCP-control) at any of the tested time points. It is noteworthy that locomotor activity was also comparable between the scPCP-control and scVeh-control at all tested time points except at day 1 and 8 of treatment where activity was significantly lower in the scPCP-control treatment group (**Figure 3.2 d and 3.3 d**). Similarly, total object exploration time in both acquisition and retention phase of dNOR were similar between scPCP-control and scVeh-control treatment groups at all tested time points, but at dNOR-8 rats in the scPCP-control group spent significantly less time exploring objects in the acquisition phase of the test. This, in addition to finding of locomotor activity at this time point can suggest a general disinterest in object exploration.

The lack of robust scPCP-induced deficit in this study is unusual. Behavioural deficit in NOR performance has been frequently reported after 7 days of WO from PCP treatment (Grayson et al., 2007; Snigdha et al., 2010; Snigdha et al., 2011a; McLean et al., 2011; Horiguchi et al., 2012) and appears to persist for at least 6 weeks (McKibben et al., 2010; McAllister et al., 2015) following PCP treatment cessation, a time line that corresponds to the duration of the study presented in this chapter. In our laboratory, scPCP-induced deficit has been observed for as long as 6 months post-treatment (Personal communication). In comparison to their male counterparts, female Lister Hooded rats perform better in the NOR (Sutcliffe et al., 2007; Sutcliffe,

2011). Female Lister Hooded rats are also reported to be more sensitive to the behavioural effects of acute (Grayson and Neill, 2004) and scPCP treatment (Grayson et al., 2007; Janhunen et al., 2015) and show a more robust deficit in the NOR performance compared with males (Grayson, 2012). Performance of female rats in the NOR is independent of the stage of the oestrous cycle (Sutcliffe et al., 2007). However, previous reports have shown that prior treatment with oestradiol benzoate (5 and 10 µg/kg) protected the female Lister Hooded rats against acute PCP-induced cognitive deficit (Sutcliffe et al., 2008). Unless oestrogen levels were higher than normal prior to or during PCP dosing regimen in this study, it is unlikely that the strain and sex of the animals in this experiment could explain the finding of the lack of scPCP deficit in NOR performance.

Although not measured using the ataxia and stereotypy behavioural scales, general observations during the PCP dosing treatment showed an acute effect of PCP on behaviour of the rats. As defined by (Sturgeon et al., 1982) rats treated with PCP exhibit signs of ataxia including the impairment of antigravity reflex, awkward jerky movements and frequent falling on the back and sides while moving. In this study, the ataxic effects of PCP were most pronounced upon the first three days of dosing and were not noticeably present by the last day of dosing. Therefore, the lack of robust scPCP-induced deficit cannot be explained by the route of delivery or the PCP itself not being viable.

As mentioned in the introduction of this chapter, the susceptibility of the scPCP treated rats to distraction might underlie the deficit in NOR performance (Grayson et al., 2014) . This is also consistent with clinical findings suggesting that patients with schizophrenia are more susceptible to environmental distractions (Cellard et al., 2007; Anticevic et al., 2011). This susceptibility is thought to be associated with hypodopaminergia in the PFC (Seamans et al., 1998; Durstewitz et al., 2000), a phenomenon also observed in scPCP treated animals (Jentsch et al., 1997). In accordance with the standard operating procedure in our laboratory, all animals were placed in an unfamiliar box during the 1-min ITI, hence were introduced to distraction levels consistently found effective in the studies reported by our laboratory (Grayson et al., 2007; Snigdha et al., 2010; Grayson et al., 2014). Yet, the expected susceptibility to distraction appeared to be absent in the scPCP treated rats in this study. The NOR testing time points were separated by 7 days (except for the NOR-22 and NOR-WO-1 which occurred on two consecutive days) and no same set of objects were presented on two consecutive sessions, therefore, it is unlikely that practice effect has contributed to the lack of robust scPCP induced deficit.

It is important to note that prior to the scPCP dosing regimen, animals used in this study were handled for habituation purposes. This was in contrast with the standard practice in our laboratory. In this study, handling was implemented only to habituate animals to the experimenter and to dosing holding position in order to reduce stress during treatment. Animals were further handled daily for AP dosing and weighing purposes. Systematic daily handling (a form of enrichment) (Pritchard et al., 2013) in adulthood is known to reverse behavioural deficits observed in the isolation reared rats and to alter their sensitivity to the psychostimulants (Weiss et al., 1999; Jones et al., 2011; Pritchard et al., 2013). Recent unpublished

findings from our laboratory highlight the influence of handling on effectiveness of the scPCP dosing regimen. In this study rats were handled for two weeks (10 minutes/animal/day) prior to scPCP treatment. Results show that the scPCP-handled animals successfully discriminate between the novel and the familiar object in the same manner as controls while non-handled scPCP treated rats are unable to perform the task (**Figure 3.8**). It is plausible that the handling implemented for habituation purposes in this study had an effect on the strength of the scPCP-induced deficit in the NOR. It is however unlikely that daily handling involved in dosing over the course of treatment has had an enriching effect as dosing is distressing to the animals (Stuart and Robinson, 2015). Nonetheless, their influence on these findings cannot be reliably excluded.

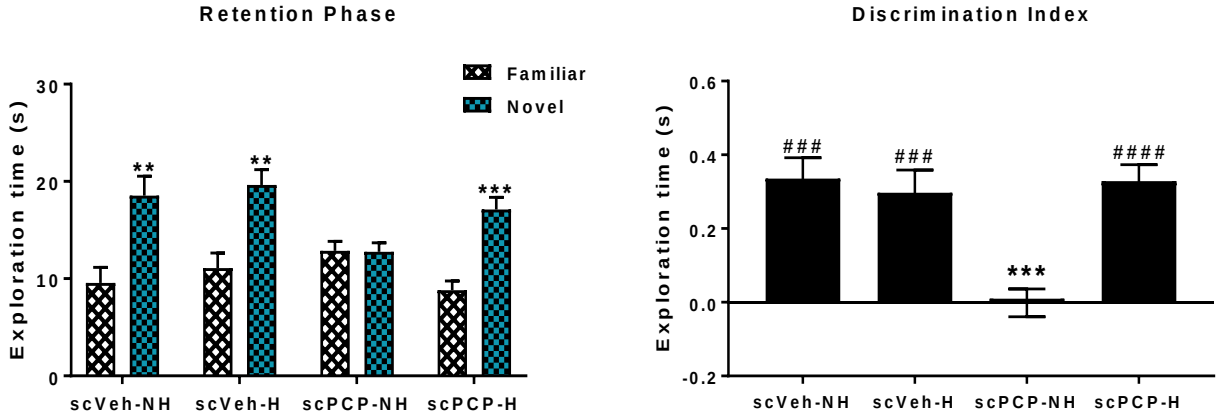

Figure 3.8. Protective effect of systematic handling against scPCP treatment in NOR performance. (A) represents the retention phase of the NOR task. Rats that were handled (10 mins/day for 14 days) prior to scPCP treatment explored the novel object significantly more than the familiar object. This effect was absent in the non-handled scPCP treated animals **p<0.01, ***p<0.001 (B) Ratio of novel to total object exploration time. Rats in all treatment groups but scPCP-NH showed a significant preference for the novel object. ***p<0.001 scPCP-NH in comparison to all other treatment groups; ### p<0.001, ####p<0.0001 vs. zero. NOR Data were analysed as previously described in section 3.4. Data are presented as Mean ± SEM, (n=10/group). H: Handled; NH: Non-Handled. Data were obtained from a study conducted by researchers in our laboratory as part of an independent project.

Caging conditions have also been reported to influence the behavioural responses of rats reared in isolation (Weiss et al., 1999). A recent study showed a significant difference in the influence of open cage (most common housing system) and individually ventilated caging (IVC) systems on the poly (I:C) evoked immune response in pregnant mice. Moreover, the efficacy of the maternal immune activation to induce cognitive deficits in adult offspring was influenced by different housing conditions (Mueller et al., 2018). Influence of long-term housing in an enriched environment (group housing in two tiered IVC as in this study) on behavioural performance of scPCP treated rats is not well studied. Given that this study adhered to the same standard cage and housing conditions as previous work from this laboratory, the influence of environment and cage conditions on the findings can be excluded. Environmental conditions (lighting,

temperature and humidity) in the testing environment remained consistent across all experimental time points. Therefore, it is unlikely that these findings are explained by alterations in the environmental conditions.

To ensure consistency within behavioural scoring, all tapes were scored by 6 other individuals (blind to treatment) from our laboratory. Results found no significant difference in the scores obtained from different individuals (data not presented). This suggests that these findings cannot be explained by poor scoring technique. In addition to the variables discussed above, the inconsistencies in the behavioural findings could also be attributed to the performance variability in the one-trial dNOR task. This is inherent to the nature of this task as it exploits spontaneous behaviour of the animals and their natural tendency to explore novelty (Dere et al., 2007; Ameen-Ali et al., 2015). Therefore, this test is sensitive to factors such as handling, external noise and stress which could confound the outcome of the test (Chan et al., 2018). Within the context of the experimental design in this study, it is difficult to discern the extent to which test-performance variability or the scPCP effectiveness (or lack thereof) contributes to the observed findings.

The interpretation of the findings of this study were made more difficult due to the limitations of study design and statistical analysis. The study should have considered the inclusion of more appropriate control groups (such as scVeh-Hal and scVeh-Olz) in order to create a balanced design. Based on the exclusion criteria outlined in **Section 3.3.**1, a large number of rats were excluded from the analysis of behavioural findings at each tested time point (**Table 3.1**). In an ideal design, rats excluded from one time point should have been excluded from analysis throughout the study and a repeated measures longitudinal statistical analysis should have been employed. However, since different rats were excluded at different time points, their exclusion from all analysis would have significantly reduced the sample size in each treatment group, further compromising the power of statistical analysis. As such, it was decided to exclude rats per tested time point in order to maintain the power of statistical analysis.

This study has other limitations which need to be addressed. The scPCP-induced deficit in NOR performance should have been examined in all treated groups prior to AP treatment. Repeated exposure to PCP enhances locomotor activity upon an acute challenge with psychostimulants (PCP or amphetamine). This locomotor activity is shown to be substantially more robust in scPCP-treated animals compared to an acute PCP insult to a vehicle/naïve rat (Johnson et al., 1998; Phillips et al., 2001; Janhunen et al., 2015). This phenomenon (scPCP induced sensitisation) could have been examined prior to the AP treatment to confirm whether the scPCP treatment had been successful. As discussed in detail in **Chapter 1.4 and 1.5.3**, reduced expression of parvalbumin (PV) (a hallmark of schizophrenia pathophysiology) is also apparent in the hippocampus and the prefrontal cortex of scPCP-treated rats (Cochran et al., 2003; Abdul-Monim et al., 2007). PV levels could have been measured in the collected brain samples from animals in this study to determine the effectiveness of scPCP treatment. Given the rapid metabolic rate in rodents (Kapur et al., 2003), the method of drug delivery used in this study fails to provide a constant and stable level of AP in the plasma of treated rats. This could be problematic from a translational perspective

as it is not comparable to clinical practice. Since dosing the animals 2-3 times a day for long-term is not feasible, an alternative method of drug delivery, such as osmotic minipumps, must be considered. Furthermore, the dNOR task used in this study is not sensitive to detect any potentially negative neurocognitive effects associated with AP treatment. Therefore, use of a different behavioural paradigm is an important consideration. By adopting a different route of drug delivery and introducing a more sensitive behavioural paradigm, next chapter will address some of the methodological limitations faced here.

Chapter 4

Study 2 - An investigation into the influence of long-term treatment with haloperidol on cognition – improved methodology

4.1. Introduction

This chapter focuses on addressing some of the methodological caveats identified in the previous study. Methodological modifications were implemented in the choice of behavioural tests and route of drug delivery.

Therapeutic efficacy of many antipsychotics (APs) is highly correlated with their affinity for dopamine D2 receptors (D2R) (Meltzer, 2013; Mauri et al., 2014). In clinic, optimum antipsychotic effect of haloperidol, a typical AP, is observed at doses between 2-5 mg/day which is associated with 65-80% D2R occupancy (Farde et al., 1992; Kapur et al., 1996; El Hage et al., 2015). In healthy human subjects, mean plasma elimination half-life of haloperidol is approximately 14.5-36.7 hours upon a single dose when administered orally, however, elimination half-life can vary depending upon the route of administration (Kudo and Ishizaki, 1999; de Leon et al., 2004). The time course of haloperidol dissociation from the D2R is slower than its elimination from the plasma and tends to remain stable for at least 27 hours upon a single dose (Nordstrom et al., 1992). A mean half-life of up to 8.3 days and clinically relevant D2R occupancy levels for up to 15 days have been reported following chronic (4 weeks) of treatment in patients with schizophrenia (Baron et al., 1989; de Leon et al., 2004).

With a half-life of 1.5 hours, plasma and brain metabolism of haloperidol in rodents is considerably faster than humans (Kapur et al., 2003; Cheng and Paalzow, 1992). Repeated daily injections of haloperidol at clinically relevant doses produce clinically comparable levels of D2R occupancy at the time of injection which reaches minimal/undetectable levels in 24 hours (Kapur et al., 2003). Therefore, once daily administration of haloperidol, even in the long-term, may not be representative of clinical practice as drug tissue distribution cannot reach a steady state. This issue can be overcome by using osmotic minipumps as an alternative route of drug delivery. The osmotic minipump delivers compounds at a predetermined rate over 24h, hence counteracting the fast metabolism of APs in rodents. Findings suggest that upon implanting osmotic minipumps in male Sprague Dawley rats, the D2R occupancy and plasma concentration of haloperidol remains in the clinically relevant range when tested at 2 (Samaha et al., 2007), 7 (Kapur et al., 2003), 14 (Samaha et al., 2007), 21 (McCormick et al., 2010) and 28 days (4 weeks) (Vernon et al., 2011; Vernon et al., 2014) of treatment.

The route of drug administration was one of the methodological shortcomings of the study presented in the last chapter. Here, osmotic minipumps were used to deliver haloperidol at a steady rate for the duration of 28 days. The dose of haloperidol (0.5 mg/kg/day) was chosen based on previous studies suggesting 75-80% D2R occupancy, with plasma concentration of approximately 7.4 ± 1.7 nM (Vernon et al., 2011; Kapur et al., 2003; McCormick et al., 2010).

In the previous study, the classic one-trial novel objects recognition (NOR) task, referred to as the disrupted NOR (dNOR) in this thesis, was employed to investigate the neurocognitive effects of haloperidol and olanzapine. The dNOR can reliably test the efficacy of treatment intended to reverse manipulation-induced

deficits in performance (Lyon et al., 2012). In the sub-chronic phencyclidine (scPCP) model for cognitive impairments in schizophrenia, this is manifested as restoration of the scPCP-induced deficit in performance of the dNOR task by atypical but not typical APs (Grayson et al., 2007; Cadinu et al., 2017; also see **Chapter 1.7.2** and **Table 1.2** for more detail). However, the dNOR is less sensitive/reliable in detecting potentially negative effects of AP treatment on cognition, as scPCP treated animals are already impaired in performance of this task and lose the ability to discriminate between the novel and the familiar object. In an effort to address this issue, in addition to dNOR, this study employed another variation of the NOR paradigm whereby the animals are left in the NOR testing box during the inter-trial-interval (ITI) period (continuous NOR- cNOR). Evidence from our laboratory suggests that the scPCP treated rats are able to distinguish between the novel and the familiar object when left undisturbed in the NOR testing box during the ITI period (Grayson et al., 2014). Therefore, this test is better suited to detect whether APs impair performance upon long-term treatment.

In order to establish the effectiveness of the scPCP dosing regimen, dNOR was conducted prior to the osmotic minipump implant. This was further validated by assessing scPCP-induced sensitisation to d-amphetamine (Amph). Acute treatment with dopamine releasing agents such as Amph is associated with elevated locomotor activity. This response is substantially more robust in rats previously treated with PCP compared to naïve animals (Phillips et al., 2001; Johnson et al., 1998; Janhunen et al., 2015). It is well documented that scPCP dosing regimen induces a hyper-responsive state in the mesolimbic dopaminergic system, an effect which is possibly downstream to cortical dopaminergic hypofunction (Jentsch et al., 1997; Jentsch et al., 1998; Jentsch and Roth, 1999). This hyper-responsiveness is associated with elevated mesolimbic dopamine release in response to dopamine enhancing substances and translates into hyper-locomotor activity in rats (Balla et al., 2003; Jentsch et al., 1998; Janhunen et al., 2015; Beninger et al., 2010). Examining the hypersensitivity of locomotor activity to acute effect of Amph, therefore, offers a way to determine the effectiveness of the scPCP treatment (Janhunen et al., 2015).

Hyper-locomotor activity is considered as an index with translational relevance to psychosis (Castellani and Adams, 1981; Adams and Moghaddam, 1998; Janhunen et al., 2015). Treatment with haloperidol has been reported to attenuate (Phillips et al., 2001) or inhibit (Samaha et al., 2007) psychostimulant-induced hyper-locomotor activity, supporting the efficacy of haloperidol in treating the positive symptoms associated with schizophrenia (Jones et al., 2011). In this study, the effect of acute Amph on activity levels of haloperidol treated rats was also examined which served as a preliminary measure of osmotic minipump functional integrity and haloperidol treatment efficacy. The confounding effect of handling prior to scPCP dosing regimen was highlighted in **Chapter 3**. It was hypothesised that the handling implemented for habituation purposes prior to scPCP dosing regimen may have contributed to the inconsistencies in the results of the previous chapter. In the present study, element of handling prior to scPCP dosing was eliminated to limit the potential involvement of this variable on the behavioural outcome.

4.2. Materials and Methods

4.2.1. Animals

A total of 45 adult female Lister Hooded rats (Charles Rivers, UK) with starting weight of 190-230 g were used in this experiment. For details on housing conditions and food and water availability please refer to **Chapter 2.1.**

4.2.2. Drug Administration

4.2.2.1. Sub-Chronic PCP/Vehicle Treatment

Rats were randomised to receive vehicle (0.9% Saline; intraperitoneal injections (i.p.), n=20) or PCP (2 mg/kg; i.p., n=25) twice a day for 7 days followed by a 7-day washout (WO) period. This is the standard operating protocol used in our laboratory which is described in more detail in **Chapter 2.3.**

4.2.2.2. Chronic Haloperidol treatment – Osmotic minipump drug delivery

Sub-chronic vehicle (scVeh) and scPCP treated rats were randomised to receive vehicle (β-hydroxypropylcyclodextrin, 20% w/v, acidified by ascorbic acid to pH 6; scVeh-control: n = 10, scPCP-control: n = 10) or haloperidol (0.5 mg/kg, acidified to pH 6; scVeh-Hal: n = 10, scPCP-Hal: n=15). The vehicle and haloperidol treatments were delivered over 28 days via osmotic minipumps (2ML4 model, Alzet) implanted subcutaneously under anaesthetised conditions. Minipumps were implanted 14 days after scPCP/scVeh treatment (see **Section 4.3** for more detail). For details of drug preparations, osmotic minipump implant surgical procedure as well as peri and post-operative care refer to **Chapter 2.2, Chapter 2.5,** and **Chapter 2.5.5,** respectively. In this study, post-operative care involved daily monitoring of weight until the last day of treatment. It is noteworthy that the sample size for the scPCP-Hal treatment group was deliberately larger to maintain statistical power in this principle treatment group in case of exclusion.

4.2.3. Behavioural Task

4.2.3.1. NOR Test

Throughout this study, performance of the animals in both the dNOR and cNOR tests was examined at different time points. The detailed protocol for these two tests is provided in **Chapter 2.4.1**.

4.2.3.1. Locomotor Activity (LMA)

In this study, LMA response to an acute dose of Amph (1 mg/Kg) was tested to examine:

1. scPCP induced Amph sensitisation: To ensure that scPCP treatment had been effective and had persisted up to the testing time point

2. Effect of Amph in scVeh and scPCP rats chronically treated with haloperidol. This served as a preliminary measure of osmotic minipump functional integrity and haloperidol treatment efficacy in inhibiting scPCP-induced Amph sensitisation.

Details of the LMA test protocol and its apparatus are provided in **Chapter 2.4.2.**

4.3. Experimental design

On the last day of WO from the scPCP/scVeh treatment, performance of the animals was tested in the dNOR (dNOR-1) task. This was to ensure that the scPCP dosing regimen had induced the expected dNOR deficit before proceeding with the study. Performance of the animals was also tested in the cNOR (cNOR-1). This was to obtain a baseline measure of task performance prior to long-term treatment with APs. These two testing sessions (dNOR-1 and cNOR-1) were separated by 1 day. In the following week (14 days post scPCP/scVeh treatment) osmotic minipumps were implanted subcutaneously, under anaesthetised conditions. The day of the implant marked the first day of treatment. Post-implant, rats were left to recover until tested again in the dNOR and cNOR tests on day 12 (dNOR-2) and day 14 (cNOR-2) of treatment.

On day 17 of treatment locomotor activity response of the rats to an acute dose of Amph was tested in the LMA automated boxes. One day prior to testing rats were habituated to the LMA box for one hour. On the day of testing, rats were placed in the box for 30 minutes to obtain a baseline measure of activity. Rats were then randomly treated with an acute dose of vehicle (0.9% saline, i.p.) or Amph (1 mg/kg; i.p.) and immediately placed back into the box. The activity was monitored every 5 minutes for a further 60 minutes. There were 8 separate treatment groups in the automated LMA test: scVeh-Veh-Veh (n=4), scVeh-Veh-Amph (n=5), scVeh-Hal-Veh (n=5), scVeh-Hal-Amph (n=5), scPCP-Veh-Veh (n=4), scPCP-Veh-Amph (n=5), scPCP-Hal-Veh (n=7), scPCP-Hal-Amph (n=8).

A final set of NOR tests were conducted on day 26 (dNOR-3) and day 28 (last day, cNOR-3) of treatment. Given the short interval between the NOR pair testing sessions, different sets of objects were used in each session to maintain rats' interest in object exploration and prevent the potential influence of practice effect on behaviour. At the experimental endpoint, rats were sacrificed via transcardial perfusion and brain tissue and blood samples were collected. These procedures are described in detail in **Chapter 2.7** and **Chapter 2.8**, respectively. Blood samples were then processed for analysis using HPLC to determine the levels of haloperidol concentration in the plasma. A brief protocol/methodology of this procedure is provided in **Chapter 2.8.2**. A summary of the experimental timeline and tissue preparation is provided in **Figure 4.1**.

4.3.1. Exclusion Criteria

At all tested time points, behavioural trials were excluded from analysis if animals explored objects for one or less than one second or jumped onto the edge of the NOR box. Based on these criteria, 2 rats were excluded from dNOR-1. At dNOR-2, one rat was sacrificed due to complications with minipump surgery (swelling and accumulation of fluids around implant site). Out of the remaining animals, 1 was excluded at dNOR-2, 3 were excluded at cNOR-2. Another rat was culled prior to the automated LMA test and the dNOR-3 and cNOR-3. From the remaining animals 3 were excluded from dNOR-3 and 4 were excluded from cNOR-3.

NOR testing session	Treatment				Total Excluded
	scVehicle (n=20)		scPCP (n=25)		
dNOR-1	-		2		2
cNOR-1	-		-		-
	scVeh-control (n=10)	scVeh-Hal (n=10)	scPCP-control (n=10)	scPCP-Hal (n=15)	
dNOR-2	1	-	-	1	2
cNOR-2	1	2	1	-	4
dNOR-3	1+3	-	1	-	5
cNOR-3	1	-	1	-	2

Table 4.1. Summary of the number of rats excluded per NOR testing session in Chapter 4. Prior to dNOR-2, 1 rat from the scVeh-control treatment group was sacrificed due to complications with minipump surgery. This is represented by the red numbers across the last 4 NOR testing sessions. Similarly, one rat from the scPCP-control group was sacrificed prior to dNOR-3. This is also represented by the red numbers in the remaining NOR sessions.

4.4. Statistical Analyses

Object exploration times in the acquisition and the retention phase were analysed using mixed-design two-way (task and treatment) ANOVAs to determine the main effect of task (exploring left/right identical object in acquisition phase and novel/familiar in retention phase; within-subjects variable) and treatment (between-subjects variable). Planned paired Student's t-tests were employed to compare between novel vs. familiar object exploration time within each treatment group when appropriate. Independent samples Student's t-tests or planned Bonferroni pair-wise comparisons (scVeh-control vs. scVeh-Hal; scVeh-control vs. scPCP-control; scPCP-control vs. scPCP-Hal; scVeh-Hal vs. scPCP-Hal) were employed to compare total object exploration times between the treatment groups. Discrimination index (DI) and NOR-locomotor activity (LMA) data were analysed by an independent Student's t-test or a one-way ANOVA, followed by planned Bonferroni pair-wise comparisons (scVeh-control vs. scVeh-Hal; scVeh-control vs. scPCP-control; scPCP-control vs. scPCP-Hal; scVeh-Hal vs. scPCP-Hal). DI data were further compared against zero using Mann-Whitney U test. For planned Bonferroni pair-wise comparisons, the significance threshold (α value) was set

at 0.05. Adjusted *p*-values were calculated by multiplying the uncorrected *p*-value (obtained from LSD planned comparisons) by the number of comparisons. In case of the study presented in this chapter, the unadjusted *p*-values were multiplied by 4 (number of planned comparisons). This is the method recommended by IBM SPSS to adjust for multiple comparisons using Bonferroni corrections (IBM SPSS Statistics Support, 2016a). The same format of analysis was repeated for the NOR data at all time points. The area under the curve (AUC) for the total LMA (obtained from automated LMA boxes) was calculated using GraphPad Prism (Version 8.0.2) and was analysed using a one-way ANOVA, followed by LSD *post-hoc* analysis. All Data are presented as Mean ± SEM. All statistical analyses were conducted using IBM SPSS (Version 23). For details on tests of normality and homogeneity of variance on NOR and automated-LMA data, refer to **Chapters 2.4.1.5** and **2.4.2.5**, respectively.

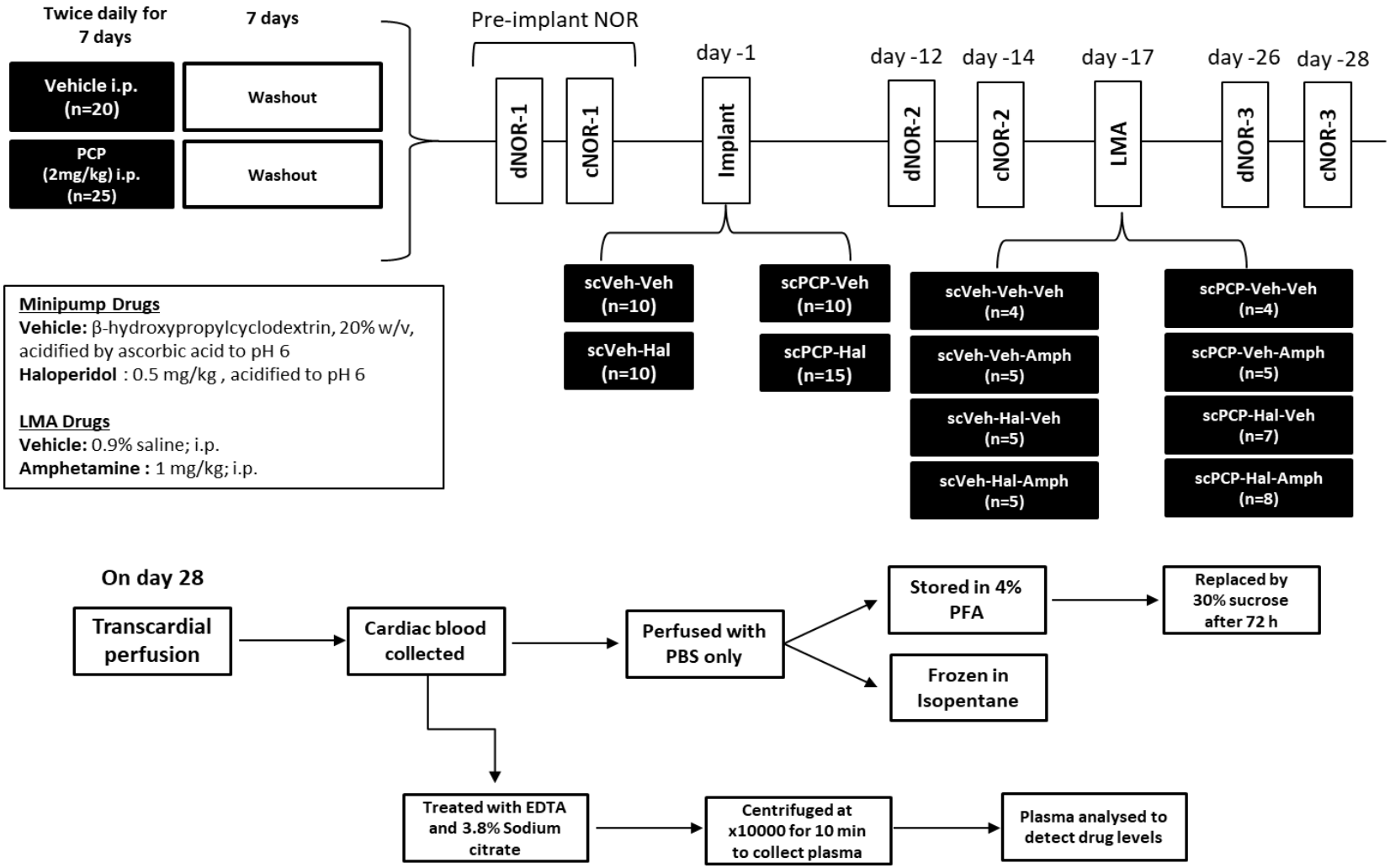

Figure 4.1. A schematic summary of the experimental timeline, tissue preparation and storage for Chapter 4. Subsequent to washout from the scPCP/scVeh treatment baseline behavioural performance was assessed in dNOR -1 and cNOR-1. Osmotic minipumps, containing haloperidol or vehicle, were then implanted in the following week. Performance in the dNOR and cNOR test was re-tested at indicated intervals throughout the treatment period. Locomotor activity of the animals in response to an acute dose of Amph was also assessed. Blood and brain samples were collected on the last day of treatment (day 28) following transcardial perfusion.

4.5. Results

4.5.1. Haloperidol concentration in blood plasma

Cardiac blood samples were collected from all rats on the last day of treatment (day 28) as described in **Chapter 2.7**. These samples were processed to measure haloperidol concentration in blood plasma as described in **Chapter 2.8.2**. Results of this analysis are presented in **Table 4.2**. According to these findings, the scVeh-control and scPCP-control groups show traces of haloperidol contamination. The concentration of haloperidol in the "control" groups is approximately 50% of plasma concentration in the "experimental" treatment groups (i.e. scVeh-Hal and scPCP-Hal). It can therefore be suggested that the scVeh-control and scPCP-control groups were both exposed to long-term treatment with a lower dose of haloperidol (approximately 0.25 mg/kg/day). This dose-response relationship is supported by other research groups showing approximately half haloperidol plasma concentration at 0.25 mg/kg compared to 0.5 mg/kg following 7 days or 8 weeks of treatment delivered via osmotic minipumps in male Sprague Dawley rats (Kapur et al., 2003; Vernon et al., 2012). Hereafter, the "scVeh-control" and "scPCP-control" treatment groups will be referred to as the scVeh-Hal-Low and scPCP-Hal-Low treatment groups, respectively. As such, the "scVeh-Hal" and "scPCP-Hal" treatment groups will be referred to as the scVeh-Hal-High and scPCP-Hal-High treatment groups, respectively.

Treatment group	Haloperidol Plasma Concentration (ng/ml)
scVeh-Hal-Low (n=9)	**9.57 ± 0.85**
scVeh-Hal-High (n=10)	**18.23 ± 2.37**
scPCP-Hal-Low (n=9)	**7.53 ± 0.58**
scPCP-Hal-High (n=14)	**14.70 ± 0.95**

Table 4.2. Haloperidol concentration in blood plasma. 1 rat from the scVeh-Hal-low and scPCP-Hal-low treatment group was sacrificed prior to treatment endpoint (28 days post-implant) due to complications with osmotic minipump surgery. Plasma samples from these two rats were not analysed. Haloperidol plasma concentration of 1 rat in the scPCP-Hal-high treatment group was below detection level, hence n=14. Data are presented as Mean ± SEM.

4.5.2. Pre-Minipump implant dNOR (dNOR-1)

4.5.2.1. Acquisition Phase

Rats in both the scVeh and scPCP treatment groups spent similar time exploring the left/right identical objects ($F_{1, 41}$= 0.002, p=0.96) (**Figure 4.2 a**). Treatment had no significant effect on total object exploration time ($F_{1,41}$ =2.90, p=0.09). Planned independent Student's t-test also did not detect a significant difference in the total object exploration time between the treatment groups (t_{41} = 1.77, p=0.08) (**Table 4.3**). There was no significant task x treatment interaction ($F_{1,41}$=0.06, p=0.8) on total object exploration time.

4.5.2.2. Retention Phase

Across both treatment groups, there was a significant difference in the novel/familiar object exploration times ($F_{1,41}$= 5.67, p<0.05). Paired Student's t-test *post-hoc* analysis within individual treatment groups showed that neither the scVeh (t_{19} = 1.54, p=0.13) nor the scPCP (t_{22} = 1.91, p=0.07) treatment group explored the novel object significantly more than the familiar object (**Figure 4.2 b**). Treatment had no significant effect on total object exploration time ($F_{1,41}$= 1.65, p= 0.20). Total object exploration time was higher in the scVeh compared to the scPCP treatment group but this did not reach significance (t_{41} = 1.44, p=0.15) (**Table 4.3**). There was no significant task x treatment interaction ($F_{1,41}$= 0.09, p=0.76) on total object exploration time.

4.5.2.3. Discrimination Index

The mean DI was lower in the scPCP in comparison to the scVeh treatment group. Based on the results of the independent Student's t-test, this difference did not reach significance (t_{41} = 0.82, p=0.42). Results of the Mann-Whitney U test showed that the DI for both scVeh (U=130, p<0.01) and the scPCP (U=104, p<0.0001) treatment group was significantly superior to zero (**Figure 4.2 c**).

4.5.2.4. Locomotor Activity

Results of an independent Student's t-test did not detect a significant difference in the LMA between the scVeh and scPCP treated group (t_{41} = 0.83, p=0.41) (**Figure 4.2 d**).

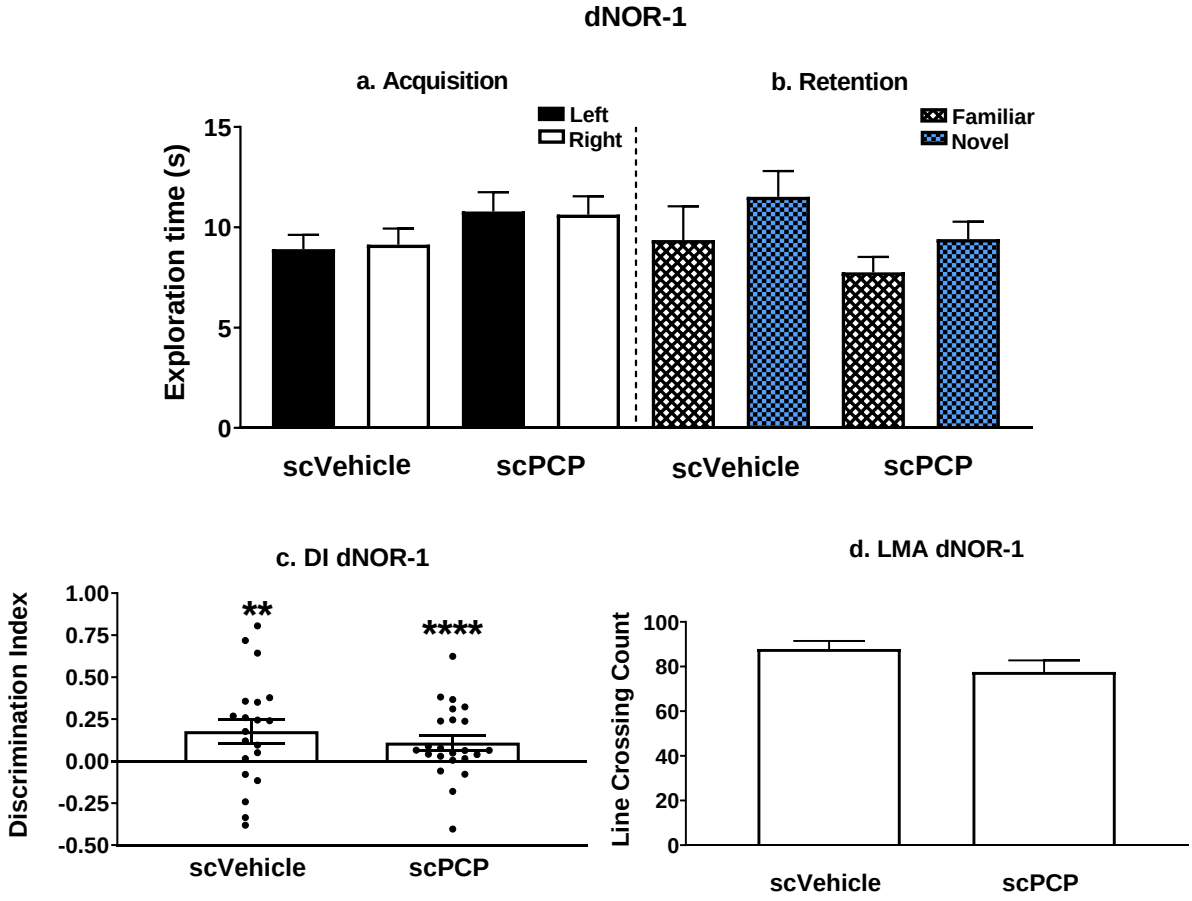

Figure 4.2. Pre-minipump dNOR performance (dNOR-1). (a) Rats in both scVeh and scPCP treatment groups spent similar time exploring the right and the left object in the acquisition phase (b) The expected scPCP-induced deficit was present in the scPCP treated rats, however the scVeh treated rats also failed to successfully perform the task (c) Ratio of novel to total object exploration time was not significantly different between treatment groups. **p<0.01, ****p<0.0001 vs. zero. Each dot represents the DI score for 1 rat (d) Number of line crossings, representing the locomotor activity during both the acquisition and the retention phase of the NOR, were not significantly different between the scVeh and the scPCP treatment groups. Data are presented as Mean ± SEM, (n=20-23/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVehicle (n=20)	18.03 ± 1.27	20.87 ± 2.65
scPCP (n=23)	20.64 ± 1.46	15.79 ± 1.59

Table 4.3. Total object exploration times in each phase of dNOR-1. The total object exploration time was not significantly different between treatment groups in both the acquisition and the retention phase. Data are presented as Mean ± SEM, (n=20-23 /group).

4.5.3. Pre-Minipump implant cNOR (cNOR-1)

4.5.3.1. Acquisition Phase

Rats in both scVeh and scPCP treatment groups spent similar time exploring the left/right identical objects ($F_{1, 43}$= 0.04, p=0.84) (**Figure 4.3 a**). Treatment had no significant effect on the total object exploration time ($F_{1,43}$ =0.27, p=0.61). Planned independent Student's t-test also did not detect a significant difference in total object exploration time between the treatment groups (t_{43} = -0.52, p=0.60) (**Table 4.4**). There was no significant task x treatment interaction ($F_{1,43}$ =0.03, p=0.87) on total object exploration time.

4.5.3.2. Retention Phase

Across both treatment groups, there was a significant difference in the novel/familiar object exploration times ($F_{1,43}$ = 64.05, p<0.0001). Paired Student's t-test *post-hoc analysis* showed that within both the scVeh (t_{19} = 4.44, p<0.001) and the scPCP (t_{24} = 7.05, p<0.0001) treatment group rats explored the novel object significantly more than the familiar object (**Figure 4.3 b**). There was no significant main effect of treatment on total object exploration time ($F_{1,43}$= 0.04, p= 0.85). Planned independent Student's t-test also showed no significant difference between the treatment groups in total object exploration time (t_{43} = -0.18, p=0.85) (**Table 4.4**). There was no significant task x treatment interaction ($F_{1,43}$ = 1.64, p=0.21) on total object exploration time.

4.5.3.3. Discrimination Index

Results of the independent Student's t-test showed no significant difference in the DI between the scVeh and scPCP treated group, however, this was higher for the scPCP treated group (t_{43} = -1.12, p=0.27) (**Figure 4.3 c**). Results of the Mann-Whitney U test revealed that the DI for both scVeh (U=26, p<0.0001) and the scPCP (U=26, p<0.0001) treatment group was significantly superior to zero. The results of the paired t-test reflect the findings of the object exploration time in the retention phase.

4.5.3.4. Locomotor Activity

The LMA was lower for the scPCP compared to the scVeh treatment group. Results of the independent Student's t-test showed that this difference did not reach significance (t_{43} = 0.88, p=0.38) (**Figure 4.3 d**).

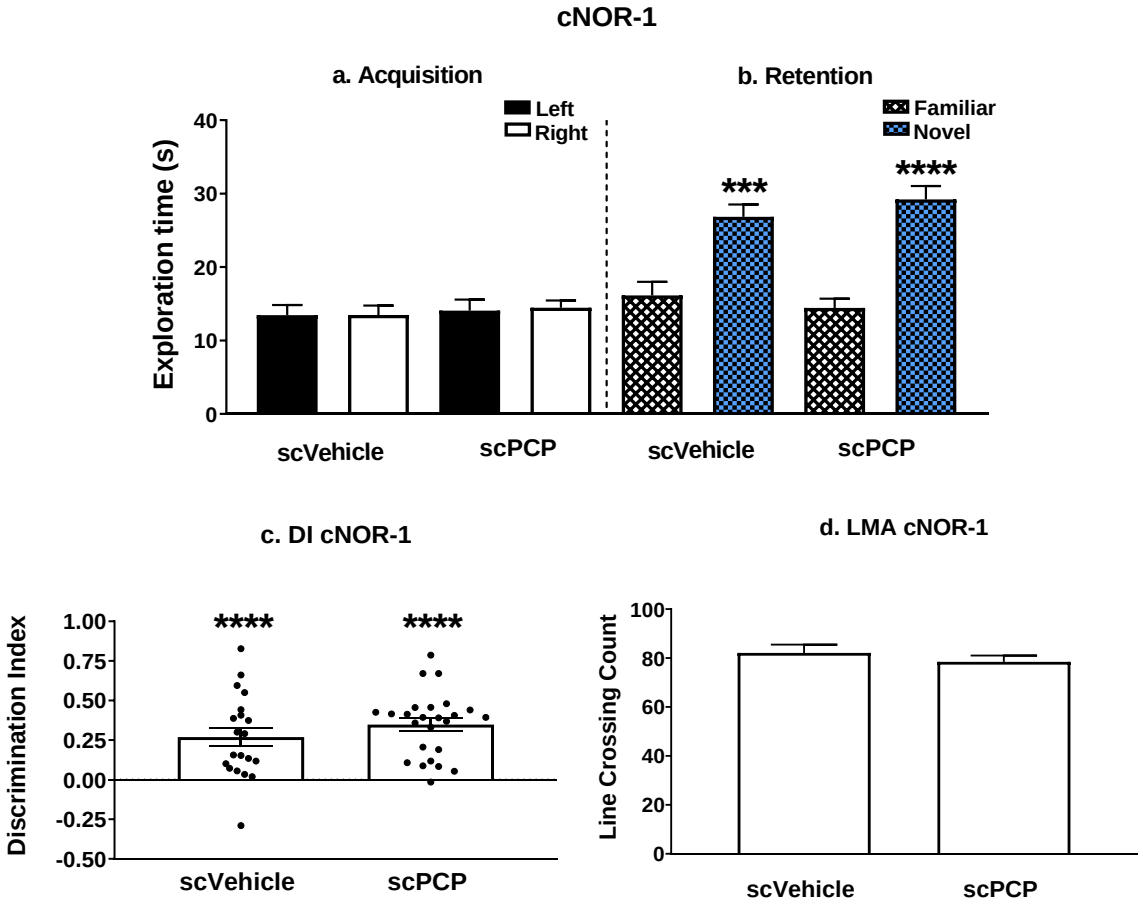

Figure 4.3. Pre-minipump cNOR performance (cNOR-1). (a) Rats in both scVeh and scPCP treatment groups spent similar time exploring the right and the left object in the acquisition phase and (b) explored the novel object significantly more than the familiar object ***p<0.001, ****p<0.0001 (c) Ratio of novel to total object exploration time was not significantly different between treatment groups. ****p<0.0001 vs. zero (d) Number of line crossings, representing the locomotor activity during both the acquisition and the retention phase, were not significantly different between the treatment groups. Data are presented as Mean ± SEM, (n=20-25/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVehicle (n=20)	26.92 ± 2.19	40.97 ± 2.59
scPCP (n=25)	28.53 ± 212	43.63 ± 2.34

Table 4.4. Total object exploration times in each phase of cNOR-1. The total object exploration time was similar between the treatment groups in both the acquisition and the retention phase. Data are presented as Mean ± SEM, (n=20-25 /group).

4.5.4. NOR performance on day 12 of treatment (dNOR-2)

4.5.4.1. Acquisition Phase

Rats in all treatment groups spent similar time exploring the left/right identical objects ($F_{1, 39} = 3.55$, p=0.07) (**Figure 4.4 a**). There was no significant main effect of treatment on total object exploration time ($F_{3,39} = 1.29$, p=0.29). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total object exploration time between the treatment groups (**Table 4.5**). There was no significant task x treatment interaction ($F_{3,39} = 0.69$, p=0.56) on total object exploration time.

4.5.4.2. Retention Phase

Across all treatment groups, there was no significant difference in the novel/familiar object exploration times ($F_{1,39} = 3.29$, p=0.07). Paired Student's t-test *post-hoc* analysis also did not detect a significant difference in novel/familiar object exploration times within individual treatment groups (**Figure 4.4 b**). There was also no significant main effect of treatment ($F_{3,39} = 0.54$, p= 0.66). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total object exploration time between the treatment groups in the retention phase (**Table 4.5**). There was also no significant task x treatment interaction ($F_{3,39} = 0.42$, p=0.74) on total object exploration time.

4.5.4.3. Discrimination Index

Results of a one-way ANOVA revealed no significant effect of treatment on the DI ($F_{3,39} = 1.12$, p=0.35). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the DI between the treatment groups. Results of the Mann-Whitney U test revealed that the DI for neither of the treatment groups (scVeh-Hal-Low (U=45, p=0.12), scVeh-Hal-High (U=45, p=0.06), scPCP-Hal-Low (U=45, p=0.06), scPCP-Hal-High (U=90, p=0.48)) was significantly different from zero (**Figure 4.4 c**). These results reflect the findings of the object exploration time in the retention phase.

