Exercise in the subchronic phencyclidine rat model for schizophrenia: mechanisms and effects on cognitive deficits

A thesis submitted to the University of Manchester for the degree of

Doctor of Philosophy in the Faculty of Biology, Medicine and Health

2020

Lisa M. Heaney

Division of Pharmacy and Optometry

School of Health Sciences

Blank page

Table of Contents

Main text: 32,715 words

Total: 53,202 words

Table of Figures

Table of tables

Abbreviations

5-HT_{2A}	5-hydroxytryptamine 2A receptor
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
AP	Action potential
BDNF	Brain-derived neurotrophic factor
BPRS	Brief psychiatric rating scale
BrdU	Bromodeoxyuridine
BSA	Bovine serum albumen
CF	Conversion factor
CNS	Central nervous system
CRP	C-reactive protein
CRT	Cognitive remediation therapy
D_2	Dopamine D 2 receptor
DCX	Doublecortin
DI	Discrimination index/indices
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FGA	First-generation antipsychotic
fMRI	Functional magnetic resonance imaging
GABA	Y-aminobutyric acid
GAD_{67}	Glutamate decarboxylase/ glutamic acid decarboxylase 67
GAT_1	GABA transporter 1
I.p.	Intraperitoneal or intraperitoneally
IGF	Insulin-like growth factor
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-1ra	Interleukin 1 receptor agonist
IL-1β	Interleukin-1 beta
IL-6	Interleukin 6
IQ	Intelligence quotient
ITI	Inter-trial interval
kDa	Kilodalton
LMA	Locomotor activity
LSD	Lysergic acid diethylamine
LTD	Long-term depression
LTP	Long-term potentiation
M	Mean
M	Molar/molarityity
MAGUK	Membrane-associated guanylate kinase
MATRICS	Measurement and treatment research to improve cognition in schizophrenia
MD	Mean difference
MetS	Metabolic syndrome
mM	Millimolar/millimolarity

NAA	N-acetylaspartate or N-acetylaspartic acid
NICE	National institute for clinical excellence
NIH	National Institute of Health
NIHM	National Institute of Mental Health
Nm	Nanometres
NMDA	N-methyl-D-aspartate
NOR	Novel object recognition (test)
P1/P2	Pellet 1/pellet 2
PANSS	Positive and negative symptom scale
PCP	Phencyclidine
PET	Positron emission tomography
PFC	Prefrontal
PMSF	Phenylmethylsulfonyl fluoride
PPI	Prepulse inhibition
PSD	Post-synaptic density
PSD_{95}	Postsynaptic density protein 95
RMW	Relative molecular weight
Rpm	Revolutions per minute
RT	Room temperature
S1/S2	Supertant 1/supernatant 2
SAP_{90}	Synapse-associated protein 90
scPCP	Subchronic phencyclidine (treatment)
SE	Standard error
SEM	Standard error of the mean
SGA	Second-generation antipsychotic
$SNAP_{25}$	Synaptosomal-associated protein 25 kDa
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment protein
SNP	Single nucleotide polymorphism
SNP	Single nucleotide polymorphism
SPSS	Statistical package for social sciences
TGF-β	Transforming growth factor-β
TNF-R	Tumour necrosis factor receptor
TNF-α	Tumour necrosis factor-α
t-SNARE	Target SNARE
TURNS	Treatment units for research and neurocognition in schizophrenia
VEGF	Vascular endothelial growth factor

Abstract

Exercise in the subchronic phencyclidine rat model for schizophrenia: mechanisms and effects on cognitive deficits

Schizophrenia is associated with substantial cognitive deficits that dramatically reduce patients' quality of life. Now recognised as a core feature of the disorder, cognitive impairments best predict functional outcome. However, the drugs used to treat schizophrenia do not improve cognition, and many have cardiometabolic side-effects that contribute substantially to the reduced life expectancy of people with the disease. Drug development targeting cognition in schizophrenia to date has been unsuccessful. Non-pharmacological interventions to improve cognition, such as cognitive remediation therapy, have had limited success. Therefore, treatments to ameliorate the cognitive impairments in schizophrenia remain an urgent, unmet clinical need.