4.5.4.4. Locomotor Activity

Results of the one-way ANOVA revealed a significant effect of treatment on the LMA across all treatment groups ($F_{3,39} = 3.53$, p<0.05). However, planned Bonferroni comparison did not detect any significant pair-wise differences in the LMA between the treatment groups (**Figure 4.4 d**).

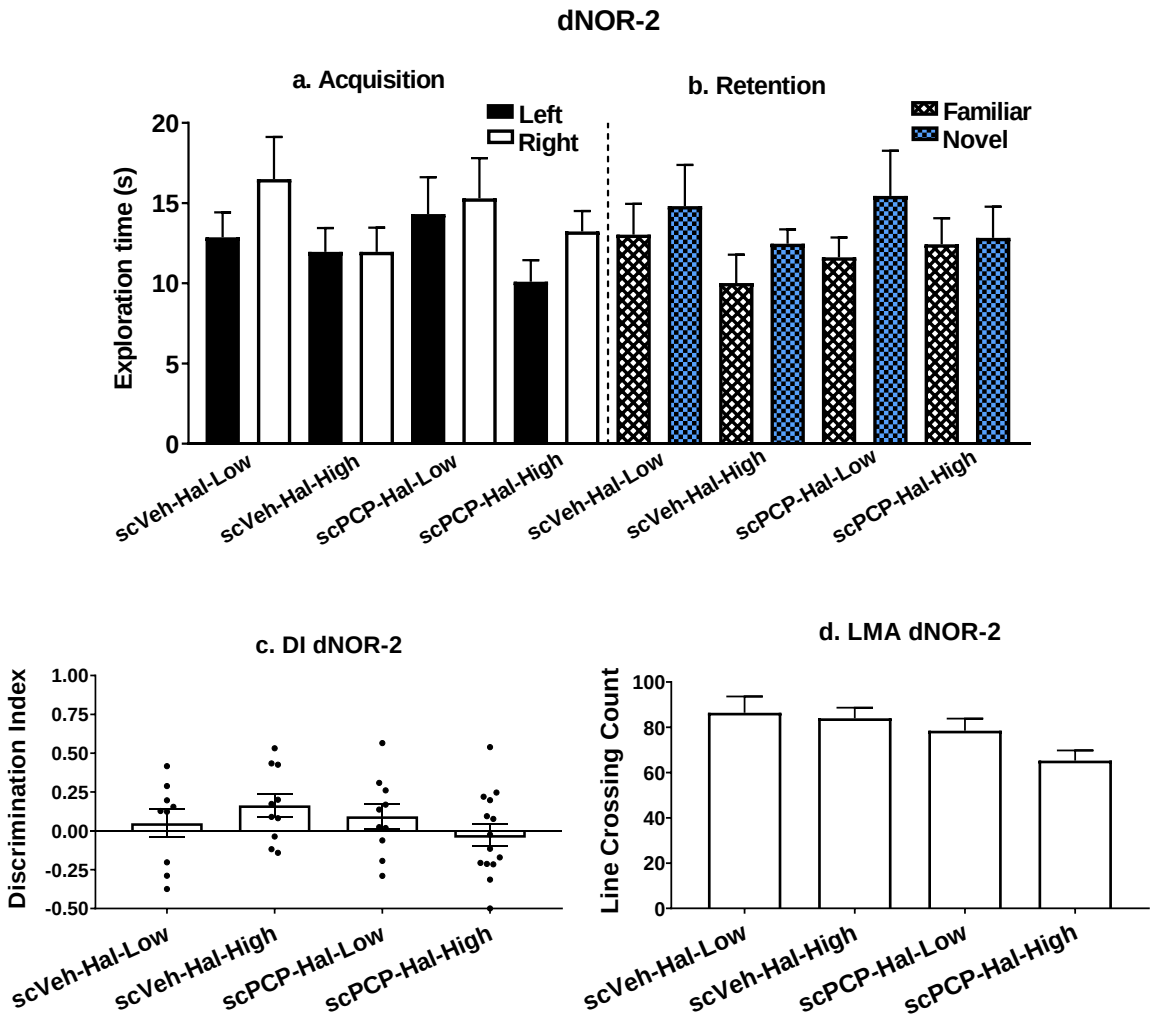

Figure 4.4. dNOR performance on day 12 of treatment (dNOR-2). (a) Rats in all treatment groups spent similar time exploring the left/right objects in the acquisition phase and (b) novel/familiar objects in the retention phase (c) Ratio of novel to total object exploration time was not significantly different between treatment groups. DI was also not significantly different from zero in any of the treatment groups. Each dot represents DI score for one rat (d). Number of line crossings, representing locomotor activity during both the acquisition and the retention phase, were not significantly different between the treatment groups. Data are represented as Mean ± SEM, (n=9-14/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVeh-Hal-Low (n=9)	29.36 ± 3.49	27.84 ± 3.22
scVeh-Hal-High (n=10)	23.28 ± 2.53	21.85 ± 2.44
scPCP-Hal-Low (n=10)	29.61 ± 3.89	27.06 ± 3.33
scPCP-Hal-High (n=14)	23.34 ± 2.12	25.26 ± 3.10

Table 4. 5. Total object exploration times in each phase of dNOR-2 on day 12 of treatment. The total object exploration time was not significantly different between treatment groups in both the acquisition and the retention phase. Data are presented as Mean ± SEM, (n=9-14/group).

4.5.5. NOR performance on day 14 of treatment (cNOR-2)

4.5.5.1. Acquisition Phase

Rats in all treatment groups spent similar time exploring the left/right identical objects ($F_{1, 37} = 0.50$, p=0.48) (**Figure 4.5 a**). There was no significant effect of treatment on total object exploration time ($F_{3,37} = 0.51$, p=0.68). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total object exploration time between the treatment groups (**Table 4.6**). There was no significant task x treatment interaction ($F_{3,37} = 1.09$, p=0.36) on total object exploration times.

4.5.5.2. Retention Phase

Across all treatment groups, there was a significant difference in the novel/familiar object exploration times ($F_{1,37} = 10.92$, p<0.01). Paired Student's t-test *post-hoc* analysis showed that only rats in the scPCP-Hal-Low treatment group (t_8=4.40, p<0.01) explored the novel object significantly more than the familiar object. This effect was absent in all other treatment groups (**Figure 4.5 b**). There was no significant main effect of treatment ($F_{3,37} = 0.11$, p= 0.95) on total object exploration time. Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total object exploration time between the treatment groups (**Table 4.6**). There was also no significant task x treatment interaction ($F_{3,37} = 0.25$, p=0.86) on total object exploration times.

4.5.5.3. Discrimination Index

Results of a one-way ANOVA revealed no significant effect of treatment on the DI ($F_{3,37} = 0.51$, p=0.68). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the DI between the treatment groups. Results of the Mann-Whitney U test showed that the DI of the scVeh-Hal-High (U=30, p<0.05) and scPCP-Hal-Low (U=15, p<0.001) but not the scVeh-Hal-Low (U=60, p=0.61) and scPCP-Hal-High (U=75, p=0.09) treatment groups was significantly superior to zero (**Figure 4.5 c**).

4.5.5.4. Locomotor Activity

Results of the one-way ANOVA revealed a significant effect of treatment on LMA across all treatment groups ($F_{3,37} = 3.68$, p=0<0.05). Planned Bonferroni comparisons revealed that the LMA was significantly higher in the scVeh-Hal-High group in comparison to the scPCP-Hal-High (p=0.012). No other significant pair-wise differences were detected in the LMA between the treatment groups (**Figure 4.5 d**).

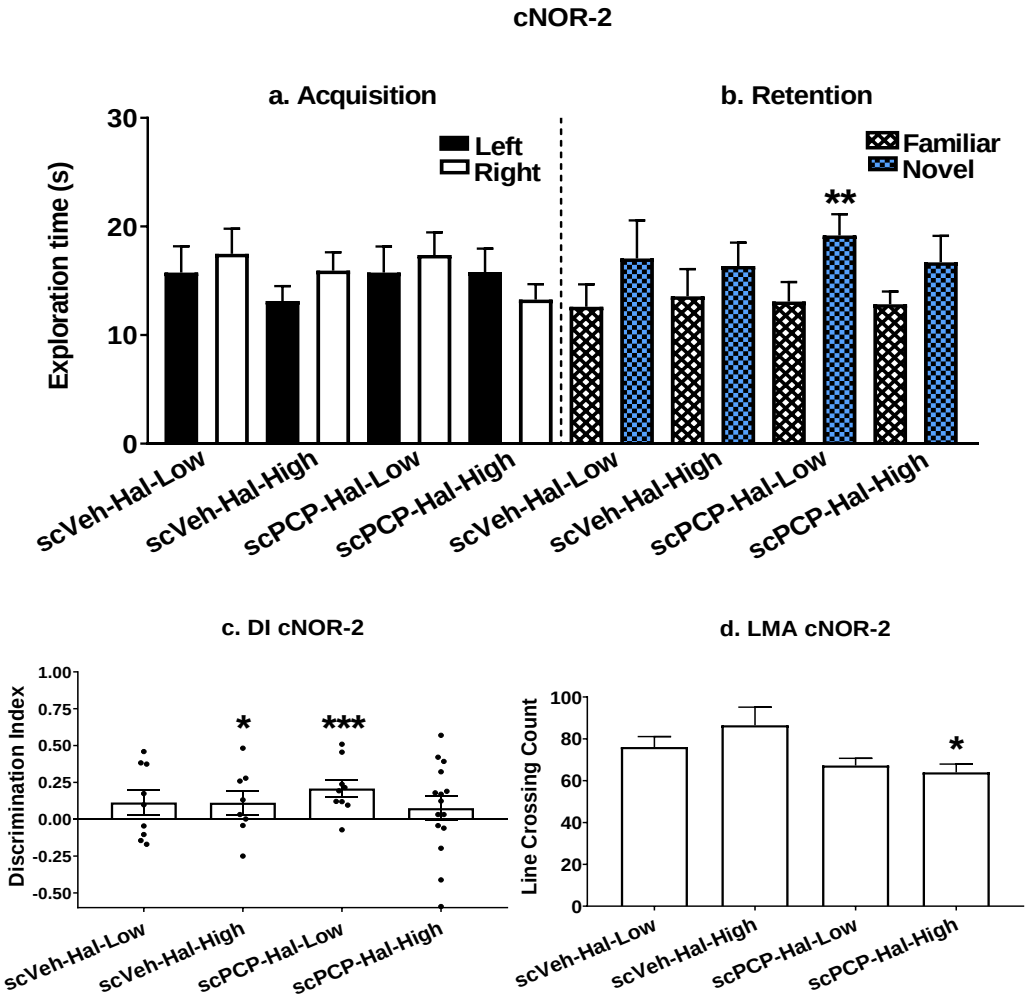

Figure 4.5. cNOR performance on day 14 of treatment (cNOR-2). (a) Rats in all treatment groups spent similar time exploring the left/right objects in the acquisition phase while (b) only rats in the scPCP-Hal-Low group explored novel object significantly more than the familiar object **p<0.01 (c) Ratio of novel to total object exploration time was not significantly different between treatment groups. *p<0.05, ***p<0.001 vs. zero. Each dot represents the DI score for 1 rat (d) Number of line crossings representing locomotor activity * p<0.05 vs. scVeh-Hal-High. No other significant pair-wise differences were detected in the LMA between the treatment groups. Data are presented as Mean ± SEM, (n=8-15/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVeh-Hal-Low (n=9)	33.23 ± 4.5	29.6 ± 4.9
scVeh-Hal-High (n=8)	29.07 ± 2.35	29.93 ± 4.1
scPCP-Hal-Low (n=9)	33.13 ± 2.94	32.26 ± 3.48
scPCP-Hal-High (n=15)	29.08 ± 2.79	29.53 ± 2.85

Table 4.6. Total object exploration times in each phase of cNOR-2 on day 14 of treatment. The total object exploration time was similar between treatment groups in both the acquisition and the retention phase. Data are presented as Mean ± SEM, (n=8-15/group).

4.5.6. NOR performance on day 26 of treatment (dNOR-3)

4.5.6.1. Acquisition Phase

Rats in all treatment groups spent similar time exploring the left/right identical objects ($F_{1, 36} = 1.29$, $p=0.26$) (**Figure 4.6 a**). There was no significant main effect of treatment on total object exploration time ($F_{3,36} =2.26$, $p=0.09$). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total object exploration times between the treatment groups (**Table 4.7**). There was no significant task x treatment interaction ($F_{3,36} =0.47$, $p=0.70$) on total object exploration times.

4.5.6.2. Retention Phase

Across all treatment groups, there was a significant difference in the novel/familiar object exploration times ($F_{1,36} = 50.56$, $p<0.0001$). Paired Student's t-test *post-hoc* analysis showed that rats in the scVeh-Hal-Low ($t_5=3.54$, $p<0.05$), scVeh-Hal-High ($t_9=2.94$, $p<0.05$), scPCP-Hal-Low ($t_8=4.12$, $p<0.01$) and scPCP-Hal-High ($t_{14}=4.98$, $p<0.001$) treatment groups explored the novel object significantly more than the familiar object (**Figure 4.6 b**). Treatment did not have a significant effect on total object exploration time ($F_{3,36} = 0.04$, $p= 0.98$). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in total object exploration time between the treatment groups in the retention phase (**Table 4.7**). There was no significant task x treatment interaction ($F_{3,36} = 0.61$, $p=0.61$) on total object exploration times.

4.5.6.3. Discrimination Index

Results of a one-way ANOVA revealed no significant effect of treatment on the DI ($F_{3,36} =0.51$, $p=0.67$). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the DI between the treatment groups. Results of the Mann-Whitney U test showed that the DI in the scVeh-Hal-Low ($U=0$, $p<0.0001$), scVeh-Hal-High ($U=20$, $p<0.05$), scPCP-Hal-Low ($U=10$, $p<0.01$) and the scPCP-Hal-High ($U=10$, $p<0.001$) treatment groups was significantly superior to zero (**Figure 4.6 c**). Results of the Mann-Whitney U test reflect the findings of the object exploration time in the retention phase.

4.5.6.4. Locomotor Activity

Results of a one-way ANOVA revealed no significant effect of treatment on the LMA ($F_{3,36} =1.79$, $p=0.16$). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the LMA between the treatment groups (**Figure 4.6 d**).

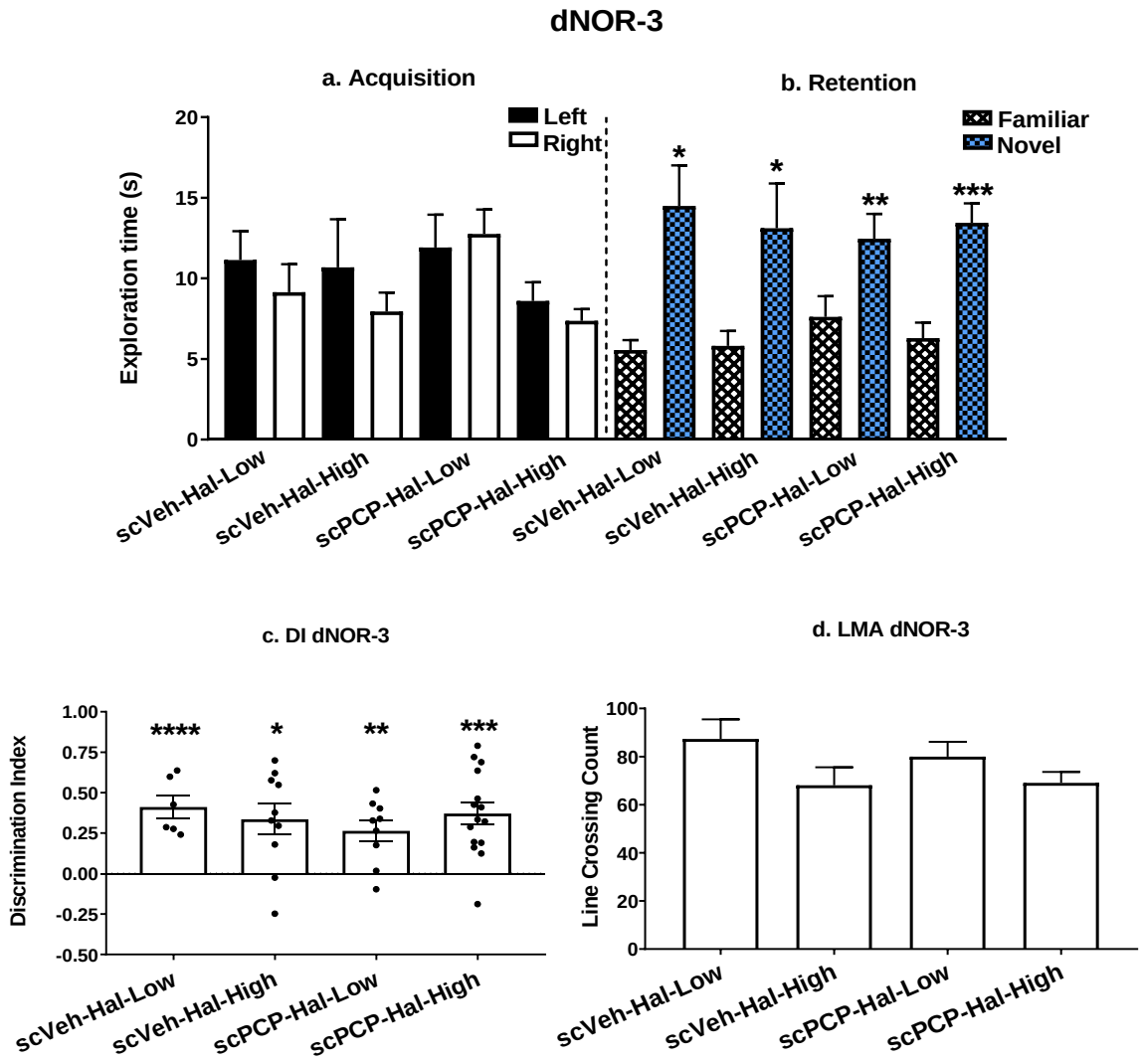

Figure 4.6. dNOR performance on day 26 of treatment (dNOR-3). (a) Rats in all treatment groups spent similar time exploring the left/right objects in the acquisition phase while (b) rats in all treatment groups explored the novel object significantly more than the familiar object *p<0.05, **p<0.01, ***p<0.001 (c) Ratio of novel to total object exploration time was similar between the treatment groups. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. zero. Each dot represents the DI score for 1 rat (d) Number of line crossings were not significantly different between the treatment groups. Data are presented as Mean ± SEM, (n=6-15/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVeh-Hal-Low (n=6)	20.28 ± 2.29	20.05 ± 2.66
scVeh-Hal-High (n=10)	18.63 ± 3.73	18.92 ± 3.31
scPCP-Hal-Low (n=9)	24.67 ± 2.46	20.07 ± 2.45
scPCP-Hal-High (n=15)	15.09 ± 2.06	18.42 ± 1.40

Table 4.7. Total object exploration times in each phase of dNOR-3 on day 26 of treatment. The total object exploration time was similar between treatment groups in both the acquisition and the retention phase. Data are presented as Mean ± SEM, (n=6-15/group).

4.5.7. NOR performance on day 28 of treatment (cNOR-3)

4.5.7.1. Acquisition Phase

Rats in all treatment groups spent similar time exploring the left/right identical objects ($F_{1, 39} = 0.77$, p=0.38) (**Figure 4.7 a**). There was no significant effect of treatment ($F_{3,39} = 1.42$, p=0.25) on total object exploration times. Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the total object exploration time between the treatment groups (**Table 4.8**). There was no significant task x treatment interaction ($F_{3,39} = 1.11$, p=0.36) on total object exploration times.

4.5.7.2. Retention Phase

Across all treatment groups, there was a significant difference in the novel/familiar object exploration times ($F_{1,39} = 8.61$, p<0.01). However, paired Student's t-test *post-hoc* analysis did not detect a significant difference in novel/familiar object exploration times within individual treatment groups (**Figure 4.7 b**). There was also no significant main effect of treatment ($F_{3,39} = 0.89$, p= 0.45) on total object exploration times. Planned Bonferroni comparisons did not detect any significant pair-wise differences in total object exploration time between the treatment groups in the retention phase (**Table 4.8**). There was no significant task x treatment interaction ($F_{3,39} = 0.49$, p= 0.68) on total object exploration times.

4.5.7.3. Discrimination Index

Results of a one-way ANOVA revealed no significant effect of treatment on the DI ($F_{3,39} = 0.41$, p=0.74). Planned Bonferroni comparisons also did not detect any significant pair-wise differences in the DI between different treatment groups. Results of the Mann-Whitney U test also showed that the DI for neither of the treatment groups ((scVeh-Hal-Low (U=45, p=0.12); scVeh-Hal-High (U=45, p=0.06); scPCP-Hal-Low (U=45, p=0.12); scPCP-Hal-High (U=105, p=0.74)) was significantly different from zero (**Figure 4.7 c**). These results reflect the findings of the total object exploration time in the retention phase.

4.5.7.4. Locomotor Activity

Results of the one-way ANOVA revealed no significant effect of treatment on the LMA ($F_{3,39} = 2.55$, p=0.07). Planned Bonferroni comparisons did not detect any significant pair-wise differences in the LMA between the treatment groups (**Figure 4.7 d**).

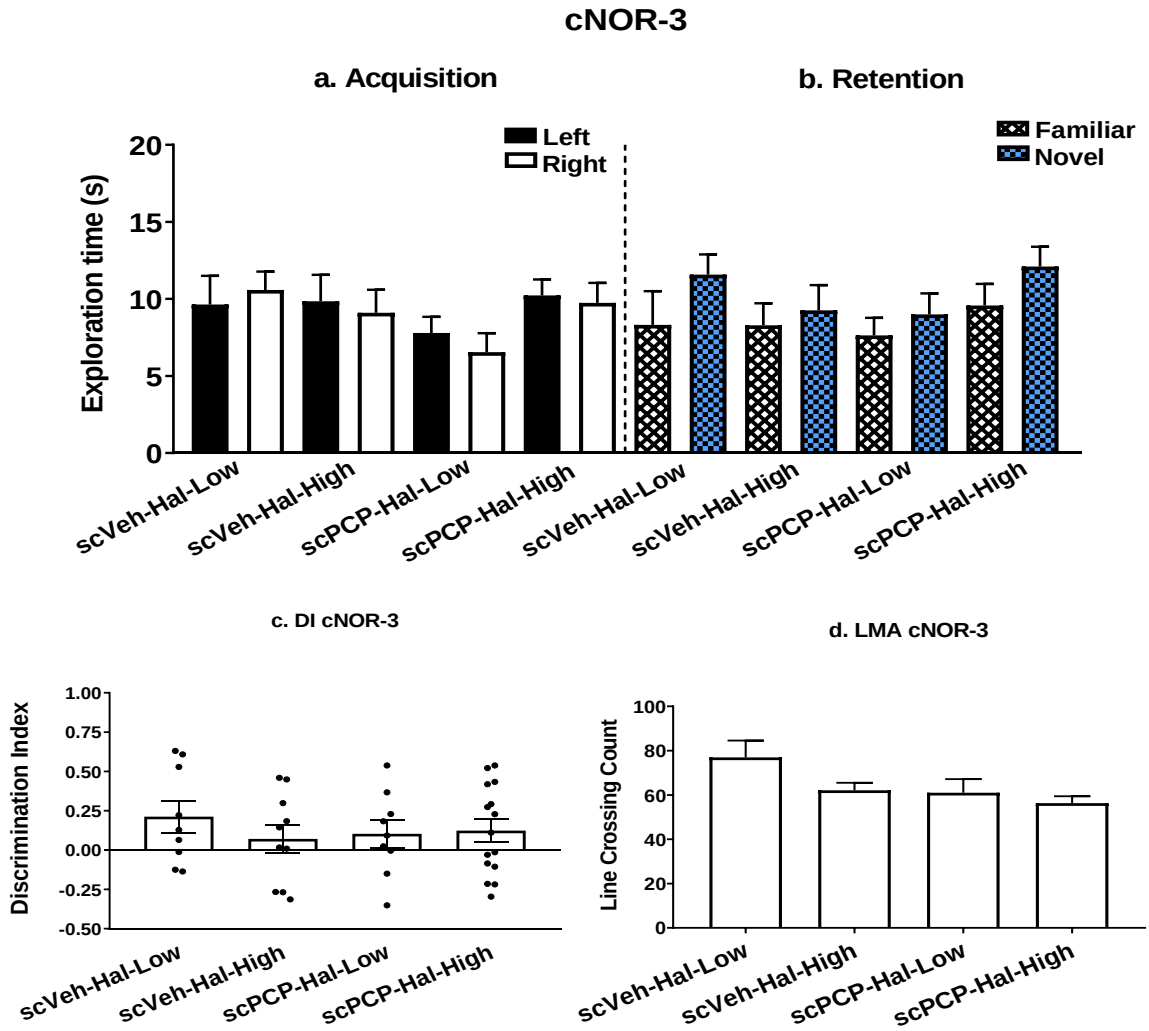

Figure 4.7. cNOR performance on day 28 of treatment (cNOR-3). (a) Rats in all treatment groups spent similar time exploring the left/right objects in the acquisition phase and (b) the novel/familiar object in the retention phase (c) Ratio of novel to total object exploration time was similar between treatment groups. DI for neither of the treatment groups was significantly different from zero. Each dot represents DI score for 1 rat. (d). Number of line crossings, representing locomotor activity in both the acquisition and the retention phase, were not significantly different between the treatment groups. Data are presented as Mean ± SEM, (n=9-15/group).

Treatment	Mean total object exploration times (s)	
	Acquisition phase	Retention phase
scVeh-Hal-Low (n=9)	20.28 ± 2.29	20.05 ± 2.66
scVeh-Hal-High (n=10)	19.34 ± 3.06	17.84 ± 2.67
scPCP-Hal-Low (n=9)	14.34 ± 2.02	16.64 ± 2.08
scPCP-Hal-High (n=15)	19.98 ± 1.85	21.67 ± 2.17

Table 4.8. Total object exploration times in each phase of cNOR-3 on day 28 of treatment. The total object exploration time was similar between the treatment groups in both the acquisition and the retention phase. Data are presented as Mean ± SEM, (n=9-15 /group).

4.5.8. Effect of Haloperidol on scPCP-induced Amph Sensitisation: Area Under the Curve (AUC) for total LMA count

The LMA before and after Amph challenge is presented in **Figure 4.8 A**. A one-way ANOVA on AUC over the 1-hour period following the acute challenge revealed a significant effect of treatment ($F_{7,35}$ =11.51, p<0.0001). LSD *post-hoc* analysis detected that, following an acute treatment with Amph, the AUC was significantly higher for the scVeh-Hal-Low-Amph group in comparison to the scVeh-Hal-Low-Veh (p<0.01) and the scVeh-Hal-High-Veh (p<0.0001) treatment groups. The AUC for the scVeh-Hal-Low-Amph group was also higher than scVeh-Hal-High-Amph treatment group but it did not reach significance (p=0.14). The AUC for scVeh-Hal-High-Amph was significantly higher than the scVeh-High-Low-Veh (p<0.05) and scVeh-Hal-High-Veh (p<0.01). The scVeh-Hal-Low-Veh group was also not significantly different from the scVeh-Hal-High-Veh treatment group (p=0.39) (**Figure 4.8 B1**).

The AUC was significantly higher for the scPCP-Hal-Low-Amph in comparison to the scPCP-Hal-Low-Veh (p<0.0001), scPCP-Hal-High-Veh (p<0.0001) and scPCP-Hal-High-Amph (p<0.01) treatment groups. The AUC was also significantly higher for the scPCP-Hal-High-Amph group in comparison to the scPCP-Hal-High-Veh (p<0.0001) and the PCP-Hal-Low-Veh (p<0.05) treatment groups. The latter was also not significantly different from the scPCP-Hal-High-Veh group (p=0.48) (**Figure 4.8 B2)**. The AUC for the scPCP-Hal-Low-Amph treatment group was higher in comparison to the scVeh-Hal-Low-Amph treatment group, but this did not reach significance (p=0.054). There was no significant difference between any other scVeh and scPCP treatment groups (**Figure 4.8 B1 and B2**). A summary of these results is provided in **Table 4.10.**

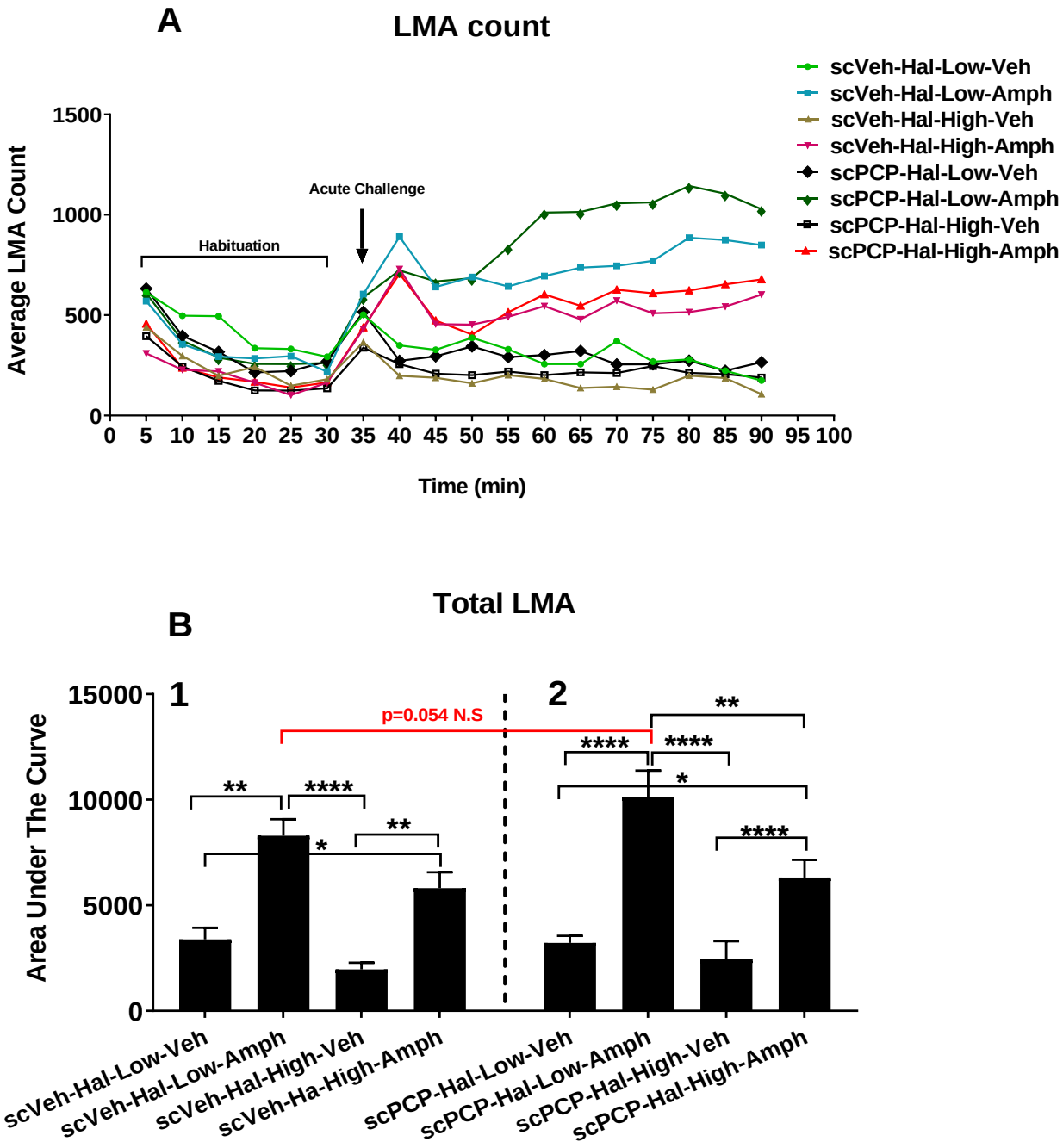

Figure 4.8. Locomotor activity in response to acute Amph challenge on day 17 of treatment with haloperidol. (A) Represents the average of the number of times rats broke the beam in 5-minute bins over a 30-min habituation period and 1h post-acute Amph challenge (B) Represents the area under the curve (AUC) for scVeh (B1) and scPCP (B2) treatment groups. Black lines show the result of the LSD *post-hoc* comparison within the scVeh and scPCP treatment groups separately. *p<0.05, **p<0.01, ****p<0.0001. The red line shows the result of comparison between scPCP-Hal-Low-Amph and its control counterpart, the scVeh-Hal-Low-Amph treatment group. AUC was higher for the latter but did not reach significance (p=0.054). Data are presented as Mean ± SEM (scVeh-Hal-Low-Veh: n=4; scVeh-Hal-Low-Amph: n=5; scVeh-Hal-High-Veh: n=5; scVeh-Hal-High-Amp n=5; scPCP-Hal-Low-Veh: n=4; scPCP-Hal-Low-Amph: n=5; scPCP-Hal-High-Veh: n=7; scPCP-Hal-High-Amp n=8). **N.S.**: Not significant

NOR-day	Treatment group	Acquisition Phase (Did rats have a preference for the left or the right object?)	Retention phase (Did rats explore the novel object significantly more than the familiar?)	Discrimination Index (compared vs. zero and compared between groups)	Locomotor activity
dNOR-1 (pre-implant)	scVehicle	NO	NO	DI>0: $p<0.01$	N.S.
	ScPCP		NO	DI >0: $p<0.0001$	
cNOR-1 (pre-implant)	scVehicle	NO	Yes: $p<0.001$	DI >0: $p<0.0001$	N.S.
	ScPCP		Yes: $p<0.0001$	DI >0: $p<0.0001$	
dNOR-2 (treatment day 12)	1: scVeh-Hal-Low	NO	NO	DI >0, N.S. against zero, $p=0.12$	N.S. compared to 2 and 3
	2: scVeh-Hal-High		NO	DI >0, N.S. against zero, $p=0.06$	N.S. compared to 1 and 4
	3: scPCP-Hal-Low		NO	DI >0, N.S. against zero, $p=0.06$	N.S. compared to 1 and 4
	4: scPCP-Hal-High		NO	DI <0, N.S. against zero, $p=0.48$	N.S. compared to 2 and 3
cNOR-2 (treatment day 14)	1: scVeh-Hal-Low	NO	NO	DI >0, N.S. against zero, $p=0.61$	N.S. compared to 2 and 3
	2: scVeh-Hal-High		NO	DI >0, $p<0.05$	N.S. compared to 1
	3: scPCP-Hal-Low		Yes: $p<0.01$	DI >0, $p<0.001$	N.S. compared to 1 and 4
	4: scPCP-Hal-High		NO	DI >0, N.S. against zero, $p=0.09$	Sig lower than 2 ($p=0.012$)
dNOR-3 (treatment day 26)	1: scVeh-Hal-Low	NO	Yes: $p<0.05$	DI >0, $p<0.0001$	N.S. compared to 2 and 3
	2: scVeh-Hal-High		Yes: $p<0.05$	DI >0, $p<0.05$	N.S. compared to 1 and 4
	3: scPCP-Hal-Low		Yes: $p<0.01$	DI >0, $p<0.01$	N.S. compared to 1 and 4
	4: scPCP-Hal-High		Yes: $p<0.001$	DI >0, $p<0.001$	N.S. compared to 2 and 3
cNOR-3 (treatment day 28)	1: scVeh-Hal-Low	NO	NO	DI >0, N.S. against zero, $p=0.12$	N.S. compared to 2 and 3
	2: scVeh-Hal-High		NO	DI >0, N.S. against zero, $p=0.06$	N.S. compared to 1 and 4
	3: scPCP-Hal-Low		NO	DI >0, N.S. against zero, $p=0.12$	N.S. compared to 1 and 4
	4: scPCP-Hal-High		NO	DI >0, N.S. against zero, $p=0.74$	N.S. compared to 2 and 3

Table 4.9. Summary of dNOR and cNOR findings at all tested time points. For the discrimination index, p values represent comparisons against zero. According to the results, there was no significant pair-wise difference in the discrimination index between the treatment groups at any of the tested time points. For the LMA, results of planned comparisons are summarised. **N.S.**: Not significant.

scVehicle group comparisons	Results	ScPCP group comparisons	Results
(1) scVeh-Hal-Low-Amph vs. (2) scVeh-Hal-Low-Veh	Yes: $p<0.01$, 1>2	(1) scPCP-Hal-Low-Amph vs. (2) scPCP-Hal-Low-Veh	Yes: $p<0.0001$, 1>2
(1) scVeh-Hal-Low-Amph vs. (3) scVeh-Hal-High-Veh	Yes: $p<0.0001$, 1>3	(1) scPCP-Hal-Low-Amph vs. (3) scPCP-Hal-High-Veh	Yes: $p<0.0001$, 1>3
(1) scVeh-Hal-Low-Amph vs. (4) scVeh-Hal-High-Amph	NO: $p=0.14$, 1>4	(1) scPCP-Hal-Low-Amph vs. (4) scPCP-Hal-High-Amph	Yes: $p<0.01$, 1>4
(3) scVeh-Hal-High-Veh vs. (2) scVeh-Hal-Low-Veh	NO: $p= 0.39$, 2>3	(3) scPCP-Hal-High-Veh vs. (2) scPCP-Hal-Low-Veh	NO: $p=0.48$, 2>3
(4) scVeh-Hal—High-Amph vs. (2) scVeh-Hal-Low-Veh	Yes: $p<0.05$, 4>2	(4) scPCP-Hal-High-Amph vs. (2) scPCP-Hal-Low-Veh	Yes: $p<0.05$, 4>2
(4) scVeh-Hal-High-Amph vs. (3) scVeh-Hal-High-Veh	Yes: $p<0.01$, 4<3	(4) scPCP-Hal-High-Amph vs. (3) scPCP-Hal-High-Veh	Yes: $p<0.0001$, 4>3
scVeh-Hal-Low-Amph vs. scPCP-Hal-Low-Amph	NO: $p=0.054$, scPCP>scVeh	N.S. difference between any other scVeh and scPCP treatment groups	

Table 4.10. Summary of the Locomotor activity in response to acute Amphetamine challenge. Locomotor activity in response to Amph was marginally higher in the scPCP-Hal-Low-Amph treatment group compared to scVeh-Hal-Low-Amph. Analysis revealed that this effect approached significance. (scVeh-Hal-Low-Veh: n=4; scVeh-Hal-Low-Amph: n=5; scVeh-Hal-High-Veh: n=5; scVeh-Hal-High-Amp n=5; scPCP-Hal-Low-Veh: n=4; scPCP-Hal-Low-Amph: n=5; scPCP-Hal-High-Veh: n=7; scPCP-Hal-High-Amp n=8)

4.6. Discussion

In this chapter, the influence of long-term (28 days) treatment with haloperidol on cognition was studied in the context of improved methodology. By introducing osmotic minipumps as a new route of drug delivery and the cNOR as a new behavioural paradigm, this study aimed to address some of the methodological limitations identified in the previous chapter. Time and financial constraint meant that in contrast to chapter 3, only one AP agent could be investigated in this study. Haloperidol was specifically chosen since its neurocognitive effects remain more controversial compared to other APs in clinical practice (see **Chapter 1.7.1** for a detailed discussion on this point). In this study, however, a longer treatment duration was selected (28 days) in comparison to the study presented in **Chapter 3** (22 days) (equating to approximately 3 and 2.5 human years, respectively (Sengupta., 2013)) to better represent treatment duration in clinical practice.

Table 4.9 provides a summary of the findings of dNOR and cNOR tests conducted throughout this study. Following WO from the scPCP/scVeh treatment and prior to the osmotic minipump implant, performance was tested in the dNOR-1 and cNOR-1 tasks to assess the baseline behavioural phenotype of the animals. At dNOR-1 and cNOR-1, rats in both scVeh and scPCP treatment groups spent similar time exploring the identical objects in the acquisition phase, suggesting no preference for object location (right or left). When tested in the dNOR-1, rats in the scPCP treatment group, failed to distinguish between the novel and the familiar object. This is in agreement with previous findings from our laboratory (Grayson et al., 2007) and others (McKibben et al., 2010), reporting a scPCP-induced deficit in dNOR performance. However, the expected preference for the novel object was also absent in the scVeh treatment group when tested in the dNOR-1 (**Figure 4.2 b**).

As discussed in the previous chapter, the DI values range between -1 and +1, with positive values suggesting a preference for the novel object. In dNOR-1, the mean DI value for both the scVeh and scPCP treatment groups was significantly greater than zero, suggesting that both treatment groups had a strong preference for novelty. The mean DI value was however, higher in the scVeh in comparison to the scPCP group, but this did not reach statistical significance. The apparent deficit in performance of the scVeh group, manifested as similar novel vs. familiar object exploration times, can be explained by the performance of a number of animals showing a preference for the familiar over the novel object (negative DI values). This is depicted in **Figure 4.2 c**, where each dot represents the DI value for a single animal. Indeed, this can be attributed to the natural variability in behaviour. As previously shown in our laboratory (Grayson et al., 2014), the expected scPCP-induced deficit in the NOR task was absent when animals remained in the NOR testing box during the ITI period (cNOR-1). Similarly, the scVeh treated animals were also able to perform the task when tested in the cNOR-1. The mean DI value for both scVeh and scPCP treatment groups was significantly greater than zero, suggesting a strong preference for novelty (**Figure 4.3 c**). There was no significant difference in the DI between these two treatment groups. It is essential to note that the apparent deficit in dNOR-1 performance in the scVeh treatment group might confound the interpretation of the

effectiveness of the scPCP treatment. Nevertheless, the decision to continue with the study was based on the successful performance of both scVeh and scPCP treatment groups in the cNOR-1.

Behavioural performance of the animals in dNOR and cNOR tests post minipump implant must be interpreted with consideration towards the blood plasma analysis. Cardiac blood plasma samples obtained on the last day (day 28) of treatment were analysed to determine haloperidol plasma concentration. Results revealed traces of haloperidol in the "scVeh-control" (9.57± 0.85 ng/ml; denoted as scVeh-Hal-Low) and "scPCP-control" (7.53 ± 0.58 ng/ml; denoted as scPCP-Hal-Low) treatment groups (see **Section 4.5.1** and **Table 4.2**). The source of this contamination was traced back to drug preparation in the laboratory. The concentration of haloperidol (0.5 mg/kg/day) in the "scVeh-Hal" (18.23± 2.37 ng/ml; denoted as scVeh-Hal-High) and "scPCP-Hal" (14.71± 0.96 ng/ml; denoted as scPCP-Hal-High) experimental groups was considerably higher than expected. An average range of 2.40 ± 1.01 ng/ml and 4.7 ± 0.32 ng/ml has been previously reported for haloperidol dose of 0.25 mg/kg and 0.5 mg/kg, respectively when tested in male Sprague-Dawley rats, following 7 days (Kapur et al., 2003) and 8 weeks (Vernon et al., 2012) of treatment delivered via osmotic minipumps. This is while, plasma level of 23.3 ± 1.5 has been associated with haloperidol dose of 2 mg/kg when tested in male Sprague Dawley rats, following 8 weeks of treatment delivered via osmotic minipumps (Vernon et al., 2012). Higher plasma levels of haloperidol in the female Lister Hooded rats used in this study is consistent with clinical findings suggesting that females show a higher plasma concentration than males for the same dose of the drug (Seeman, 2004; Weston-Green et al., 2010). To the best of my knowledge there are no reports of haloperidol plasma concentration comparison between male and female rodents. Therefore, it is difficult to draw firm conclusions based on the findings reported in the present study. Indeed, the dose of haloperidol delivered in this study should have been confirmed by analysing the residual volume of drug left in the minipump at experimental endpoint. This would have confirmed whether higher haloperidol plasma concertation found in this study is biologically relevant or whether it has bee due to miscalculations regarding dose during drug preparation.

Following osmotic minipump implant, behavioural performance of the animals was tested in dNOR and cNOR on days 12 (dNOR-2), 14 (cNOR-2), 26 (dNOR-3) and 28 (cNOR-3). As expected, rats in all treatment groups spent similar time exploring the identical objects at all tested time points. When tested on day 12 of treatment rats in the scVeh-Hal-Low and scPCP-Hal-Low treatment group were unable to successfully perform the dNOR-2 task (**Figure 4.4 b**). However, when tested on day 26 of treatment both scVeh-Hal-Low and scPCP-Hal-Low treatment groups explored the novel object significantly more than the familiar object in the dNOR-3 (**Figure 4.6 b**). On day 14 of treatment, only rats in the scPCP-Hal-Low group were able to successfully perform in the cNOR-2 (**Figure 4.5 b**). This ability was absent in both scVeh-Hal-Low and scPCP-Hal-Low treatment groups on the last day of treatment in cNOR-3 (**Figure 4.7 b**). Rats in the scVeh-Hal-High and scPCP-Hal-High treatment groups showed a deficit in performance of the dNOR-2 task when tested on the day 12 of treatment. The same group of rats were able to perform the task when

tested on day 26, in the dNOR-3 paradigm. Both scVeh-Hal-High and scPCP-Hal-High treatment groups were unable to perform in the cNOR-2 and cNOR-3 (**Table 4.9**).

As represented in the DI figures, the mean DI values for all treatment groups were above zero at all tested time points post minipump implant except for the scPCP-Hal-High treatment group at dNOR-2, where the DI mean was below zero (no significant difference). This suggests that rats in all but one treatment group show a preference towards the novel rather than the familiar object. Similar to the findings of the previous study (**Chapter 3**), results of DI comparisons against zero reflected the same pattern as the object exploration time in the retention phase. Accordingly, this novelty preference was significantly higher than zero (chance) at time points where rats explored the novel object significantly more than the familiar object. Similarly, the novelty preference was not significantly different from zero at time points where rats spent similar time exploring the novel and the familiar object (with exception to the scVeh-Hal-High treatment group at cNOR-2 where DI was significantly higher than zero). Importantly, there was no significant difference in the DI between the any of the treatment groups at any tested time point post minipump implant (refer to **Table 4.9** for a summary of these findings). Collectively, the findings of the DI measure suggest that while rats in some treatment groups showed a significant preference for novelty (compared to zero), the extent of novelty preference is similar between all treatment groups at all tested time points.

The outcome of these behavioural tests is highly inconsistent. Previous findings from our laboratory suggest that acute treatment with haloperidol (0.05 and 0.075 mg/kg; i.p.) does not rescue the scPCP-induced deficit in the dNOR test (Grayson et al., 2007). Similarly, 7 (Nagai et al., 2011) and 14 (Ozdemir et al., 2012) days of treatment with haloperidol at (1 mg/kg/day) fails to rescue manipulation-induced deficit in the dNOR. Ozdemir and colleagues (2012) also reported a significant impairment in dNOR performance of healthy mice upon 14 days of treatment with haloperidol (these studies are discussed in detail in **Chapter 1.7.2 and Chapter 3**). While these findings are consistent with the performance of rats in all Hal-Low and Hal-High treatment groups at dNOR-2 (all groups failed to perform the dNOR-2 task), it fails to explain the successful performance of rats in all treatment groups in dNOR-3. Therefore, the ability of the scVeh/scPCP-Hal-Low and scVeh/scPCP-Hal-High treatment groups to successfully perform in dNOR-3 is unlikely to be due to the effects of haloperidol treatment. If viewed independent of the dNOR, the pattern of results from the cNOR-2 and cNOR-3 suggests impairment in the performance of the task in the scVeh-Hal-High and scPCP-Hal-High treatment groups. A similar deficit is also apparent in both scVeh (at cNOR-2 and cNOR-3) and scPCP (at cNOR-3 only) treatment groups that were exposed to lower dose of haloperidol due to contamination. However, considering the inconsistencies in the outcome of the dNOR tests it is also plausible that the apparent impairment in performance of the cNOR-2 and cNOR-3 is due to similar inconsistencies. In addition, the findings of the dNOR-3 and cNOR-3 were further confounded because of interventions implemented in the automated-LMA study conducted on day 17 of treatment. This is further discussed in the next page.

Considering the short interval between the NOR pair testing sessions, different sets of objects were presented to the animals to minimise practice effect and over-familiarisation with objects (**Figure 2.2** and **Table 2.2**). Furthermore rat-object allocations were carefully counterbalanced (**Figure 2.3**). Therefore, the apparent deficit in performance of cNOR-2 and cNOR-3 cannot be explained by a general sense of familiarity with the objects. Indeed, this is reflected in the total object exploration times which fall within a similar range for dNOR-2 and cNOR-2 as well as dNOR-3 and cNOR-3. Furthermore, the LMA was similar between all treatment at all tested time points, except at cNOR-2, where LMA was significantly lower in the scPCP-Hal-High treatment group in comparison to the scVeh-Hal-High treatment group. Collectively, the findings of total object exploration time and LMA confirm that animals' underperformance is not due to sedation and a general disinterest to move in the NOR test box. External and environmental variables including the time of testing, room temperature and lighting and home-cage cleaning times, were all kept consistent throughout the study and are unlikely to have contributed to the observed effects.

Hyper-locomotor activity in response to psychostimulants such as Amph in scPCP-treated rats is well documented in the literature (Jentsch et al., 1998; Balla et al., 2003; Beninger et al., 2010; Janhunen et al., 2015). In this study, the scPCP-induced Amph sensitisation was tested, primarily to ensure that the scPCP treatment had been effective. Based on previous findings (Jentsch et al., 1998; Balla et al., 2003; Beninger et al., 2010; Janhunen et al., 2015), it was hypothesized that the total LMA in the "scPCP-control" groups will be significantly higher than the "scVeh-control" treatment groups. Given the presence of contamination in the treatment groups previously designated as "controls", the validity of this hypothesis cannot be reliably confirmed in this study. However, the finding of marginally higher (p=0.054) LMA count in the scPCP-Hal-Low compared to the scVeh-Hal-Low treatment group in response to Amph (1mg/kg; i.p.), may point towards the effectiveness of the scPCP dosing regimen (**Figure 4.8 B**; Refer to **Table 4.10** for a summary of the findings). It must be noted that with sample sizes as low as 4 in some treatment groups, the LMA study was statistically under powered. It is possible that in case of higher power and sample size, a robust Amph-induced hyper-locomotor activity would have been observed in the scPCP-Hal-Low-Amph treatment group. The confounding effect of small sample size could have been eliminated by implementing a cross-over design, so that all rats receiving an acute dose of Amph would receive an acute dose of Veh and vice-versa. This would have also eliminated the potentially confounding effect of Amph treatment on dNOR-3 and cNOR-3 performance, which were assessed after the automated-LMA study.

In the present study, total LMA count of the scVeh and scPCP rats contaminated with low-dose haloperidol (0.25 mg/kg/day; see **Section 4.5.1**) for 17 days at the time of Amph injection was significantly higher than their respective control groups (scVeh-Hal-Low-Veh and scPCP-Hal-Low-Veh). A similar effect was observed when comparing total LMA count of the scVeh-Hal-High-Amph and scPCP-Hal-High-Amph treatment group with their respective control (scVeh/scPCP-Hal-High-Veh). Elevated LMA in response to dopamine releasing agents is a well-validated model of psychosis, as psychostimulants such as Amph exacerbate psychotic symptoms in patients with schizophrenia (Adams and Moghaddam, 1998; Phillips et

al., 2001). Both typical and atypical APs have previously been reported to attenuate or inhibit the LMA response to psychostimulants (Phillips et al., 2001; Samaha et al., 2007). It is also understood that prolonged treatment with APs is accompanied by reduction or complete loss of treatment efficacy, manifested as enhanced sensitivity to dopamine releasing agents such as Amph (dopamine super-sensitivity phenomenon) (Seeman et al., 2005). For instance, in a study of healthy male Sprague Dawley rats, continuous treatment with haloperidol (0.25 and 0.75 mg/kg/day- minipump delivery) blocked the Amph-induced LMA for up to 2 days of treatment. Treatment efficacy was lost when tested on day 12-13 of treatment and the LMA response to Amph was elevated to control levels and was similar in rats treated with low or high dose of haloperidol (Samaha et al., 2007). In the present study, however, the total LMA count was higher in the scVeh/scPCP-Hal-Low-Amph treatment groups in comparison to scVeh/scPCP-Hal-High-Amph treatment groups. This effect reached significance in the scPCP-Hal-Low-Amph compared to scPCP-Hal-High-Amph only (**Figure 4.8 B** and **Table 4.10**). This suggest that, in contrast to the findings of Samaha and colleagues (2007), treatment with high-dose of haloperidol (0.5 mg/kg/day) may have been more efficacious than its lower dose in reducing the effect of Amph treatment on LMA. Furthermore, while Samaha and colleagues (2007) report a complete loss of treatment efficacy, it appears that this effect was more subtle and gradual in rats treated with high dose of haloperidol. Discrepancies between the findings of the present study and that of Samaha et al (2007) could be due to differences in the sex and strain of rats used in these studies. It is essential to note that, due to contamination and lack of appropriate control groups, these findings must be interpreted with caution and are subject to further assessments.

Indeed, the presence of contamination and the consequent lack of appropriate control treatment groups strongly undermines the conclusive power of the behavioural findings of this study. In the present study, the effectiveness of the scPCP treatment could not be confidently confirmed by the findings of the dNOR-1 test. Although rats in the scPCP treatment group could not perform the dNOR-1 task, a similar deficit in novel object detection was present in the scVeh treatment group, confounding the findings of the dNOR-1 test. Nevertheless, the findings of the LMA test (marginally higher total LMA count in the scPCP-Hal-Low-Amph vs. scVeh-Hal-Low-Amph treatment group) may suggest that the scPCP treatment has been effective. It is noteworthy that, in contrast to the previous study (**Chapter 3**), in the present study rats were not handled for habituation purposes prior to the scPCP dosing regimen. They were however, handled for weighing purposes on a daily basis 7 days prior to osmotic minipump implant until the end of the study. In spite of this, the scPCP induced deficit appears to have been established. This observation further highlights the significance of the protective effect of handling against scPCP treatment (see **Chapter 3**). Nevertheless, the influence of daily handling cannot be reliably excluded. It is plausible that in the absence of handling the observed scPCP effect would have been more robust.

As discussed in detail in **Chapter 1.3.1**, the NOR test is an ethologically relevant behavioural paradigm which exploits the natural tendency of rodents to explore novelty. Performance in this test does not depend on lengthy training periods or acquisition of rules and illuminates the influence of motivation to retrieve food

reward on performance. However, given its spontaneous nature, this task is more sensitive to external factors that might influence spontaneous exploratory behaviour including stress, noise and handling (Chan et al., 2018). Therefore, performance in the NOR test tends to be more variable than performance in a rule-dependent tasks (Lyon et al., 2012), leading to ambiguous findings that can be misinterpreted (Chan et al., 2018). As also alluded to in the previous chapter, this could explain the inconsistencies in the findings of the dNOR and cNOR in the present study.

One way to overcome this variance is to increase the number of trials in a day. This is readily achievable by using the continual trial NOR task. Initially introduced by (Albasser et al., 2010) and later adapted by Ameen-Ali et al (2012), this task allows for the same animal to be tested several times and minimises the element of handling during task performance. Briefly, animals are initially placed in an E-shaped holding area with doors that connect the central and the side arms to an E-shape object holding area. Rats then shuttle through the central arm to the object-holding area where they explore two identical objects (A; sample/acquisition phase) for 2-3 minutes. Rats then go back to the holding area through the side-arms for the duration of the inter-trial interval followed by test/retention phase of the task. During this phase, the animals are presented with a duplicate of the familiar (A) and a novel (B) object which they explore for 2-3 minutes, followed by the return to the holding area. Animals are let back into the object area which now contains a familiar (B) and a novel object (C). The procedure continues until rats complete a set number of trials. A similar setting has been applied so that animals are presented with a sample phase each time as it is more comparable to a traditional NOR task (Ameen-Ali et al., 2012). This task could have provided a more reliable paradigm for studying the objectives of the present study. Alternatively increasing the inter-trial delay also could have increased the reliability of the test (Lyon et al., 2012).

Although the dNOR and cNOR behavioural findings are inconclusive, this study provided two lines of evidence that can positively contribute to current knowledge. The first finding was the unexpected high levels of haloperidol in the plasma of the treated groups. Previous studies have reported lower haloperidol plasma concentration for a dose of 0.25 mg/kg (2.40 ± 1.01 ng/ml) and 0.5 mg/kg (4.7 ± 0.32 ng/ml) drug plasma concentrations in male Sprague-Dawley rats following 7 days (Kapur et al., 2003) to 8 weeks (Vernon et al., 2012) of treatment delivered via osmotic minipumps, respectively. This study showed that, in line with clinical observations, the plasma levels of the drug are higher in females in comparison to males for the same dose of the drug (Seeman, 2004; Weston-Green et al., 2010). This is an important finding as the doses of treatments should be adjusted for each sex. The second finding was the gradual loss of treatment efficacy in Hal-High treatment groups compared to Hal-Low. Where previous studies in male rats show a complete loss of haloperidol efficacy (both at low and high doses) to block Amph-induced hyper locomotor activity at early stages of treatment, this study showed a more subtle loss of efficacy at later stages in treatment which could be attributed to strain and sex differences in rats used. However, making definitive conclusions for the latter point is beyond the limits of the data presented here as in this study only one time point was considered and appropriate treatment groups were lacking.

The first two experimental chapters discussed in this thesis aimed to provide insight into the effect of long-term treatment with haloperidol and olanzapine on cognition. The next two chapters aim to characterise the neural correlates of cognition, specifically in the ventral hippocampus (v.Hipp) – medial prefrontal cortex (mPFC) pathway in the scPCP model for cognitive impairments for schizophrenia. This is an essential step towards understanding the influence of long-term treatment with haloperidol on the v.Hipp-mPFC pathway in this well validated animal model.

Chapter 5

Study 3 – Characterising the synaptic properties of ventral hippocampus - medial prefrontal cortex pathway in the sub-chronic PCP model for schizophrenia

5.1. Introduction

As previously described in **Chapter 1.9**, the hippocampal formation (HF) and prefrontal cortex (PFC) independently contribute to a range of mnemonic and cognitive functions (Eichenbaum, 2017; Godsil et al., 2013). Most importantly, the functional interaction between these two brain regions allows for complex computation of higher-order cognitive functions. For instance, functional interaction between HF-PFC is reported in humans and rodents during working memory tasks (Anderson et al., 2010; Benchenane et al., 2010; O'Neill et al., 2013; Meyer-Lindenberg et al., 2005; Sigurdsson and Duvarci, 2015), episodic memory (Veyrac et al., 2015; Gerlach et al., 2011) and context-dependent learning and memory retrieval (Barch and Ceaser, 2012; Brown et al., 2014; Milad et al., 2007; Navawongse and Eichenbaum, 2013). Their interaction is also essential for contextual emotional regulation and successful completion of a goal-directed task (Godsil et al., 2013; Jin and Maren, 2015; Numan, 2015).

The functional relatedness of these two brain regions is reflected in their anatomy (discussed in detail in **Chapter 1.9**). HF and the PFC are strongly connected through direct and indirect pathways (Eichenbaum, 2017). In both rodents and primates, a unidirectional monosynaptic connection from the area CA1/subiculum of the ventral hippocampus (vHipp) to the mPFC provides fast communication between these two regions (Jay et al., 1989; Jay and Witter, 1991). While there are no direct connections between the dorsal hippocampus (dHipp) to the mPFC, the latter strongly influences hippocampal function via bidirectional connections to the parahippocampal regions including the entorhinal cortex (Vertes, 2004) and the perirhinal cortex (Agster and Burwell, 2009) and via the thalamic nuclei (Cassel et al., 2013) allowing the formation of a functional loop between all these regions (Jin and Maren, 2015).

Executive processes mediated by the PFC exert a top-down cognitive control on memory processing in the HF (Blumenfeld et al., 2011; Preston and Eichenbaum, 2013; Rich and Shapiro, 2009) while the hippocampus generates contextual and spatial representations of events that can modulate the ongoing cognitive task (Colgin, 2011). Neurons in the dHipp can rapidly form specific contextual and spatial representations of a specific event (Komorowsky et al., 2013). As such the dHipp assumes the role of a comparator (Duncan et al., 2012). Accordingly, the dHipp can create a match/mis-match signal by comparing the mPFC-mediated cognitive strategy in response to an event with the contextual representations of previous experiences (Kumaran and Maguire, 2006; Numan, 2015). The resulting match/mis-match signal is then communicated to the mPFC via vHipp, whereby the cognitive strategies can be modified and consolidated (Komorowski et al., 2013; Preston and Eichenbaum, 2013; Eichenbaum, 2017).

Emerging evidence suggests that the vHipp plays an important role in synchronising the interaction between the mPFC and dHipp during memory related paradigms (O'Neill et al., 2013) and facilitates goal-directed behaviours (Floresco et al., 1997; Taylor et al., 2016). In addition, interaction between vHipp and mPFC is essential for processing contextual fear memory and extinction (Hugues and Garcia, 2007) to guide

avoidance behaviour (Padilla-Coreano et al., 2016). Given its strong anatomical connections to the amygdala, vHipp may also provide any affective component of memory to the mPFC (Moser and Moser, 1998). The glutamatergic vHipp innervations to the mPFC are restricted to the prelimbic (Prl), infralimbic and medial orbital areas (Jay et al., 1989; Jay and Witter, 1991; Degenetais et al., 2003). Stimulation of vHipp neurons produces an excitatory response in the mPFC (Laroche et al., 1990; Burette et al., 1997) that is primarily mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) (Jay et al., 1992). Indeed, the ability of vHipp neurons to modulate synaptic weight in the mPFC is well characterised in rodents (described in detail in **Chapter 1.10**). Paired-pulse stimulation (PPS) of vHipp neurones can induce short-term synaptic facilitation at the mPFC synapses (Laroche et al., 1990). Tetanic stimulation of vHipp can establish NMDA receptor (NMDAR) mediated (Jay et al., 1995) long-term synaptic potentiation (LTP) in the mPFC that can persist for several hours in anaesthetised rats (Laroche et al., 1990) and for several days in awake freely moving rats (Taylor et al., 2016; Jay et al., 1996). This LTP was found to be readily reversible (undergo depotentiation) under specific stimulation patterns (Burette et al., 1997). Furthermore, long-term synaptic depression (LTD) has also been observed in the mPFC neurons upon repeated series of short high-frequency trains (5 pulses at 250 Hz) applied at 1 Hz to the vHipp (Takita et al., 1999). As described in **Chapter 1.8**, processes of synaptic plasticity are fundamental to processing of cognitive information. Dynamic modulation of synaptic weight in the vHipp-mPFC pathway further highlights the significance of this pathway in higher-order cognitive functions.

In schizophrenia research, much attention has been focused on the HF and PFC as localised structural and functional abnormalities in these brain regions are core features of the disease pathophysiology (Bast, 2011; Colgin, 2011; Arnold et al., 2015; van Erp et al., 2016; Bahner and Meyer-Lindenberg, 2017; van Erp et al., 2018). In addition, considerable evidence from functional imaging studies suggests that the interaction between HF-PFC is strongly compromised in patients with schizophrenia during resting state (Zhou et al., 2007a; Zhou et al., 2007b; Zhou et al., 2008) and memory-related tasks (Meyer-Lindenberg et al., 2005; Benetti et al., 2009). These aberrant functional interactions which are independent of disease stage are also observed in first episode patients and in individuals at risk of developing psychosis (Benetti et al., 2009; Godsil et al., 2013). Such abnormal activities could explain the cognitive impairments and abnormal emotional regulation associated with the disease (Milad et al., 2007; Gerlach et al., 2011; Godsil et al., 2013). Emerging evidence also highlights the significance of these disruptions in emergence of psychosis (Samudra et al., 2015; Reilly et al., 2017).

Disruption in HF-PFC functional coupling and synaptic plasticity has also been reported in neurodevelopmental (e.g. maternal immune activation) (Dickerson et al., 2010; Dickerson et al., 2012) and genetic (Sigurdsson et al., 2010; Sigurdsson, 2016) models for schizophrenia and pharmacological models of psychosis (acute NMDAR antagonist) (Blot et al., 2015). Reduced connectivity between these regions is also reported in the sub-chronic phencyclidine (scPCP) model of the disease (Dawson et al., 2014; Dawson et al., 2015) which is compatible findings in schizophrenia patients (Samudra et al., 2015). Of the available

functional imaging studies in humans and electrophysiological studies in animals, most have focused on the interaction between the mPFC and dHipp, interruption of which is consistent with deficits in the formulation of cognitive strategies as well as interruptions in the comparator system in patients with schizophrenia (Numan, 2015). However, the extent to which vHipp contributes to these abnormalities is less well characterised.

Amongst modulatory neurotransmitters, noradrenaline plays an important role in regulating the aforementioned cognitive domains mediated by HF-PFC functional interaction (Ramos and Arnsten., 2007; Do-Monte et al., 2010; Xing et al., 2016; Borodovitsyna et al., 2017). Dysregulation in noradrenergic transmission has been implicated in the pathophysiology of schizophrenia and the emergence of cognitive impairments associated with the disease (Yamamoto and Hornykiewicz., 2004). Although post-mortem and metabolite findings of noradrenaline dysregulation are inconsistent in these patients (Yamamoto and Hornykiewicz., 2004; Borodovitsyna et al., 2017), treatment with compounds that reduce noradrenaline availability such as clonidine and guanfacine (α_2-adrenoceptors agonists) and propranolol (β-adrenoceptors antagonist) have been reported to improve cognitive impairments and positive symptoms associated with the disease, respectively (Arnsten., 2004; Miyamoto et al., 2005).

Indeed, noradrenaline strongly influences synaptic plasticity in the vHipp-mPFC pathway (Lim et al., 2010). For instance, systemic injection of idazoxan (α_2-adrenoceptor antagonist; 4 mg/kg; i.p.) significantly enhanced LTP in the mPFC of anaesthetised rats by elevating mPFC noradrenaline levels. Contrary to this, LTP was inhibited upon systemic injection of clonidine (α_2-adrenoceptor agonist; 1mg/kg; i.p.) by significantly reducing the availability of noradrenaline (Lim et al., 2010). However, the influence of β-adrenoceptors on processes of synaptic plasticity in this pathway is less well characterised. Understanding the effects of β-adrenoceptors is important as their activation is involved in mediating cognitive processes that require contextual and emotional appraisal of stimuli (such as fear extinction memory), which is one of the core functions of the vHipp-mPFC pathway (Ramos and Arnsten., 2007; Hugues and Garcia., 2007; Do-Monte et al., 2010).

In order to address these gaps in knowledge, this study aimed to investigate and characterise the synaptic properties of the vHipp-mPFC pathway in the sub-chronic PCP (scPCP) model for cognitive impairments in schizophrenia. To this end, the ability of the synapses to establish short-term synaptic plasticity (STP), LTP and its subsequent reversal (depotentiation) as well as LTD was investigated. This study employed two recording protocols to effectively characterise potential excitability differences between the scPCP and controls. This study also examined the influence of β-adrenoceptors on synaptic plasticity of the vHipp-mPFC pathway by blocking its effect using propranolol.

5.2. Materials

5.2.1. Animals

53 adult female Lister Hooded rats (Charles Rivers, UK) with starting weight of 190-230 g and average age of 11 weeks were used in this study. This study lasted for approximately 14 weeks. Housing and general husbandry conditions were identical to those described in **Chapter 2.1.**

5.2.2. Sub-Chronic PCP/Vehicle Treatment

All rats were randomised to receive vehicle (Veh; 0.9% Saline, intraperitoneal injections (i.p.), n=25) or PCP (2 mg/kg; i.p., n=28) twice a day for 7 days followed by a 7-day washout (WO) period. This is the standard operating protocol used in our laboratory, which is described in more detail in **Chapter 2.3.**

5.3. Experimental Procedures and timeline

On the last day of WO period from the scPCP/scVeh treatment, performance of animals was tested in the disrupted novel object recognition task (dNOR-1) to ensure that the scPCP treatment had been effective (refer to **Chapter 2.4.1** for a detailed description of the dNOR protocol). This test was repeated at study mid-point (dNOR-2; 9-weeks post scPCP treatment) in a subset of animals that had not yet undergone electrophysiological recording. Following dNOR-1, animals were subjected to electrophysiological recording procedures under general anaesthesia, details of which are described in **Chapter 2.6.** Details of the recording protocol are also described in **Chapter 2.6** and a schematic summary of the experimental procedures and timeline of this study are presented in **Figure 5.1.** Briefly, a recording multi-electrode array (2x16 electrode, linear channel configuration) was placed in the Prl region of the mPFC (B+3.2 mm, ML= 1.5 mm, 10˚ from midline, depth=4 mm) and the stimulating electrode was placed in the ventral CA1 (B-6.5 mm, ML= 5.5 mm, depth=4.4-5.5 mm). A spontaneous recording (30 mins), followed by recording during sensory stimulation (toe-pinch every 30s for 20min) in a subset of rats was obtained from the mPFC prior to stimulation of the vHipp. These data were obtained for another experimenter and will not be presented in this thesis. Following spontaneous and sensory-evoked recording, synaptic connectivity was examined in all rats using an input-output protocol (I/O) through electrical stimulation of CA1 (vHipp) and recording in Prl. At this point rats were divided into cohort 1 (n=33) and cohort 2 (n=20) to be tested in different recording protocols. Baseline STP was examined in both cohorts using a PPS protocol. Next, induction of long-term synaptic plasticity was examined. By application of high-frequency stimulation (HFS) to the vHipp the ability of the vHipp-mPFC synapses to support LTP was examined in cohort 1 (n=33; scPC*P*=18, scVeh=15). This was followed by low-frequency stimulation (LFS) of the vHipp (45 mins post HFS) in the same group, to examine the subsequent reversal of LTP (depotentiation) in the vHipp-mPFC pathway. STP was re-examined post-HFS and post-LFS **(protocol A)**. In cohort 2 (n=20; scPC*P*=10, scVeh=10), rats were first exposed to LFS to determine the capacity for LTD which was then followed by HFS (30 mins post LFS) to

test for reversal of any LFS-induced effects/or induction of LTP. In this cohort, 5 rats from each treatment group were treated with an acute dose of propranolol (Pro; β-adrenoceptor antagonist;10 mg/kg; i.p.), 30 minutes prior to the LFS. The dose selected for propranolol treatment is commonly used in behavioural studies examining the effect of β-adrenoceptors on fear extinction memory in rats (Do-Monte et al., 2010). This pre-treatment time was selected based on evidence suggesting that propranolol concentration is highest in cortical areas at 30 minutes post-administration (Elghozi et al., 1979; Goh and Manahan-Vaughan., 2013). STP was re-examined post-LFS and post-HFS (**protocol B)**. At the end of recording all rats underwent transcardial perfusion under terminal anaesthesia and brain tissue was collected and processed as detailed in **Chapter 2.8. Figure 5.1** represents a schematic summary of the experimental procedures and timeline of this study.

5.3.1. Exclusion Criteria

At all tested time points, behavioural trials were excluded from analysis if animals either explored objects for one or less than one second or jumped onto the edge of the NOR test box. Based on these criteria only 1 rat was excluded from the analysis of dNOR-1 behavioural task. Exclusion criteria for electrophysiological analysis were based on *post-hoc* electrode placement confirmation. Accordingly, rats were excluded from final analysis if either stimulating or recording electrode missed the regional target (See **Chapter 2.8.3** for examples of histological electrode placement confirmation). Based on this criterion, 10 rats were excluded from the final analysis. Furthermore, 2 rats died under anaesthesia, prior to recording. Accordingly, 19 scVeh and 22 scPCP treated rats were included in the final analysis of the electrophysiological data. **Table 5.1** provides a detailed summary of these exclusions for both behavioural and electrophysiological studies presented in Chapter 5.

5.4. Statistical Analyses

Data obtained from the behavioural tasks were analysed using mixed-design two-way (task and treatment) ANOVAs to examine the main effect of task (exploring left/right identical object in acquisition phase and novel/familiar in retention phase; within-subjects variable) and treatment (between-subjects variable). Planned paired Student's t-tests were employed to compare between novel/familiar object exploration time within individual treatment groups when appropriate. Independent Student's t-test was also employed to compare total object exploration time between treatment groups. DI data were analysed using an independent samples t-test. Mann-Whitney U test was also used to compare mean DI values against zero. Electrophysiological data were analysed using mixed-design or repeated measures two-way ANOVAs followed by *post-hoc* Bonferroni analysis when appropriate. For Bonferroni pair-wise comparisons, the significance threshold (α value) was set **at 0.05**. Adjusted *p*-values were calculated by multiplying the uncorrected *p*-value (obtained from LSD planned comparisons) by the number of comparisons (number of comparisons are defined in appropriate sections throughout the results section in this chapter). This is the method recommended by IBM SPSS Statistics Support (2016a) to adjust for multiple comparisons using

Bonferroni correction. All statistical analyses (except for the assessment of long-term plasticity which were analysed in GraphPad Prism Version 8.0.2) were conducted using IBM SPSS (Version 23). For details on tests of normality and homogeneity of variance on NOR and the electrophysiological data, refer to **Chapter 2.4.1.5** and **Chapters 2.6.5.1** and **2.6.5.2**, respectively.

<table>
<tr><th rowspan="2">NOR testing session</th><th colspan="4">Treatment</th><th rowspan="2">Total Excluded</th></tr>
<tr><th colspan="2">scVehicle (n=25)</th><th colspan="2">scPCP (n=28)</th></tr>
<tr><td>dNOR-1</td><td colspan="2">1</td><td colspan="2">-</td><td>1</td></tr>
<tr><td></td><td colspan="2">scVehicle (n=8)</td><td colspan="2">scPCP (n=11)</td><td></td></tr>
<tr><td>dNOR-2</td><td colspan="2">-</td><td colspan="2">-</td><td>-</td></tr>
<tr><td rowspan="2">Electrophysiology
(Exclusion from both cohorts based on criterion)</td><td colspan="2">scVehicle (n=25)</td><td colspan="2">scPCP (n=28)</td><td></td></tr>
<tr><td colspan="2">6</td><td colspan="2">6</td><td>12</td></tr>
<tr><td>Number of rats included in final analysis of electrophysiological data</td><td colspan="2">Total scVehicle (n=19)
cohort 1 (n=11)
cohort 2 (n=8)</td><td colspan="2">Total scPCP (n=22)
Cohort 1 (n=12)
Cohort 2 (n=10)</td><td></td></tr>
<tr><td>I/O (both cohorts)</td><td colspan="2">8 excluded due to expressing a positive going response</td><td colspan="2">2 excluded due to expressing a positive going response</td><td>10</td></tr>
<tr><td>Cohort 1 (STP)</td><td colspan="2">1 died prior to post-HFS PPS and 1 died prior to post-LFS PPS</td><td colspan="2">-</td><td>See Section 5.5.3.1 and Figure 5.4</td></tr>
<tr><td>Cohort 1 (HFS/LFS)</td><td colspan="2">2</td><td colspan="2">1 excluded due to recording technical issues</td><td>3</td></tr>
<tr><td></td><td>scVeh-control (n=4)</td><td>scVeh-Pro (n=4)</td><td>scPCP-control (n=5)</td><td>scPCP-Pro (n=5)</td><td></td></tr>
<tr><td>Cohort 2 (STP)</td><td>-</td><td>-</td><td>-</td><td>1 died prior to post-HFS PPS</td><td>See Section 5.5.4.1 and Figure 5.6</td></tr>
<tr><td>Cohort 2 (LFS/HFS)</td><td>-</td><td>-</td><td>-</td><td>1, 1 excluded due to recording technical issues</td><td>2</td></tr>
</table>

Table 5.1. Summary of the number of rats excluded from behavioural and electrophysiological assessments in Chapter 5. dNOR-2 was conducted approximately 9 weeks post scPCP treatment on the specified number of rats that had not yet undergone electropysiological recordings. Rats that were excluded from the I/O analysis due to expressing a positive going response were included in other stages of the analysis. In cohort 1 (STP) , 2 rats died under anaeshesia prior to post-LFS recordings. These two rats were excluded from analysis when comparing between baseline, post-HFS and post-LFS STP (see section 5.5.3.1) and when analysing the effect of HFS/LFS. Similarly, 1 rat from cohort 2 (STP) died under anaesthesia prior to post-HFS recordings. This rat was also excluded from analysis when comparing between baseline, post-LFS and post-HFS STP (see section 5.5.4.1) and when analysing the effect of LFS/HFS.

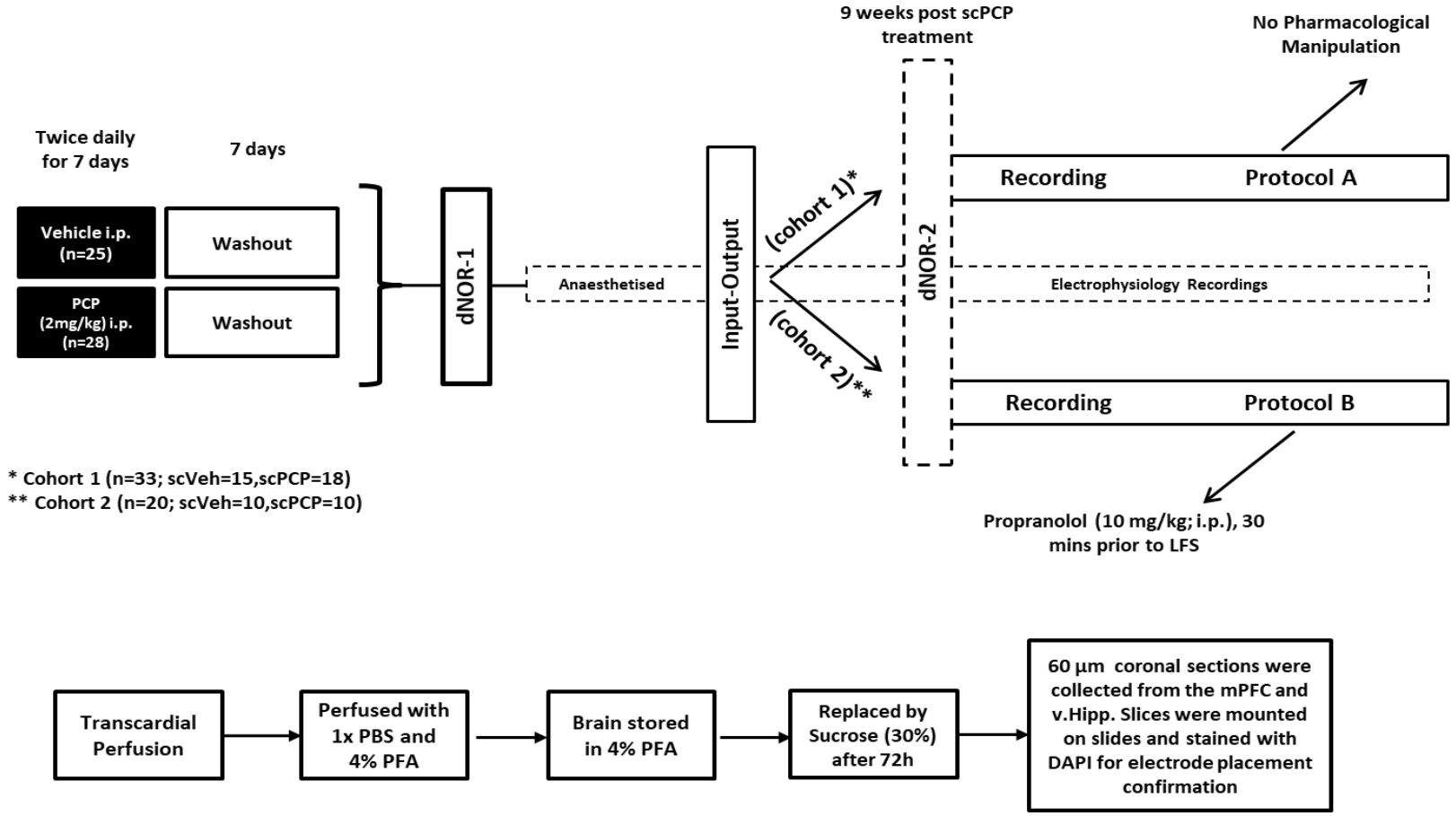

Figure 5.1. A schematic summary of the experimental timeline, tissue preparation and storage for Chapter 5. All rats were tested in dNOR1, following which they underwent acute electrophysiological recording under anaesthetised conditions. All rats were exposed to the same input-output recording protocol after which they were separated into cohort 1 and cohort 2 and followed recording **protocol A** and recording **protocol B**, respectively. Performance in the dNOR task was re-tested 9 weeks post-scPCP treatment in a subset of rats that had yet not undergone electrophysiological recording (dNOR-2). Brain samples were collected following transcardial perfusion and tissue was prepared as previously described.

5.5. Results

5.5.1. scPCP-induced deficit in dNOR performance was present after WO period and was still evident 9 weeks post-scPCP treatment

5.5.1.1. Acquisition Phase

Two-way (treatment and task) ANOVA showed that scVeh and scPCP rats spent similar time exploring the left/right identical objects at both dNOR-1 ($F_{1, 50}$ = 0.03, p=0.86) (**Figure 5.2 A1**) and dNOR-2 ($F_{1, 17}$ = 0.01, p=0.91) (**Figure 5.2 B1**). Treatment had no significant effect on total object exploration time at dNOR-1 ($F_{1,50}$ =0.20, p=0.65) and dNOR-2 ($F_{1,17}$=0.01, p=0.91). Independent Student's t-test also did not detect a significant difference between the treatment groups in the total object exploration time at dNOR-1 (t_{50}=-0.45, p=0.65) and dNOR-2 (t_{17}=-0.11, p=0.90) (**Table 5.2**). There was also no significant treatment x task interaction at dNOR-1: ($F_{1,50}$ =0.00, p=0.96) and dNOR-2 ($F_{1,17}$ =1.89, p=0.18) on total object exploration time.

5.5.1.2. Retention Phase

In both scVeh and scPCP treatment groups, there was a significant difference in the novel/familiar object exploration times at both dNOR-1 ($F_{1,50}$ = 13.96, p<0.0001) and dNOR-2 ($F_{1,17}$ = 14.09, p<0.01). Paired Student's t-test *post-hoc* analysis showed that rats in the scVeh treatment group explored the novel object significantly more than the familiar at dNOR-1 (t_{23}=4.05, p<0.0001) (**Figure 5.2 A2**) and dNOR-2 (t_{7}=3.08, p<0.05) (**Figure 5.2 B2**). This effect was absent in the scPCP treatment group at both tested time points (**Figure 5.2 A2 and B2**). There was no significant main effect of treatment on total object exploration times at either of the tested time points: dNOR-1 ($F_{1,50}$ = 0.10, p= 0.74) and dNOR-2 ($F_{1,17}$ = 0.38, p= 0.54). Independent Student' t-test also did not detect a significant difference between the treatment groups in the total object exploration time at dNOR-1 (t_{50}=-0.32, p=0.74) and dNOR-2 (t_{17}=0.62, p=0.54) (**Table 5.2**). There was a significant task x treatment interaction on total object exploration time at dNOR-1 ($F_{1,50}$ = 5.11, p<0.05) but not at dNOR-2 ($F_{1,17}$ = 1.77, p=0.20).

5.5.1.3. Discrimination Index

Results of independent Student's t-test revealed that the mean DI value was significantly lower in the scPCP in comparison to the scVeh treatment group at both dNOR-1: (t_{50}=3.46, p<0.01) and dNOR-2 (t_{17}=2.28, p<0.05). Results of the Mann-Whitney U test showed that the mean DI value for the scVeh (U=78, p<0.0001) but not the scPCP (U=286, p=0.15) rats was also significantly superior to zero at dNOR-1 (**Figure 5.2 A3**). Similarly, at dNOR-2, mean DI value for scVeh (U=12, p<0.01) but not scPCP (U=36, p=0.07) was significantly superior to zero (**Figure 5.2 B3**).

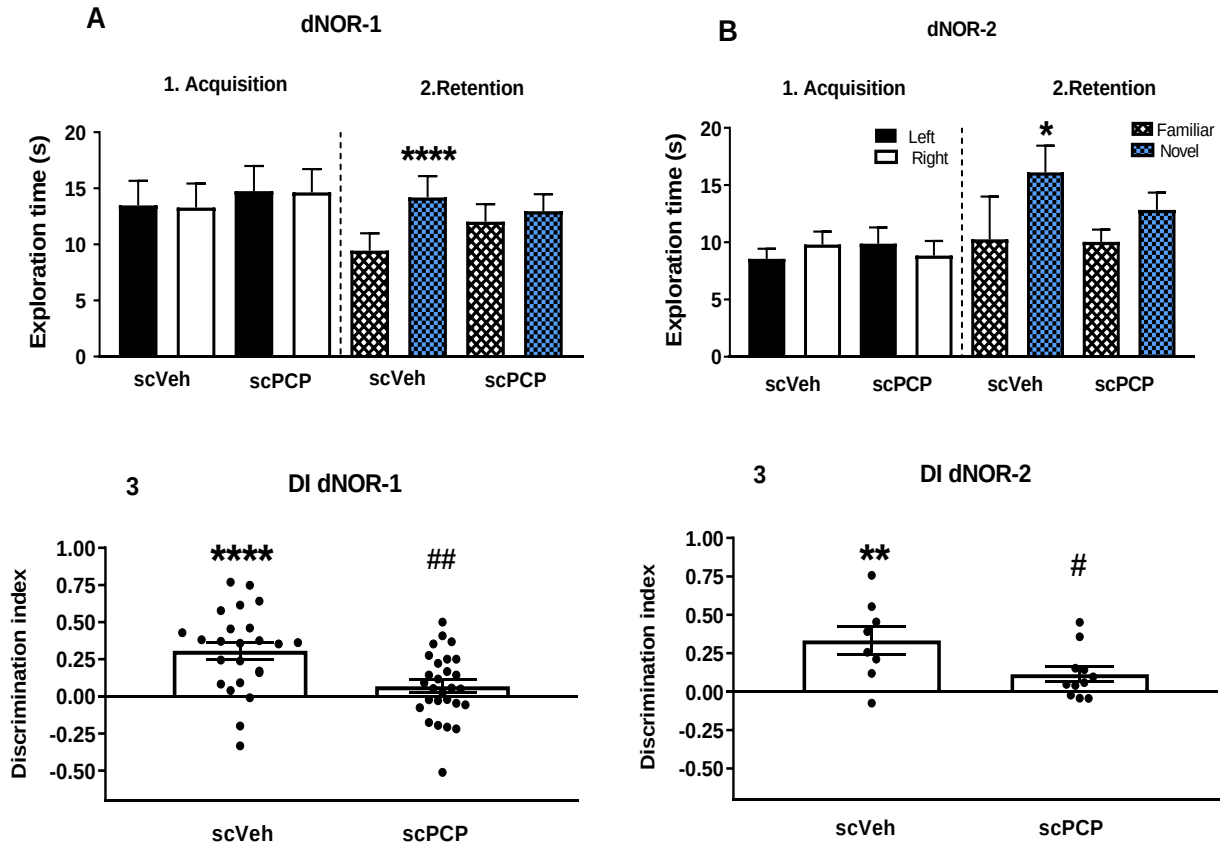

Figure 5.2. Performance in the dNOR task 1 week (A: dNOR-1) and 9 weeks (B: dNOR-2) post-scPCP treatment. (A1 and B1) rats in both treatment groups spent similar time exploring the identical objects at both tested timepoints (A2 and B2) scVeh treated rats explored the novel object significantly more than the familiar object at both tested time points. This effect was absent in the scPCP treated rats at both tested time points. *p<0.05, ****p<0.0001 (A3 and B3) Ratio of novel to total object exploration time. #p<0.05, ##p<0.01 vs. scVeh; **p<0.01, ****p<0.0001 vs. zero. Data are presented as Mean ± SEM. dNOR-1 (n=24-28/group), dNOR-2 (n=8/11group).

NOR	Treatment	Mean total object exploration times (s)	
		Acquisition phase	Retention phase
dNOR-1	scVehicle (n=24)	26.75 ± 4.13	23.63 ± 3.15
	ScPCP (n=28)	29.38 ± 4.13	24.98 ± 2.79
dNOR-2	scVehicle (n=8)	18.35 ± 1.56	26.37 ± 5.94
	ScPCP (n=11)	18.72 ± 2.48	22.87 ± 2.21

Table 5.2. Total object exploration time in each phase of dNOR-1 and dNOR-2. The total object exploration time was not significantly different between the scVeh and scPCP treatment groups at the acquisition and the retention phase of the dNOR at either tested time points. Data are presented as Mean ± SEM, dNOR-1 (n=24-28/group), dNOR-2 (n=8-11/group).

5.5.2. Synaptic connectivity in the vHipp-mPFC pathway is weaker in the scPCP treated rats

In order to assess the synaptic connectivity of vHipp-mPFC pathway 20 pairs of pulses (PP; P1 and P2) were applied to the vHipp at a range of currents intensities to evoke population field excitatory post-synaptic potentials (fEPSPs) in the mPFC. The 20 paired responses were then averaged and the slope and amplitude of the mean P1 responses were measured; comparing these against stimulus intensity and group (scVeh vs. scPCP) revealed any changes in synaptic functional integrity in the vHipp-mPFC. From the scVeh treatment group, 8 rats expressed a positive going response (possibly due to electrodes residing outside the region of maximum response negativity) while the remaining 11 expressed negative going responses. From the scPCP treatment group, 2 rats expressed a positive going response and was excluded from the analysis of I/O response. Therefore, in the analysis of the I/O responses, there were 11 rats in the scVeh and 20 in the scPCP treatment groups.

A two-way (treatment and current intensity) ANOVA on slope showed that current intensity ($F_{2.13,62.04}$= 27.52, p<0.0001) and treatment ($F_{1, 29}$ = 9.16, p<0.01) had a significant effect on response slope. There was also a significant treatment x current intensity interaction on response slope ($F_{2.13,62.04}$= 7.08, p<0.01). Analysis of simple effects using independent (α=0.05, p-value adjusted for 8 comparisons) and paired Student's t-tests (α=0.05, p-value adjusted for 28 comparisons per treatment group) adjusted by Bonferroni corrections for multiple comparison revealed that in both treatment groups, response slope increased as a function of current intensity, however there was no significant difference between response slope at one current intensity compared to the one before in either treatment groups. In both treatment group response slope was steepest at 800µA. This reached significance in comparison to 100µA (p<0.05) only in the scVeh treatment group and in comparison to 100µA (p<0.0001) and 300µA (p<0.01) in the scPCP treatment group. At both treatment groups, response slope was least steep at 100µA. This reached significance in comparison to 300-700µA (p<0.0001) in the scPCP treatment group only. Response slope was steeper in scVeh compared to the scPCP at 300-800µA treatment group. This effect did not reached significance following Bonferroni corrections for multiple comparisons (**Figure 5.3 A**).

A two-way (treatment and current intensity) ANOVA on amplitude showed a significant main effect of current intensity on response amplitude ($F_{2.44, 70.83}$ = 35.88, p<0.0001). There was however no significant main effect of treatment ($F_{1,29}$ = 0.00, p=0.94) and no significant treatment x current intensity interaction ($F_{2.44, 70.83}$ = 0.44, p=0.87) on response amplitude. Similar to slope measures, the response amplitude increased as a function of current intensity. Bonferroni pair-wise comparisons showed that across both treatment groups response amplitude was significantly higher at 200µA vs. 100µA (p<0.01), 300µA vs. 200µA (p<0.01), 400µA vs. 300µA (p<0.01). There were no other significant pair-wise differences between response size at consecutive current intensities. The amplitude of the fEPSP was highest at 700µA for both scVeh and scPCP treatment groups. This reached significance in comparison to 100-300µA (p<0.0001). Response

size was also significantly lower at 100µA compared to 200µA (p<0.01) and all other current intensities (p<0.0001) (**Figure 5.3 B**).

Collectively, results suggest that in both scVeh and scPCP treatment groups, slope and amplitude of the fEPSP response increases as a function of current intensity. This effect reaches a plateau at 600-700 µA for the slope of scPCP treatment group and the amplitude measures of both treatment groups. The strength and size of the response is highest at 800µA and 700µA respectively in both treatment groups. Rats sub-chronically treated with PCP show a deficit for input strength of synaptic connectivity (measured by slope of fEPSP) in the vHipp-mPFC pathway. Response slope in the scPCP was generally lower than scVeh treatment group at 300-800µA, but this effect did not reach significance following Bonferroni corrections for multiple comparisons. There was no significant difference in response amplitude (size) between the treatment groups.