Exercise has emerged as a potential intervention to reduce cognitive impairment in schizophrenia. While there is growing clinical evidence that exercise improves cognition, how it does so is poorly understood. Animal models are useful tools for investigating disease, and the subchronic phencyclidine (scPCP) rat model for schizophrenia is well-established and widely used to model schizophrenia-like cognitive deficits. However, research examining the effect of exercise on cognition in this model is lacking.

This thesis presents a body of work examining the effect of voluntary exercise on cognition in the scPCP rat model for schizophrenia. The studies described within it demonstrate a novel exercise protocol can improve the novel object recognition (NOR) deficit present in this model. Furthermore, the pro-cognitive effects of wheel running endured for at least two weeks of sedentary behaviour but by four weeks, the NOR deficit had returned. Brain tissue was collected and analysed for synaptic and γ-aminobutyric acid (GABA)ergic markers with western blotting. Two significant relationships were reported but these results were deemed inconclusive. Additionally, brain-derived neurotrophic factor (BDNF) was measured in plasma but no effect of exercise was found.

For the first time, exercise was shown to improve cognition in the scPCP rat model for schizophrenia. Furthermore, this effect lasted for at least two weeks after exercise cessation. Pathological changes were not observed, but the robust behavioural changes produced will provide the opportunity to explore the pro-cognitive mechanisms of exercise in an animal model for schizophrenia.

Declaration

I declare that no portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other University or institute of learning.

Copyright statement

i) The author of this thesis (including any appendices and/or schedules to this thesis) owns certain copyright or related rights in it (the "Copyright") and s/he has given The University of Manchester certain rights to use such Copyright, including for administrative purposes.

ii) Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made **only** in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.

iii) The ownership of certain Copyright, patents, designs, trademarks and other intellectual property (the "Intellectual Property") and any reproductions of copyright works in the thesis, for example graphs and tables ("Reproductions"), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.

iv) Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IP Policy (see http://documents.manchester.ac.uk/DocuInfo.aspx?DocID=24420), in any relevant Thesis restriction declarations deposited in the University

Library, The University Library's regulations (see http://www.library.manchester.ac.uk/about/regulations/) and in The University's policy on Presentation of Theses

Acknowledgements

I would like to start by thanking Dr Mike Harte for ensuring I became an independent and self-sufficient researcher while under his guidance. I would also like to thank him for his support while writing my thesis. I would also like to thank Prof. Jo Neill for allowing me to experience her dynamic lab and learn so much from so many incredible people during my PhD. Special thanks go to Giovanni, Jo O., Naz, Bushra, Matt, Chlöe, Ambreen, Katie, Will, Ruth, Andy, Vicki, Michelle, Tom, Daniela, Syeda, Antonio, Marianne, Toby, Ben, Anna, Harry, Hagar, Adam, Olivia, Linzi, Jen and Sam. A shout goes to honorary members of the lab, Jess and Matt. I know I've forgotten someone and I'm sorry in advance. Huge thanks to Dr Laura Brown for such an enjoyable experience teaching her undergrads and for always being my reference.

I want to thank Adam for being generally an amazing person, but also for bringing me cups of tea and making sure I was eating and sleeping during write-up. You deserve at least a BSc in neuroscience for the information you've acquired, particularly when Jane and I were talking shop. I'd like to thank Jane for being a brilliant housemate, even if wine consumption was a little more than the recommended guidelines.

Thank you to my mum and dad, for allowing me to move home in my 20s to do my master's. I have taken the hint that, shortly after I left, you turned my room into a sewing studio. Thanks to Liam for offering excellent editing tips, even if he did owe me a favour, and Laura for doing so much to find me gainful employment.

Thank you to all my friends who are still my friends, despite being abandoned for at least a year and some for much more. I have been so excited to come out of hibernation and see you all. That was until COVID-19 had other ideas. I just want to put it out there that I was self-isolating before it was trendy. Special thanks for eternal patience go to Kate, Louise, Meredith, Brian, Gemma, Mel, Oktawia, Califia, Helyn, Leila, Hayley, Donna, Chen, Martin, and Jacqui. I've missed so many people, but I'm excited about catching up with you all. I have a lot of video calls to make!

Lastly, I'd like to remember Kittie Brewer and Jonathan Linnell. We lost you while I was doing this PhD and the world is poorer for it. You are sorely missed by all the people who loved you, of which there are many, including me.