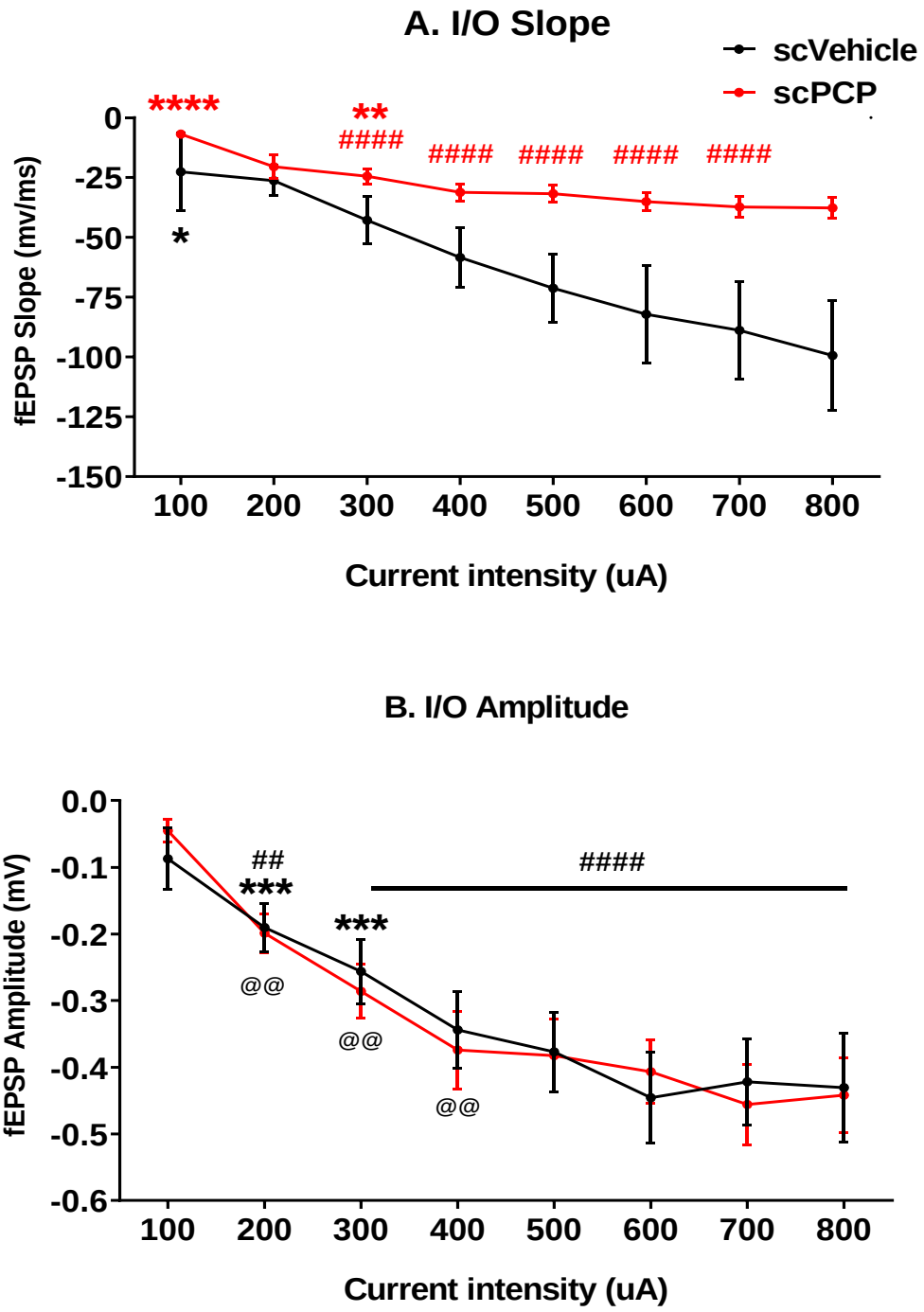

Figure 5.3. Current input-output response relationship. (A) Represents the fEPSP slope and (B) amplitude of negative going responses as a function of current intensity in scVeh and scPCP treatment groups. Measurements were obtained from the P1 of average waveform. All comparisons were made by two-way (treatment and current intensity) ANOVA followed by *post-hoc* Bonferroni test to detect any pair-wise differences. (A) *p<0.05, **p<0.01, ****p<0.0001 vs. 800µA; ####p<0.0001 vs 100µA (red symbols for scPCP only; black symbols for scVeh only) (B) ***p<0.001 vs. 700µA; ##p<0.01, ####p<0.0001 vs. 100µA; @@p<0.01 comparisons of response size at one current intensity vs. one before. In (B), all symbols represent comparisons with treatment grouped together. Data are presented as Mean ± SEM, (n=11-20/group).

5.5.3. Cohort 1 (Recording Protocol A)

5.5.3.1. Short-term synaptic plasticity is similar for the scPCP and scVeh treated rats

In order to assess the ability of the system to support STP, 20 PP at varying inter-pulse intervals (IPI) (25, 50, 100, 150, 200, 500 and 1000ms) were applied to the vHipp, using the current intensity that elicited half the maximum amplitude calculated from the I/O curve (Max response amplitude at 700µA: (-0.43 ± 0.25)). This was usually at approximately 300µA (-0.27 ± 0.17). The paired-pulse protocol was executed at 3 time points for each experimental subject: prior to application of high-frequency stimulation (baseline), after application of HFS (post-HFS) and after LFS (post-LFS). This was to determine both the pre-or post-synaptic locus of LTP expression and to examine the behaviour of synapses in response to high and low frequency stimulation. Slope and amplitude were first measured from an average waveform from P1 and P2 and then P2 measures were normalised to P1 to calculate percentage change as a paired-pulse index (PPI: PPI>0 represented PP facilitation (PPF); PPI<0 represented PP depression (PPD)) (see **Figure 5.4 B1** for an example of PPF and PPD).

A mixed-design two-way (treatment x IPI) ANOVA showed a significant main effect of IPI on slope PPI at baseline ($F_{2.77,58.29} = 4.27$, $p<0.05$) and post-LFS ($F_{2.41,45.81} = 5.97$, $p<0.01$) but not post-HFS ($F_{2.50,50.15} = 2.69$, $p=0.06$). There was no significant difference in slope PPI between the scVeh and scPCP treatment groups at baseline ($F_{1,21} = 0.11$, $p=0.74$), post-HFS ($F_{1,20} = 0.10$, $p=0.75$) and post-LFS ($F_{1,19} = 1.30$, $p=0.26$). There were also no significant treatment x IPI interaction on slope measurements at baseline ($F_{2.77,58.29} = 0.61$, $p=59$), post-HFS ($F_{2.50,50.15} = 0.64$, $p=0.56$) and post-LFS ($F_{2.41,45.81} = 1.15$, $p=0.33$) (**Figure 5.4 A1, A2, A3**). Under all tested conditions, response slope was highest at 50ms IPI for both scVeh and scPCP treatment groups *Post-hoc* Bonferroni comparisons (α=0.05, *p*-value adjusted for 21 comparisons/recording condition) did not detect any significant pair-wise differences in baseline slope PPI between 50ms IPI and any other IPIs (**Figure 5.4 A1**). Pair-wise also comparisons did not detect any significant differences in post-HFS slope PPI between 50ms IPI and any other IPI. However, it detected that post-HFS, slope PPI was significantly higher at 50ms IPI and 100ms IPI in comparison to 500ms IPI ($p<0.05$) across both treatment groups (**Figure 5.4 A2**). However, post-LFS, slope PPI at 50ms IPI was significantly higher in comparison to all other IPIs (100 and 500 ms IPI: $p<0.01$, 150, 200 and 1000 ms IPI: $p<0.05$) but not 25ms IPI (**Figure 5.4 A3**).

A mixed design two-way (treatment x IPI) ANOVA showed a significant main effect of IPI on amplitude PPI at baseline ($F_{3.20,67.23} = 4.9$, $p<0.01$) and post-LFS ($F_{2.95,56.17} = 5.04$, $p<0.01$) but not post-HFS ($F_{3.62,72.49} = 2.13$, $p=0.054$). There was no significant difference in amplitude PPI between the scVeh and scPCP treatment groups at baseline ($F_{1,21} = 0.08$, $p=0.76$), post-HFS ($F_{1,20} = 0.28$, $p=0.60$) and post-LFS ($F_{1,19} = 0.87$, $p=0.36$). There were also no significant treatment x IPI interaction on amplitude PPI measurements at the baseline ($F_{3.20,67.23} = 0.25$, $p=0.28$), post-HFS ($F_{3.62,72.49} = 0.80$, $p=0.56$) and post-LFS ($F_{2.95,56.17} = 1.28$, $p=0.28$) (**Figure 5.4 B1, B2, B3**). Mean amplitude PPI was highest at 50ms IPI across both treatment

groups. *Post-hoc* Bonferroni pair-wise comparisons (α=0.05, *p*-value adjusted for 21 comparisons per recording condition) detected that this reached significance in comparison to 500 ($p<0.05$) IPI. In addition, baseline amplitude PPI was significantly lower at 500ms ($p<0.05$) and 1000ms ($p<0.01$) IPIs compared to 100ms IPI (**Figure 5.4 B1**). Post-HFS, Amplitude PPI was highest at 100ms IPI but this did not reach significance in comparison to any other IPIs. Pair-wise comparisons however, detected that amplitude PPI was significantly higher at 150ms IPI in comparison to 500ms IPI ($p<0.05$) (**Figure 5.4 B2**). Post-LFS, amplitude PPI was highest at 25ms IPI but this did not reach significance compared to any other IPIs. Pair-wise comparisons detected that amplitude PPI was significantly lower at 500ms in comparison to 50ms and 100ms ($p<0.05$) and 150ms ($p<0.01$) IPIs (**Figure 5.4 B3**).

Repeated measures two-way (IPI and stimulation pattern) ANOVAs were also conducted separately for scVeh and scPCP groups to examine the main effect of stimulation pattern on STP. Results revealed no significant effect of stimulation pattern on the PPI for scVeh (slope: ($F_{2,16}= 1.62$, $p=0.22$), amplitude: ($F_{2,16} =1.25$, $p=0.31$)). Please note that 2 rats from the scVeh treatment group died under anaesthesia prior to post-HFS and post-LFS. These 2 rats were excluded from all tested conditions for this section of analysis. Stimulation pattern also did not have a significant effect on the PPI in the scPCP (slope: ($F_{2,22}= 2.35$, $p=0.12$), amplitude: ($F_{2,22} =2.59$, $p=0.09$)) treatment group. This suggests that for each treatment group, there is no significant difference in the slope and amplitude PPI across all three PPS conditions.

Collectively these findings show that slope and amplitude PPI are highest at short (25ms and 50ms) IPIs and decrease at higher (100-1000ms) IPIs. The strength (as measured by slope) and size (as measured by amplitude) of STP is similar between the scVeh and scPCP treatment groups at each tested condition. Furthermore, for scVeh and scPCP treatment groups, there was no significant difference in the slope and amplitude PPI across all tested conditions, indicating a post-synaptic locus for LTP expression in both treatment groups.

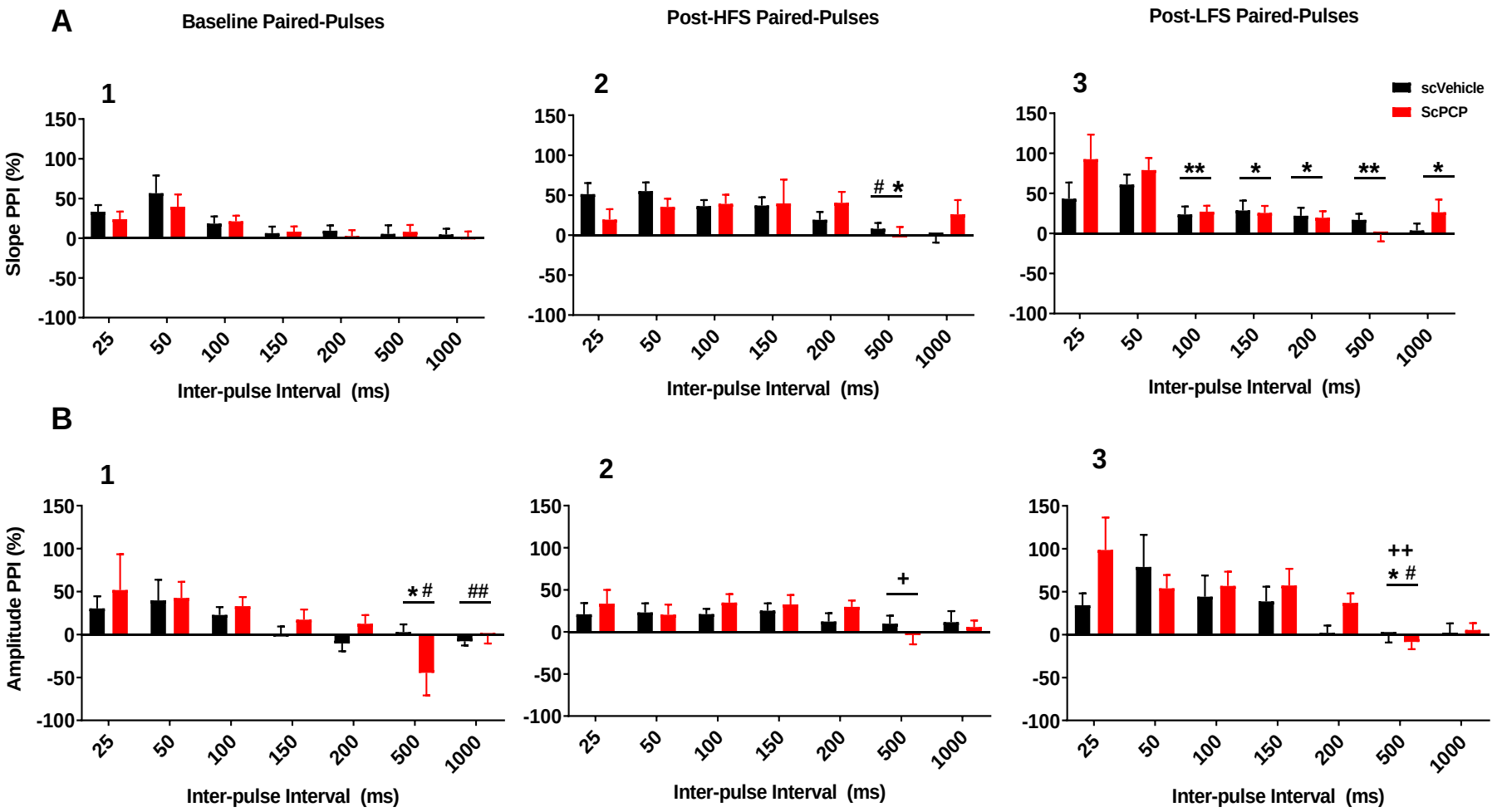

Figure 5.4. Short term plasticity using Paired-Pulse stimulation for cohort 1 (Recording protocol A). (1) Baseline, (2) post-HFS and (3) post-LFS PPI for slope (A1-3) and amplitude (B1-3). STP is predominantly manifested as PPF in mPFC synapses upon PPS of the vHipp. Data were analysed by two-way (treatment and IPI) ANOVAs. There was no significant difference between the scVeh and scPCP treated rats under each tested condition. *Post-hoc* Bonferroni pair-wise comparisons between slope and amplitude PPI at various IPIs for both treatment groups are represented on the graphs. *p<0.05, **p<0.01 vs. 50 ms IPI; #p<0.05, ##p<0.01 vs. 100 ms IPI, +p<0.05, ++p<0.01 vs. 150 ms IPI. Data are presented as Mean ± SEM, (scVeh n=11 and scPCP n=12 at baseline. Post-HFS, 1 rat from the scVeh group died under anaesthesia, therefore post-HFS scVeh n=10, scPCP=12; Post-LFS, another rat from the vehicle group died under anaesthesia, therefore post-LFS scVeh n=9 and scPCP n=12).

5.5.3.2. HFS-induced LTP is stronger in the scPCP in comparison to scVehicle treatment group while the effect of LFS in reversing LTP was stronger in scVeh in comparison to scPCP.

In order to assess the ability of synapses in supporting LTP, HFS (5 trains of 20 pulses at 200Hz) was applied to the system. Furthermore, the effect of repetitive LFS (900 PPS, IPI=5 ms, IBP=1 s) on previously potentiated synapses was examined. It was hypothesised that this LFS pattern might reverse the LTP of the system the extent of which might differ in scVehicle and scPCP treated rats. The behaviour of the system was monitored at baseline (pre-HFS), post-HFS and post-LFS by the application of 30-60 PPS (IPI=50 ms, IBP=30s) from which the slope and the amplitude of the first pulse (P1) were measured and analysed.

Mixed-design two-way (treatment x stimulation pattern) ANOVA showed a significant main effect of stimulation pattern ($F_{2,116} = 204.7$, $p<0.0001$) and treatment ($F_{1,118} = 6.25$, $p<0.05$) on the slope of the fEPSP response. No significant interaction between these factors was detected ($F_{2,116} = 2.10$, $p=0.12$). *Post-hoc* Bonferroni pair-wise comparisons (α=0.05, p-value adjusted for 3 comparisons per treatment group) suggest that post-HFS, response slope for scVeh and scPCP treatment group was significantly higher in comparison to baseline measures ($p<0.0001$), suggesting that in both treatment groups, LTP could be successfully established. After application of LFS, response slope was significantly lower in comparison to baseline ($p<0.0001$) and post-HFS ($p<0.0001$) in the scVeh treatment group (**Figure 5.5 A1 and C1**). This represents not just depotentiation of LTP but a significant depression of the response below baseline levels. Similarly, in the scPCP treatment group, response slope was significantly lower post-LFS in comparison to baseline ($p<0.05$) and post-HFS ($p<0.0001$) (**Figure 5.5 A2 and C1**). Further *post-hoc* Bonferroni analysis (α=0.05, p-value adjusted for 3 comparisons) showed that post-HFS, slope potentiation was greater in the scPCP compared to the scVeh treatment group but this does not reach significance (**Figure 5.5 C2**). However, post-LFS, response slope was significantly higher in the scPCP group in comparison to scVeh treatment group ($p<0.05$) (**Figure 5.5 C3**).

A separate mixed-design two-way ANOVA (interval x treatment) was conducted to assess differences between the last 10 minutes of baseline, the first, second and third 10 minute interval of post-HFS and the first 10 minutes of post-LFS. Results revealed a significant interaction between these factors ($F_{4,152} = 3.77$, $p<0.01$). Simple effects were analysed using paired (α=0.05, p-value adjusted for 10 comparisons per treatment group) and independent *(*α=0.05, p-value adjusted for 3 comparisons) Student's t-test corrected for multiple comparisons by Bonferroni analysis. Results revealed that in the scVeh treatment group, response slope in the last 10 minutes of post-HFS was significantly lower than the first 10 minutes post-HFS ($p<0.01$). There were no other significant pair-wise differences between other post-HFS 10 minute intervals. Moreover, all three post-HFS intervals were significantly higher than baseline ($p<0.0001$) and post-LFS ($p<0.0001$). In the scPCP treatment group, response slope was significantly lower at the second compared to first 10 minute interval post-HFS ($p<0.05$), however, response increased again at the last 10 minutes of post-HFS recording and this did not reach significance compared to the first two 10-minute

intervals. All three intervals were significantly greater than baseline (first and third: $p<0.0001$; last: $p<0.01$). Further analysis. The last 10-minute interval post-HFS was significantly higher in the scPCP compared to the scVeh treatment group ($p<0.05$), while there was no significant difference between the other post-HFS intervals between the treatment groups.

A similar format of analysis was performed on the amplitude measurements. Two-way (treatment x stimulation pattern) ANOVA showed a significant main effect of stimulation pattern ($F_{2,116} = 242.6$, $p<0.0001$), treatment ($F_{1,118} = 11.75$, $p<0.001$) as well as a significant interaction ($F_{2,116} = 51.85$, $p<0.0001$), between these factors on amplitude of the fEPSP response. Simple effects were analysed using paired (α=0.05, p-value adjusted for 3 comparisons per treatment group) and independent *(*α=0.05, p-value adjusted for 3 comparisons) Student's t-test corrected for multiple comparisons by Bonferroni analysis. Results of Bonferroni pair-wise comparisons on amplitude detected a similar pattern of results as slope measures. As such, response amplitude for was significantly higher post-HFS in comparison to baseline ($p<0.0001$) for both scVeh and scPCP treatment group (**Figure 5.5 B1, B2 and D1**). In the scVeh treatment group, response amplitude was significantly lower post-LFS in comparison to baseline ($p<0.01$) and post-HFS ($p<0.0001$) (**Figure 5.5 B1, D1**). Similarly, in the scPCP treatment group, response amplitude was significantly lower post-LFS in comparison to baseline ($p<0.0001$) and post-HFS ($p<0.0001$) (**Figure 5.5 B2 and D1**). Further analysis showed that the amplitude of the response in the scPCP treatment group was significantly higher in comparison to the scVeh treatment group at post-HFS ($p<0.0001$) and significantly lower post-LFS ($p<0.05$) (**Figure 5.5 D2 and D3**).

A separate mixed-design two-way ANOVA (interval x treatment) was conducted to assess differences between the last 10 minutes of baseline, the first, second and third 10 minute intervasl of post-HFS and the first 10 minutes of post-LFS. Results revealed a significant interaction between these factors ($F_{4,152} = 27.56$, $p<0.0001$). Simple effects were analysed using paired (α=0.05, p-value adjusted for 10 comparisons per treatment group) and independent *(*α=0.05, p-value adjusted for 3 comparisons) Student's t-test corrected for multiple comparisons by Bonferroni analysis. Results detected that in the scVeh treatment group, response amplitude in the third post-HFS 10 minute interval was significantly lower than the second ($p<0.05$). There were no other significant pair-wise differences between the post-HFS 10 minute intervals with each other. In the scVeh treatment group, the first post-HFS 10 minute interval but not the second and third was significantly higher than baseline ($p<0.05$). In the scPCP treatment group all three 10-minute intervals post-HFS were significantly greater than baseline ($p<0.0001$) and were not significantly different from each other. All three post-HFS 10-minute intervals were significantly greater in comparison to their scVeh counterparts ($p<0.0001$).

Collectively, these results suggest that LTP can be established in the scPCP and the scVeh treatment groups. The magnitude of this potentiation is greater in the scPCP treatment group. Post-HFS, response size was significantly more potentiated in the scPCP compared to the scVeh. When comparing the whole duration of post-HFS recording, there was no significant difference in response slope between treatment groups. However, response slope was significantly steeper in the last 10 mins of post-HFS in the scPCP compared to the scVeh treatment group. LFS of previously potentiated vHipp-mPFC synapses, completely reversed the effect of LTP to values significantly below the baseline for both slope and amplitude measures, suggesting an LTD-like pattern of activity. However, the induced LTD was significantly less robust in the slope measures of the scPCP treatment group compared to control. This is while the opposite effect is observed in amplitude measures. Together, these data might suggest that the vHipp-mPFC pathway is more excitable in the scPCP compared to the scVeh treatment group.

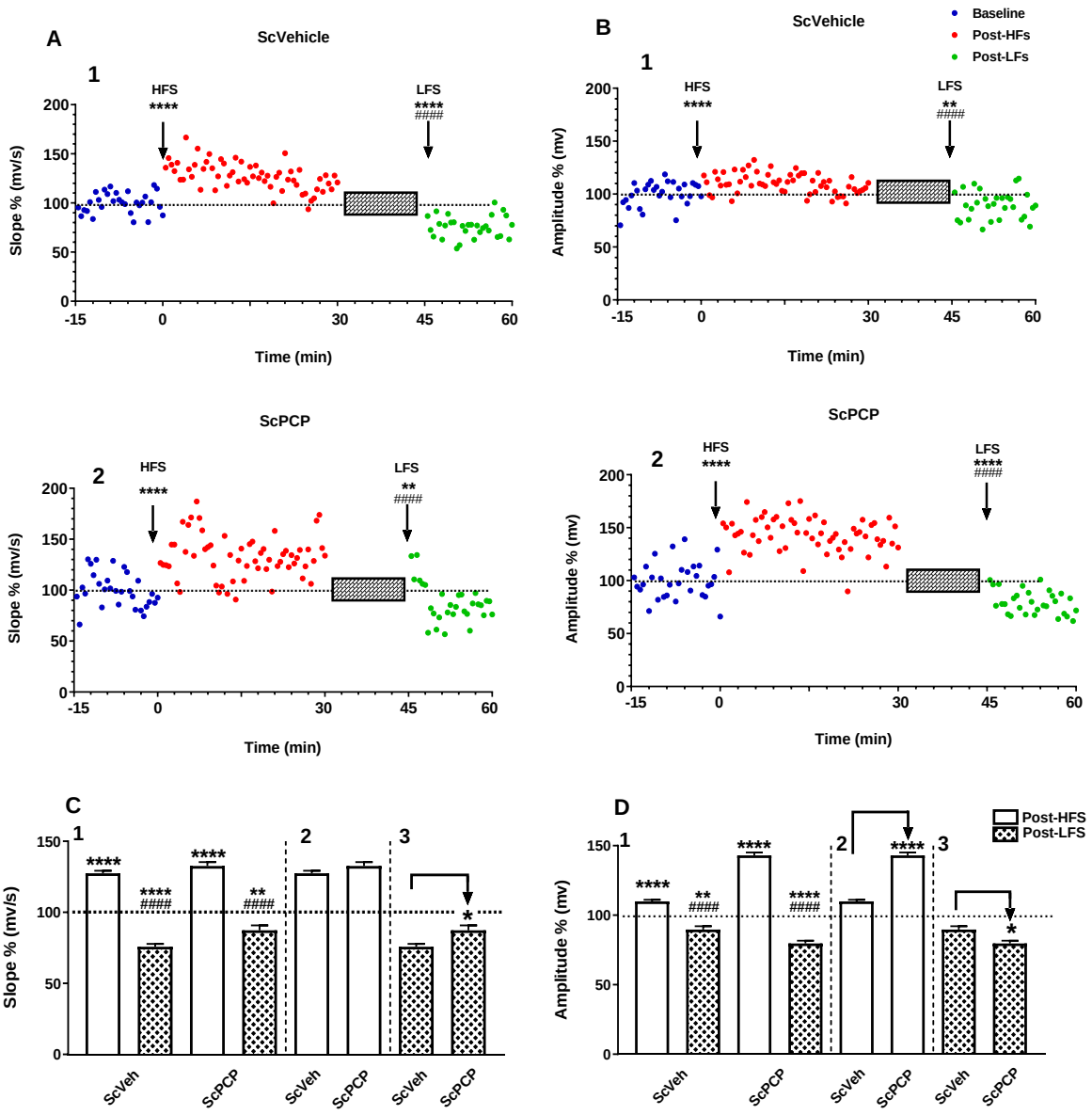

Figure 5.5. LTP induction and reversal in scVeh and scPCP treated rats. P1 slope (A) and amplitude (B) during the baseline (pre-HFS), post-HFS and post-LFS periods, separately for each treatment group (hatched horizontal bars represent the delay between the end of post-HFS recording and application of LFS. Panels C1 and D1 present the same data as A and B, respectively. Panels C2 and C3 as well as D2 and D3 show treatment comparisons within post-HFS and post-LFS conditions separately. Data were analysed with a two-way ANOVA followed by Bonferroni *post-hoc* pair-wise comparisons. For A1-2, B1-2, C1 and D1 **p<0.01, ****p<0.0001 vs. baseline; ####p<0.0001 vs. post-HFS. For C2, C3, D2 and D3: *p<0.05, ****p<0.0001. Data are presented as the Mean ± SEM % change to pre-HFS baseline, (scVeh n=9, scPCP n=11).

5.5.4. Cohort 2 (Recording Protocol B)

5.5.4.1. Short-term plasticity was similar for propranolol treated scVeh and scPCP rats in comparison to controls

Two-way (treatment x IPI) ANOVA showed a significant main effect of IPI on slope PPI at baseline (pre-LFS) ($F_{3.28,46.01}$= 16.96, p<0.0001), post-LFS ($F_{3.42,47.97}$= 4.73, p<0.01) and post-HFS ($F_{3.11,40.53}$= 6.63, p<0.01). There was no significant main effect of treatment on slope PPI at baseline ($F_{3.14}$= 1.47, p=0.26), post-LFS ($F_{3,14}$= 0.15, p=0.92) and post-HFS ($F_{3,13}$= 0.65, p=0.59). *Post-hoc* Bonferroni analysis (α=0.05, p-value adjusted for 6 comparisons/recording condition) also did not detect any significant pair-wise differences in slope PPI between the treatment groups (scVeh-control, scVeh-Pro, scPCP-control, scPCP-Pro). There were also no significant treatment x IPI interaction for slope at baseline ($F_{9.86,46.01}$ = 1.19, p=0.32), post-LFS ($F_{10.28,47.97}$= 0.69, p=0.73) and post-HFS ($F_{9.35,40.53}$= 0.54, p=0.84). Slope PPI was highest at 50ms IPI under all tested conditions (**Figure 5.6 A1, A2, A3**). *Post-hoc* Bonferroni analysis (α=0.05, p-value adjusted for 21 comparisons/recording condition) detected that at baseline, this reached significance in comparison to all other IPIs (25,100,150,500 ms p<0.01; 200,1000 ms p<0.0001). Furthermore, baseline slope PPI was significantly lower at 1000ms IPI compared to 100ms and 200ms (p<0.05) and 150ms IPIs (p<0.01) (**Figure 5.6 A1**). No significant pair-wise differences were detected in post-LFS slope PPI between IPIs across all treatment groups (**Figure 5.6 A2**). Pattern of results for post-HFS slope PPI was similar to baseline. As such slope PPI was significantly higher at 50ms IPI compared to 150 and 1000 ms IPI (p<0.05). Furthermore, post-HFS slope PPI was significantly lower at 1000ms IPI in comparison to 25ms and 150ms IPIs (p<0.05) (**Figure 5.6 A3**).

Two-way (Treatment x IPI) ANOVA showed a significant main effect of IPI on amplitude PPI at baseline ($F_{2.58,36.15}$= 12.72, p<0.0001), post-LFS ($F_{3.21,\ 45.05}$= 7.68, p<0.0001) and post-HFS ($F_{3.45,44.89}$ = 8.38, p<0.0001). There was no significant main effect of treatment on amplitude PPI at baseline ($F_{3,14}$ =1.41, p=0.28) and post-LFS ($F_{3,14}$ =2.78, p=0.08). Post-HFS, there was a significant main effect of treatment on amplitude PPI ($F_{3,13}$= 3.89, p<0.05). *Post-hoc* Bonferroni analysis (α=0.05, p-value adjusted for 6 comparisons/recording condition) did not detect any significant pair-wise differences in amplitude PPI between treatment groups at any of the tested conditions (**Figure 5.6 B1, B2, B3**). There was no significant treatment x IPI interaction on amplitude PPI at baseline ($F_{7.74,\ 36.15}$= 1.31, p=0.27), post-LFS ($F_{9.65,45.05}$= 1.00, p=0.45) and post-HFS ($F_{10.36,44.89}$ = 1.10, p=0.38). Amplitude PPI was also highest at 50ms IPI at baseline. *Post-hoc* Bonferroni analysis (α=0.05, p-value adjusted for 21 comparisons/recording condition) detected that this reached significance in comparison to 200ms (p<0.01), 500-1000ms (p<0.001) IPI. Amplitude PPI was also significantly lower at 500ms IPI compared to 100-150ms (p<0.01) and 200ms IPI (p<0.001). Similarly, amplitude PPI was significantly lower at 1000 ms IPI compared to 100 ms and 150 ms IPI (p<0.01) and 200 ms IPI (p<0.05) (**Figure 5.6 B1**). Post-LFS, amplitude PPI was highest at 100ms IPI and this reached significance in comparison to 500ms (p<0.01) and 1000ms (p<0.001). Furthermore, amplitude PPI

was significantly lower at 500ms IPI compared to 200ms IPIs (p<0.01). Similar pattern emerged for responses at 1000ms IPI where the amplitude PPI was significantly lower in comparison to 50ms (p<0.05) and 150-200ms IPI (p<0.01) (**Figure 5.6 B2**). Post-HFS, amplitude PPI was highest at 50ms IPI. This reached significance compared to 500ms and 1000ms IPI (p<0.01). PPI for two latter IPIs was significantly lower in comparison to 100ms and 150ms IPI (p<0.01) (**Figure 5.6 B3**).

Repeated measures two-way ANOVA (IPI and stimulation pattern) were also conducted separately for each treatment group to examine the main effect of stimulation pattern on STP. Results revealed no significant effect of stimulation pattern on the PPI for scVeh-control (slope: ($F_{1.00,3.02}$= 1.15, p=0.36), amplitude: ($F_{2,6}$ =0.57, p=0.59)), scVeh-Pro (slope: ($F_{2,6}$= 0.00, p=0.99), amplitude: ($F_{2,6}$ =0.57, p=0.59)), scPCP-control (slope: ($F_{2,8}$ = 0.57, p=0.58), amplitude: ($F_{2,8}$ =2.11, p=0.18)) and scPCP-Pro (slope: ($F_{2,6}$= 1.29, p=0.34), amplitude: ($F_{2,6}$ =2.73, p=0.14)) treatment groups. This suggests that for each treatment group, there is no significant difference in the slope and amplitude PPI across all three PP recording conditions. (Please note that in the scPCP-Pro treatment group, 1 rat died under anaesthesia prior to post-HFS. This rat was excluded from baseline and post-LFS in this stage of the analysis).

Reflecting a similar pattern as cohort 1, findings in cohort 2 also showed that the slope PPI was highest at 50ms IPI in all tested conditions and was comparatively lower at shorter (25ms) and longer (100-1000ms) IPIs. A similar pattern of results emerged for amplitude PPI where at baseline and post-HFS, amplitude PPI was highest at 50ms IPI. However, post-LFS, amplitude PPI was highest at 100ms IPI. Results collectively suggest that the strength and the size of STP is similar for the propranolol-treated scVeh and scPCP in comparison to each other and controls at each tested condition. Furthermore, for each treatment group, there was no significant difference in the slope and amplitude PPI across all tested conditions, indicating a post-synaptic locus for the effects of LFS and HFS.

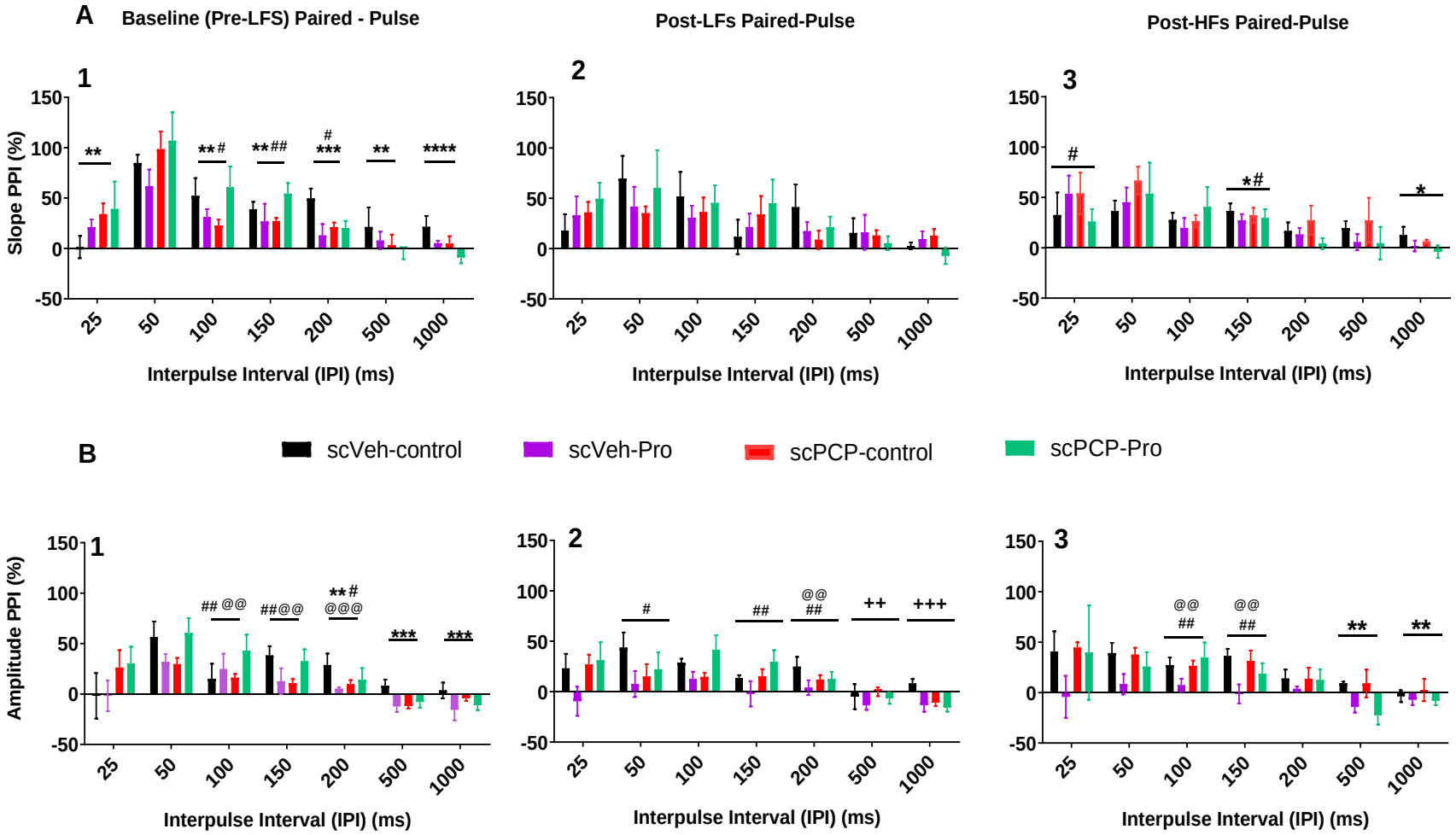

Figure 5.6. Short term plasticity is similar for the propranolol treated scVeh and scPCP rats in comparison to controls (cohort 2; recording protocol B). Baseline (1), post-LFS (2) and post-HFS (3) PPI for slope (A1-3) and amplitude (B1-3). STP is predominantly manifested as PPF in mPFC synapses upon PPS of the vHipp. Data were analysed by two-way (treatment and IPI) ANOVAs followed by Bonferroni *post-hoc* analysis. There were no significant pair-wise differences in slope and amplitude PPI between the treatment groups at any of the tested condition. There was no significant difference in slope and amplitude IPI across all three tested conditions within each treatment group. Pair-wise comparisons for slope and amplitude PPI at various IPIs across all treatment groups are presented on the graphs. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. 50ms IPI; +<0.05, ++p<0.01, +++p<0.001 vs. 100ms IPI; IPI @@p<0.01, @@@p<0.001 vs. 500ms IPI #p<0.05, ##p<0.01 vs. 1000ms IPI. Data are presented as Mean ± SEM, (scVeh-control n=4, scVeh-Pro n=4, scPCP-Veh=5, scPCP-Pro n=5 at baseline and post-LFS. From this treatment group 1 rat died prior to post-HFS. Therefore, post-HFS data from 4 rats were analysed in the scPCP-Pro treatment group).

5.5.4.2. LFS significantly potentiated previously non-potentiated vHipp-mPFC synapses in scPCP rats and this was attenuated by β-adrenoceptor antagonist (propranolol)

Two-way (treatment and stimulation pattern) ANOVA showed a significant main effect of stimulation pattern ($F_{2,232}$= 68.46, p<0.0001) and treatment ($F_{3,116}$= 27.10, p<0.0001) as well as a significant treatment x stimulation pattern interaction ($F_{6,232}$= 14.12, p<0.0001) on slope of the fEPSP response. Simple effects were analysed using paired (α=0.05, p-value adjusted for 3 comparisons per treatment group) and independent (α=0.05, p-value adjusted for 6 comparisons per stimulation pattern) Student's t-test corrected for multiple comparisons by Bonferroni analysis. Results did not detect a significant difference between post-LFS and baseline response slope in the scVeh-control, scVeh-Pro and scPCP-Pro treatment groups (**Figure 5.7 A1, A2, A4 and C1**). In contrast, LFS significantly potentiated response slope compared to baseline (p<0.01) in the scPCP-control treatment group (**Figure 5.7 A3 and C1**). Bonferroni comparisons further detected that in the scVeh-control treatment group, response slope was significantly higher post-HFS compared to post-LFS (p<0.01) but it was not significantly different from baseline (**Figure 5.8 A1 and C1**). No significant pair-wise differences were detected in response slope between the three tested conditions in the scVeh-Pro treatment group (**Figure 5.7 A2 and C1**). In the scPCP-control treatment group, response slope post-HFS was significantly higher compared to baseline (p<0.0001) and post-LFS (p<0.0001) (**Figure 5.7 A3 and C1**). Similarly, response slope in the scPCP-Pro treatment group was significantly higher post-HFS in comparison to baseline (p<0.0001) and post-LFS (p<0.0001) (**Figure 5.7 A4 and C1**).

Further analysis showed that post-LFS, response slope was significantly higher in the scPCP-control compared to the scVeh-control (p<0.0001), scVeh-Pro and scPCP-Pro (p<0.001) treatment groups. There were no other significant pair-wise differences between the treatment groups post-LFS. Post-HFS, response slope was significantly lower in the scVeh-control in comparison to the scPCP-control and scPCP-Pro (p<0.0001) treatment groups. There was no significant difference in response slope post-HFS between scVeh-Pro and its control. Furthermore, response slope post-HFS was significantly lower in the scVeh-Pro treatment group in comparison to the scPCP-control (p<0.0001) and scPCP-Pro (p<0.001) treatment groups. Post-HFS response slope was significantly lower in the scPCP-Pro in comparison to scPCP-control treatment group (p<0.0001) (**Figure 5.7 C2 and C3**).

Two-way (treatment and stimulation pattern) ANOVA showed a significant main effect of stimulation pattern ($F_{2,232}$= 15.46, p<0.0001) and a main effect of treatment ($F_{3,116}$= 11.73, p<0.0001) as well as a significant treatment x stimulation pattern interaction ($F_{6,232}$= 18.46, p<0.0001) on amplitude measurements. Reflecting a similar pattern of results as the slope measurements, analysis of simple effects (as analysed for the slope measures) using Bonferroni corrections for multiple pair-wise comparisons, did not detect any significant differences between post-LFS response amplitude and baseline in the scVeh-control, scVeh-Pro and scPCP-Pro treatment groups (**Figure 5.7 B1, B2, B4 and D1**). In contrast, in the scPCP-control treatment group, post-LFS response amplitude was significantly lower than baseline (p<0.001) (**Figure 5.7 B3 and**

B1). Bonferroni pair-wise comparisons further detected that in the scVeh-control treatment group, post-HFS response amplitude was significantly lower than baseline (p<0.0001) and post-LFS (p<0.01) (**Figure 5.7 B1 and D1**). The opposite effect was observed in the scVeh-Pro treatment group, where post-HFS response amplitude was significantly higher in comparison to baseline (p<0.0001) and post-LFS (p<0.001) (**Figure 5.7 B2 and D1**). In the scPCP-control treatment group, post-HFS response amplitude was significantly higher in comparison to baseline and post-LFS (p<0.0001) (**Figure 5.7 B3 and D1**). In the scPCP-Pro no significant pair-wise differences were detected in the response amplitude between the tested conditions (**Figure 5.7 B4 and D1**).

Further analysis showed that post-LFS response amplitude was significantly lower in scPCP-Veh compared to the scVeh-Veh (p<0.05), scVeh-Pro (p<0.0001) and scPCP-Pro (p<0.05) treatment groups. Post-HFS response amplitude was significantly lower in the scVeh-Veh in comparison to scVeh-Pro (p<0.0001), scPCP-control (p<0.0001) and scPCP-Pro (p<0.001) and in the scPCP-Pro group compared to the scVeh-Pro and scPCP-control treatment groups (p<0.0001) (**Figure 5.7 D2 and D3**).

In summary, LFS of the previously non-potentiated synapses significantly potentiated response slope in the scPCP-control treatment group, but not in any other treatment group. In fact, in the scVeh-control group, response slope was reduced to below baseline but this did not reach statistical significance. HFS further potentiated response slope in the scPCP-control treatment group, an effect that was absent in all other treatment groups. In the scVeh-control treatment group, HFS potentiated response slope to values significantly greater than post-LFS. There was however, no significant difference between post-HFS and baseline slope measures in this treatment group. Following LFS, response amplitude in the scVeh-control treatment group was also reduced to values below the baseline, but this effect was not significant. In the scPCP-control treatment group a similar effect was observed. In this treatment group however, response size was significantly lower than baseline. No significant differences were observed in response amplitude following LFS in the scVeh-Pro and scPCP-Pro treatment groups. Following HFS, response amplitude in the scVeh-control treatment group was significantly reduced to values below the baseline, while they were potentiated to values significantly greater than baseline in the scPCP-control treatment group. A similar effect was also observed in amplitude measures of the scVeh-Pro treatment group while these measures remained unchanged in the scPCP-Pro treatment group compared to baseline.

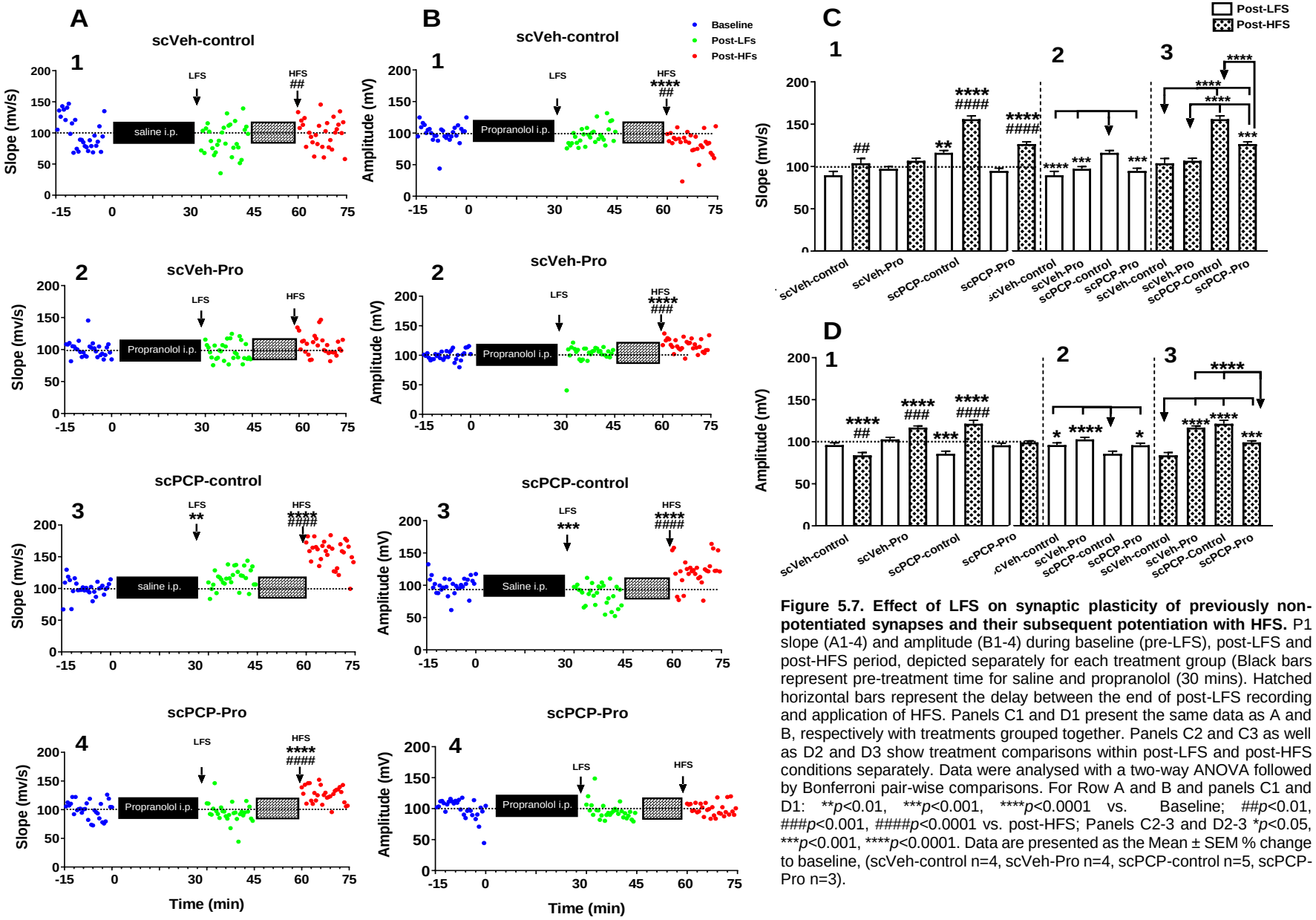

Figure 5.7. Effect of LFS on synaptic plasticity of previously non-potentiated synapses and their subsequent potentiation with HFS. P1 slope (A1-4) and amplitude (B1-4) during baseline (pre-LFS), post-LFS and post-HFS period, depicted separately for each treatment group (Black bars represent pre-treatment time for saline and propranolol (30 mins). Hatched horizontal bars represent the delay between the end of post-LFS recording and application of HFS. Panels C1 and D1 present the same data as A and B, respectively with treatments grouped together. Panels C2 and C3 as well as D2 and D3 show treatment comparisons within post-LFS and post-HFS conditions separately. Data were analysed with a two-way ANOVA followed by Bonferroni pair-wise comparisons. For Row A and B and panels C1 and D1: **p<0.01, ***p<0.001, ****p<0.0001 vs. Baseline; ##p<0.01, ###p<0.001, ####p<0.0001 vs. post-HFS; Panels C2-3 and D2-3 *p<0.05, ***p<0.001, ****p<0.0001. Data are presented as the Mean ± SEM % change to baseline, (scVeh-control n=4, scVeh-Pro n=4, scPCP-control n=5, scPCP-Pro n=3).

5.6. Discussion

Intact functional interaction between the HF and mPFC is essential for processing higher-order cognitive functions including episodic memory and the process of decision making involved in guiding goal directed behaviours. Schizophrenia is associated with impairments in a range of cognitive domains, including those mediated by the HP-PFC pathway (see introduction as well as **Chapter 1.2**). While many of the functional imaging techniques confirm disturbed functional coupling between dHipp and mPFC in patients with schizophrenia (Meyer-Lindenberg et al., 2005; Zhou et al., 2007a; Zhou et al., 2007b; Zhou et al., 2008; Bahner and Meyer-Lindenberg, 2017), the role of vHipp remains relatively less explored (Benetti et al., 2009). Given the strong anatomical connection between vHipp-mPFC, and the role of vHipp in synchronising the activity of dHipp and mPFC (**Chapter 1.10**), it is likely that changes in synaptic plasticity of the vHipp-mPFC pathway have major implications in processing cognitive functions. In this study, the synaptic properties of the vHipp-mPFC pathway were investigated in the scPCP-model for cognitive impairments associated with schizophrenia.

Prior to electrophysiological recordings, behavioural phenotype of animals was examined in the dNOR task, following 7 days of WO from scPCP treatment. This was to ensure that scPCP treatment had been effective. The behavioural test was repeated at experimental midpoint (9 weeks post-scPCP dosing) to ensure that the scPCP-induced deficit had persisted throughout the course of the study. Electrophysiological recordings explored properties of synaptic connectivity, STP and long-term plasticity in the vHipp-mPFC using two recording protocols (**A and B**), results of which are discussed below.

5.6.1. scPCP-induced deficit in dNOR performance was present after the WO period and was still evident 9 weeks post-scPCP treatment

At both tested time points, animals in both scVeh and scPCP treatment groups spent similar time exploring the identical objects in the acquisition phase, suggesting no preference for the location of the object in the NOR test box (i.e. right vs. left). In agreement with previous findings from our laboratory (Grayson et al., 2007; Grayson et al., 2014) and others (McAllister et al., 2015; McKibben et al., 2010) scPCP treatment induced a deficit in the retention phase of the dNOR-1. While the scVeh treated animals explored the novel object significantly more than the familiar in the retention phase, this ability was absent in the scPCP treated rats (**Figure 5.2 A2**). The scPCP-induced deficit was still evident in object exploration times in dNOR-2, 9 weeks post-scPCP treatment (**Figure 5.2 B2**). The scPCP-induced deficit is also reflected in the DI. As discussed in previous chapters, the DI values range between -1<0<+1, with positive values indicating a preference for novelty. The mean DI value in the scVeh but not the scPCP treatment group was significantly greater than zero at dNOR-1 (**Figure 5.2 A3**) and dNOR-2 (**Figure 5.2 B3**), indicating a robust deficit in novelty preference in the scPCP treated rats which persists after 9 weeks of treatment. DI was also significantly higher in the scVeh in comparison to the scPCP treatment group at both tested time points,

further supporting the scPCP-induced deficit in dNOR-1 (**Figure 5.2 A3**) performance which was still present when tested 9 weeks post-scPCP treatment at dNOR-2 (**Figure 5.2 B3**).

5.6.2. Synaptic connectivity in the vHipp-mPFC pathway is weaker in the scPCP treated rats.

Comparisons of fEPSP response slope (an index of response strength) and amplitude (an index of response size) at increasing current intensities (100-800µA) revealed that in both scVeh and scPCP treatment groups, response strength and size increased as a function of current intensity (though not in a strictly linear fashion). This effect appears to have reached a plateau at 700µA and 800µA for slope measures in the scPCP treatment group and in amplitude measures for both scVeh and scPCP (**Figure 5.3 A and B**). The strength of synaptic connectivity in the vHipp-mPFC pathway was significantly weaker in the scPCP in comparison to scVeh treatment group (significant main effect of treatment). A trend towards reduced strength in synaptic connectivity in the scPCP compared to scVeh treatment group was observed at 300-800µA current intensities, however, these effects did not reach significance following corrections for multiple comparisons (**Figure 5.3 A**).

As discussed in depth in **Chapter 1.10**, the basal excitatory transmission in the vHipp-mPFC (as evoked by stimulation patterns employed in assessing synaptic connectivity in this study) is primarily mediated through the AMPARs (Jay et al., 1992). Involvement of NMDARs in mediating basal transmission is unlikely, since its blockade with D-AP5 (NMDAR antagonist) has previously been shown not to affect responses evoked by low-frequency stimulations similar to those employed here (Jay et al., 1992). In addition, in keeping with its role in long-term potentiation in the vHipp-mPFC pathway (Jay et al., 1995), involvement of NMDAR would have been associated with long-lasting alterations in synaptic weight, which were absent in responses observed following stimulation with paired-pulses at varying current intensities in this segment of the study. Therefore, reduced synaptic strength in this pathway in the scPCP treated rats may suggest that the AMPAR-mediated current is weaker in the scPCP treated rats in comparison to controls. Reduced AMPAR-mediated current in the scPCP treated rats is supported by indirect lines of evidence. In a behavioural study, systemic injection of ampakines CX546 and CX516, selective AMPAR modulators which increase AMPAR-mediated currents, were reported to rescue the scPCP-induced deficit in NOR performance (Damgaard et al., 2010).

Post-mortem studies have produced mixed findings on changes in AMPAR sub-unit expression levels in the PFC tissue of patients with schizophrenia (Rubio et al., 2012). Furthermore, to date, there are no studies investigating AMPAR expression alterations in the scPCP treated rats. Therefore, whether altered AMPAR expression contributed to the reduced vHipp-mPFC synaptic strength cannot be confidently confirmed. A more likely explanation of reduced synaptic strength in the scPCP treated rats is the scPCP-induced loss of dendritic spines in layer 2 and 3 of the mPFC (Elsworth et al., 2011) on which the vHipp glutamatergic projections form synapses (Jay and Witter, 1991). Indeed, reduced excitatory input into layer II and III of the PFC is also well documented in schizophrenia (Hoftman et al., 2017).

In contrast to the findings of synaptic strength, response size was similar between the scVeh and scPCP treatment group (**Figure 5.3 B**). This finding is in agreement with Nomura et al (2016) who reported no significant difference in the AMPA-mediated current amplitude measured at various current intensities in hippocampal slice preparations of mice pre-treated with scPCP or scVeh (Nomura et al., 2016). Amplitude of the evoked response in the mPFC is closely regulated by the inhibitory local GABAergic circuitry. The vHipp projections to mPFC form synapses on an important subclass of GABAergic interneurons expressing parvalbumin (PV) calcium binding protein (Gabbott et al., 2002). The critical position of these PV-interneurons (soma and axon initial segment) (Gonzalez-Burgos and Lewis, 2008) which are also recruited via AMPAR activation (Gonzalez-Burgos et al., 2015), in addition to other inhibitory mechanisms enables the vHipp to regulate response size through feed-forward and feed-back processes (Tierney et al., 2004).

Reduced expression of PV is consistently reported in the dHipp and mPFC of scPCP-treated rats (Reynolds and Neill, 2016) which is in line with reduced PV expression in patients with schizophrenia. It is therefore, expected for the inhibitory input to be reduced to the pyramidal neurons (Gonzalez-Burgos et al., 2015), leading to larger response size in the scPCP treated rats. The amplitude findings from the I/O recordings in this study in face of reduced PV expression, may suggest that other regulatory inhibitory mechanisms might also be involved in mediating response amplitude in the scPCP-treated rats. This has been confirmed by evidence derived from studies in brain-derived neurotrophic factor (BDNF) IV promoter (contributes to transcription of BDNF) knockout (KO) mice (Sakata et al., 2009). BDNF-IV KO mice exhibit significant reduction in the expression of PV interneurons in the mPFC, accompanied by reduced inhibitory input to the layer V of the mPFC (Sakata et al., 2009; Sakata et al., 2013). Whole cell and voltage-clamp recordings, however, revealed that this did not have an effect on the amplitude and the frequency of the spontaneous excitatory response (Sakata et al., 2009). This supports involvement of other inhibitory systems or compensatory mechanisms in regulating response size. It is also plausible that AMPAR expression is increased on the inhibitory interneurons as part of a compensatory mechanism to restore inhibitory/ excitatory balance, keeping response size as control level. Confirming the involvement of these suggested mechanisms in the observed effects is subject to further experiments.

5.6.3. Cohort 1 (Recording Protocol A)

5.6.3.1. Short-term synaptic plasticity is similar for scVehicle and scPCP treated rats

STP in the vHipp-mPFC pathway was examined at baseline, post-HFS and post-LFS by delivering PPS of varying IPI to the vHipp. PPI calculations in each tested condition revealed that across all IPIs, STP was predominantly present in form of synaptic facilitation (PPI>0) (in both slope and amplitude) in the mPFC. At baseline and post-LFS tested conditions, response slope facilitation was highest at 50ms IPI and decreased at longer (100-1000ms) IPIs in both scPCP and scVeh treated animals. Post-HFS also response slope PPI was highest at 50 ms IPI but PPI did not decrease at longer IPIs. Statistical analysis did not detect a significant difference in slope facilitation between the scPCP and scVeh treatment at baseline, post-HFS

and post-LFS, suggesting that the mechanisms of excitatory neurotransmitter release and STP were not significantly altered by scPCP treatment. It is noteworthy that although no significant difference was detected between the scVeh and the scPCP treatment groups, baseline slope facilitation appeared to be lower in the scPCP treatment group compared to controls at 25ms and 50ms IPI (**Figure 5.4 A1**). This effect could be explained by scPCP-induced elevation in glutamate release in the mPFC following stimulation of the vHipp (Arvanov and Wang., 1999; Ninan et al., 2003; Amitai et al., 2012).

In comparison to the pyramidal neurons, GABAergic interneurons are more sensitive to the effects of NMDA receptor antagonism. In the presence of acute low-dose MK-801 (0.1 mg/kg; i.p.), the firing rate of inhibitory interneurons is reduced, resulting in disinhibition of pyramidal neurons (Homayoun and Moghaddam, 2007) and excess glutamate release in the mPFC. Indirect measures of disinhibition have also been reported through microdialysis studies, whereby, an acute PCP challenge in PCP-naïve rats and rats repeatedly treated with PCP (2 mg/kg/day, for 5 consecutive days, route of administration not specified) increased glutamate efflux in the mPFC (Amitai et al., 2012). As extensively discussed in **Chapters 1.4 and 1.11,** cortical disinhibition in response to NMDAR antagonists is in part mediated by the vHipp (Suzuki et al., 2002; Jodo et al., 2005). It is therefore plausible that due to the effect of scPCP treatment, PPS of the vHipp is accompanied by excess glutamate release in the mPFC, resulting in reduced PPF at short IPIs. Indeed, the potential contribution of other brain regions such as thalamus in mediating this disinhibitory effect must also be considered (Kiss et al 2011a; Kiss et al., 2011b).

In the present study, synaptic facilitation was also observed in response amplitude in all tested conditions. Pattern of results for amplitude facilitation resembles those of slope facilitation reported above. Amplitude facilitation was highest at 25ms and 50ms IPI in all tested conditions (**Figure 5.4 B1-3**). At baseline and post-LFS, but not post-HFS, amplitude facilitation decreased at longer IPIs. In fact, at baseline, 500ms and 1000ms IPIs were associated with PPD in both scVeh and scPCP treated rats (**Figure 5.4 B1**). There was no significant difference in amplitude facilitation at any IPI between the scVeh and scPCP treatment group at baseline, post-HFS and post-LFS.

These findings are consistent with Nomura et al (2016) who also observed no significant difference in the amplitude facilitation in the scPCP and scVeh mouse hippocampal slices. In contrast, several lines of evidence have reported a significant reduction in PPF in amplitude measures. For instance, PPS (40ms IPI) of the forceps minor was associated with a significant reduction in amplitude PPF in mPFC slices of rats previously treated with scPCP (Arvanov and Wang., 1999; Ninan et al., 2003). In these studies, reduced amplitude PPF was accompanied by a significant increase in excitatory post-synaptic current variance, collectively pointing towards elevated evoked glutamate release in the mPFC (Malinow and Tsien., 1990; Arvanov and Wang., 1999; Ninan et al., 2003). A significant reduction in vHipp-mPFC amplitude facilitation (at 100ms IPI) has also been reported in anaesthetised rats following an acute systemic injection of MK-801 (0.05 mg/kg; intravenous), an effect that was also attributed to elevated glutamate release in the mPFC (Kiss et al 2011a; Kiss et al., 2011b). Findings of elevated glutamate release in these studies could also be

explained by the mechanisms of disinhibition described above. Discrepancies between the amplitude findings of this study and those of others could be attributed to the differences in dosing regimen (scPCP vs. acute MK-801) (Kiss et al 2011a; Kiss et al., 2011b) and the circuitry under investigation (Arvanov and Wang., 1999; Ninan et al., 2003). Collectively, the finding of no significant difference in amplitude facilitation observed in the present study in addition to findings of unchanged response size (amplitude) from the I/O recordings may suggest that the inhibitory mechanisms regulating response amplitude in the vHipp-mPFC pathway have remained intact, while those regulating response slope may be affected.

Whether synaptic protein machinery involved in presynaptic glutamatergic release is affected in the scPCP model remains unclear. Synaptotagmin and synaptosomal-associated protein of 25kDa (SNAP-25) play key roles in the process of neurotransmitter release (Sudhof, 2012). Synaptotagmin acts as a calcium sensor and triggers the chain of events that lead to the activation of the SNAR protein complex of which SNAP-25 is a key member, leading to the process of vesicle docking and neurotransmitter release (Sudhof., 2012). Previous observations have shown marked reduction in expression of synaptotagmin and synaptosomal-associated protein of 25kDa (SNAP-25) in rat cortical cell cultures exposed to PCP over 48 h (Adachi et al., 2013). A trend towards reduction of SNAP-25 in the mPFC was also reported in adult male Sprague-Dawley rats chronically treated with MK-801 (0.1 mg/kg; i.p.) (Liu et al., 2018). This is while, the expression of SNAP-25 in the mPFC remained unchanged in mice sub-chronically treated with MK-801 (0.3 mg/kg/day; i.p.) (Ozdemir et al., 2012).

The pre- or post-synaptic locus of expression of LTP remains a matter of intense debate amongst neuroscientists (Lisman, 2009) with evidence supporting recruitment of pre-synaptic (Kullmann and Siegelbaum, 1995; Yang and Calakos, 2013) or post-synaptic mechanisms (Luscher and Malenka, 2012) or both in expression of LTP (Padamsey and Emptage, 2014). Processes of STP are best understood for presynaptic loci (Zucker and Regehr, 2002). Therefore, examination of release probability through assessment of STP has been used as a method to determine pre/post-synaptic nature of LTP expression. Reduction in the neurotransmitter probability of release (reduced PPF possibly due to depleted neurotransmitter resources) following HFS, usually supports a presynaptic locus for LTP (Yang and Calakos, 2013) while no change in PPF is associated with post-synaptic LTP mechanism (Shin et al., 2011). As such, based on the results of the present study, it can be suggested that the locus of LTP expression was post-synaptic since there was no significant difference between the slope and amplitude PPI in the scVeh and scPCP treatment group post-HFS compared to baseline (**Figure 5.4 A1 and A2, B1 and B2**). A close observation of the pattern of post-LFS slope and amplitude PPI shows that both measures had increased in scPCP and scVeh treatment groups (**Figure 5.4 A1 and 3 B1 and B3**). While post-LFS slope and amplitude PPI were not significantly different from post-HFS and baseline, the involvement of presynaptic mechanisms in supporting the effects of LFS cannot be reliably excluded.

On its own, this approach is insufficient to determine the pre- or post-synaptic nature of LTP expression. As mentioned previously, processes of STP are influenced by post-synaptic processes that can change

PPI without altering neurotransmitter probability of release. For example, increased expression of AMPAR following LTP is a post-synaptic event that influences PPI (Kullmann and Siegelbaum, 1995; Luscher and Malenka, 2012; Yang and Calakos, 2013). Whether these post-synaptic events are involved in eliciting observed responses in the present study requires more in-depth analysis using slice electrophysiology to assess AMPA mediated current prior to and after HFS and LFS. Other methods such as monitoring release probability as a function of progressive reduction in NMDAR-mediated current in presence of non-competitive antagonist and visualisation of presynaptic vesicles using specialised dyes can also be used to determine the locus of expression of HFS and LFS. Future investigations might consider using one or combination of these techniques to determine the nature of LTP and LTD expression in the vHipp-mPFC pathway.

5.6.3.2. HFS-induced LTP is stronger in the scPCP in comparison to scVehicle treatment group while the effect of LFS in reversing LTP was stronger in scVeh in comparison to scPCP.

The ability of the vHipp-mPFC pathway to support LTP was investigated by HFS (5 trains of 20 pulses delivered at 200 Hz) of the vHipp. HFS significantly potentiated the response slope in comparison to baseline measures in both scPCP and scVeh treated rats (**Figure 5.5 A1, A2 and C1**). This potentiation was higher in the scPCP treated rats and reached significance in the last 10 minutes of the post-HFS recording in comparison to controls (**Figure 5.5 C2**).

As extensively described in **Chapter 1.10**, induction of LTP in the vHipp-mPFC pathway is dependent on NMDAR. This is supported by the failure to induce LTP in the presence of NMDAR selective antagonist D-AP5 (Jay et al., 1995). Functional NMDAR are a heteromeric complex protein structures comprised of two obligatory GluN1 subunit and two GluN2 (GluN2A-D) (of the same or difference subtype subunits) or a combination of GluN2 and GluN3 (GluN3A-B) (Traynelis et al., 2010). In the mPFC, the GluN2B subtype strongly influences synaptic plasticity. Specific blockade of GluN2B containing NMDAR in the mPFC inhibits LTP formation upon stimulation of the vHipp afferent projections (Monaco et al., 2015; Flores-Barrera et al., 2014). Chronic administration of PCP (10 mg/kg/day; i.p. for 30 days) (Lindahl and Keifer, 2004) and MK-801 (0.1 mg/kg/day; i.p. for 21 days) (Liu et al., 2018) in Sprague-Dawley rats significantly reduces the expression of GluN1 and GluN2B subunits in the mPFC. In addition, chronic treatment with PCP also significantly reduces receptor protein expression in the CA3 region of the hippocampus (Lindahl and Keifer, 2004).

In light of these findings, the ability of the scPCP treated animals to support LTP (recorded over 30 mins post-HFS) in the vHipp-mPFC is interesting. Of greater interest is the stronger potentiation in scPCP compared to control animals. The process of disinhibition, which was discussed earlier, could account for this finding. In this case, the reduction in the NMDA receptor subunits could be explained as the effort of the system to restore excitatory/inhibitory balance. This is a likely explanation as post-HFS, the amplitude of the response was also potentiated in the scPCP treated rats and this significantly higher than the scVeh

treated animals, suggesting reduced inhibition (**Figure 5.5 B1, B2, D1 and D2**). A similar effect is also observed in other animal models for cognitive deficits associated with schizophrenia. For instance, HFS (2 sets of 10 trains (250Hz, 200ms) delivered 6 mins apart) of the vHipp in MAM treated rats, induced a robust LTP in response amplitude in the mPFC which was significantly higher in comparison to saline treated rats (Goto and Grace, 2006) (**Figure 5.8 C and D**). Similarly, acute systemic injection of MK-801 (0.1 mg/kg) followed by HFS (2 sets of 50 stimuli delivered at 250Hz, repeated 10 times at 0.1Hz, delivered 6 mins apart) of the vHipp induced a gradual and significant potentiation in the vHipp-mPFC response, which was significantly higher than the effects of HFS in saline treated rats (Blot et al., 2015) (**Figure 5.8 A and B**). These similarities could point towards the presence of a common underlying mechanism associated with vHipp-mPFC hyper-excitability.

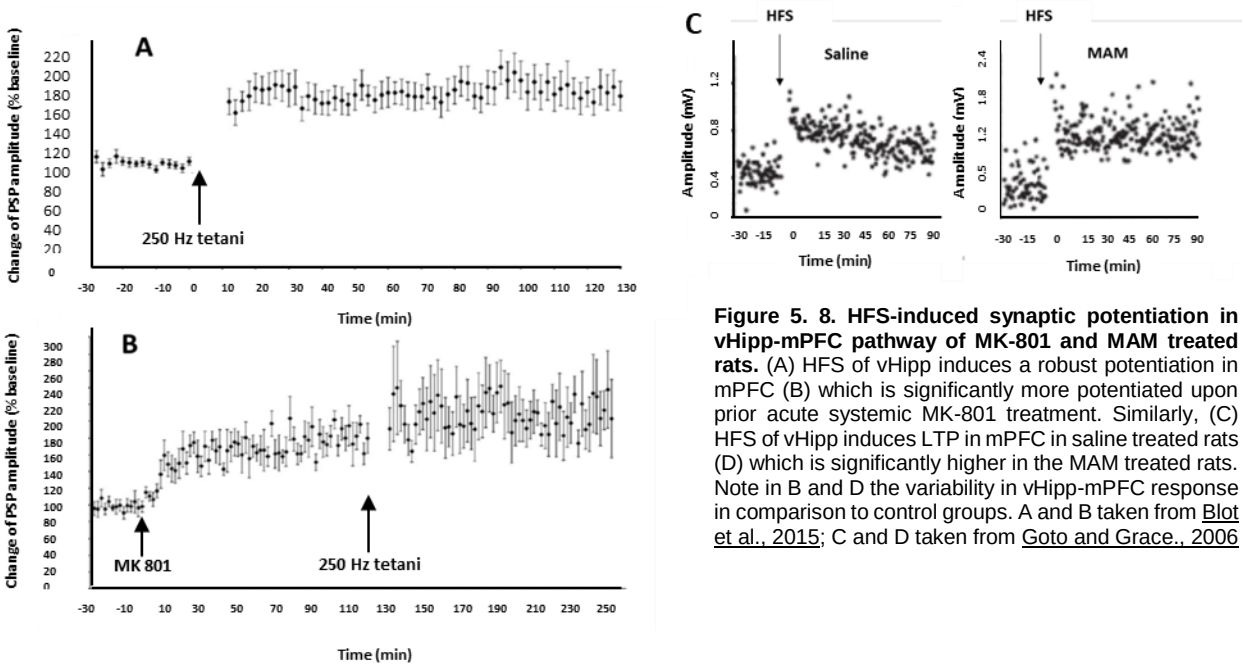

Figure 5. 8. HFS-induced synaptic potentiation in vHipp-mPFC pathway of MK-801 and MAM treated rats. (A) HFS of vHipp induces a robust potentiation in mPFC (B) which is significantly more potentiated upon prior acute systemic MK-801 treatment. Similarly, (C) HFS of vHipp induces LTP in mPFC in saline treated rats (D) which is significantly higher in the MAM treated rats. Note in B and D the variability in vHipp-mPFC response in comparison to control groups. A and B taken from Blot et al., 2015; C and D taken from Goto and Grace., 2006

Furthermore, compensatory NMDA receptor hyperactivity could also account for these findings. This is supported by an *in vitro* study of mPFC pyramidal neurons in male Sprague-Dawley rats sub-chronically treated with PCP (2mg/kg/twice daily for 7 days). In this study, Ninan and colleagues (2003) reported a shift in the concentration-response curve of NMDA but not AMPA receptors to the left as well as a depolarisation of the resting membrane potential in the pyramidal neurons. This may suggest that post-PCP treatment, pyramidal neurons in the mPFC are hyperactive, hence may be hyperplastic (Ninan et al., 2003). Other studies also support this hypothesis. For instance, stimulation of forceps minor or application of NMDA to mPFC slices (10 nM and 20 nM) from rats pre-treated with scPCP (2mg/kg/twice daily for 7 days; i.p. followed by 48-60h or 1 week of WO), significantly enhanced NMDAR-mediated current (Arvanov and Wang, 1999). Authors suggest that the excess glutamate released in response to scPCP treatment acts primarily on the AMPA receptors to depolarise membrane potential, allowing for NMDAR to be activated

(Arvanov et al., 1997; Arvanov and Wang, 1999). Given the differences between *in vivo* and *in vitro* preparations, these findings should be interpreted with caution.

Subsequent attempts to reverse LTP were successful in both scPCP and scVeh treatment groups. Application of LFS (900 PP at 1 Hz) to previously potentiated synapses significantly reduced the slope and the amplitude of the response to values below baseline in both scPCP and scVeh treated animals (**Figure 5.5 A1, A2 and C1**). This represents not just depotentiation of LTP but a significant depression of response (LTD) slope and amplitude. The magnitude of LTD was significantly greater in slope measures in the scVeh treated animals in comparison to the scPCP group (**Figure 5.5 C3**) and in amplitude for the scPCP treatment group compared to control (**Figure 5.5 D3**). Based on these findings it can be hypothesised that, in addition to hyper-excitability at glutamatergic synapses, inhibitory mechanisms that regulate response size are also hyper-excitable, leading to a greater reduction in response amplitude upon LFS (see below for details).

LFS- induced depression in slope and amplitude measures to values below baseline, points towards the ability of the system to completely reverse LTP. Given the relatively short post-LFS recording period (15 mins), it is difficult to determine the effectiveness of this stimulation protocol in inducing LTD. The same LFS protocol (900 PP, 5ms IPI at 1HZ) applied to previously potentiated vHipp synapses has been found to a significantly reduce response amplitude to baseline, followed by a gradual potentiation in response amplitude to values significantly above baseline. A similar LFS protocol (900 single pulses at 1 Hz or 450 PPs, 35ms IPI at 1 Hz) applied to the vHipp has been shown to induce a robust transient depression in previously potentiated synapses at the mPFC, whereby, the observed depression in response recovered 10-30 minutes post-LFS to values observed post-HFS (Burette et al., 1997). This suggests that the LFS protocol has been ineffective in inducing LTD and depotentiation in the vHipp-mPFC pathway. It is therefore plausible that upon longer recording period post-LFS, a different pattern of activity could have emerged in this study.

The processes of long-term synaptic plasticity are Ca^{2+} dependent. In excitatory neurons, the magnitude of Ca^{2+} entry during synaptic activation determines the polarity of any change in synaptic weight (LTP, LTD or no change). While LTP induction requires a significant increase in the concentration of post-synaptic Ca^{2+}, lower levels of Ca^{2+} are required for induction of LTD (Bliss and Cooke, 2011; Luscher and Malenka, 2012). Based on the findings presented here, it can be hypothesised that, for the same level of HFS, the concentration of Ca^{2+} entering pyramidal neurons is higher in scPCP mPFC during HFS, leading to a slightly higher response slope and stronger LTP. In the hippocampus, HFS of inhibitory interneurons results in activation of NMDARs, leading to LTD (Gaiarsa et al., 2002; Gaiarsa and Ben-Ari, 2006). Whether a similar mechanism is involved in inhibitory mechanisms regulating response size in the mPFC is not known and cannot be confidently determined in this study. However, the general pattern of the results suggests that LTD in GABAergic interneurons, in addition to the general reduced inhibition in the scPCP treated rats, might lead to the observation of higher response amplitude following HFS. During LFS of previously

potentiated synapses, Ca^{2+} entry threshold is reduced to the level of LTD (the theory of sliding threshold for long-term synaptic plasticity also known as the MCB rule) (Bienenstock et al., 1982; Stanton, 1996) in both scVeh and scPCP treatment groups. However, it appears that for the same level of LFS, the magnitude of response slope LTD was significantly lower in the scPCP rats, suggesting a dysregulation in Ca^{2+} concentration, which also results in significant potentiation of inhibitory mechanisms regulating response size (manifested as significant depression in response amplitude). It must be noted that the processes of long-term synaptic plasticity for inhibitory interneurons are complex and the mechanisms of induction and expression vary greatly between neural circuits (Castillo et al., 2011; Maffei, 2011). While these mechanisms are studied in detail in the circuitries of the visual cortex, ventral tegmental area and hippocampus (Maffei, 2011), they are considerably less well understood in the PFC. In order to dissect this circuitry in both physiologically healthy animals and in disease model, detailed examinations using slice preparations are warranted.

One striking consistency in investigations of the vHipp-mPFC pathway plasticity, is the high variability in evoked neuronal activity. As depicted in **Figure 5.5 A2 and B2**, baseline measures of vHipp-mPFC response are more variable in the scPCP treatment group in comparison to controls. A similar pattern is observed in the MAM-treated (**Figure 5.8 B**) (Goto and Grace, 2006) and MK-801 treated rats (**Figure 5.8 D**) (Blot et al., 2015) in comparison to controls. This general variability may also contribute to the disturbances in synaptic plasticity in these models. NMDARs are pre/post-synaptic activity coincidence detectors and play an important role in optimising the signal-to-noise ratio. Abnormal potentiation of vHipp-mPFC synaptic plasticity in response to HFS in the scPCP treated rats could reflect reduced signal-to-noise ratio in the mPFC, resulting in the aberrant association between stimuli, a prominent feature of psychosis and cognitive impairments associated with schizophrenia (Goto et al., 2010; Blot et al., 2015). This, in addition to findings of disturbed HF-PFC synchrony and functional connectivity in both humans (Meyer-Lindenberg et al., 2005; Bahner and Meyer-Lindenberg, 2017; Sigurdsson and Duvarci, 2015) and disease models (Goto and Grace, 2006; Dickerson et al., 2010; Sigurdsson et al., 2010; Dickerson et al., 2012; Dawson et al., 2014; Dawson et al., 2015; Sigurdsson, 2016) adds further support to the significance of this pathway in the pathophysiology of the disease.

5.6.4. Electrophysiology – Cohort 2

5.6.4.1. Short-term plasticity was similar for propranolol treated groups in comparison to controls

In this cohort, animals were separated into 'intention-to-treat' groups to provide better comparison with post-LFS and post-HFS measures. Although some variability is apparent in the baseline PPI, the general pattern of results remains consistent. Similar to the observations in cohort 1, baseline PPS of the vHipp at varying IPIs evoked slope and amplitude facilitation in the mPFC in rats in cohort 2 and both slope and amplitude PPI decreased as a function of IPI (**Figure 5.6 A1-3 and B1-3**). Analysis did not detect any significant differences in the slope and amplitude PPI between the treatment groups at any of the tested conditions.

In this cohort slope and amplitude PPI were also highest at 50ms IPI under all tested conditions except for post-LFS amplitude PPI which was highest at 100ms IPI.

At baseline slope PPI at 50ms was significantly higher than PPI at all other IPIs (**Figure 5.6 A1**) while amplitude measure at 50ms IPI was only significantly higher in comparison to 200-1000 ms IPI. The baseline slope findings in cohort 2 are in contrast to cohort 1 where slope PPI at 50ms IPI did not reach significance in comparison to any other IPIs. In addition, while baseline slope PPI at 25ms and 50 ms IPI appeared lower in the scPCP treatment group compared to control in cohort 1, this trend was absent in cohort 2. After LFS, the slope and amplitude PPI had decreased in the short IPIs, so that post-LFS, slope PPI at 50ms IPI was not significantly different compared to PPI at any other IPIs in cohort 2. Post-LFS, however, amplitude facilitation was highest at 100 ms IPI and this reached significance in comparison to 500 and 1000 ms IPIs (**Figure 5.6 A2 and B2**). After HFS, the pattern of slope and amplitude PPI results was similar to the baseline measures (**Figure 5.6 A3 and B3**). It is noteworthy that for each treatment group, there was no significant difference in the slope and amplitude PPI across any of the tested conditions. Stability of the PPF in slope and amplitude, suggests that the mechanisms involved in mediating the LFS and HFS effects may be post-synaptic and are independent of the β-adrenergic mediated mechanisms. Yet, given the emergence of similar pattern of results as baseline in post-HFS condition, influence of pre-synaptic mechanism cannot be reliably excluded (see section **5.6.3.1** for more relevant detail).

5.6.4.2. LFS significantly potentiated previously non-potentiated vHipp-mPFC synapses in scPCP rats and this was attenuated by β-adrenoceptor antagonist (propranolol)

A simple change in the recording protocol revealed an interesting set of results that further highlighted the excitability differences in the vHipp-mPFC in the scPCP and scVeh treatment groups. Application of LFS (900 PPS, 5 ms IPI, 1Hz) to previously non-potentiated synapses in the scVeh-control treatment group reduced response slope (**Figure 5.7 A1, C1**) and size (**Figure 5.7 B1, D1**) to values below the baseline, however, this effect was not statistically significant. Burette et al (1997) had previously reported no significant change in response amplitude following LFS (same protocol as used in this study) of previously non-potentiated synapses (Burette et al., 1997). They further reported a slow-rising potentiation in response size that became significant approximately 15-30 minutes after LFS. In the present study, post-LFS responses were recorded for 15 minutes only. It is plausible that upon longer recording duration, similar effects as reported by Burette et al (1997) would have been observed in this study. In this study, subsequent application of HFS did not induce a significance change in response slope of the scVeh-control treatment group in comparison to baseline but significantly increased response slope in comparison to post-LFS. This is while HFS significantly reduced response size to values below the baseline and post-LFS in this treatment group (**Figure 5.7 A1, B1**). This is in contrast with Burette et al (1997) who reported a significant potentiation in response size post-HFS, when applied 60min post-LFS. It is therefore, plausible that at the time of

application of HFS in this study (15 mins post-LFS), the synapses were still under a strong inhibitory lock preventing potentiation following HFS.

LFS of the previously non-potentiated synapses in the scPCP-control treatment group induced a significant potentiation in response slope, which was significantly higher in comparison to all other treatment groups (**Figure 5.7 A3, C1, and C3)**. Interestingly, LFS of previously non-potentiated synapses induced a significant reduction in response size in the scPCP-control, suggesting an underlying potentiation of inhibitory mechanisms that regulate response size (**Figure 5.7 B3, D1**). Subsequent HFS further potentiated response slope and amplitude in the scPCP-control treatment group (**Figure 5.7 A3 and B3**).

In light of the observed effects in the scVeh-control group and the findings of Burette et al (1997), these findings highlight significant alterations in the excitability threshold, leading to the hyper-excitability of the vHipp-mPFC pathway in the scPCP-control treatment group. They show that in addition to the hyper-excitability of the glutamatergic responses, the inhibitory mechanisms may also be hyper-excitable and the LTP induced in the inhibitory mechanisms are less stable and readily reversible. As discussed in the previous sections, the hyper-excitability in glutamatergic synapses could be explained by reduced inhibition (disinhibition) in the scPCP treatment group, as well as the compensatory hyper-responsiveness of the NMDARs. Furthermore, these findings might point towards disturbances in the regulation of calcium concentration threshold, maintenance of which is essential for determining the polarity of the response in glutamatergic synapses (see **Section 5.6.2.3** for more relevant detail). In addition to these factors, alterations in the noradrenergic system could also explain these observations in the scPCP-Veh treatment group.

In the presence of an acute dose of β-adrenoceptors antagonist (propranolol), LFS did not induce any change in the response slope and size of the scPCP treatment group. Subsequent HFS, also did not induce any change in the response amplitude compared to baseline, while significantly potentiating response slope to levels above baseline (**Figure 5.7 A4 and B4**). Previous studies have reported an increase in NMDAR-mediated current in pyramidal neurons upon stimulation of the β-adrenoceptors in slice preparations of mPFC (Ji et al., 2008). Several lines of evidence suggest that activation of β-adrenoceptors increases the post-synaptic excitability of glutamatergic neurons in the presence of strong cortical depolarization in the mPFC of rats (Mueller et al., 2008; Marzo et al., 2010). Furthermore, these receptors increase glutamatergic fEPSP current and frequency (Kobayashi et al., 2009) and regulate resting membrane potential of pyramidal neurons in the mPFC (Grzelka et al., 2017). It is plausible that over-activation of β-adrenoceptors contributed to the observed effects in the scPCP-Veh treatment group, while their blockade with propranolol modifies the excitability threshold. In the scVeh treatment group, acute treatment with propranolol did not significantly alter response slope and size in response to LFS, an effect which is similar to the scVeh-control and scPCP-Pro treatment groups. Subsequent HFS, did not have an effect on response slope while significantly potentiating response size to values above the baseline. This is in contrast to the observed effect in the scVeh-control treatment whereby response size significantly decreased in response to HFS.

This is also showing a different pattern in comparison to the scPCP treatment group, where treatment with propranolol potentiated response slope and not size. Collectively, these results suggest that the effect of the noradrenergic system in regulating the inhibitory mechanisms that regulate response slope and its size are differentially affected in the scPCP treatment group compared to the scVeh.

β-adrenoceptors play an important role in modulating synaptic weight in response to contextual and emotional information. Activation of these receptors is essential for consolidation and retrieval of fear extinction memory, which refers to the elimination of fear response upon repeated exposure to fear-conditioning stimuli. This behavioural paradigm relies on appraisal and integration of contextual and emotional information, which is one of the core functions of the vHipp-mPFC pathway (Hugues and Garcia., 2007; Do-monte et al., 2010). The findings of repeated LFS in presence and absence of propranolol in the scPCP treatment group might, by extension, point towards deficits in stimulus appraisal which may have functional consequences such as deficits in fear extinction memory (Pollard et al., 2012).

5.7. Conclusion

To the best of my knowledge, this is the first study to examine the synaptic properties of the vHipp-mPFC pathway in the scPCP model for cognitive impairments associated with schizophrenia. Investigations into this pathway, which is heavily involved in cognition, is essential for better understanding the neural pathophysiology of cognitive deficits associated with the disease.

Collectively, these investigations suggest that the strength of the glutamatergic input from the vHipp to the mPFC is compromised following scPCP treatment, while the size of this connectivity remains unchanged. Findings of STP and long-term synaptic plasticity point towards hyper-excitability of the vHipp-mPFC pathway following scPCP treatment, a process that may involve loss or reduction of inhibitory control over glutamatergic responses, compensatory hyper-activation of the NMDARs and alterations in excitability threshold. Results further highlight potential alterations in the noradrenergic system which plays an important role in regulation of synaptic plasticity in the vHipp-mPFC pathway (Lim et al., 2010) and in the mPFC micro-circuitry (Wang et al., 2013; Zhou et al., 2013). It is important to note that in addition to the noradrenergic system, dopamine also strongly modulates synaptic plasticity in the vHipp-mPFC pathway (Gurden et al., 1999; Gurden et al., 2000; Jay et al., 2004). Neither noradrenergic nor dopaminergic neurotransmitter systems can act independently since alterations in the availability levels of one system affects the levels of the other (Lim et al., 2010; Xing et al., 2016). As such, the influence of alterations in dopaminergic transmission on observed effects cannot be excluded. However, given the scope of this study, it is difficult to assess the nature of noradrenaline and dopamine collaboration (synergistic or antagonistic) on processes of synaptic plasticity in the vHipp-mPFC pathway (Xing et al., 2016).

This study has several potential limitations. During assessments of long-term plasticity, responses to HFS and LFS were monitored for maximum of 30 minutes to cover the window for initial protein synthesis (Bliss and Cooke., 2011). A longer observation period (1-2h, which is common in electrophysiological studies)

would have provided more certainty about the stability of the observed effects. Given the long duration of the study (approximately 14 weeks), the electrophysiological recordings were obtained from animals with an age range of 12 to 25 weeks. It is well established that the natural process of aging alters some electrophysiological properties of neurons (such as input resistance and LTP induction and maintenance) in the mPFC (Chang et al., 2005) and hippocampus (Lynch., 2004). As such, the wide age-range of the animals used in this study could potentially confound the interpretation of the findings. A staggered experimental design would have been beneficial in minimising the potential effect of age on the experimental output.

Nevertheless, these preliminary investigations characterised the basic electrophysiological properties of the vHipp-mPFC in the scPCP model. The following chapter will address the effect of long-term treatment with haloperidol, a typical antipsychotic, on the synaptic properties of this pathway in the scPCP model.

Chapter 6

Study 4 - An investigation into the effect of long-term haloperidol treatment on synaptic properties of the vHipp-mPFC Pathway

6.1. Introduction

Synaptic plasticity, the processes by which synaptic strength is modulated (Goto et al., 2010), is integral to functional interaction between brain regions and processing of cognitive functions (Uhlhaas and Singer, 2010; Park et al., 2014; Price et al., 2014) (see **Chapter 1.8** for review). Synaptic plasticity is regulated by the major excitatory glutamatergic neurotransmitter system and modulated by a number of neurochemical substances, including dopamine (Goto et al., 2010; Price et al., 2014). Imbalances in these and other neurotransmitter systems are at the core of schizophrenia pathophysiology (Howes et al., 2015) (see **Chapter 1.4** for review) and along with dysregulation of synaptic plasticity (Daskalakis et al., 2008; Frantseva et al., 2008) (see **Chapter 1.11** for review), are thought to underlie the cognitive impairments associated with the disease.

Substantial evidence suggests that antipsychotics (APs) modify synaptic plasticity (Gemperle et al., 2003; Gemperle and Olpe, 2004; Matsumoto et al., 2008; Xu and Yao, 2010; Shim et al., 2012) through their direct and indirect action on the glutamatergic and dopaminergic neurotransmission (Arvanov et al., 1997; Arvanov and Wang, 1999; Lopez-Gil et al., 2007; Lum et al., 2018; Burger et al., 2005). It is thought that these modulations underlie the neurocognitive effects of APs. However, as previously discussed in **Chapter 3** and **Chapter 1.7** , the neurocognitive effects of long-term treatment with APs, especially haloperidol, remain controversial. Randomised clinical trials (with up to 3 year follow up) point towards neurocognitive improvement in patients treated with haloperidol (Harvey et al., 2005; Crespo-Facorro et al., 2009; Ayesa-Arriola et al., 2013). However, these improvements are small and most often strongly correlated with improvement in the positive symptoms, suggesting that haloperidol may not have a direct beneficial effect on cognition (Harvey et al., 2005; Crespo-Facorro et al., 2009; Ayesa-Arriola et al., 2013). In fact, naturalistic studies point towards significant cognitive decline as a function of accumulative treatment with both typical and atypical AP agents (Husa et al., 2014; Husa et al., 2017). Consistent with these findings, results of the pre-clinical studies also report that acute (Grayson et al., 2007; McLean et al., 2008; Paine and Carlezon, 2009; Snigdha et al., 2010) and longer-term (i.e. 14 days) (Hashimoto et al., 2005; Ozawa et al., 2006; Nagai et al., 2011; Ozdemir et al., 2012) treatment with haloperidol is ineffective in rescuing manipulation induced cognitive deficits in the animal models for schizophrenia while significantly impairing cognition after 90 days (approximately 7.5 human years) of treatment (Terry et al., 2002; Terry et al., 2006; Sengupta, 2013) (reviewed in **Chapter 1.7.2** and **Chapter 3**).

Across the literature, processes of long-term potentiation (LTP) have been suggested as the physiological processes underpinning learning and memory. Several lines of evidence suggest that while haloperidol increases NMDA receptor (NMDAR) and reduces AMPA receptor (AMPAR) mediated currents (Arvanov et al., 1997; Arvanov and Wang, 1999; Gemperle et al., 2003) it does not influence LTP. For instance, bath application of haloperidol (30-100 µM) was found to significantly reduce the strength of excitatory transmission in layer V of prelimbic (Prl) slices in male Sprague-Dawley rats. However, this was not associated with alterations in LTP induction and maintenance (Gemperle et al., 2003). Similarly, Xu et al

(2009) also reported no change in slice LTP induction in layer V of the Prl/infralimbic (IL) cortex upon stimulation of layer II-III in haloperidol treated mice. This was the case for mice acutely (1 mg/kg; i.p.) or chronically (14 days, 0.5 mg/kg/day; i.p.) treated with haloperidol (Xu et al., 2009). *In vivo*, acute treatment with haloperidol (1 mg/kg; i.p.) also did not influence LTP induction and maintenance in the mPFC upon stimulation of vHipp under anaesthetised conditions (Matsumoto et al., 2008). Contrary evidence has also been reported, whereby bath application of haloperidol (2 μM) to medial prefrontal cortex (mPFC) slices of physiologically healthy mice, was found to completely abolish the induction of spike-timing dependent LTP in layer V upon stimulation of layer II-III under intact GABAergic transmission (Xu and Yao, 2010).