Preface

Abstracts

Heaney L.M., Gonzales A, Neill J.C., Yung A.R., Allen, S., Harte MK. Gym rats: exercise rescues the cognitive deficit in the subchronic PCP rat model for schizophrenia. *SIRS Meeting 2018*

Funding

2017	Human Brain Project School, Austria	Award for accommodation, subsistence and travel to present a talk and a poster.
2017	ECNP Junior Scientists Workshop, France	Award for travel and full accommodation to give a talk during the workshop and to present a poster
2015	BAP travel bursary, UK	To attend BAP conference
2014	Medical Research Council, UK	PhD, including £36k consumables budget
2014	The University of Manchester	4 year President's Doctoral Scholarship (£1k p.a.)

Conferences attended

2018	SIRS meeting, Florence
2017	BAP summer meeting, London
2016	BAP summer meeting, Brighton
2016	IEPA meeting, Milan
2015	BAP summer meeting, Bristol

Teaching

2018	Higher Education Academy	Fellow award (FHEA)
2015 - 2018	The University of Manchester	BSc Psychology - Individual differences seminars and marking BSc Psychology – Introduction to cognition seminars and marking
2017		MPharm 'medicines in practice' course featured lecture; exam question based on content

Public engagement activity

2018	Performing live experiments, 'Look Around You' screening at the Odeon, Manchester. Manchester Science Week, in association with MOSI
2017	'Schizophrenia: from lab bench to clinical practice', Pint of Science, Manchester (sold out event)
2016	Presenter, PubHD – PhD research in the pub

2016	Designed slide show for digital display, presented at stall. Brain Box, Manchester Day. Town hall, Manchester
2015, 2016	Stand-up comedy about research topic, Bright Club 'The Big One' and Bright Club 'Pieces', both Manchester
2015	Stand-up comedy about research, Science Show-Off opening the British Science Association's Science Communication Conference, Manchester
2015	'Science Speed Dating', year nine pupils, Rochdale
2014 – 2015	'Meet a scientist', Science Grrl, Lancaster Science Festival, Manchester Science Week

Prizes

2017	Poster in top five, MRC/BBSRC PGR conference, The University of Manchester
2015	Best first-year talk, Pharmacy and Optometry School PGR Conference, The University of Manchester

Previous education

2012	Manchester Metropolitan University	MSc by research, psychology (merit)
2007	The University of Oxford	MA (Oxon)

Previous Research experience

2013 – 2014	Manchester Mental Health and Social Care Trust (NHS)	Research coordinator, 'INSITE' suicide prevention therapy trial
2013 – 2014	The University of Manchester	Intern research assistant, 'What Makes a Mother', fMRI study involving mothers and babies

Chapter 1 Introduction

1.1 Schizophrenia and cognitive deficits

Schizophrenia is a severe and disabling mental illness (van Os and Kapur 2009) that affects around 1% of the population (Ripke 2014). It is characterised by positive and negative symptoms and profound cognitive deficits. Positive symptoms include hallucinations and delusional thinking, and negative symptoms manifest as behaviours such as avolition and social withdrawal. Antipsychotics are the first-line treatment for someone experiencing symptoms of schizophrenia (NICE 2014). These drugs are somewhat effective, in that 33.5% of people show an improved score of at least 50% on the positive and negative symptom scale (PANNS), or the brief psychiatric rating scale (BPRS), within four to six weeks of treatment (Samara et al. 2019). However, the most consistent improvements are seen for positive symptoms (Milev et al. 2005) and antipsychotics show minimal usefulness for the treatment of negative or cognitive symptomology (Conn et al. 2008; Leucht et al. 2009).

There is evidence that the negative and cognitive domains of schizophrenia are connected; the severity of one correlates with the other but neither correlate with positive symptoms (Bilder et al. 2000; O'Leary et al. 2000; Milev et al. 2005). First-generation antipsychotics (FGAs) share a common pharmacological effect in that they have a high affinity for dopamine D_2 receptors and are slow to dissociate once bound (Miyamoto 2005). It was hoped that the second-generation antipsychotics (SGAs), developed in the 1990s, would have a 'wider spectrum of efficacy' (Leucht et al. 2009). This was partly due to their interaction with both the dopaminergic and

the serotonergic neurotransmitter systems, with a particularly high affinity for $5HT_{2A}$ receptors (Miyamoto et al. 2005). Unfortunately, their superiority has not been demonstrated (Lieberman et al. 2005; Leucht et al. 2009; Lally and MacCabe 2015) and SGAs are similar to FGAs, in that they offer little improvement for negative symptoms and cognitive deficits (Lieberman et al. 2005; Keefe et al. 2007; Davies et al. 2008; Lally and MacCabe 2015; Ribeiro et al. 2018). Furthermore, any modest improvements in the negative or cognitive domains may be due to a reduction in positive symptoms (Leucht et al. 2017) or secondary negative symptoms (Kirschner et al. 2017; Leucht et al. 2017).