According to the studies reported here (more extensively reviewed in **Chapter 1.12**), the interference of haloperidol with LTP under acute and in some cases long-term treatment conditions is minimal. By extension this could partly explain the ineffectiveness of haloperidol in rescuing manipulation induced cognitive deficits in animal models for schizophrenia. As recently reviewed by Price et al (2014), the majority of studies investigating the influence of haloperidol and other APs on synaptic plasticity have been conducted in slice preparations of medial prefrontal cortex (mPFC) obtained from physiologically healthy rodents. While these studies may elucidate the mechanism of action of APs in local circuitries that mediate cognitive function, they are substantially limited in their translational validity both to disease state and to *in vivo* alterations of synaptic plasticity upon AP treatment. Furthermore, since treatment with APs tends to be long-term, research into the influence of long-term treatment with APs is much needed.

In order to address this gap in current understanding, this study aimed to investigate the effects of long-term (28 day, approximately 3 human years) haloperidol treatment on synaptic plasticity of the ventral-hippocampus (vHipp)-mPFC pathway in the sub-chronic phencyclidine (scPCP) model of cognitive impairments for schizophrenia. The significance of this pathway in association with schizophrenia was discussed in detail in **Chapter 5** as well as **Chapter 1.9.** Briefly, the interaction between these two anatomical regions is essential for mediating higher-order cognitive processes such as episodic memory and its emotional regulation as well as executive function, all of which are impaired in patients with schizophrenia (Kalkstein et al., 2010; Millan et al., 2012; Preston and Eichenbaum, 2013; Eichenbaum, 2017).

Thus far, studies reported in this thesis have been unsuccessful in determining the long-term neurocognitive effects of treatment with haloperidol. Secondary to investigating the effects of haloperidol on synaptic plasticity, this study aimed to re-examine the long-term neurocognitive effects of this compound. Prior to implanting osmotic minipumps, the behavioural phenotype of the animals was tested in the disrupted novel object recognition task (dNOR). This was to determine whether the scPCP dosing regimen was effective in inducing cognitive deficits. The behaviour of the animals was also tested in the continuous NOR (cNOR) at baseline and again on the last day of treatment (day 28). This was to determine potentially detrimental neurocognitive effects of haloperidol.

6.2. Materials and Methods

6.2.1. Animals

A total of 30 adult female Lister Hooded rats (Charles Rivers, UK) with starting age of 11 weeks and starting weight of 190-230 g were used in this experiment. Housing and general husbandry conditions were identical to those described in **Chapter 2.1.**

6.2.2. Drug Administration

6.2.2.1. Sub-Chronic PCP/Vehicle Treatment

Rats were randomised to receive vehicle (0.9% Saline, intraperitoneal injections (i.p.), n=10) or PCP (2 mg/kg; i.p., n=20) twice a day for 7 days followed by a 7-day washout (WO) period. This is the standard operating protocol used in our laboratory which is described in more detail in **Chapter 2.3.**

6.2.2.2. Chronic Haloperidol treatment – Osmotic minipump drug delivery

Sub-chronic vehicle (scVeh) and scPCP treated rats were randomised to receive vehicle (β-hydroxypropylcyclodextrin, 20% w/v, acidified by ascorbic acid to pH 6; scVeh-control: n = 5, scPCP-control: n = 10) or haloperidol (0.5 mg/kg, acidified to pH 6; scVeh-Hal: n = 5, scPCP-Hal: n=10) (for details of drug preparation, refer to **Chapter 2.2.**). Similar to **Chapter 4**, in this study also, the vehicle and haloperidol treatments were delivered over 28 days via osmotic minipumps (2ML4 model, Alzet) implanted subcutaneously under anaesthetised conditions. The implant surgical procedure as well as peri- and post-operative care are described in detail in **Chapter 2.5.**

6.3. Experimental Procedures and timeline

On the last day of WO from the scPCP/scVeh treatment, performance of the animals was tested in the dNOR-1. Two days later, baseline behavioural performance was also assessed in the cNOR (cNOR-1) task (A detailed protocol for these two paradigms is provided in **Chapter 2.4.1**). In the following week (14 weeks post scPCP/scVeh treatment), the osmotic minipumps were implanted subcutaneously under anaesthetised conditions. The day of the implant marked the first day of treatment. Rats were then left in their home cage for the full duration of drug treatment (handled only 7 days prior to and after osmotic minipump implant for weighing purposes). On day 28, rats were re-tested in the cNOR (cNOR-2) and were immediately subjected to terminal electrophysiological recording procedures under anaesthetised condition. Details of the electrophysiological procedure and the recording protocol (**Recording protocol A**) are described in **Chapter 2.6**. Briefly, the recording electrode (2x16, polytrode channel configuration) was placed in the Prl region of the mPFC (B+3.2 mm, ML= 1.5 mm, 10˚ from midline, depth=4 mm) and the stimulating electrode was placed in the ventral CA1 (B-6.5mm, ML= 5.5mm, depth=4.4-5.5 mm).

Spontaneous activity and activity in response to sensory stimulations (toe-pinch) were obtained from the mPFC prior to stimulation of the vHipp. These data were obtained for another experimenter and will not be presented in this thesis. Following the spontaneous recording, synaptic connectivity and short-term synaptic plasticity (STP) were examined using an input-output and paired-pulse stimulation (PPS) protocol, respectively. Next, high-frequency stimulation (HFS; 5 trains of 20 pulses at 200 Hz) was delivered to the vHipp to study LTP in the vHipp-mPFC pathway. This was followed by low-frequency stimulation (LFS; 900 paired-pulses at 1Hz, 5ms inter-pulse interval) of the vHipp (45 mins post HFS), to examine the subsequent reversal of LTP in this pathway. STP was also examined post-HFS and post-LFS **(Recording Protocol A)**. At the end of recording all rats underwent transcardial perfusion under terminal anaesthesia and brain tissue was collected and processed as detailed in **Chapter 2.8.** A summary of the experimental timeline and tissue preparation is provided in **Figure 6.1.** Please note that in this study, scPCP/scVeh dosing, behavioural testing, minipump implants and electrophysiological recording procedures were staggered across 3 cohorts of 10 rats. This was implemented to minimise the potentially confounding effect of age on electrophysiological recordings (Lynch., 2004; Chang et al., 2005). Animals were approximately 11 weeks old at the time of scPCP/scVeh dosing. At the time of electrophysiological recordings, animals in each cohort were 20-23 weeks old.

6.3.1. Exclusion Criteria

At all tested time points, behavioural trials were excluded from analysis if animals explored each object (right vs. left; novel vs. familiar) for one or less than one second or jumped onto the edge of the NOR test box. Based on these criteria only one rat from the scPCP-Hal treatment group was excluded from the analyses of the cNOR-2. Exclusion criteria for electrophysiological analysis were based on *post-hoc* electrode placement confirmation. Accordingly, rats were excluded from final analysis if either stimulating or recording electrode missed the regional target (See **Chapter 2.8.3** for examples of histological electrode placement confirmation). Based on this criterion, one rat from the scPCP-control treatment group was excluded from the final analysis. Moreover, one rat was excluded from the scVeh-control and scVeh-Hal treatment groups as the appropriate field excitatory post-synaptic potential (fEPSP) response and shape could not be obtained during the experiment. Accordingly, 4 scVeh-control, 4 scVeh-Hal, 9 scPCP-control and 10 scPCP-Hal treated rats were included in the final analysis of the electrophysiological data. **Table 6.1** provides a detailed summery of the number of rats excluded from each treatment group during the behavioural and electrophysiological assessments presented in this chapter.

6.4. Statistical analyses

Data obtained from the behavioural task were analysed using mixed-design, two-way (task and treatment) ANOVAs to examine the main effect of task (exploring left/right identical object in acquisition phase and novel/familiar in retention phase; within-subjects variable) and treatment (between-subjects variable). Paired Student's t-tests were employed to compare between novel vs. familiar object exploration time within

each treatment group when appropriate. Independent samples Student's t-test or planned Bonferroni pair-wise comparisons (scVeh-control vs. scVeh-Hal; scVeh-control vs. scPCP-control; scPCP-control vs. scPCP-Hal; scVeh-Hal vs. scPCP-Hal; α-value=**0.05**, *p*-values adjusted for 4 comparisons) were employed to compare total object exploration times between treatment groups. Discrimination index (DI) data were analysed using independent samples Student's t-test or a one-way ANOVA, followed by planned Bonferroni pairwise comparisons (scVeh-control vs. scVeh-Hal; scVeh-control vs. scPCP-control; scPCP-control vs. scPCP-Hal; scVeh-Hal vs. scPCP-Hal) when appropriate. Mann-Witney U test was also used to compare mean DI values against zero. Electrophysiological data were analysed using mixed-design or repeated measures two-way ANOVAs followed by *post-hoc* Bonferroni comparisons when appropriate. For all Bonferroni *post-hoc* comparisons the α-value was set at **0.05**. Adjusted *p*-values were then calculated by multiplying the unadjusted *p*-value by the number of comparisons (number of comparisons are reported in relevant sections throughout the results section). This is the method recommended by IBM SPSS Statistics Support (2016a) to adjust for multiple comparisons using Bonferroni correction. All statistical analyses (except for the assessment of long-term plasticity which were analysed in GraphPad Prism Version 8.0.2) were conducted using IBM SPSS (Version 23). For details on tests of normality and homogeneity of variance on NOR and the electrophysiological data, refer to **Chapter 2.4.1.5** and **Chapters 2.6.5.1** and **2.6.5.2**, respectively.

NOR testing session	**Treatment**				**Total Excluded**
	scVehicle (n=10)		**scPCP (n=20)**		
dNOR-1	**-**		**-**		**-**
cNOR-1	**-**		**-**		**-**
	scVeh-control (n=5)	**scVeh-Hal (n=5)**	**scPCP-control (n=10)**	**scPCP-Hal (n=10)**	
cNOR-2	**-**	**-**	**-**	**1**	**1**
Electrophysiology (Exclusion based on criterion)	**1**	**1**	**1**	**-**	**3**
Number of rats included in final analysis of electrophysiological data	**scVeh-control (n=4)**	**scVeh-Hal (n=4)**	**scPCP-control (n=9)**	**scPCP-Hal (n=10)**	
I/O (Excluded due to expressing a positive going response)	**-**	**1**	**2**	**2**	**5**
STP	**-**	**-**	**-**	**-**	**-**
HFS/LFS	**-**	**1** excluded due to technical issues with recordings	**-**	**-**	**1**

Table 6.1. Summary of the number of rats excluded from behavioural and electrophysiological assessments in Chapter 6. Please note that rats excluded during the I/O recordings were later included in the analysis of STP and HFS/LFS (long-term plasticity). Therefore, the number of animals per treatment group for analysis of STP and HFS/LFS is the same as the number of animals included in the analysis of electrophysiological data presented in this table.

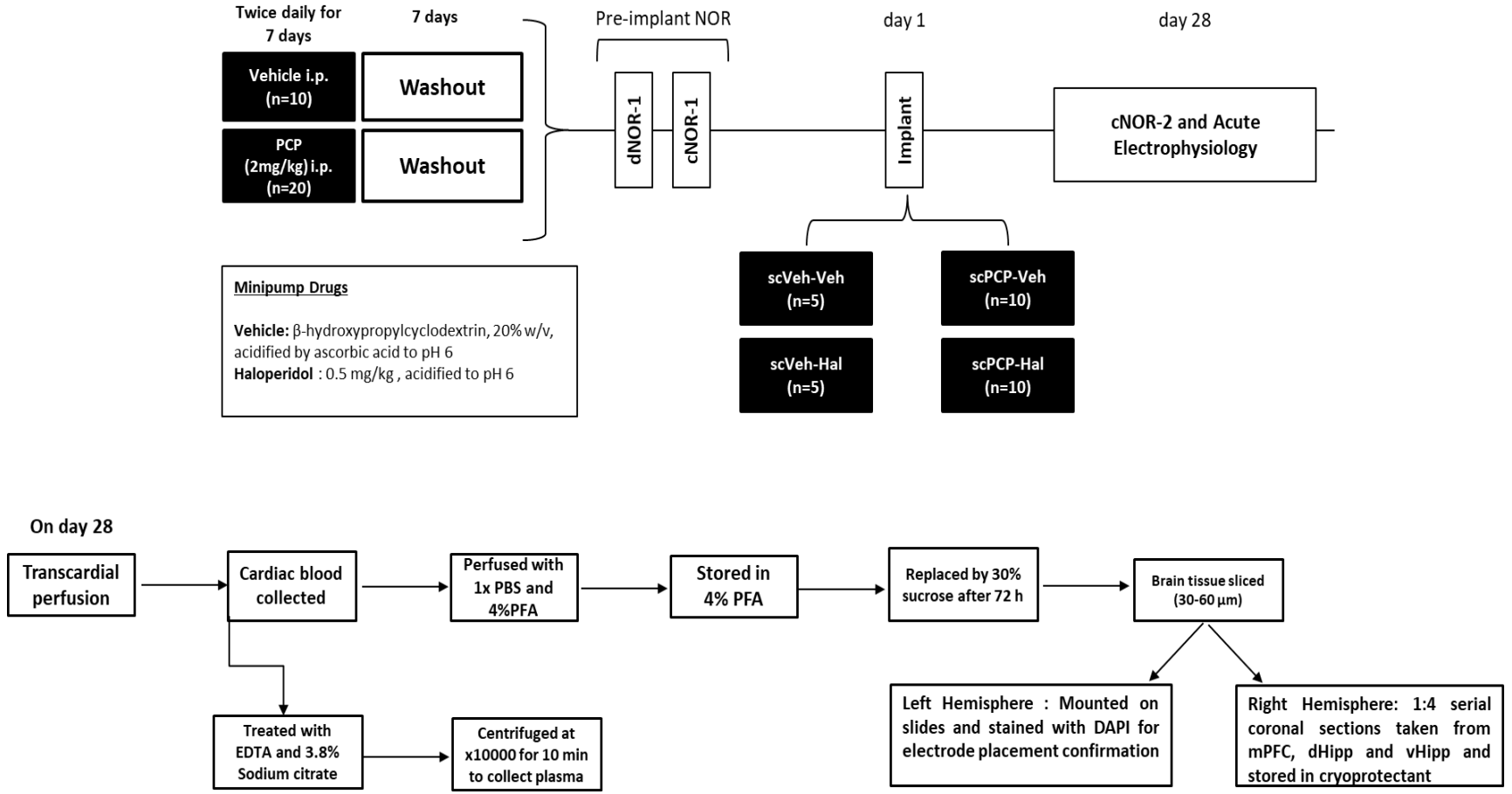

Figure 6.1. A schematic summary of the experimental timeline, tissue preparation and its storage for Chapter 6. Rats were treated with scPCP/scVeh (twice daily for 7 days) followed by a 7-day washout period. Baseline behavioural assessment obtained using dNOR and cNOR-1 prior to osmotic minipump implant. cNOR assessment was repeated on last day of treatment followed by acute electrophysiological recording under anaesthetised conditions. Culling technique and the process of tissue sample collection is described.

6.5. Results

6.5.1. scPCP-induced deficit in dNOR performance was present in treated rats while performance in cNOR-1 remained intact

6.5.1.1. Acquisition Phase

Two-way (task and treatment) ANOVA showed that rats in both scVeh and scPCP treatment groups spent similar time exploring the identical objects in the acquisition phase of dNOR ($F_{1, 28}$ = 1.52, p=0.22) (**Figure 6.2 A1**) and cNOR-1 ($F_{1, 28}$= 0.06, p=0.80) (**Figure 6.2 B1**). Treatment had a significant effect on total object exploration time in the acquisition phase of dNOR ($F_{1, 28}$ = 5.93, p<0.05) where this was significantly higher for the scPCP compared to the scVeh treatment group (t_{28}=-2.43, p<0.05). There was no significant difference in total object exploration time between the treatment groups in cNOR-1 ($F_{1, 28}$ = 0.16, p=0.68) (**Table 6.2**). Similarly, there was no significant task x treatment interaction on total object exploration time in dNOR ($F_{1, 28}$ = 3.82, p=0.06) and cNOR-1 ($F_{1, 28}$= 0.27, p=0.61).

6.5.1.2. Retention Phase

Two-way (task and treatment) ANOVA detected a significant difference in the novel/familiar object exploration time in the retention phase of dNOR ($F_{1, 28}$ = 12.64, p<0.01) and cNOR-1 ($F_{1, 28}$ = 9.96, p<0.01). Planned paired Student's t-test detected that rats in the scVeh (t_9=2.96, p<0.05) treatment group at dNOR (**Figure 6.2 A2**) and rats in both scVeh (t_9=2.40, p<0.05) and scPCP (t_{19}=2.41, p<0.05) treatment groups at cNOR-1 (**Figure 6.2 B2**) explored the novel object significantly more than the familiar object. This effect was absent in the scPCP treatment group when tested in dNOR-1 (t_{19}=1.50, p=0.14) (**Figure 6.2 A2**). There was no significant effect of treatment on total object exploration times at the dNOR ($F_{1, 28}$ = 0.04, p=0.84) and cNOR-1 ($F_{1, 28}$ = 0.28, p=0.60) in the retention phase. Independent Student's t-test also did not detect a significant difference in total object exploration time between the scVeh and scPCP treatment groups at dNOR (t_{28}=-0.20, p=0.84) and cNOR-1 (t_{28}=0.53, p=0.60) (**Table 6.2**). Similarly, there was no significant task x treatment interaction on total object exploration time in the dNOR ($F_{1, 28}$ = 3.77, p=0.06) and cNOR-1 ($F_{1, 28}$ = 0.04, p=0.83).

6.5.1.3. Discrimination Index

Results of independent sample t-test detected that in the dNOR, the difference in the DI between the scVeh and scPCP treatment group approached significance ($t_{12.04}$=1.97, p=0.07) and was marginally higher for the scVeh compared to the scPCP treated rats. Results of the Mann-Whitney U test detected that for both scVeh (U=40, p<0.01) and scPCP (U=100, p<0.01) mean DI values were significantly greater than zero (**Figure 6.2 A3**). Analyses did not detect a significant difference in the DI of scVeh and scPCP treated rats in the cNOR-1 (t_{28}=0.07, p=0.94). Furthermore, the mean DI of the scVeh (U=60, p<0.05) but not scPCP (U=160, p=0.24) was significantly greater than zero in cNOR-1 (**Figure 6.2 B3**).

6.5.2. Long-term treatment with haloperidol did not impair performance on cNOR-2 in scVehicle and scPCP treatment groups.

6.5.2.1. Acquisition Phase

Similar to previous findings, a two-way (task and treatment) ANOVA detected no significant effect of task on object exploration time suggesting that rats in all treatment groups spent similar time exploring the right and left object ($F_{1, 25} = 0.89$, $p=0.35$) (**Figure 6.2 C1**). The effect of treatment on total object exploration time approached significance ($F_{3,25} = 2.99$, $p=0.05$). Planned Bonferroni comparisons did not detect any significant pair-wise differences in total object exploration times between the treatment groups (**Table 6.2**). There was no significant task x treatment interaction on total object exploration time ($F_{3, 25} = 2.08$, $p=0.12$).

6.5.2.2. Retention Phase

Two-way (task and treatment) ANOVA detected a significant difference in novel/familiar object exploration times ($F_{1, 25} = 30.63$, $p<0.0001$). Planned paired Students t-test showed that rats in the scVeh-control ($t_4=2.86$, $p<0.05$), scVeh-Hal ($t_4=4.60$, $p<0.05$), scPCP-control ($t_9=3.85$, $p<0.01$) and scPCP-Hal ($t_8=3.71$, $p<0.01$), explored the novel object significantly more than the familiar object (**Figure 6.2 C2**). Treatment had a significant effect on total object exploration time ($F_{3, 25} = 4.55$, $p<0.05$). Planned Bonferroni comparisons revealed that total object exploration time was significantly higher in the scPCP-control treatment group when compared to the scVeh-control ($p=0.02$). No other significant pair-wise differences were detected (**Table 6.2**). There was also no significant task x treatment interaction on total object exploration time ($F_{3, 25} = 1.48$, $p=0.24$).

6.5.2.3. Discrimination Index

Results of a one-way ANOVA showed that there was no significant effect of treatment on DI across all treatment groups ($F_{3, 25} = 0.11$, $p=0.95$). Results of the Mann-Whitney U test showed that the mean DI values in the scVeh-control ($U=0$, $p<0.001$), scVeh-Hal ($U=11$, $p<0.05$), scPCP-control ($U=11$, $p<0.01$) and scPCP-Hal ($U=0$, $p<0.0001$) treatment groups was significantly greater than zero (**Figure 6.2 C3**).

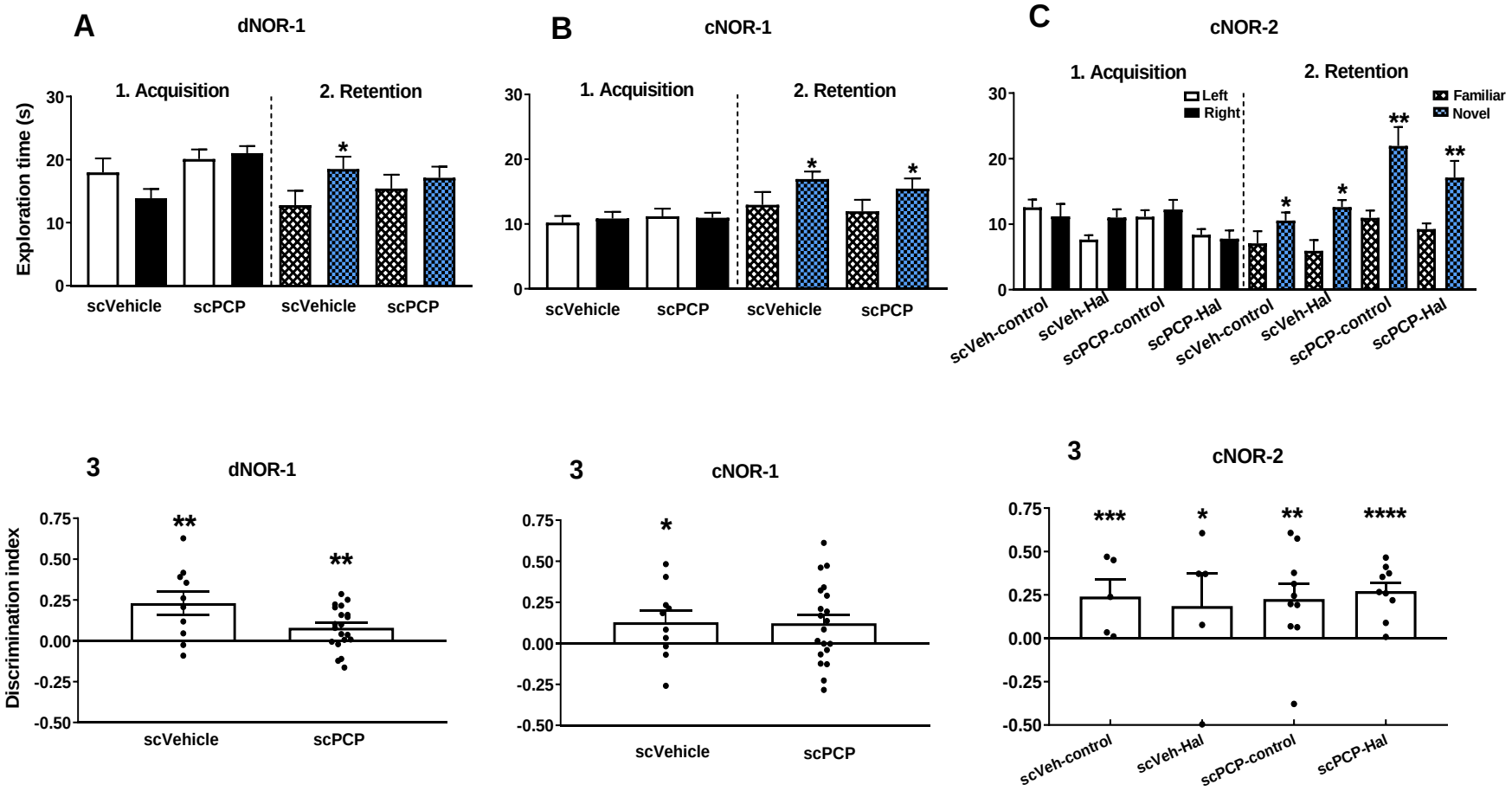

Figure 6.4. Performance in the dNOR and cNOR-1 at baseline and cNOR-2 after 28 days of treatment with haloperidol. (A1, B1, C1) At all tested timepoints, rats in all treatment groups spent similar time exploring the identical objects in the acquisition phase (A2) scVeh rat but not the scPCP rats explored the novel objects significantly more than the familiar in dNOR while in (B2) cNOR-1 both treatment groups were able to discriminate between the novel from the familiar object. (C2) rats in all treatment groups were able to perform the task after 28 days of treatment with haloperidol *p<0.05, ** p<0.01 novel vs. familiar (A3, B3, C3) Ratio of novel to total object exploration time was not significantly different between any of the treatment group at all tested time points. *p<0.05, ** p<0.01, ***p<0.001, **** p<0.0001 vs. zero. Data are presented as Mean ± SEM. dNOR and cNOR-1 (n=10-20/group), cNOR-2 (n=5-10/group)

NOR	Treatment	Mean total object exploration times (s)	
		Acquisition phase	Retention phase
dNOR	scVehicle (n=10)	31.83 ± 2.74	31.28 ± 3.77
	ScPCP (n=20)	41.12 ± 2.31 *	32.51 ± 3.81
cNOR-1	scVehicle (n=10)	21.07 ± 1.86	29.92 ± 2.78
	ScPCP (n=20)	22.17 ± 1.65	27.43 ± 3.00
cNOR-2	scVeh-control (n=5)	23.76 ± 2.58	17.63 ± 2.90
	scVeh-Hal (n=5)	18.66 ± 1.38	18.53 ± 2.3
	scPCP-control (n=10)	23.36 ± 2.36	32.88 ± 3.35 *
	ScPCP-Hal (n=9)	16.16 ± 1.64	26.35 ± 2.99

Table 6.2. Total object exploration times in each phase of dNOR, cNOR-1 and cNOR-2. Total object exploration times were compared separately for each timepoint. dNOR *p<0.05 vs scVeh; cNOR-2 *p<0.05 vs scVeh-control. There were no other significant pair-wise differences in total object exploration time between other treatment groups in cNOR-1 and cNOR-2. Data are presented as Mean ± SEM. dNOR and cNOR-1 (n=10-20/group), cNOR-2 (n=5-10/group)

6.5.3. Long-term treatment with haloperidol does not alter synaptic connectivity in the vHipp-mPFC pathway

In order to assess the influence of long-term treatment with haloperidol on synaptic connectivity of the vHipp-mPFC pathway, 20 pairs of pulses (PP; P1 and P2) were applied to the vHipp at a range of current intensities to evoke population field EPSPs (fEPSP) in the mPFC. The 20 paired responses for each intensity were then averaged and the slope and amplitude of the mean P1 responses were measured; comparing these against stimulus intensity (100-800µA) and group (scVeh-control, scVeh-Hal, scPCP-control, scPCP-Hal) revealed any changes in synaptic connectivity in the vHipp-mPFC. One rat from the scVeh-Hal, 2 rats from the scPCP-control and 2 rats from the scPCP-Hal treatment groups expressed a positive going response and were excluded from the analysis of I/O response. Hence the analysis was based on the negative going responses only.

A mixed-design two-Way (treatment and current intensity) ANOVA detected a significant effect of current intensity on response slope ($F_{1.99,35.82}$ = 10.63, p<0.0001). There was, however, no significant effect of treatment ($F_{3, 18}$ = 0.82, p=0.50) and no significant treatment x current intensity interaction ($F_{5.97,35.82}$ = 0.89, p=0.50) on response slope. Mean response slope was steepest at 800µA. *Post-hoc* Bonferroni pairwise comparisons (α=0.05, p-value adjusted for 28 comparisons) showed that this reached significance in comparison to 100 (p<0.01), 200, 300 and 500 µA (p<0.05). Furthermore, response slope was least steep at 100µA and this reached significance compared to all other current intensities (300-400 µA: p<0.05; 500 and 700 µA p<0.01) but not at 200 and 600µA. The current intensity inducing half of the maximum response slope was at approximately at 300µA. At this current intensity response slope was significantly lower in comparison to 700 µA (p<0.05) (**Figure 6.3 A**).

A mixed-design two-way (treatment and current intensity) ANOVA detected a significant effect of current intensity on response amplitude ($F_{2.87,51.72} = 52.16$, $p<0.0001$). However, there was no significant effect of treatment ($F_{3,18} = 2.56$, $p=0.09$) and no significant treatment x current intensity interaction ($F_{8.62,51.72} = 1.06$, $p=0.40$) on response amplitude. Similar to slope measures, response amplitude was highest at 800µA (-0.68 ± 0.06). *Post-hoc* Bonferroni pairwise comparisons (α=0.05, *p*-value adjusted for 28 comparisons) showed that this reached significance in comparison to 100-300 µA ($p<0.0001$), 400 µA ($p<0.01$) and 500µA ($p<0.05$). The amplitude of the response at 100µA was significantly lower in comparison to all other current intensities ($p<0.0001$). The current intensity inducing half of the maximum response was at approximately 300µA (- 0.37 ± 0.06). At this current intensity, response amplitude was significantly higher than 200µA ($p<0.05$), 500-600µA ($p<0.01$) and 700µA ($p<0.0001$) (**Figure 6.3 B**).

Collectively, these results show that there were no statistically significant differences in response slope and amplitude between the treatment groups, suggesting that the strength and size of synaptic connectivity may have remained intact upon long-term haloperidol treatment. Mean response slope and amplitude measure were highest at 800µA. Except for slope measures in the scVeh-control and the scPCP-control treatment groups which were highly variable, response amplitude and slope increased as a function of current intensity. Response amplitude was significantly higher at 200 vs. 100µA ($p<0.0001$) and 200µA vs. 300µA ($p<0.05$) (**Figure 6.3 B**). There were no other significant pair-wise differences between response slope and amplitude measure at one current intensity compared to one before.

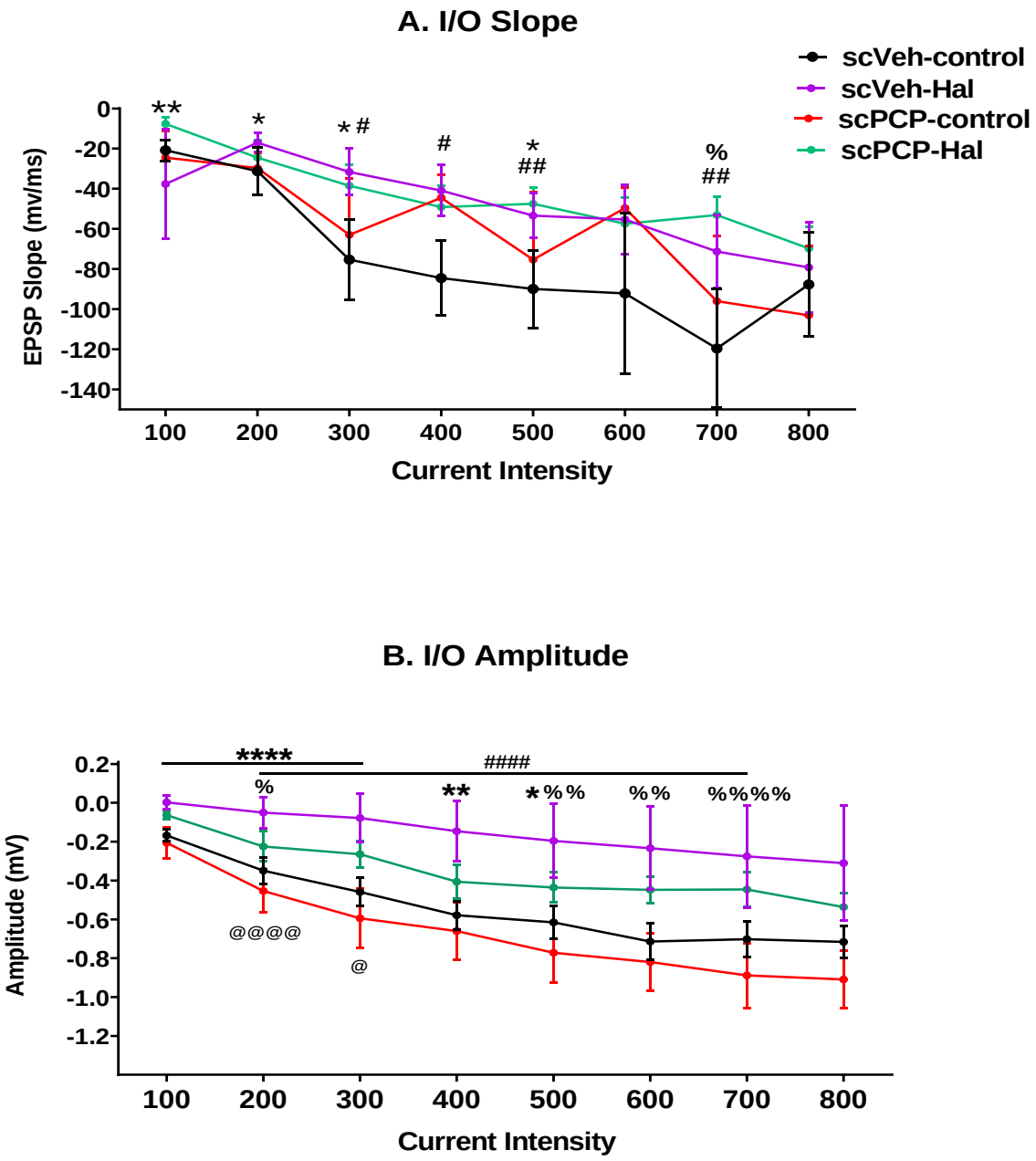

Figure 6.5. Current input-output response relationship. Represents the fEPSP (A) slope and (B) amplitude of the negative going responses as a function of current intensity. Measurements were obtained from the P1 of average waveform. All comparisons were made by two-way (treatment and current intensity) ANOVAs followed by *post-hoc* Bonferroni comparisons to detect pair-wise differences. There were no significant pair-wise differences in response slope and amplitude between the treatment groups. Symbols on graphs show current intensity pair-wise comparisons across all treatment groups #p<0.05, ##p<0.01, #### p<0.0001 vs. 100 µA; % p<0.05, %% p<0.001, %%%% p<0.0001 vs. 300 µA; *p<0.05, **p<0.01, ****p<0.0001 vs. 800µA; ,@@p<0.01,@@@@p<0.0001 for response at one current intensity vs. one current intensity before. Data are presented as Mean ± SEM, (scVeh-control n=4, scVeh-Hal n=3, scPCP-control n=7, scPCP-Hal n=8)

6.5.4. Haloperidol induces hyper-excitability in the vHipp-mPFC pathway manifested as increased paired-pulse facilitation

In order to assess the effect of long-term treatment with haloperidol on STP, 20 PP at varying inter-pulse intervals (IPI) (25, 50, 100, 150, 200, 500 and 1000 ms) were applied to the vHipp, using the current intensity that elicited half the maximum response calculated from the I/O curve. This was usually at 300µA (Amplitude: -0.37 ± 0.06). Similar to the previous study, the paired-pulse protocol was executed at 3 time points for each subject: prior to HFS (baseline), after application of HFS (post-HFS) and after LFS (post-LFS). This was to examine the behaviour of haloperidol treated circuits in response to HFS and LFS. Slope and amplitude were first measured from an average waveform from P1 and P2 and then P2 measures were normalised to P1 to calculate percentage change as a paired-pulse index (PPI; PPI>0 represented PP facilitation (PPF); PPI<0 represented PP depression (PPD)).

Mixed-design two-way (treatment x IPI) ANOVAs showed a significant main effect of IPI on slope PPI at baseline ($F_{4.41,101.59}$ = 13.01, p<0.0001), post-HFS ($F_{2.79,64.17}$= 14.72, p<0.0001) and post-LFS ($F_{2.65,61.15}$= 8.51, p<0.0001). Treatment had a significant effect on slope PPI at baseline ($F_{3,23}$ =3.47, p<0.05), post-HFS ($F_{3,23}$ = 6.42, p<0.01) and post-LFS ($F_{3,23}$= 3.22, p<0.05). There was however no significant treatment x IPI interaction on slope PPI at baseline ($F_{13.25,101.59}$= 1.29, p=0.26), post-HFS ($F_{8.37,64.17}$ = 0.59, p=0.78) and post-LFS ($F_{.97,61.15}$= 0.27, p=0.97). Slope PPI was highest at 50ms IPI under all tested conditions. *Post-hoc* Bonferroni pair-wise comparisons (α=0.05, p-value adjusted for 21 comparisons/recording condition) detected that baseline slope PPI was significantly higher at 50ms IPI compared to all other IPIs (p<0.001) except at 25ms IPI. Furthermore, baseline slope PPI was significantly lower at 500ms (p<0.05) and 1000ms IPI (p<0.01) in comparison to 150ms IPI (**Figure 6.4 A1**). Post-HFS, slope PPI at 50ms IPI was significantly higher than 150 ms IPI (p<0.05) and 200-1000 ms IPI (p<0.001) but was not significantly different from PPI at 25 and 100 ms IPI. Furthermore, post-HFS slope PPI was significantly lower at 500ms and 1000ms in comparison to 100ms (p<0.0001) and 150ms (p<0.01) IPIs (**Figure 6.4 A2**). Post-LFS slope PPI was significantly higher at 50ms IPI compared to 150ms (p<0.05) and 200-1000ms IPIs (p<0.001) but was not significantly different from PPI at 25ms and 100ms IPI (**Figure 6.4 A3**). Slope PPI was higher in the haloperidol treated groups compared to controls. *Post-hoc* Bonferroni pair-wise comparisons (α=0.05, p-value adjusted for 6 comparisons/recording condition) detected that slope PPI was significantly higher in the scPCP-Hal in comparison to the scPCP-control group at baseline (p<0.05), post-HFS (p<0.01) and post-LFS (p<0.05). There was however no significant difference between the scVeh-Hal treatment group and its control under any of the tested conditions. (**Figure 6.4 A1-3**).

Mixed-design two-way (treatment x IPI) ANOVAs showed a significant main effect of IPI on amplitude PPI at baseline ($F_{3.28,75.47}$ = 3.86, p<0.05), Post-HFS ($F_{3.11,71.55}$ = 6.09, p<0.01) and Post-LFS ($F_{2.44,56.09}$= 5.56, p<0.01). Treatment also had a significant effect on the amplitude PPI at baseline ($F_{3,23}$ =4.93, p<0.01), post-HFS ($F_{3,23}$ =4.25, p<0.05) and post-LFS ($F_{3,23}$ =3.22, p<0.05). There were also no significant treatment x IPI

interaction on amplitude PPI at baseline ($F_{9.84,75.47}$ = 1.03, p=0.43), post-HFS ($F_{9.33,71.55}$ = 0.65, p=0.75) and Post-LFS ($F_{7.31,56.09}$= 0.73, p=0.64). Amplitude PPI was highest at 50ms IPI under all tested conditions. *Post-hoc* Bonferroni pair-wise comparisons (α=0.05, p-value adjusted for 21 comparisons/recording condition) detected that this reached significance in comparison to 500ms IPI (p<0.01) at baseline (**Figure 6.4 B1**), 150-200ms IPI (p<0.05) and 500-1000ms (p<0.001) post-HFS (**Figure 6.4 B2**) and in comparison to 200 (p<0.05), 500-1000ms IPI (p<0.01) post-LFS (**Figure 6.4 B3**). No other significant pair-wise differences were detected between IPIs at baseline. Post-HFS amplitude PPI was significantly lower at 1000ms IPI in comparison to 150 (p<0.05) and 200 ms (p<0.01) IPIs. Similarly, post-LFS amplitude IPI was significantly lower at 500 ms and 1000 ms IPI in comparison to 150 ms IPI (p<0.05). Amplitude PPI was highest in the haloperidol treatment groups compared to controls. Bonferroni pair-wise comparisons (α=0.05, p-value adjusted for 6 comparisons/recording condition) further detected that baseline amplitude PPI was significantly higher in the scPCP-Hal in comparison to the scVeh-control treatment group (p<0.05) (**Figure 6.4 B1**). Post-HFS, amplitude PPI was found to be significantly higher in the scPCP-Hal treatment group in comparison to the scPCP-control treatment group (p<0.05). No significant pair-wise differences were detected in amplitude PPI between the treatment groups post-LFS (**Figure 6.4 B2**).

Repeated measures two-way (IPI and stimulation pattern) ANOVAs were also conducted separately for scVeh and scPCP groups to examine the main effect of stimulation pattern on STP. Results revealed no significant effect of stimulation pattern on the PPI for scVeh-control (slope: ($F_{2,6}$= 0.35, p=0.71), amplitude: ($F_{2,6}$ =4.49, p=0.06)), scVeh-Hal (slope: ($F_{2,6}$= 0.52, p=0.61), amplitude: ($F_{2,6}$ =2.81, p=0.13)), scPCP-control (slope: ($F_{2,16}$ = 0.58, p=0.56), amplitude: ($F_{2,16}$=2.19, p=0.14)) and scPCP-Hal (slope: ($F_{2,18}$= 1.31, p=0.29), amplitude: ($F_{1.21,10.90}$ = 2.16, p=0.14)) treatment group. This suggests that for these treatment groups, there is no significant difference in slope and amplitude PPI across all the PPS recording conditions.

These results demonstrate that slope and amplitude facilitation are largest at short IPIs (50ms) and decrease as IPI increases. Results also show that scPCP-Hal slope PPI was significantly higher compared to its control under all tested conditions. Amplitude PPI for this group was also significantly higher in the scPCP-Hal group compared to scVeh-control and baseline and compared to scPCP-control post-HFS. Slope and amplitude PPIs were also higher in the scVeh-Hal treatment group compared to its control but these did not reach statistical significance. Collectively, results suggest that haloperidol treatment induces a hyper-excitability in both scPCP and scVeh treatment groups manifested as higher slope and amplitude facilitation in comparison to controls. Slope and amplitude PPI of each treatment group was similar across all three tested conditions, suggesting that the effects of HFS and LFS are predominantly post-synaptic.

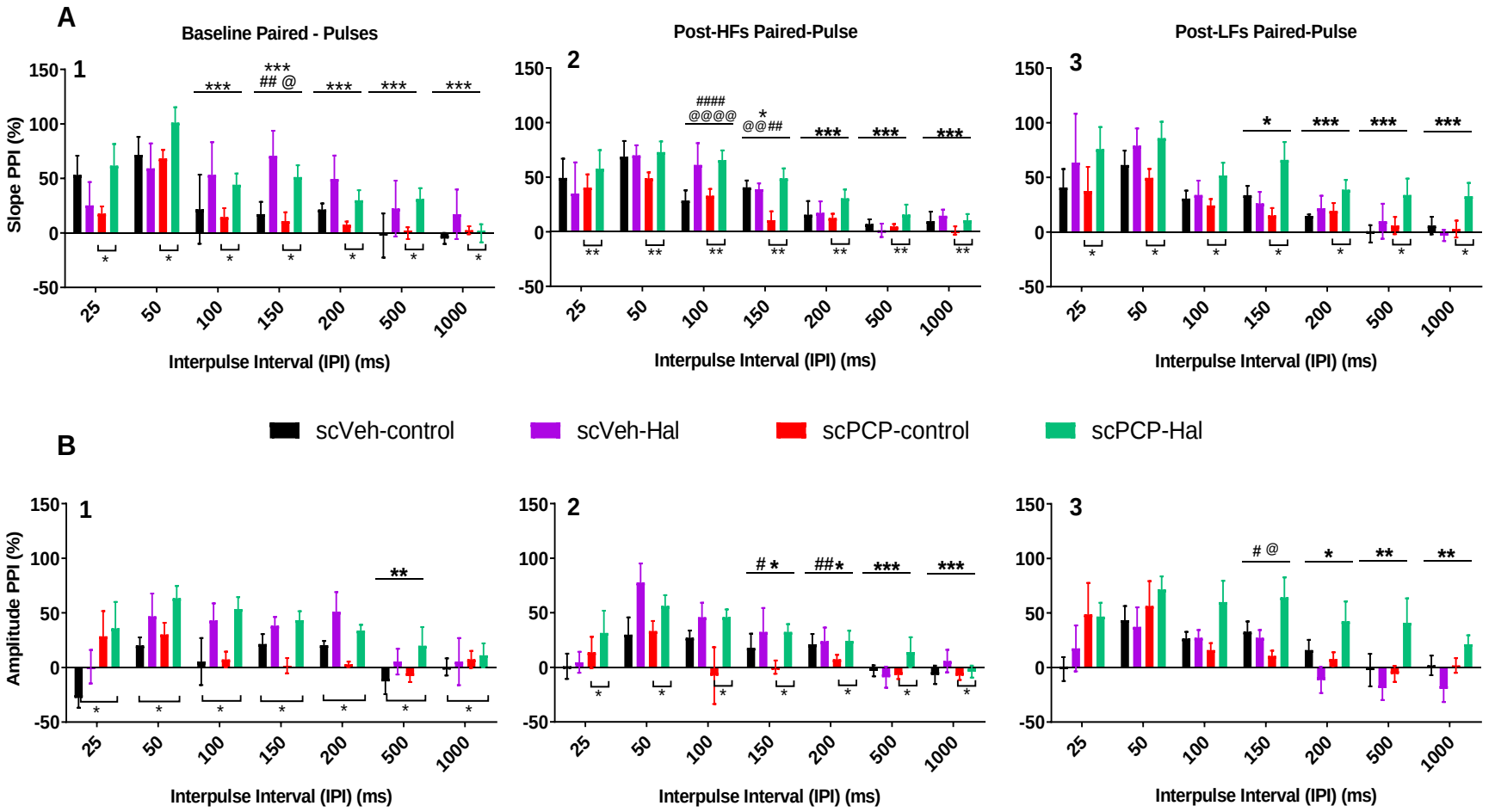

Figure 6.6. Short term plasticity using Paired-Pulse stimulation. (1) Baseline, (2) post-HFS and (3) post-LFS PPI for slope (A1-3) and amplitude (B1-3). STP is predominantly presented as PPF in the mPFC upon PPS of vHipp. Data were analysed by a two-way (treatment and IPI) ANOVA. Straight lines above graph bars represent pairwise comparisons of slope and amplitude PPI between different IPIs across all treatment groups. *p<0.05, p**<0.01, *** p<0.001 vs. 50 ms IPI; @ p<0.05, @@ p<0.01, @@@@ p<0.0001 vs. 500 ms IPI; # p<0.05, ## p<0.01, #### p<0.0001 vs. 1000 ms IPI. Brackets indicate pair-wise comparisons of slope and amplitude PPI between treatment groups at each IPI. *p<0.05, **<0.01. Data are presented as Mean ± SEM (scVeh-control n=4, scVeh-Hal n=4, scPCP-control n=9, scPCP-Hal n=10)

6.5.5. Long-term treatment with haloperidol potentiates LTP and prevents LTD-like activity to be established in the scPCP treated rats

In order to assess the effect of long-term haloperidol treatment on long-term synaptic plasticity, HFS (5 trains of 20 pulses at 200Hz) was applied to the vHipp to induce LTP in the mPFC. Furthermore, the effect of repetitive LFS (900 PPS, IPI=5 ms, IBP=1 s; applied 45 mins post-HFS) on previously potentiated synapses was examined. The behaviour of the system was monitored at baseline (pre-HFS), post-HFS and post-LFS by the application of 30-60 PPS (IPI=50 ms, IB*P*=30 s) from which the slope and the amplitude of the first Pulse (P1) was measured and analysed. Analysis was performed on the mean slope and amplitude responses obtained from the whole baseline, post-HFS and post-LFS recording duration.

A mixed deign two-way (treatment x stimulation pattern) ANOVA showed a significant main effect of stimulation pattern ($F_{2,117}$= 225.4, p<0.0001), treatment ($F_{3,351}$= 26.15, p<0.0001) and a significant interaction ($F_{6,351}$= 21.49, p<0.0001) between these factors on the slope of the fEPSP response. Simple effects were analysed using paired (α=0.05, p-value adjusted for 3 comparisons per treatment group) and independent *(*α=0.05, p-value adjusted for 6 comparisons per stimulation pattern) Student's t-test corrected for multiple comparisons by Bonferroni analysis. Results revealed that post-HFS response slope was significantly potentiated in all treatment groups in comparison to baseline (scVeh-control p<0.001, scVeh-Hal, scPCP-control, scPCP-Hal p<0.0001), showing that LTP was established by HFS in all treatment groups (**Figure 6.5 A1-4, C1**). Following LFS, scVeh-control response slope was reduced in comparison to post-HFS, but this effect did not reach statistical significance. Post-LFS response slope was also not significantly different from baseline in this treatment group (**Figure 6.5 A1, C1**). In the scPCP-control group, post-LFS response slope was reduced to values below post-HFS and baseline slope measures (LTD-like activity). While this effect reached significance in comparison to post-HFS (p<0.0001), it approached significance in comparison to baseline measures (p=0.07) (**Figure 6.5 A3, C1**). In both scVeh-Hal and scPCP-Hal treatment groups, post-LFS response slope was significantly reduced compared to post-HFS (p<0.0001) while it was not significantly different from baseline (depotentiation) (**Figure 6.5 A2 and A4, C1**).

Further analysis showed that post-HFS, response slope potentiation was stronger in the haloperidol treated groups and this effect reached significance in the scVeh-Hal group in comparison to the scVeh-control (p<0.0001) and scPCP-control (p<0.0001) and in the scPCP-Hal treatment group in comparison to the scPCP-control (p<0.0001) and scVeh-control (p<0.0001) treatment groups. Response slope potentiation was also significantly stronger in the scPCP-Hal in comparison to the scVeh-Hal treatment group (p<0.01). Response slope potentiation was also significantly higher in the scPCP-control in comparison to the scVeh-control treatment group (p<0.01) (**Figure 6.5 C2**). Post-LFS, slope of the response was significantly lower scPCP-control group in comparison to scVeh-control and scPCP-Hal (p<0.0001) and scVeh-Hal (p<0.05) treatment groups (**Figure 6.5 C3**).

Two-way (treatment x stimulation pattern) ANOVA showed a significant main effect of stimulation pattern ($F_{2, 117}$= 52.43, p<0.0001), treatment ($F_{3,351}$ = 123.3, p<0.0001) and a significant treatment x stimulation pattern interaction ($F_{6, 351}$= 32.20, p<0.0001) on the amplitude of the fEPSP response. Simple effects were analysed using paired (α=0.05, p-value adjusted for 3 comparisons per treatment group) and independent (α=0.05, p-value adjusted for 6 comparisons per stimulation pattern) Student's t-test corrected for multiple comparisons by Bonferroni analysis. Results did not detect a significant difference in response amplitude between the stimulation patterns in the scVeh-control treatment group (**Figure 6.5 B1, D1**). In the scVeh-Hal treatment group, post-HFS response amplitude was significantly higher in comparison to baseline (p<0.0001). In the same group, post-LFS response amplitude was also significantly higher compared to baseline (p<0.0001) and significantly lower compared to post-HFS (p<0.001) (**Figure 6.5 B2, D1**). In the scPCP-control treatment group, post-HFS response amplitude was not significantly different in comparison to baseline. However, response amplitude was significantly lower post-LFS in comparison to baseline measures (p<0.0001) and post-HFS (p<0.0001) (**Figure 6.5 B3, D1**). In the scPCP-Hal treatment group, the post-HFS amplitude of the response was significantly higher in comparison to baseline measures (p<0.0001). In this treatment group, post-LFS response amplitude was also significantly higher in comparison to baseline (p<0.01) but this was not significantly different from post-HFS (**Figure 6.5 B4 and D1**).

Further analysis showed that post-HFS, amplitude potentiation was stronger in the haloperidol treated groups and this effect reached significance in the scVeh-Hal treatment group in comparison to the scVeh-control and scPCP-control treatment groups (p<0.0001) and in the scPCP-Hal treatment group in comparison to the scPCP-control (p<0.01) and scVeh-control (p<0.0001). Post-HFS amplitude potentiation was also significantly stronger in the scVeh-Hal treatment group in comparison to the scPCP-Hal treatment groups (p<0.0001). Response amplitude was higher in the scPCP-control compared to scVeh-control but this did not reach significance (**Figure 6.5 D2**). Post-LFS, response amplitude was also higher in the haloperidol treatment groups and this reached significance in the scVeh-Hal treatment group in comparison to scVeh-control, scPCP-control and scPCP-Hal (p<0.0001) and in the scPCP-Hal treatment group in comparison to the scPCP-control (p<0.0001) and scVeh-control (p<0.01) treatment group. Moreover, the amplitude of the response in the scPCP-control was significantly lower than scVeh-control (p<0.001) treatment group (**Figure 6.5 D3**).

These results show that following HFS, LTP was established in response slope in all treatment groups. This was accompanied by a significant potentiation in response amplitude in the scVeh-Hal and scPCP-Hal but not the scVeh-control and scPCP-control treatment groups. Potentiation in both slope and amplitude measures was significantly higher in the haloperidol treatment groups compared to controls. While slope potentiation was significantly higher in the scPCP-Hal treatment group in comparison to scVeh-Hal, the opposite effect was observed for response amplitude. Following LFS, a significant depression was observed in response slope and amplitude in the scPCP-control treatment group which was significantly

lower in comparison to all other treatment groups for both measures. In other treatment groups, response slope depotentiated to values comparable to baseline and there was no significant difference in slope measures between these treatment groups. Post-LFS, response amplitude in the scVeh-control treatment group was reduced to values below the baseline and post-HFS, but this did not reach significance in comparison to either condition. Post-LFS, response amplitude in the scVeh-Hal treatment group was also depotentiated (significantly reduced compared to post-HFS) but it remained significantly higher than baseline. In the scPCP-Hal treatment group, response amplitude was also depotentiated, but this did not reach significance in comparison to post-HFS while remaining significantly higher than baseline. Post-LFS, response amplitude was significantly higher in the haloperidol treatment groups compared to controls. This was also higher for the scVeh-Hal in comparison to the scPCP-Hal treatment group. These findings collectively point towards hyper-excitability/plasticity in response to long-term haloperidol treatment. It also suggests that haloperidol's influence on inhibitory mechanisms regulating response size is different between scVeh and scPCP treated rats.

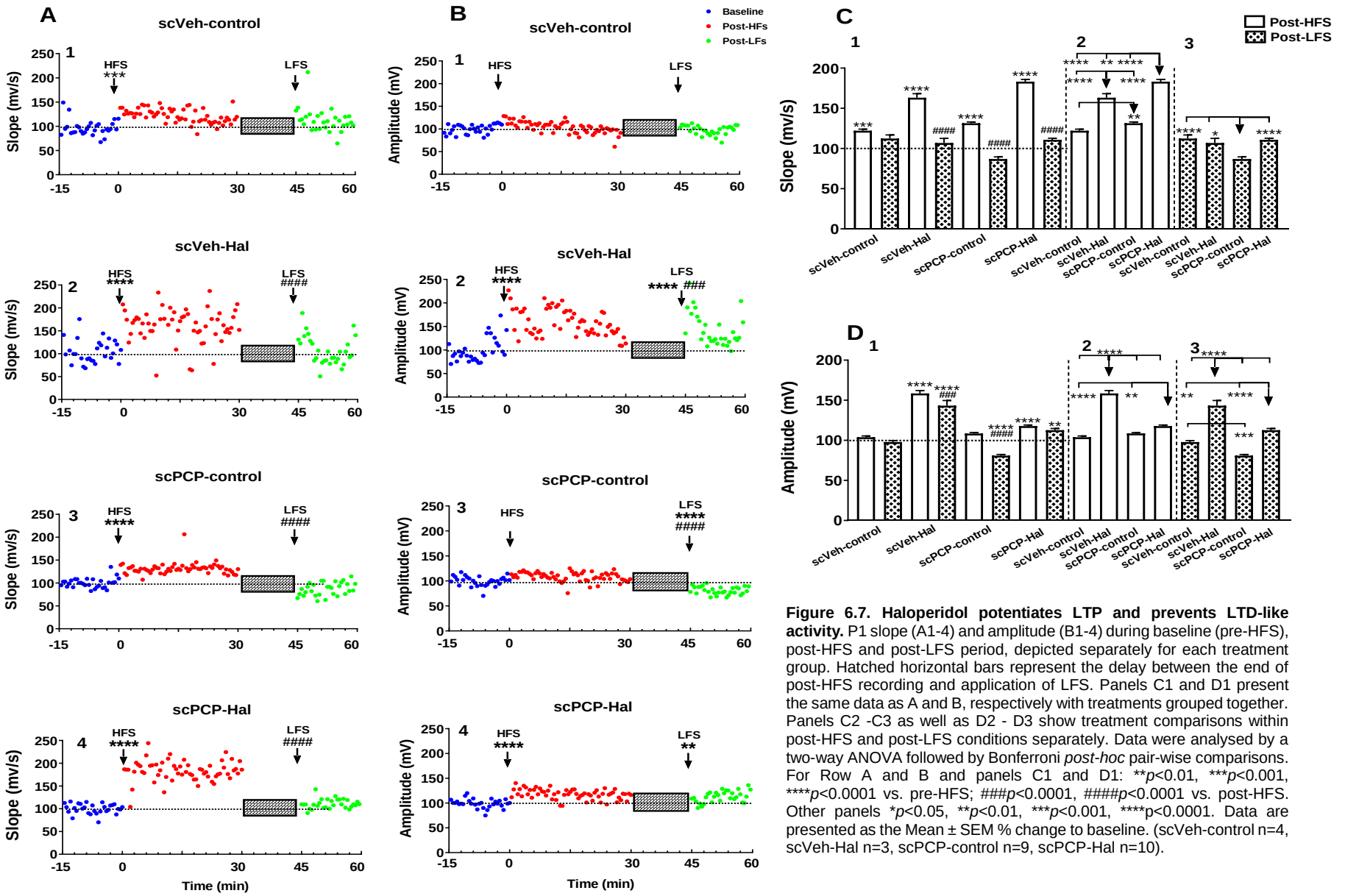

Figure 6.7. Haloperidol potentiates LTP and prevents LTD-like activity. P1 slope (A1-4) and amplitude (B1-4) during baseline (pre-HFS), post-HFS and post-LFS period, depicted separately for each treatment group. Hatched horizontal bars represent the delay between the end of post-HFS recording and application of LFS. Panels C1 and D1 present the same data as A and B, respectively with treatments grouped together. Panels C2 -C3 as well as D2 - D3 show treatment comparisons within post-HFS and post-LFS conditions separately. Data were analysed by a two-way ANOVA followed by Bonferroni *post-hoc* pair-wise comparisons. For Row A and B and panels C1 and D1: ***p*<0.01, ****p*<0.001, *****p*<0.0001 vs. pre-HFS; ###*p*<0.0001, ####*p*<0.0001 vs. post-HFS. Other panels **p*<0.05, ***p*<0.01, ****p*<0.001, ****p<0.0001. Data are presented as the Mean ± SEM % change to baseline. (scVeh-control n=4, scVeh-Hal n=3, scPCP-control n=9, scPCP-Hal n=10).

6.6. Discussion

6.6.1. Long-term treatment with haloperidol did not impair performance on the cNOR-2 in scVeh and scPCP treated rats

Following WO from the scVeh/scPCP treatment and prior to implanting the osmotic minipumps, the performance of the animals in the dNOR and cNOR-1 was assessed. As expected, rats in scVeh and scPCP treatment groups, spent similar time exploring the identical objects in the acquisition phase of both dNOR and cNOR-1, suggesting that rats showed no preference for object location (left vs. right). In agreement with previous findings from this lab (Grayson et al., 2007; Grayson et al., 2014) and others (McKibben et al., 2010) as well as findings reported in **Chapter 5** , rats in the scVeh treatment group showed a significant preference for the novel over the familiar object in the retention phase of the dNOR. This ability was absent in the scPCP treatment group. Although the mean DI values indicate a significant preference for novelty in both treatment groups (DI significantly greater than zero in both scVeh and scPCP treatment groups), the DI score of the scVeh treatment group was marginally higher than the scPCP treatment group but this did not reach significance (p=0.07) (**Figure 6.2 A1-3**). Together, these findings suggest that the scPCP treatment had been effective. In agreement with findings of Grayson and colleagues (2014), scPCP treated rats were able to distinguish between the novel and the familiar object in the cNOR-1. This was manifested as a significant preference for exploring the novel rather than the familiar object in the retention phase of the trial. Furthermore, DI values were similar for both the scVeh and the scPCP treated rats in cNOR-1 with both groups showing a strong preference for novelty (DI>0), which reached significance in the scVeh but not the scPCP treatment group in comparison to zero (**Figure 6.2 B1-3**).

Following 28 days of treatment with haloperidol (0.5 mg/kg/day; via osmotic minipump), performance of the rats was re-tested in the cNOR-2 task. Results revealed that long-term treatment with haloperidol at a clinically relevant dose did not impair the performance of the rats in this task. Similar to their respective control groups, rats in the scVeh-Hal and scPCP-Hal treatment groups explored the novel object significantly more than the familiar object (**Figure 6.2 C2**). This was further confirmed by the finding of no measurable significant difference in the DI values between the treatment groups. Indeed, rats in all treatment groups showed a strong preference for the novel over the familiar object (DI>0; **Figure 6.2 C3**), which reached significance in all treatment groups when compared against zero (**Figure 6.2 C3**). It is important to note that total object exploration time in the retention phase was significantly higher in the scPCP-control treatment group compared to the scVeh-control treatment group (**Table 6.2**). Similarly, total object exploration time appeared higher in the scPCP-Hal treatment group in comparison to the scVeh-Hal treatment group, but this did not reach significance (**Table 6.2**). Collectively, the findings of total object exploration time might indicate a general disinterest in object exploration time in the scVeh-control and scVeh-Hal treatment groups. It is noteworthy that the sample size in the scVeh-control (n=5) and scVeh-Hal (n=5) treatment groups was small. Therefore, the finding of no impairment in the performance of the

scVeh-Hal treatment group should be interpreted with caution. Nonetheless, these findings are supported by Terry and colleagues (Terry et al., 2002; Terry et al., 2006), reporting that 45 days of treatment with haloperidol (2 mg/kg/day; delivered via drinking water) does not impair performance of healthy male Wistar rats on tasks of spatial learning and memory. On the contrary, Ozdemir et al (2012) reported that 14 days of treatment with haloperidol (1 mg/kg/day; i.p.) significantly impaired the performance of healthy mice in the dNOR task. These differences could be attributed to the dose and route of drug delivery as well as gender and intra-species differences.

6.6.2. Long-term treatment with haloperidol does not alter the synaptic connectivity between vHipp-mPFC

Comparison of fEPSP response slope (an index of response strength) and amplitude (an index of response size) at increasing current intensities (100-800μA) did not detect any significant pair-wise differences between the treatment groups. This suggests that the strength and size of glutamatergic input from vHipp to the mPFC was not significantly affected by long-term haloperidol treatment. Similar to the findings of the previous study (**Chapter 5**; see **Figure 5.3 A**) response slope in the scPCP-control was less steep than scVeh-control treatment group which points towards reduced strength of synaptic connectivity (**Figure 6.3 A**). In contrast to **Chapter 5**, however, this effect did not reach significance in the present study. The strength of synaptic connectivity in the scPCP-Hal treatment group was comparable to its control group. Similarly, the strength of synaptic connectivity in the scVeh-Hal treatment group was also comparable to the scPCP-control and scPCP-Hal treatment group. Although not statistically significant, synaptic connectivity was weaker in the scVeh-Hal treatment group in comparison to scVeh-control (**Figure 6.3 A**). Reduced connectivity strength in the scVeh-Hal and scPCP-Hal treatment groups was accompanied by reduced response size while it remained similar for the scVeh and scPCP control groups (**Figure 6.3 B**).

Reduced synaptic connectivity strength in the scVeh-Hal treatment group is unlikely to be due to the alterations in the dendritic spines. This is supported by a recent study where the number of dendritic spines, shape and density remained unchanged upon 28 days of treatment with haloperidol (1 mg/kg/day; i.p.) in the mPFC of male Sprague Dawley rats (Konopaske et al., 2014). The finding of the present study could however be better explained by the effect of haloperidol on the feed-forward/feed-back inhibitory mechanisms.

Pyramidal (Carr and Sesack, 1996) and GABAergic interneurons (Krimer et al., 1997) in the mPFC are heavily innervated by dopamine afferents originating from the ventral tegmental area (VTA). Stimulation of the vHipp is associated with a transient increase in dopamine release in the mPFC (Matsumoto et al., 2008). Through its action on the D1 receptor (D1R) and D2 receptor (D2R), dopamine exerts a strong regulatory control over the micro-circuitry of the mPFC (Gao et al., 2003; Xu and Yao, 2010). Dopamine regulation of GABAergic interneurons is complex. Evidence suggests that pharmacological activation of D2R, reduces the firing rate of pyramidal neurons, by increasing the firing rate and excitability of fast-spiking (parvalbumin

(PV)-containing) interneurons in rat mPFC slices (Tseng and O'Donnell, 2004; Tseng et al., 2006). Dopamine exerts a different effect on non-fast spiking interneurons. In this case, through its action on D2R, dopamine reduces the firing rate of the interneurons, leading to hyperactivity in pyramidal neurons (Tierney et al., 2008). This effect which is also reported in the fast-spiking interneurons is counteracted by D2R antagonists such as Raclopride or Sulpride (Tierney et al., 2008). Similarly, acute D2R blockade by bath application of haloperidol was found to potentiate the GABAergic mediated responses in mPFC slices (Xu and Yao, 2010). Since dopamine regulation of GABAergic interneurons is synapse specific (Gao and Goldman-Rakic, 2003; Gao et al., 2003), it is plausible that these studies have targeted different classes of PV-containing interneurons which differentially synapse onto the pyramidal neurons (Gonzalez-Burgos and Lewis, 2012), and differentially react to dopamine. In these studies, the subclass of fast-spiking and non-fast-spiking interneurons recorded from were not reported.

The inhibitory effects of haloperidol exerted on the system might explain the reduced strength and size of synaptic input in the scVeh-Hal treatment groups. In addition, this effect could be explained by the effect of haloperidol on AMPAR-mediated current. Slice bath application of haloperidol (20-100 nM) was reported to significantly reduce the AMPAR- mediated current in layer V of the mPFC upon stimulation of the forceps minor (Arvanov et al., 1997). This is supported by similar findings by Gemperle et al (2003), who showed a significant reduction in the strength of AMPAR-mediated current in layer V of the mPFC neurons upon stimulation of layer II-III in presence of haloperidol (30-100 μM).

It appears that the inhibitory effects of haloperidol are stronger in the scVeh treatment group compared to the scPCP, since the strength of input in the scPCP-Hal treatment group remains unchanged after 28 days of treatment with haloperidol. As previously mentioned in **Chapter 5,** reduced synaptic strength in the scPCP-control treatment group could partly be due to reduced AMPAR-mediated current. As such it would have been expected that the strength of synaptic connectivity be further reduced in the scPCP-Hal treatment group. It is plausible, however, that the generally reduced inhibitory tone in the scPCP treatment group, in addition to the potential net effect of haloperidol in disinhibiting other inhibitory mechanisms, collectively counteract the effect of haloperidol on AMPAR-mediated current, maintaining the strength of connectivity at scPCP-control level. In contrast with findings of Gemperle et al (2003) and Arvanov et al (1997), other line of evidence suggest that pharmacological activation of D2Rs, reduces the AMPAR-mediated current (Tseng and O'Donnell, 2004), while its blockade with haloperidol increases AMPAR-mediated current through phosphorylation of PKA-ser845 site in the GluR1 subunit of the receptor (Hakansson et al., 2006). It can be suggested that the counteracting effect of haloperidol on AMPAR-mediated current occur within the same system, but their effect becomes apparent when the inhibitory tone is reduced. This could explain control level synaptic connectivity strength in the scPCP-Hal treatment group. Collectively, these findings demonstrate that in the disease model haloperidol does not influence the strength of synaptic connectivity beyond the effects of scPCP treatment but may have an effect on inhibitory mechanisms that regulate response size. The extent to which compensatory mechanisms such as

upregulation of D2R in the mPFC upon long-term exposure to haloperidol contribute to the observed effects in the present study cannot be determined and require more detailed studies.

6.6.3. Haloperidol induces hyper-excitability in the vHipp-mPFC pathway manifested as increased paired-pulse facilitation.

STP in the vHipp-mPFC pathway was examined at baseline, post-HFS and post-LFS by delivering PPS to the vHipp. PPI calculations in each tested condition revealed that across all IPIs, STP was predominantly present in form of PPF (PPI>0) (in both slope and amplitude) in the mPFC. Slope and amplitude facilitation were highest at short (50ms) IPI and decreased at longer IPIs in all treatment groups under all tested conditions. Similar to **Chapter 5**, pair-wise comparisons did not detect a significant difference in slope and amplitude facilitation between the scVeh-control and scPCP-control under any of the tested network conditions. However, while in **Chapter 5** slope facilitation appeared lower in scPCP compared to scVeh treatment group at short (25 ms and 50 ms) IPIs at baseline and post-HFS (**Figure 5.4 A1-3, B1-3**), this trend was absent in the findings of the present study. Amplitude facilitation under all network tested conditions were similar in both studies.

STP comparisons point towards the hyper-excitability of the haloperidol treatment groups. At 100-1000ms IPI, mean slope facilitation was higher in the scVeh-Hal treatment group compared to its scVeh-controls at baseline, but this did not reach statistical significance. This effect was absent at post-HFS and re-emerged post-LFS. Amplitude facilitation was also higher for scVeh-Hal treatment group compared to its control under all tested conditions and similar to slope measures, this also did not reach significance. Similar patterns of activity emerged for the scPCP-Hal treatment group. For this treatment group, slope facilitation was significantly higher in comparison to its control. This hyper-excitability was reflected in the amplitude facilitation of the scPCP-Hal treatment group which was higher than its control at all tested conditions, reaching significance at post-HFS only (**Figure 6.4 A1-3, B1-3**).

Repeated administration of PCP elevates evoked extracellular glutamate and reduces baseline dopamine levels in the mPFC of rats (Amitai et al., 2012). Elevated glutamate release is assumed to be due to the potent influence of PCP on the NMDA receptors located on the GABAergic interneurons, reduced activation of which induces disinhibition of pyramidal neurons (Homayoun and Moghaddam, 2007). Reduced dopamine in the mPFC could be a compensatory mechanism to potentiate action of inhibitory interneurons (Xu and Yao, 2010). It is also known that long-term treatment with haloperidol also reduces dopamine baseline levels in the mPFC (Amato et al., 2011), which could further potentiate the inhibitory effect of the GABAergic interneurons. The finding of higher slope facilitation for the scVeh-Hal and scPCP-Hal treatment groups in comparison to their controls could represent the systems efforts to restore excitatory/inhibitory balance. As mentioned previously, dopamine regulation of GABAergic interneurons is complex and synapse specific. Evidence suggests through its action on D2R, dopamine increases the activity of a subset of fast-spiking interneurons (Tseng and O'Donnell, 2004; Tseng et al., 2006). Therefore, reduced dopamine

in response to prolonged haloperidol treatment might reduce the activity of these interneurons leading to hyper-excitability in the haloperidol treated groups.

STP is often studied as an index of neurotransmitter release probability which is a purely pre-synaptic phenomenon. Previous studies have reported that long-term treatment with haloperidol over 21 days (0.5 mg/kg/day; i.p.) (Yamamoto and Cooperman, 1994), 6 weeks (1 mg/kg/day; delivered through drinking water) (Pietraszek et al., 2002) and 6 months (0.23 mg/kg/day; osmotic minipump) did not increase veratridine or K-evoked glutamate release in the mPFC. Furthermore, acute pre-treatment with haloperidol does not alter glutamate release in response to acute treatment with PCP (Arvanov and Wang, 1999; Arvanov et al., 1997; Adams and Moghaddam, 2001). Similarly, short-term treatment with haloperidol (0.5 mg/kg/day; i.p.; 7 days treatment with haloperidol followed by 7 days of haloperidol and PCP treatment; (PCP: 2mg/kg/bidaily for 7 days; i.p.) does not alter scPCP-induced elevation in electrical-evoked glutamate release (Ninan et al., 2003). Given these findings, the extent to which pre-synaptic events such as concentration of available neurotransmitter for release and release probability as well as changes in the neurotransmitter releasing machinery contribute to the observed hyper-excitability cannot be determined. Currently, there is a clear gap in understanding of the influence of long-term AP treatment on molecular markers of synaptic integrity, including synaptosomal-associated protein of 25 kDa (SNAP-25), which plays an important role in neurotransmitter release (Sudhof, 2012).

As depicted in **Figure 6.4 A1-3 and B1-3**, the slope and amplitude facilitation in the haloperidol treated groups persists at longer IPIs (200-1000 ms) under all three PP recording conditions, an effect which is stronger in the scPCP treatment group. These findings are unlikely to be due to alterations in the glutamate transporters as the density of these proteins is not affected by long-term treatment with haloperidol. This has been confirmed by three studies reporting control level glutamate transporters in the mPFC of male Sprague Dawley rats upon 21 days of treatment with haloperidol (1 mg/kg/day; p.o.) (Spurney et al., 1999), in male Wistar rats upon 4 weeks of depot injection with haloperidol (12 mg/kg/week; s.c.) (Burger et al., 2005) and in male Sprague Dawley rats upon 6 months of treatment with haloperidol decanoate (28 mg/kg/every 3 weeks for 6 months) (O'Donovan et al., 2015). Alterations in the expression level of AMPAR could account for the findings presented here. 21 days of treatment with haloperidol (0.5 mg/kg/day; i.p.) was found to significantly increase high-affinity AMPA binding sites in the mPFC of Sprague Dawley rats (McCoy et al., 1998). In the face of unchanged glutamate transporter levels, upregulation of the high-affinity AMPAR not only provides more binding sites for the excitatory neurotransmitter but also allows for longer glutamate-receptor interaction, allowing for strong slope and amplitude facilitation to be observed at longer IPIs (up to 1000ms).

A more plausible explanation for these findings is the alteration in the conductance of presynaptic $GABA_B$ receptors ($GABA_BR$). $GABA_BRs$ play a key role in regulating GABA release from the interneurons (Lacey et al., 1988; Nathan et al., 1990; Olpe et al., 1994). Electrophysiological studies using paired-pulse stimulation protocols have revealed a conductance range of 350-4000 ms for the $GABA_BRs$. During this

range of IPIs and in the presence of $GABA_BR$ agonist, the response evoked from the second pulse is smaller than the first in the pyramidal post-synaptic neuron, suggesting an increase in GABA release (Nathan et al., 1990). An opposite effect is reported in the presence of a potent antagonist, whereby response evoked from the second pulse is larger than the first (Olpe et al., 1994). Persistence of slope and amplitude PPF in longer IPIs observed in the present study, reflects the conductance range of the $GABA_BR$ and suggests that the conductance of this receptor may be reduced by haloperidol treatment.

$GABA_BR$ and D2Rs interact to activate G-protein coupled inwardly rectifying potassium channels, which potentiate $GABA_BR$-mediated inhibitory post-synaptic current (Lalive et al., 2014). This interaction is dependent on the activation of the NMDARs, so that the activation of the NMDARs leads to a depression in the $GABA_BR$-mediated current (Lalive et al., 2014; Lalive and Luscher 2016). Prolonged blockade of D2Rs, in addition to the effects of haloperidol on the NMDAR conductance (see below), could result in the depression of the $GABA_BR$-mediated current, leading to hyper-excitability of the pyramidal neurons, at the range of $GABA_BR$ conductance. Effect of scPCP treatment on the NMDAR-mediated current (as explained in **Chapter 5**) may explain why these observed effects are stronger in the scPCP-Hal treatment group in comparison to the scVeh-Hal. It is important to mention that thus far, these mechanisms have been identified and studied in the hippocampus and the VTA. Whether similar mechanisms are involved in the circuitry of the mPFC remains to be determined.

It is noteworthy that slope and amplitude PPI of each treatment group was similar across all three tested conditions, suggesting that the effects of HFS and LFS are predominantly post-synaptic. A closer observation of the pattern of slope PPI shows a trend towards higher slope facilitation post-LFS compared to post-HFS in the scPCP-Hal treatment group. As discussed in the **Chapter 5**, this may suggest a pre-synaptic locus for the effect of HFS and LFS. The extent to which post-synaptic or pre-synaptic factors contribute to the expression of LTP cannot be determined by these observations. In depth investigations into the factors mentioned here are essential for further elucidating the impact of APs on synaptic function. It must be noted that in this study, the sample size for the scVeh-control and scVeh-Hal treatment groups was small (n=4). Future research must examine these parameters using a larger sample size to reinforce statistical power.

6.6.4. Long-term treatment with haloperidol potentiates LTP and prevents LTD-like activity to be established in the scPCP treated rats

In order to assess the influence of long-term haloperidol treatment on the ability of the system to support LTP and its subsequent reversal, HFS (5 trains of 20 pulses at 200 Hz) and LFS (900 PPs, 5 ms IPI, 1Hz; applied 45 mins post-HFS) were delivered to the vHipp, respectively. Similar to the findings of **Chapter 5**, HFS significantly potentiated the strength of the response in comparison to baseline measures, suggesting that LTP could be established in all treatment groups (**Figure 6.5 A1-4, C1**). The magnitude of this potentiation was significantly higher in the haloperidol treated group in comparison to controls (**Figure 6.5**

C2), an effect that is reported to be absent upon acute treatment with haloperidol (1 mg/kg; i.p.) under similar experimental conditions (Matsumoto et al., 2008). Most importantly, slope potentiation was significantly higher for the scPCP-Hal in comparison to scVeh-Hal treatment group (**Figure 6.5 C1 and C2**). HFS was also accompanied by a significant potentiation in response amplitude in the haloperidol treated groups. In contrast with slope measures however, amplitude potentiation was significantly higher in the scVeh-Hal in comparison to the scPCP-Hal treatment group (**Figure 6.5 B1-4, D1-3**). In contrast to the previous study, however, HFS-induced amplitude potentiation was absent in both scVeh-control and scPCP-control treatment group. For the scVeh-control treatment group, this might partly be explained by the small sample size (n=4) which is smaller than the sample size in the previous study (n=9). Furthermore, in contrast to the previous study, the present study implemented a staggered experimental design to reduce the potentially confounding effect of age on electrophysiological recordings. In doing so, and due to the general setup of the experiment, rats used in this study (20-23 weeks old) were much older than the majority of rats use in the study presented in **Chapter 5** (11-25 weeks old) at the time or recording. Evidence from in-vivo electrophysiological studies points towards reduced excitability of pyramidal neurons in the CA1 region of the hippocampus (Oh et al., 2016) and decreased action potential amplitude in the mPFC (Chang et al., 2005; Rizzo et al., 2015). It is therefore plausible that the discrepancies in the amplitude findings of these two studies are due to the confounding effect of age on synaptic properties of the vHipp-mPFC pathway.

Nevertheless, these findings of haloperidol treated groups point towards a haloperidol-induced hyper-excitability in the vHipp-mPFC pathway and are in agreement with the hyper-excitability observed in the STP. *In vitro*, slice bath application of haloperidol (50 nM) has been reported to significantly facilitate the NMDAR-mediated current in pyramidal cells of the mPFC under both voltage clamp and current clamp conditions, in the presence of bicuculine ($GABA_A$ antagonist) (Arvanov et al., 1997). This has been confirmed by other *in vitro* studies of rat mPFC slices using other D2R antagonists (Tseng and O'Donnell., 2004). Although experimental conditions of such *in vitro* studies are not comparable to the present *in vivo* study, this finding might provide some explanation with regards to the stronger LTP induction in the haloperidol treatment groups. It is important to note that in these experiments an increase in the NMDAR-mediated current is not associated with an increase in excitatory neurotransmitter release (Arvanov et al., 1997; Ninan et al., 2003). Therefore, the observed hyper-excitability in the scVeh-Hal treatment group is predominantly explained by reduction of inhibitory control, possibly mediated through the NMDAR-.$GABA_B$R-D2R interactions. As discussed in the **Chapter 5**, sub-chronic treatment with PCP results in hypersensitivity/responsivity of the NMDA receptors in the mPFC (Arvanov and Wang, 1999) (Ninan et al., 2003). Reduced inhibition by haloperidol treatment in addition to the scPCP-induced depolarisation is a prime candidate mechanism for stronger LTP induction in the scPCP-Hal treatment group. The findings of the amplitude potentiation point towards the differential effect of haloperidol treatment on the inhibitory mechanisms that regulate response size in the scPCP and the scVeh treatment groups.

These observations are unlikely to be due to alteration in expression levels of NMDAR subunits upon long-term haloperidol treatment. As reported by Krzystanek et al (2016), 4 weeks of treatment with haloperidol (1 mg/kg/day; i.p.) did not alter the expression of NMDAR subunits in the cortex, however, it was associated with a significant reduction in the expression of NR2A subunit in the thalamus (Krzystanek et al., 2016). Given the strong connections between the thalamus and the cortex, such alterations might also influence the observed effects in the mPFC.

Application of LFS to previously potentiated synapses in the scVeh-control treatment group did not have an effect on response slope or amplitude while in the scPCP-control treatment group; it significantly reduced response slope and amplitude to values below baseline (**Figure 6.5 A1 and A3, B1and B3, C1 and D1**). In the **Chapter 5**, LTD was observed in both scVeh and scPCP treatment groups. The inconsistencies in the findings of scVeh-control treatment group between studies could be attributed to the small sample size in this treatment group in the present study. LFS depontentiated response slope in both scVeh-Hal and scPCP-Hal treatment groups to baseline (**Figure 6.5 A2 and A4, C1 and C3**). In the scVeh-Hal treatment group, response amplitude was significantly reduced in comparison to post-HFS but remained significantly superior to baseline. This is while in the scPCP-Hal treatment group, depotentiation effect was minimal and subtle. To an extent, this observation in the scVeh-Hal treatment group could be attributed to its small sample size (n=3). Therefore, results in this treatment group should be interpreted with caution. Collectively, these findings suggest that in the scVeh-Hal and scPCP-Hal treatment group the inhibitory mechanisms that regulate response size are differentially affected in response to haloperidol treatment. It can be suggested that LFS is less able to produce LTD in the scPCP-Hal treated rats, an effect that might also be explained by the influence of haloperidol on the inhibitory mechanism. More investigations are required to delineate the nature of these observations.

6.7. Conclusion

To my knowledge, this is the first study to examine the influence of long-term haloperidol treatment on synaptic properties of the vHipp-mPFC pathway in an animal model for cognitive impairments associated with schizophrenia. Collectively these investigations suggest that long-term haloperidol treatment does not significantly alter the synaptic connectivity between the vHipp-mPFC. However, the strength and amplitude of the fEPSP response was generally smaller in the scVeh-Hal in comparison to the scVeh-control treatment group. This may be due to the effect of haloperidol on reducing AMPAR-mediated current and enhancing inhibitory constraint on pyramidal firing depolarization levels. It can be hypothesized that several compensatory mechanisms including generally reduced inhibitory tone in the scPCP treatment group, prevents further reduction in the strength of the excitatory synaptic connectivity in the vHipp-mPFC pathway in the scPCP-Hal treatment group. Investigations of the STP revealed an interesting pattern of results, pointing towards haloperidol induced hyper-excitability in the vHipp-mPFC pathway, an effect which is stronger in the scPCP-Hal treatment group in comparison to its control counterpart. Disruptions in the $GABA_BR$-D2R-NMDAR interaction was hypothesized to mediate this observed effect. Long-term

haloperidol treatment also potentiated LTP in comparison to controls. This augmentation was significantly higher in slope measures in the scPCP-Hal treatment group in comparison to its control counterpart. This may result from an increase in NMDAR sensitivity as a result of haloperidol and PCP treatment.

Enhanced response strength in the scPCP-Hal treated rats upon HFS is not necessarily associated with better/worse performance in vHipp-mPFC pathway dependent behavioural paradigms. Given that the NMDARs act as coincidence detectors and optimise signal-to-noise ratio upon receiving stimuli, their over-activation in the scPCP-Hal treatment group could point towards aberrant response to relatively irrelevant stimuli. In addition to its involvement in higher-order cognitive processes, hyperactivity of the vHipp circuitry and disruptions in the vHipp-mPFC interaction has been implicated in the emergence of psychosis (Jodo, 2013; Samudra et al., 2015; Eichenbaum, 2017). Therefore, the observed hyper-excitability of the vHipp-mPFC pathway could point towards treatment failure and relapse of psychotic symptoms, a phenomenon that is observed in a sub-class of patients upon prolonged treatment with APs (**Chapters 1.1** and **3**). The dysregulation of STP and long-term synaptic plasticity may also have implications in higher-order cognitive processes. These, however, were not reflected in the behavioural paradigms employed in this study, as these tests do not depend on vHipp-mPFC interaction for successful performance (**Chapter 1.3)**.

This study has several limitations. The sample size in the scVeh-control and scVeh-Hal was very small (n=3-4 depending on the analysis; see relevant sections for details). As such, findings of relevance to these groups must be interpreted with caution. Since this study implemented a staggered design (partly to reduce the potentially confounding effect of age on electrophysiological recordings), the extent to which the findings of the scVeh-control and scPCP-control groups in the present study are comparable to those in the previous chapter may be limited. Therefore, any comparisons made between these treatment groups across studies of chapter 5 and 6 must be interpreted with caution. Most importantly, the validity of the findings of the present study is subject to the assessment of blood plasma samples collected on the last day of haloperidol treatment. This is essential to confirm that an expected haloperidol plasma concentration has been established and that there are no contaminations in the control treatment groups (See **Chapter 4**). Nevertheless, the findings of this study provide a preliminary understanding of the impact of long-term AP treatment on neural networks involved in cognition. In order to understand the underlying mechanisms that mediate these effects, many in depth studies using variety of electrophysiological and pharmacological techniques are required. Functional/behavioural consequences of these synaptic alterations must be examined using more complex behavioural paradigms that require integration of spatial, temporal and contextual information to target vHipp-mPFC interaction.

Chapter 7

General Discussion

1.7. Main Aims and Objectives

The current understanding of the neurocognitive impact of long-term treatment with antipsychotics (APs) remains limited in the clinic. Animal models of cognitive impairments associated with schizophrenia provide a paradigm for systematic investigations of these effects. Thus far, the translational validity of pre-clinical studies has been limited due to methodological shortcomings. The primary aim of this project was to investigate the neurocognitive impact of long-term treatment with haloperidol and olanzapine and to systematically address some of the methodological limitations common to pre-clinical practice (inappropriate dose and route of drug delivery). To this end, a series of behavioural studies were conducted in the well-validated sub-chronic phencyclidine (scPCP) model for cognitive impairments associated with schizophrenia.

The processes of synaptic plasticity are fundamental to information processing and ultimately cognition. Evidence from clinical studies points towards dysregulations of synaptic plasticity in patients with schizophrenia. Similarly, several pre-clinical studies have also identified plasticity and connectivity disturbances in genetic (Sigurdsson, 2016; Sigurdsson et al., 2010), neurodevelopmental (Goto and Grace., 2006) and pharmacological (Blot et al., 2015) animal models for the disease. This project aimed to characterise the synaptic properties (synaptic connectivity and short- and long-term synaptic plasticity) of the ventral hippocampus (vHipp)- medial prefrontal cortex (mPFC) pathway in the scPCP model for the first time. This pathway was selected as it is involved in mediating a range of higher-order cognitive functions including episodic memory, executive function and goal directed behaviour, deficits in which are reported in patients with schizophrenia. Substantial evidence, derived primarily from *in vitro* brain slice preparations of physiologically healthy rodents, suggest that APs alter the processes of synaptic plasticity within neural circuitries. These processes have seldom been studied *in vivo* and in disease models. Therefore, this project also aimed to investigate the impact of long-term haloperidol treatment on synaptic properties of the vHipp-mPFC pathway. To summarise, this project aimed to fulfil the three following objectives:

1. To investigate the influence of long-term haloperidol and olanzapine treatment on cognitive performance in the scPCP rat model of cognitive impairments associated with schizophrenia. This involved systematically addressing some of methodological limitations (inappropriate dose and route of drug treatment) common to pre-clinical practice to improve translational validity of the findings.
2. To characterise synaptic connectivity and plasticity in the vHipp-mPFC pathway in the scPCP rat model for cognitive impairments associated with schizophrenia.
3. To investigate the effect of long-term haloperidol treatment on the synaptic properties of the vHipp-mPFC pathway in the scPCP model.

Next, the experimental evidence and main findings derived from studies conducted in this thesis will be discussed in relation to these objectives.

7.2. An investigation into the influence of long-term treatment with haloperidol and olanzapine using a combination of behavioural techniques: Addressing methodological limitations in pre-clinical research.

Chapter 3 describes a series of behavioural studies designed to address the first aim of this project. In this study, scPCP rats were treated with haloperidol (0.1 mg/kg/day; oral administration; p.o.) or olanzapine (1.5 mg/kg/day; p.o.) for 22 days. Over this treatment period, their behaviour was assessed in the disrupted novel object recognition (dNOR) paradigm, which was used as a measure of cognition. This paradigm which is identified as a test of visual learning and memory (Young et al., 2009) is thought to provide information about elements of episodic memory as it relies on the ability to identify previously encountered elements or episodes (Morici et al., 2015). This test was selected as deficits in visual learning and memory and episodic memory are reported in patients with schizophrenia (**Chapter 1.2**). Performance in this test was first assessed on the initial day of treatment with APs and was repeated once per week until treatment end. Throughout this period, dNOR test was always conducted 90 minutes after AP treatment administration. Rats then underwent a week-long washout period during which the performance in the dNOR test was assessed on the first and the last day of the washout.

A number of studies had previously investigated the effects of acute treatment with haloperidol (Grayson et al., 2007) or olanzapine (Snigdha et al., 2010) in rescuing scPCP-induced deficit in dNOR performance. Several studies have also investigated the influence of short-term (up to 14 days) treatment with haloperidol on dNOR performance in a range of animal models for the cognitive impairments associated with schizophrenia (Nagai et al., 2011; Ozdemir et al., 2012; Ozawa et al., 2006). However, evidence for the influence of short-term treatment with olanzapine and head-to-head comparison of the long-term neurocognitive effects of both haloperidol and olanzapine on performance of scPCP rats in the dNOR task is lacking in the literature. To my knowledge, the data in **Chapter 3** represents the first study to compare the effects of acute, short-term and long-term treatment with haloperidol and olanzapine on cognitive performance in scPCP rats. Furthermore, by assessing behaviour over the washout period, this study provided the opportunity to capture potentially long-lasting “off drug” neurocognitive effects of these APs.

Results of this study were, however, inconclusive since the general performance of the animals in the dNOR tests was highly variable. Therefore, the effectiveness of the scPCP treatment in inducing a robust dNOR deficit could not be confirmed. Consequently, it was challenging to meaningfully interpret the behavioural outcome of the AP treated groups **(Table 3.8).** Critical analysis of a range of potentially confounding variables highlighted the impact of handling and the inherent variability of the one-trial dNOR in the observed effects. In this study, animals were handled prior to the scPCP dosing regimen to habituate them to the dosing holding position. An independent study recently conducted by other researchers in our laboratory points towards the protective effect of regular handling against the scPCP-induced deficit in dNOR. In this study, rats were handled for two weeks (10 minutes/animal/day) prior to sub-chronic treatment with PCP. Results show that handled scPCP-treated animals successfully discriminated between

novel and familiar objects in the same manner as controls, while non-handled scPCP treated rats could not, showing the expected deficit in object recognition memory (**Figure 3.8**).

It is noteworthy that the handling implemented in **Chapter 3** did not follow a systematic pattern/duration, nor did it occur every day. Nevertheless, it remains a possibility that this is the reason for the lack of a robust dNOR deficit in this study. Currently, there is no evidence for the influence of regular handling, which is considered a form of enrichment (Pritchard et al., 2013), on rescuing the scPCP-induced dNOR deficit. It is however, plausible that similar to the isolation rearing model (Pritchard et al., 2013; Weiss et al., 1999), the scPCP model is also sensitive to the effects of handling. Therefore, daily handling of the rats (for dosing purposes) could have also contributed to the observed effects.

Another potential explanation for the inconsistencies in the results of **Chapter 3** is the inherently variable nature of the one-trial dNOR paradigm. This will be discussed in more detail at a later point in this section. Results from the study presented in **Chapter 3** suffered from another experimental limitation commonly seen in pre-clinical studies. This was the poor choice of AP route of delivery to the animals for the duration of the study. As discussed in depth in **Chapters 3** and **4**, the rate of metabolism of haloperidol and olanzapine is 4-6 times higher in rodents in comparison to humans (Kapur et al., 2003). Therefore, once-daily administration of these compounds, even in the long-term, may not be representative of clinical practice, as drug-tissue distribution cannot reach a steady state.

Chapter 4, describes a set of experiments designed specifically to address the methodological limitations of the study described in **Chapter 3**. This included elimination of handling prior to scPCP treatment and the introduction of osmotic minipumps as an alternative route for drug delivery. In this study, haloperidol (0.5 mg/kg/day) was delivered via the osmotic minipump over 28 days. This is only the second study to use osmotic minipumps in the context of a cognitive/behavioural study in a well-validated animal model of the disease. In the first osmotic minipump study Amitai et al (2007) tested the effects of clozapine (4 mg/kg/day) in the performance of scPCP rats in the 5-CSRTT (Amitai et al., 2007).

In addition to using the dNOR paradigm, the study presented in **Chapter 4** also employed a variation of this test, known as the continuous NOR (cNOR) (Grayson et al., 2014). It was reasoned (see **Chapter 4**) that while the dNOR is reliable in testing the efficacy of drug treatments to rescue scPCP-induced deficit, the cNOR test is more sensitive to the potentially detrimental effects of AP treatment on cognition. In this study, two experimental steps were employed to ensure that the scPCP treatment had been effective. The first step was a baseline behavioural assessment using the dNOR and cNOR tasks prior to osmotic minipump implant. The second step examined the well-validated scPCP-induced sensitisation to amphetamine (Amph) to confirm effectiveness of scPCP treatment (Janhunen et al., 2015). This effect, which is manifested as hyperactivity in the scPCP treated rats, was tested on day 17 post-minipump implant in automated locomotor activity chambers. The dNOR and cNOR tests were also repeated on days 12 and 26 (for dNOR) and 14 and 28 (for cNOR) during treatment with haloperidol.

The baseline behavioural assessment revealed a deficit in the performance of dNOR-1 task in the scPCP treatment group. Since a similar deficit in novelty detection was also present in the scVeh treatment group at baseline (possibly due to natural variability in behaviour), the effectiveness of the scPCP treatment could not be confidently confirmed at this stage (**Figure 4.2**). Indeed, assessment of the effectiveness of the scPCP treatment by the automated locomotor activity study was also confounded because of haloperidol contamination in the "scVeh/scPCP-control" treatment groups. As discussed in depth in **Chapter 4**, rats in the "scVeh/scPCP-control" (denoted scVeh-Hal-Low and scPCP-Hal-Low, respectively) treatment groups were contaminated with a lower dose of haloperidol for long-term (estimated to be approximately 0.25 mg/kg/day based on the plasma concentration findings, **Table 4.2**). Nevertheless, rats in the scPCP-Hal-Low treatment group appeared to be more sensitive to the effect of Amph compared to the scVeh-Hal-Low treatment group. Although this effect did not reach significance (p=0.054), the finding of marginally higher total LMA count in the scPCP-Hal-Low-Amph treatment group compared to its counter control suggests that the scPCP treatment may have been effective (**Figure 4.8, Table 4.10**). Indeed, sample sizes as low as 4 in some treatment groups in the automated-LMA study, render the statistical analysis underpowered. It is possible that in case of higher power and sample size, a robust Amph-induced hyper-locomotor activity would have been observed in the scPCP-Hal-Low-Amph treatment group. It is noteworthy that during the course of this study, rats were weighed everyday as part of their peri- and post-operative care. It is plausible that in the absence of this level of handling, a more robust effect would have been observed in the automated locomotor activity test in the scPCP-Hal-Low treatment group in response to Amph. The potential effects of low-dose haloperidol contamination and the influence of acute Amph treatment on the locomotor activity of rats in the "scVeh/scPCP-Hal experimental" treatment groups (denoted scVeh-Hal-High and scPCP-Hal-High) will be discussed in more detail in **Section 7.5**.

In this study also, the outcome of the dNOR and cNOR tests, following osmotic minipump implant, were highly variable (**Table 4.8**). However, unlike **Chapter 3**, the study presented in **Chapter 4** could, to some extent, exclude the ineffectiveness of scPCP treatment as a source of this variability. The conclusive power of the study presented in **Chapter 4** was, however, considerably limited due to the presence of haloperidol contamination in the control treatment groups. Nevertheless, the variability in behavioural performance could not be fully explained by the effect of haloperidol contamination (see **Section 4.6** for a detailed discussion). As briefly mentioned in this chapter, the outcome of the one-trial NOR paradigm is inherently variable. Since this task exploits the spontaneous exploratory behaviour of animals, it is sensitive to factors that can interfere with explorative behaviour including animal stress levels, external noise (Ameen-Ali et al., 2015) and as observed in studies presented here also by handling. Therefore, results obtained from NOR can be ambiguous and be misinterpreted (Chan et al., 2018). The probability of variable findings increases as the test is repeated over time, an effect that was observed in studies presented in **Chapters 3** and **4**. This suggests that using the one-trial dNOR and cNOR tasks may not be suitable for repeated testing as presented in these chapters. It should be noted however, that previous work from our laboratory has been

able to successfully repeat the dNOR paradigm in an experimental design similar to **Chapter 3** (Leger et al., 2015).

As discussed in **Chapter 4**, the continual trial NOR (not to be mistaken for cNOR) is a newly developed variation of the traditional one-trial NOR whereby the same animal can be tested several times over a single session, reducing performance variability (Ameen-Ali et al., 2012). During the inter-trial interval animals are held in an inbuilt holding area which is connected to an object area. These two compartments are connected by removable doors which the animals are trained to shuttle through (**Figure 7.1**). As such, the continual trial NOR also eliminates the influence of handling on performance. Upon its validation in the scPCP model, this approach could be taken instead of the traditional NOR task as conducted in this thesis, allowing for more reliable results.

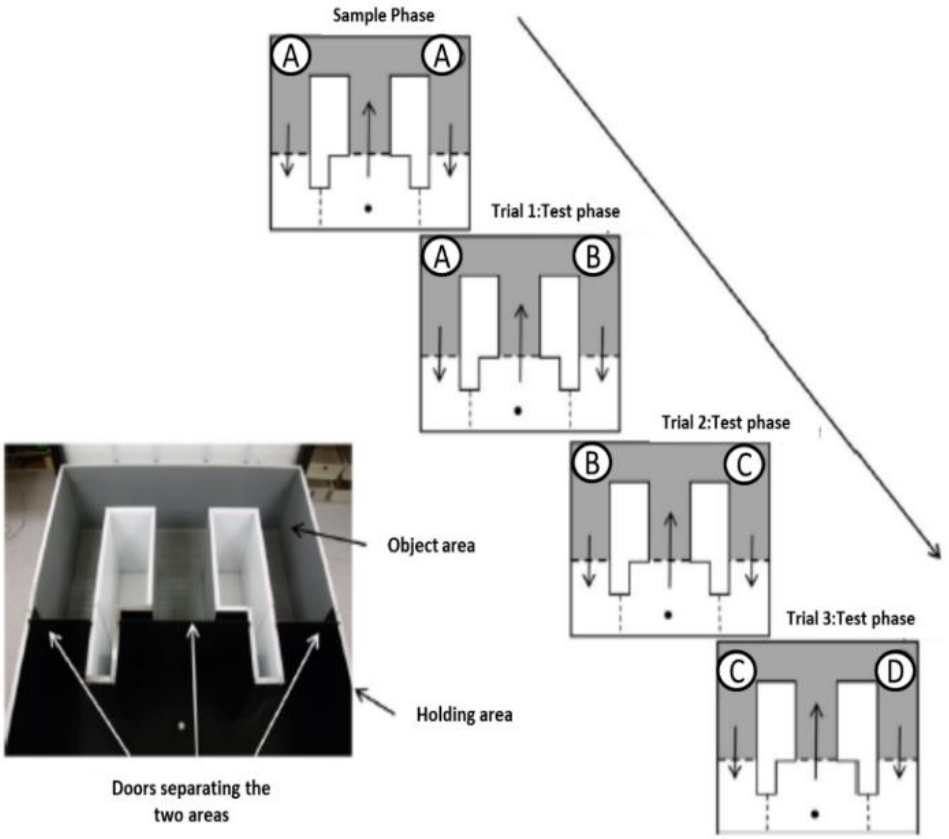

Figure 7.1. Continual trial NOR testing Box and an example of task procedure. The image on the left is a photograph of the continual trial NOR testing box. The rats are trained to shuttle through the removable doors. They are held in the holding area during the ITI and are allowed to explore objects in the object area at sample and test phase. The image on the right, represents an example of task procedure. Here, the animals are presented with two identical objects which they explore for 2-3 minutes. They return to the holding area for ITI and then are given 2-3 minutes to explore the objects in the trial 1. In each test phase a novel object is introduced while the novel object from the previous study will be considered as a familiar. Test can also be conducted with an independent sample (acquisition) phase for each trial to resemble the classic one-trial NOR. Figure adapted from Ameen-Ali et al (2015)

Collectively, studies in **Chapters 3** and **4** failed to completely fulfil the first objective of this thesis due to methodological limitations. However, **Chapter 6**, provided the opportunity for a third attempt to examine the impact of long-term treatment haloperidol on cognition. Similar to **Chapter 4**, animals in **Chapter 6** were also treated with haloperidol for 28 days which was delivered via subcutaneously implanted osmotic minipumps. In this study, behavioural performance was also assessed at baseline using the dNOR and cNOR tests, prior to osmotic minipump implant, to ensure the effectiveness of the scPCP treatment. Rats were then left in their home cage for the duration of the treatment and re-tested in the cNOR on the last day (day 28) (no daily handling except for 7 days prior and post minipump implant as part of peri- and post-operative care). Based on previous evidence from other research groups, it was hypothesised that it is unlikely that haloperidol would rescue the scPCP-induced deficit in the dNOR task (Nagai et al., 2011; Ozdemir et al., 2012; also see **Chapters 1.7.2, 3** and **4** for more detail). Therefore, this study primarily focused on determining whether long-term treatment with haloperidol would impair performance on a task scPCP rats can perform. Results confirmed that for the duration studied, haloperidol did not impair cognition on the cNOR task in the scPCP and scVeh treatment groups. To my knowledge this is the first study of the influence of long-term treatment with haloperidol on the cNOR task in the scPCP treated rats. The results of this study which fulfilled the first objective of this thesis, are in agreement with previous findings reporting no cognitive impairment in healthy rats in performance of a working memory paradigm (a variation of the Morris Water maze) after 45 days of treatment with haloperidol at a much higher dose (2 mg/kg/day; delivered through drinking water) (Terry et al., 2002; Terry et al., 2006). The findings of the behavioural study presented in **Chapter 6** must be interpreted with caution. Since the sample size of the scVeh-control and scVeh-Hal treatment groups was small, the study may have been underpowered. Future investigations must consider re-examining these effects in a larger sample size.

7.3. Characterising the synaptic properties of the vHipp-mPFC pathway is the scPCP model

Intact synaptic connectivity and dynamic alteration in synaptic weight are fundamental to accurate flow of information within and between brain regions which, in turn, are essential for cognitive processes. The significance of the vHipp-mPFC pathway in mediating cognition and its relevance to schizophrenia was thoroughly discussed in **Chapters 1.9 and 5**. Several research groups had previously examined aspects of synaptic plasticity and the functional interaction (coherence, synchrony and spike-phase coupling) between these two regions in animal models for the disease (Goto and Grace, 2006; Dickerson et al., 2010; Sigurdsson et al., 2010; Dickerson et al., 2012; Blot et al., 2015). **Chapter 5** describes the first study to investigate the synaptic properties of the vHipp-mPFC pathway in the scPCP model. Experiments described in **Chapter 6** were planned to re-examine these properties in an independent cohort in order to provide evidence for the validity of these findings.

This study revealed a significant reduction in the strength of synaptic connectivity between vHipp and the mPFC in the scPCP (n=20) treatment group compared to controls (n=11) (**Figure 5.3 A**). A trend towards

this effect was also observed in the study reported in **Chapter 6** in a new cohort of scPCP-treated animals, but this effect did not reach significance (**Figure 6.3 A**). The observed effect in **Chapter 6** could be attributed to the small sample size in the scVeh-control (n=4) and scPCP (n=7) treatment groups. Collectively these findings point towards disruption in the efficacy of information transfer between these two regions in the scPCP model. Reduced functional integration between the HF and mPFC has also been reported in the scPCP model (Dawson et al., 2014). In their study, Dawson and colleagues (2014) suggest that this finding can be attributed to the dysconnectivity of the thalamus which is key in mediating communication between the HF and mPFC (Cassel et al., 2013). The results of the study presented in **Chapter 5 and 6** are complementary to existing evidence by suggesting that altered synaptic connectivity in the only direct connection between the PFC and HF, could contribute to the alterations in functional integration between these two regions.

It must be noted that, in these studies, no synaptic connectivity abnormalities were detected in the amplitude (response size) measurements. This could suggest that while the excitatory input into the mPFC is dysfunctional, the processes of feed-forward/feed-back inhibition that regulate response size may remain intact. Parvalbumin (PV)-containing interneurons in the mPFC receive monosynaptic input from vHipp and can regulate the response through feed-forward and feed-back mechanisms (Gabbott et al., 2002; Tierney et al., 2004). scPCP treatment is associated with a significant reduction in the expression of parvalbumin (PV) (Cochran et al., 2003; Abdul-Monim et al., 2007; Jenkins et al., 2008; McKibben et al., 2010), therefore, response amplitude would have been expected to be larger in the scPCP treated rats if loss of PV in these cells renders them unable to provide normal level of inhibition. Evidence from brain-derived neurotrophic factor (BDNF) IV promoter (contributes to transcription of BDNF) knockout (KO) mice contradict this hypothesis (Sakata et al., 2009). BDNF-IV KO mice exhibit significant reduction in the expression of PV interneurons in the mPFC, accompanied by reduced inhibitory input to the layer V of the mPFC (Sakata et al., 2009; Sakata et al., 2013). Whole cell and voltage-clamp recordings, however, revealed that this did not have an effect on the amplitude and the frequency of the spontaneous excitatory response (Sakata et al., 2009). These studies support the involvement of other inhibitory systems or compensatory mechanisms in regulating response size. Indeed, sub-chronic treatment with PCP (2 mg/kg/day for 7 days followed by 7 days of washout) has been reported to reduce BDNF mRNA expression in the mPFC and hippocampus in female Lister Hooded rats (Snigdha et al., 2011b). Therefore, findings of Sakata and colleagues (2009; 2013) may, to some extent, explain the findings reported in **Chapter 5.** Nevertheless, given the species (mice vs. rats) and gender (male vs. female) differences between the studies, caution must be observed when interpreting these results.

Processes of short-term synaptic plasticity (STP) are often studied as an index of neurotransmitter release probability. Reports of acute treatment with NMDAR antagonists point towards disruptions in STP in the vHipp-mPFC pathway (Kiss et al., 2011a; Kiss et al., 2011b). While investigations of STP presented in this thesis do not reveal statistically significant differences between the scPCP and scVeh treatment groups,

there is a trend for reduced slope facilitation in the scPCP treated rats compared to controls at short (25ms and 50ms) inter-pulse intervals (IPI) at baseline (**Figure 5.4 A1**). This effect was however absent in the second cohort studied in **Chapter 5** and in an independent cohort studied **Chapter 6**, possibly due to small sample size in the scVeh/scPCP-control treatment groups (**Figure 5.6 A1**, **Figure 6.4 A1**). Nevertheless, this observation could be explained by several factors including reduced inhibition (disinhibition) in response to prolonged reduction in basal glutamatergic transmission, alteration in neurotransmitter concentration and alterations in the neurotransmitter releasing machinery, which were discussed in some detail in **Chapter 5**. It is also important to note that there was no significant difference in amplitude measures between scVeh and scPCP treatment groups within and across any of the tested network conditions, further pointing towards either an intact or a compensatory feed-forward/feed-back inhibitory mechanism. To evaluate the inhibitory processes that contribute to regulating response size, a more detailed study of localised circuitries must be conducted using *in vitro* electrophysiological techniques.

Through assessment of STP under various network conditions (baseline, post-HFS and post-LFS), these studies were able to demonstrate that the effects of high-frequency stimulation (HFS) and LFS were predominantly post-synaptic. This conclusion is based on the understanding that the involvement of presynaptic mechanisms is accompanied by alterations in the neurotransmitter probability of release and change in paired-pulse facilitation (PPF) (Yang and Calakos, 2013; Luscher and Malenka, 2012). A degree of caution must be observed when interpreting these findings. Although there was no statistical evidence of significant change in STP between any of the tested network conditions, the pattern of the data proposes the potential involvement of pre-synaptic mechanisms. Post-HFS, slope PPI for both scVeh and scPCP treated rats appeared to be slightly lower at short (25ms and 50ms) IPIs and higher at longer IPIs for both treatment groups compared to baseline, but these effects did not reach significance (**Figure 5.4 A1-3, Figure 6.4 A1-3**). Furthermore, it appears that post-LFS responses in the scPCP group but not the scVeh treatment group were higher than baseline and post-HFS in **Chapter 5** (**Figure 5.4 A1-3**), while approaching baseline levels in **Chapter 6** (**Figure 6.4 A1-3**). As briefly mentioned in **Chapters 1.8 and 5,** involvement of both pre- and post-synaptic processes in the expression of LTP is supported by previous findings (Lisman, 2009; Yang and Calakos, 2013), however, the extent to which each mechanism contributes to the observed effects reported here cannot be fully confirmed without employing approaches such as assessment of post-synaptic AMPAR expression and AMPAR-mediated current, visualisation of pre-synaptic vesicle release and quantal analysis (Kullmann and Siegelbaum, 1995; Luscher and Malenka, 2012; Yang and Calakos, 2013).

Investigations into processes of long-term synaptic plasticity collectively point towards hyper-excitability of scPCP treated rats compared to controls. As presented in **Chapter 5**, HFS of vHipp after baseline measures significantly potentiated response slope in the mPFC (induced LTP) in both the scVeh and scPCP treatment groups. This was accompanied by a significant potentiation of response amplitude. The magnitude of slope and amplitude potentiation were higher in the scPCP in comparison to controls. This effect reached

significance for amplitude measures for the whole duration of recording while reaching significance in the last 10 minute post-HFS interval for slope measures (**Figure 5.5 A, B, C, D**). These findings may be indicative of hyper-excitability, through reduced inhibition, in the scPCP rats. Subsequent LFS of previously potentiated synapses significantly reduced response slope to values below the baseline indicating not only depotentiation but a significant depression in glutamatergic transmission in both scPCP and scVeh treatment groups, the magnitude of which was significantly greater in the scVeh treatment group. This was accompanied by a significant depression in response amplitude, the magnitude of which, in contrast to slope, was significantly higher in scPCP treated rats. This may suggest that, in addition to the hyper-excitability of the glutamatergic transmission, the inhibitory mechanisms are also hyperplastic. As discussed in **Chapter 5**, concentration of Ca^{2+} entry to pyramidal neurons, determines the polarity of the response (LTP or LTD) (Luscher and Malenka., 2012). Although scPCP treated rats can support LTP and its subsequent reversal, the differences in the magnitude of these effects may be indicative of dysregulations in Ca^{2+} concentration threshold. As such, for the same HFS, more Ca^{2+} enters the neuron, resulting in higher response in scPCP treated network, while for the same LFS, Ca^{2+} concentration is not reduced to the same level of scVeh treated rats. The way in which this potential alteration in Ca^{2+} concentration regulation influences plasticity in the inhibitory mechanisms that regulate response size cannot be determined based on the outcome of investigations reported here. Understanding these properties require in depth analysis of circuitries using slice electrophysiological techniques.

A simple alteration in the recording protocol revealed an interesting set of results which further highlighted excitability differences between the scPCP and scVeh treated rats. LFS of previously non-potentiated synapses in the scVeh group, reduced response slope and amplitude to values below baseline, but this did not reach significance (**Figure 5.7 A1 and B1**). Burette et al (1997), reported no significant change in response amplitude following LFS (same protocol as studies in this thesis) of previously non-potentiated synapses (Burette et al., 1997). They also reported a gradual but significant potentiation in response size to values above baseline, approximately 15-30 minutes post-LFS. In the study presented in **Chapter 5**, responses were monitored for only 15 minutes post-LFS. It is plausible that upon a longer monitoring period, a similar effect would have been observed (as reported by Burette et al (1997)) in the scVeh treated animals. LFS of previously non-potentiated synapses in the scPCP treatment group, significantly potentiated response slope while significantly reducing response amplitude to values below baseline (**Figure 5.7 A3 and B3**). The slope findings in the scPCP treatment group further point towards the hyper-excitability of the glutamatergic synapses which might be explained by reduced excitability threshold due to dysregulation of Ca^{2+} concentration, disinhibition or a compensatory hyperactivity of the NMDARs (for relevant details see **Chapter 5**).

Subsequent HFS (15 to 20 minutes post-LFS) in the scVeh treatment group, did not have a significant effect on response slope, however, it significantly reduced response amplitude to values below baseline (**Figure 5.7 A1 and B1**). This is while HFS further potentiated response slope and amplitude in the scPCP treatment

group (**Figure 5.7 A3 and B3**). Burette et al (1997) reported a significant potentiation in response amplitude in treatment-naïve rats following HFS, applied approximately 60 minutes post-LFS. With consideration to the findings of this study, it can be suggested that under control conditions LFS potentiates inhibitory mechanisms, preventing potentiation in response to HFS. Since there was no significant difference in the STP post-HFS in comparison to post-LFS and baseline, this effect cannot be attributed to depletion in neurotransmitter stores. The findings of the scPCP-control treatment group further point towards the hyper-excitability of the glutamatergic responses. These findings may also suggest that the LTP induced in the inhibitory mechanisms that regulate response size are readily reversible and less stable in the scPCP treated rats, potentially contributing to their observed hyper-excitability. Indeed, these hypotheses cannot be definitively confirmed based on the findings of studies presented in **Chapter 5.** In addition, the nature of these alterations and their functional consequences remain largely unknown. Investigations in this thesis suggest that these alterations in processes of long-term synaptic plasticity but not STP may partly be due to changes in the noradrenergic system in response to scPCP treatment. Acute propranolol treatment significantly reduced the magnitude of LFS- and HFS-induced slope potentiation in the scPCP treated rats and normalised amplitude measures to baseline (**Figure 5.7 A2, A4, B2 and B4**).

Collectively, these investigations show that excitatory input from the vHipp to the mPFC is severely compromised following sub-chronic treatment with PCP. This is accompanied by a trend towards hyper-excitability in the scPCP treated rats which is apparent in mechanisms of STP and long-term synaptic plasticity. Results further highlight potential alterations in neuromodulatory systems, including noradrenergic systems which play an important role in regulating synaptic plasticity and cognition. As well as validating the neurophysiological properties of the scPCP model, these investigations, though preliminary, have advanced current understanding of fundamental neuronal abnormalities with relevance to schizophrenia.

As outlined in **Chapter 5**, responses to HFS and LFS are often tested over a longer period (1-2 hours). In the studies reported here, responses were monitored for maximum of 30 minutes post-HFS and post-LFS to cover the window for initial protein-synthesis. Longer observation could have provided more certainty about the stability of the observed neuronal activities. LFS following baseline recording was able to dissect some of the finer differences between scPCP and controls, suggesting inhibitory mechanism differences and dysregulations in excitability threshold. These investigations point towards differences in the stability of inhibitory potentiation and alteration in excitability threshold, with a potential involvement of the noradrenergic system. Future investigations must consider a more in-depth analysis of these processes in a larger sample size.

7.4. Influence of long-term haloperidol treatment on synaptic properties of the vHipp-mPFC pathway

Chapter 6 describes the first study to examine the influence of long-term haloperidol treatment (via osmotic minipumps) on synaptic properties of the vHipp-mPFC pathway. Investigations into synaptic connectivity did not detect any statistically significant differences between the haloperidol and control treatment groups. This could be explained by the small sample size in the scVeh-control (n=4) and scVeh-Hal (n=3) treatment groups, rendering the statistical analysis underpowered. However, as presented in **Figure 6.3 A and B**, the strength and amplitude of synaptic connectivity was considerably lower in the scVeh-Hal treatment group compared to its control. A similar effect was observed in the scPCP-Hal (n=8) treatment group but only in the amplitude measure while the strength of excitatory input was comparable to scPCP-control (n=7). Similar to the findings reported in **Chapter 5**, the strength of excitatory input in the scPCP-control appeared to be lower than the scVeh-control treatment group (did not reach significance; see **Section 7.3**). As **Chapter 6** describes in more detail, reduced synaptic vHipp-mPFC connectivity in the scVeh-Hal group is unlikely to be due to alterations in dendritic spines (Konopaske et al., 2014) but could be explained by haloperidol-induced activation of inhibitory mechanisms that regulate response strength and size. Dopamine regulation of GABAergic interneurons is complex and synapse specific. Pharmacological stimulation of D2Rs has been reported to increase the firing rate of fast-spiking interneurons leading to reduced firing rate of pyramidal neurons (Tseng and O'Donnell., 2004; Tseng et al., 2006). Dopamine exerts a different effect on non-fast spiking interneurons; in which case, the interneuron firing rate is reduced leading to hyper-excitability in pyramidal neurons (Tierney et al., 2008). In contrast with findings of Tseng and O'Donnell (2004) and Tseng et al (2006), this effect has also been reported in fast-spiking interneurons (Tierney et al., 2008) and it is counteracted by D2R antagonists such as haloperidol and raclopride (Tirney et al., 2008; Xu and Yao, 2010) (see **Section 6.6.2** for details). This could explain both reduced strength and amplitude of synaptic connectivity upon treatment with haloperidol in the scVeh rats. In addition, this effect could be explained by reduced AMPAR-mediated current in response to haloperidol treatment (Arvanov et al., 1997; Gemperle et al., 2003). Contradictory evidence has also been reported, suggesting that D2R antagonists increase AMPAR-mediated current (Tseng and O'Donnell., 2004; Hakansson et al., 2006). It can be hypothesized that both mechanisms occur in the same system while their counteracting effect becomes apparent when the inhibitory tone is reduced. This may explain why in the scPCP-Hal treatment group, the strength of synaptic connectivity is maintained at scPCP-control level. Collectively, it can be concluded that in the scPCP model, haloperidol does not influence the strength of synaptic connectivity beyond the effect of scPCP treatment, but may have an effect on inhibitory mechanisms that regulate response size.

Investigations of STP under baseline, post-HFS and post-LFS network conditions point towards hyper-excitability of the haloperidol treated scVeh and scPCP rats. Both slope and amplitude facilitation were higher in haloperidol treatment groups compared to controls under all tested conditions (with some

variations in the scVeh-Hal treatment group; see **Chapter 6** for a more detailed discussion) (**Figure 6.4 A1-3 and B1-3**). This effect reached significance for slope facilitation in the scPCP-Hal treatment group compared to its control (**Figure 6.4 A1-3**). Prolonged treatment with haloperidol is associated with reduced basal dopamine release (Amato et al., 2011), which could reduce the activity of a sub-set of fast-spiking interneurons, leading to hyper-excitability in the haloperidol treatment groups (Tseng and O'Donnell., 2004).

Interestingly, slope and amplitude facilitation were apparent at longer IPIs (150ms-1000ms) in haloperidol treatment groups, which could be attributed to the elevated expression of high-affinity AMPARs (McCoy et al., 1998) in response to prolonged haloperidol treatment. Hyperactivity at this IPI range reflects the conductance range of $GABA_BR$ (Nathan et al., 1990; Olpe et al., 1994) and suggests that the conductance of this receptor may be reduced by haloperidol treatment. As discussed in detail in **Chapter 6,** this effect could be due to haloperidol-induced dysregulation in the $GABA_BR$-D2R-NMDAR interaction. The $GABA_BR$-D2Rs interact to increase $GABA_BR$-mediated inhibitory post-synaptic current. Over-activation of the NMDARs counteracts the $GABA_BR$-D2R inhibitory effect. Prolonged blockade of D2Rs, in addition to the effect of haloperidol on NMDARs, could depress $GABA_BR$-mediated current, leading to the hyper-excitability of pyramidal neurons at $GABA_BR$ conductance range. This might explain the findings of elevated AMPAR-mediated current in response to haloperidol treatment (Tseng and O'Donnell., 2004; Hakansson et al., 2006).

In face of compromised synaptic connectivity, it is challenging to confidently interpret these findings. Furthermore, as described in **Chapter 6**, there are gaps in current understanding of the effects of long-term treatment with haloperidol that prevent confident interpretation of these results. Existing evidence suggests that long-term treatment with haloperidol does not increase veratridine or K^+-evoked glutamate release in the mPFC (Yamamoto and Cooperman, 1994; See and Lynch, 1995; Pietraszek et al., 2002; Konopaske et al., 2013). Furthermore, acute pre-treatment with haloperidol also does not alter pre-synaptic glutamate release upon acute treatment with PCP. Similarly, short-term treatment with haloperidol also does not alter the effects of scPCP treatment on evoked glutamate release (Ninan et al., 2003). Therefore, the loss of inhibitory control in addition to possible involvement of presynaptic events - concentration of neurotransmitter and release probability as well as changes in the neurotransmitter releasing machinery - could explain the hyper-excitability in the haloperidol treatment groups. Currently, there is a clear gap in understanding of the influence of long-term AP treatment on molecular markers of synaptic integrity, including synaptosomal-associated protein of 25 kDa (SNAP-25), which plays an important role in neurotransmitter release (Sudhof, 2012). Examining these factors is important in understanding the effects of APs on synaptic functions. It is important to note that in the present study, the sample size for the scVeh-control and scVeh-Hal treatment groups was small (n=4). Future research must examine these parameters using a larger sample size to reinforce statistical power.

28 days of treatment with haloperidol in both scVeh and scPCP treated rats was associated with a significant potentiation of synaptic weight and size in response to high-frequency stimulation (HFS), an effect which was significantly more pronounced for slope measures in the scPCP-Hal treatment group in comparison to scVeh-Hal and in the amplitude measures in the latter compared to the scPCP-Hal treatment group (**Figure 6.5 A1-4 and B1-4**). These findings are in agreement with the hyper-excitability observed in the pattern of STP. *In vitro* studies have also suggested that haloperidol moderately increases the NMDAR-mediated current. However, in these experiments this is not associated with an increase in excitatory neurotransmitter release (Arvanov et al., 1997; Ninan et al., 2003). Therefore, the observed hyper-excitability in the scVeh-Hal treatment group is predominantly explained by loss of inhibitory control, possibly mediated through $GABA_BR$-D2R-NMDAR interaction. This, in addition to the scPCP-induced depolarisation of membrane potential (see **Section 7.3** and **Chapter 5**), allows for a more pronounced LTP in the scPCP-Hal treatment group. A hypothesis for the potential functional consequence of this hyper-excitability is provided in **Section 7.5**. Application of LFS to previously potentiated synapses in the scVeh-Hal and scPCP-Hal, depotentiated response slope to baseline (**Figure 6.5 A2 and A4, C1 and C3**). A similar effect was observed for amplitude measures, but depotentiation was more robust in the scVeh-Hal treatment group compared to scPCP-Hal (**Figure 6.5 B2 and B4, D1 and D3**). It is important to note that the validity of these findings is subject to the assessment of blood plasma samples collected on the last day of haloperidol treatment. Analysis of plasma samples is essential to confirm that an expected haloperidol plasma concentration has been established (i.e. the minipumps were functioning as expected) and that there are no traces of in the control treatment groups (See **Chapter 4**). Nevertheless, these preliminary findings collectively suggest that long-term treatment with haloperidol may compromise glutamatergic synaptic connectivity in the vHipp-mPFC pathway. Haloperidol further disrupts the process of STP and long-term plasticity by primarily reducing inhibitory control in the system. Further studies are required to dissect the underlying mechanisms of these observed effects. It is interesting to investigate these alterations at various time-points under haloperidol treatment. Furthermore, it is essential to investigate the significance of these alterations in cognition. This is discussed in more detail in the following section.

7.5. Additional Findings and Limitations

While the main findings of this project in support of each objective are outlined in the previous sections, this section focuses on the additional observations and findings which further add to current understanding and underlies knowledge gaps that need to be addressed in the future.

One of the challenges faced throughout this project was the choice of clinically relevant dose of AP treatment for female rats. A few research teams have examined the D2R occupancy and plasma concentration levels of a range of APs at varying doses delivered via sub-cutaneous injections (Kapur et al., 2003), oral dosing (Barth et al., 2006) or osmotic minipump (Kapur et al., 2003; Samaha et al., 2007; McCormick et al., 2010; Vernon et al., 2011; Vernon et al., 2012). All of these studies have been conducted

in physiologically healthy male rats. Since there are currently no reports of AP plasma concentration comparison between male and female rodents, dose-response studies in male rats inform treatment dose selection in studies with female rodents. Indeed, studies reported in this thesis also relied on these resources as a reference for the choice of haloperidol and olanzapine treatment dose.

This is one of the most confounding limitations of pre-clinical research which hinders the translational validity of findings. In clinical practice, it is well established that females show a higher plasma concentration than males for the same dose of the drug (Seeman, 2004; Weston-Green et al., 2010). **Chapter 4** presented findings that are in agreement with these clinical observations. As mentioned before, in this study female rats were treated with haloperidol (0.5 mg/kg/day) for 28 days. This dose of haloperidol delivered through osmotic minipumps is associated with a plasma concentration of 4.7± 0.32 ng/ml in male Sprague-Dawley rats following 8 weeks of treatment (Vernon et al., 2011; Vernon et al., 2012). Analysis of plasma samples collected from female rats on the last day of treatment (day 28) revealed an unexpectedly high level of haloperidol in the plasma of the rats (18.23± 2.37 ng/ml for the scVeh-Hal-High treatment group and 14.71± 0.96 ng/ml for the scPCP-Hal-High treatment group). This level of haloperidol concentration is associated with higher doses of this compound (approximately 2 mg/kg/day) in male rats (Vernon et al., 2011; Vernon et al., 2012). **Chapter 6**, followed the same experimental design as the study presented in **Chapter 4**. Similarly, plasma samples were collected on the last day of treatment, however, they were not further processed due to time and operational constraints. Analysis of those samples will further elucidate the validity of these preliminary findings. Nevertheless, these findings highlight the importance of acknowledging sex differences as a factor in pre-clinical research and the need to provide evidence to inform future practice.

Locomotor activity in response to an acute Amph challenge was studied for two reasons. One, as described in **Section 7.2**, was to ensure that the scPCP treatment had been effective. The second purpose was to examine the efficacy of haloperidol to inhibit Amph-induced hyperactivity in the haloperidol treated scVeh and scPCP rats. Prior to analysis of the plasma samples obtained from rats in **Chapter 4**, the locomotor activity study also served as an indirect measure of osmotic minipump functional integrity. As discussed in **Chapter 4**, hyperactivity in response to psychostimulants such as Amph is considered as an index of translational validity for psychosis. Several lines of evidence support the effectiveness of both typical and atypical APs in attenuating or inhibiting psychostimulant-induced hyperactivity (Phillips et al., 2001; Samaha et al., 2007; Goff et al., 2017; Yin et al., 2017) which supports the predictive validity of these treatments for psychosis management.

In the study presented in **Chapter 4**, acute Amph challenge in the scVeh-Hal-Low and scPCP-Hal-Low treatment groups was associated with a significant elevation in locomotor activity when compared to their respective controls (scVeh-Hal-Low-Veh and scPCP-Hal-Low-Veh, respectively). A similar effect was observed in the scVeh/scPCP-Hal-High treatment groups in comparison to their controls (scVeh-Hal-High-Veh and scPCP-Hal-High-Veh, respectively; **Figure 4.8, Table 4.10**). Collectively, these findings point

towards a significant reduction in haloperidol efficacy, at both low (0.25 mg/kg/day) and high (0.5 mg/kg/day) doses, to block the behavioural effects of Amph after 17 days of treatment. As previously described in **Chapter 4**, this observation could be explained by the phenomenon of dopamine super-sensitivity, which refers to the enhanced sensitivity to dopamine and dopamine releasing agents (such as Amph) in response to long-term treatment with AP agents (Seeman et al., 2005). As previously discussed in **Chapter 1.1**, pre-clinical studies suggest that dopamine super-sensitivity could explain the high rate of relapse upon abrupt treatment discontinuation as well as antipsychotic-induced psychosis in some patients upon prolonged treatment (Goff et al., 2017; Yin et al., 2017).

It is also important to note that, in the study presented in **Chapter 4**, total LMA count in the scVeh/scPCP-Hal-Low-Amph treatment group was higher than scVeh/scPCP-Hal-High-Amph treatment group. This effect reached significance for the scPCP treatment group only. While previous studies in physiologically healthy male rats show a complete loss of haloperidol efficacy (0.25 and 0.75 mg/kg/day- minipump delivery) in blocking the Amph-induced hyperactivity at early stages of treatment (efficacy present at day 2 but lost at day 12-13 post treatment) (Samaha et al., 2007), the findings of **Chapter 4** point towards a more subtle and gradual loss of treatment efficacy in female rats. It further suggests that in contrast to the findings of Samaha and colleagues (2007), treatment with high-dose of haloperidol (0.5 mg/kg/day) may have been more efficacious than its lower dose in reducing the effect of Amph treatment on LMA. Collectively, these findings may be highlighting sex differences in treatment efficacy which can have major implications in clinical practice. Due to the contamination and lack of appropriate treatment groups, the study presented in this thesis is limited in its ability to confirm this hypothesis with certainty. A time-course dose-response study involving both male and female rats (scPCP and healthy controls) treated with haloperidol or other APs would greatly contribute to the understanding of these observed effects.

Results of the study in **Chapter 6** also provide indirect evidence for the loss of haloperidol efficacy upon prolonged treatment. As previously described in **Chapter 6**, in addition to its involvement in cognitive impairments, imbalances in the interaction between the vHipp and mPFC also contribute to the emergence of incoherent thoughts in psychosis (Jodo, 2013; Samudra et al., 2015; Eichenbaum, 2017). It could be argued that psychosis is associated with deficits in segregating relevant from irrelevant stimuli (Howes and Kapur, 2009; Grace, 2016). NMDARs, which mediate LTP in the vHipp-mPFC projections, are coincidence detectors of pre- and post-synaptic activity and they optimise signal-to-noise ratio (salient vs. non-salient) upon receiving stimuli. The hyperplasticity observed in the scPCP-Hal treated rats (see **Section 7.4**) could highlight dysregulation of signal filtering and information transfer from vHipp to mPFC, due to over-activation of NMDAR. It is known that acute treatment with PCP disinhibits the pyramidal neurons in the HF, resulting in excess release of glutamate in the mPFC (Suzuki et al., 2002; Jodo et al., 2005; Jodo, 2013). Hyperactivity of the HF has a positive association with psychosis (Grace, 2016). It is plausible, therefore, that in the long-term, disinhibitory effects of haloperidol contributes to this hyperactivity, manifesting as hyper-plasticity of the vHipp-mPFC pathway, eventually leading to psychosis. In order to assess this

hypothesis, a series *in vitro* and *in vivo* electrophysiological studies are required to study the flow of information from vHipp to mPFC at different stages of treatment with haloperidol. It will also be important to conduct a time-course study to examine whether the relative influence of haloperidol on synaptic properties of the vHipp-mPFC pathway change as a function of treatment stage.

One of the major limitations of studies presented in this thesis was the reliance on one cognitive task. In humans, episodic memory is defined as the conscious recollection of past events or episodes in relation to its specific temporal and contextual elements (Tulving, 2002). The process of recollection (interchangeably called 'recognition' in the literature) is defined as the ability to identify previously encountered episodes and is fundamental to episodic memory (Suzuki and Naya, 2014). Collectively, recollection and episodic memory rely upon the interaction between the hippocampal formation (HF) and the prefrontal cortex (PFC). It is argued that the NOR task, in its classic form and at short inter-trial intervals (Dere et al., 2007), relies on a familiarity-based rather than a recollection-based strategy (Morici et al., 2015). This is further supported by substantial evidence from lesion studies, which point towards the strong involvement of the perirhinal cortex but not the hippocampus and the PFC in NOR task performance (Barker et al., 2007; Barker and Warburton, 2011).

Variations of the NOR task, including 'object-in-place' and 'temporal order memory', rely on integration of spatial, contextual and temporal information and involve the hippocampus and the PFC (Barker et al., 2007; Barker and Warburton, 2011). Therefore, these variations might be a better test for examining higher-order cognitive processes such as episodic memory. In recent years, advances have been made in developing episodic memory testing paradigm in rodents. For instance, the What-Where-Which task (Eacott and Norman, 2004), incorporates all the elements of episodic memory (what items, where in space and in which context) and relies on the process of conscious recollection of previous experience (Seel et al., 2018). Given that this thesis aimed to characterise the vHipp-mPFC pathway, known to be integral to episodic memory processing, it would have been very interesting to have used a more relevant behavioural task such as What-Where-Which rather than the traditional NOR paradigm. In addition, it is known that in patients with schizophrenia, familiarity-based strategies are relatively preserved. While this project showed that long-term haloperidol treatment does not impair similar processes in rats, its examination on more a complex task would have provided a better measure for the functional consequences of haloperidol-induced alteration in synaptic plasticity.

Indeed, the electrophysiological studies were also hindered by certain limitations which could compromise interpretation of the findings. One of these limitations was the wide age range of rats used in the study presented in **Chapter 5**. All rats used in this study were treated with scPCP at the same time, while electrophysiological recordings were obtained over an average of 14 weeks. Therefore, at the time of recording, rats were between 12 to 25 weeks old. Given the influence of age on intrinsic neuronal properties in the hippocampus and the mPFC (Chang et al., 2005; Rizzo et al., 2015; Oh et al., 2016), it would have been more appropriate to employ a staggered design approach to limit the age range and to minimise the

confounding effect of age on subsequent electrophysiological recordings. Indeed, the study presented in **Chapter 6**, used a staggered design, partly to limit the effect of age on recordings (rats in this study were 20-23 weeks old at the time of recording). Consistent with reduced excitability in pyramidal neurons in the CA1 region of the hippocampus as well as reduced action potential amplitude in the mPFC (Chang et al., 2005; Rizzo et al., 2015; Oh et al., 2016), the HFS-induced potentiation in response amplitude was absent in the scVeh-control and scPCP-control treatment group in **Chapter 6**. This finding is in contrast to the effects observed in **Chapter 5**. Indeed, the extent to which age contributed to the observed effects cannot be confidently determined in these studies and must be further investigated. As alluded to in the previous sections, the small sample size of treatment groups in **Chapter 6** could also explain, in part, some of the discrepancies in the findings of the electrophysiological studies. Indeed, future investigations must consider using a larger sample size in order to maintain the statistical power. Given these limitations, any comparison made between the study presented in **Chapters 5** and **6,** must be interpreted with caution.

7.6. Future Directions and Concluding Remarks

This project attempted to overcome some of the methodological limitations of pre-clinical research in schizophrenia. These studies were able to successfully overcome the issue of fast metabolic rate of APs, however, they failed to address dose-response sex differences which are essential for choosing appropriate drug dose. Investigations into these factors will be invaluable in informing future studies and the promotion of good practice.

The electrophysiological studies reported here, primarily focused on the examination of synaptic connection strength and plasticity properties within the vHipp-mPFC pathway in the scPCP model. In addition to synaptic plasticity, network oscillatory activities are also essential for timely representation and transfer of information within functional networks and are fundamental to cognition. Throughout the studies reported in **Chapters 5** and **6**, relevant data required for analysis of oscillatory behaviour of the vHipp-mPFC pathway were also collected and are currently under analysis. Results of these investigations will further advance understanding of network alterations of relevance to the cognitive impairments associated with schizophrenia and will provide insight into the effects of long-term haloperidol treatment on network activity. Future investigations must consider examining these elements using different APs and novel cognitive enhancers. Equally important is the study of oscillatory activities and functional interaction of vHipp-mPFC in awake scPCP treated animals during performance of cognitive tasks. Substantial evidence supports the disruption of HF-PFC functional interaction in patients with schizophrenia. Investigating these properties in the scPCP model, will not only validate the model with its relevance to cognitive impairments of the disease but will also provide a new target for the study of effects of APs and novel treatments.

Throughout the course of this project, a substantial volume of brain tissue was collected and stored for subsequent analysis. Samples collected from rats in **Chapter 3**, are currently in preparation to be analysed for proteins markers of synaptic integrity (SNAP-25 and post-synaptic density of 95 kDa; PSD-95) as well

as PV in the PFC and HF. Based on previous findings (Cochran et al., 2003; Abdul-Monim et al., 2007; Jenkins et al., 2010), it is expected for the PV expression to be reduced in both structures. Since the effectiveness of scPCP treatment could not be reliably confirmed through behavioural assessments in this study (see **Section 7.2**), the findings from post-mortem analysis might be able provide a definitive answer. In case the effectiveness of scPCP treatment can be reliably validated through analysis of PV, subsequent analysis of SNAP-25 and PSD-95 will be novel findings significantly contributing to current understanding in alterations of markers of synaptic integrity in an animal model for schizophrenia and in response to prolonged AP treatment. Thus far, most reports on expression levels of PV have focused on the dorsal hippocampus (dHipp). Brain samples collected form the study presented in **Chapter 6**, are currently in preparation for immunohistochemistry analysis of PV in the vHipp, dHipp and the mPFC. Findings of this analysis will also contribute to better validating the scPCP model and expanding on the potential mechanisms involved in the pathophysiology of schizophrenia.

In conclusion, the studies presented in this thesis were able to successfully fulfil the aims and objective outlined for this project. Collectively, these studies investigated the influence of haloperidol on cognitive performance and showed no cognitive impairments in association with its prolonged treatment. For the first time, this project characterised the basic synaptic properties of the vHipp-mPFC pathway in the scPCP model and further expanded these findings by examining the influence of haloperidol treatment on synaptic plasticity in this pathway. Subject to the future work suggested here, the scPCP model has the potential to greatly contribute to the understanding of the schizophrenia disease process, identify novel targets for treatment and advance efforts in drug discovery with the aim of treating the cognitive impairments associated with the disease.

Chapter 8

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