There is little controversy that the negative and cognitive symptoms of schizophrenia are more disabling and persistent than the positive symptoms (Green, Kern, et al. 2004; Milev et al. 2005; Albert et al. 2011). The cognitive deficits associated with schizophrenia are a core feature of the disease (Keefe 2008; Mesholam-Gately et al. 2009; Kaneko 2018). They contribute more to poor functional outcomes (Green 1996; Milev et al. 2005) and carer burden (Hofer et al. 2005; Milev et al. 2005; Hunter and Barry 2012) than positive symptoms (Green 2006; Vierling-Claassen et al. 2008). Whilst negative symptoms do somewhat correlate with functioning, cognitive performance is the superior predictor, consistently accounting for around 20-60% of the variance in both global and specific functional performance (Green 1996). Cognitive impairments are associated with social and occupational disability in those with schizophrenia (Green 1996; Harvey et al. 1998). The subsequent loss of productivity contributes the most to the indirect (non-healthcare) costs of schizophrenia (Harvey and Keefe 2001; Ko et al. 2003; Kitchen et al. 2012).

People with schizophrenia perform around one to two standard deviations below controls during cognitive tasks (Hoff et al. 2005; Keefe 2008; Meier et al. 2014; Firth, Cotter, et al. 2017). Impairments are present before disease onset (Reichenberg and Harvey 2007) and those who develop schizophrenia demonstrating mild cognitive impairment in childhood (Dickson et al. 2012). In their teens and early 20s, these individuals are an average of 0.5 standard deviations (the equivalent of eight IQ points) below controls (Woodberry et al. 2008). A 2014 meta-analysis found that those with first-episode or chronic schizophrenia demonstrate poor cognitive performance between 0.9 and 1.4 standard deviations below their peers (Meier et al. 2014). This reduction is equivalent to a 14 - 21 point decrease in IQ, an impairment that remains relatively stable until the onset of age-related cognitive decline (Fucetola et al. 2000; Heaton et al. 2001; Hoff et al. 2005; Meier et al. 2014). There is also robust evidence that asymptomatic relatives display cognitive impairments (Egan et al. 2001; Snitz et al. 2006), particularly in the domains most affected by schizophrenia (Sitskoorn et al. 2004; Snitz et al. 2006). Furthermore, a higher schizophrenia polygenic risk score is associative with lower IQ (Hubbard et al. 2015). These findings show that the cognitive deficits of schizophrenia are a core feature of the disorder and may have a strong genetic component.

In conclusion, impaired cognition in schizophrenia is a crucial focus for research and treatment, presenting an opportunity to substantially reduce the human and financial burden of the illness (Sevy and Davidson 1995; Harvey and Keefe 2001; Ko et al. 2003; Green, Nuechterlein, et al. 2004; Barnett et al. 2010; Neill et al. 2010; Kitchen et al. 2012; Liemburg et al. 2013).

1.2 Schizophrenia and brain alterations

Schizophrenia is associated with alterations in brain structure and function. Neuroimaging studies have provided insight into structural changes in schizophrenia (Wright et al. 2000; Fayed et al. 2019). The most robust findings are that people with schizophrenia have smaller total brain volume, reduced volume of the thalamus, enlarged ventricles, and thinning of the frontal, parietal, and temporal lobes (Konick and Friedman 2001; Haijma et al. 2013; Doostdar et al. 2019). For the most part, this is independent of antipsychotic treatment (Pantelis et al. 2003; Brugger and Howes 2017). Additionally, multiple reviews and meta-analyses agree that hippocampal and parahippocampal areas demonstrate reduced volumes (Pantelis et al. 2003; Vita et al. 2006; Steen et al. 2014; Fayed et al. 2019). These regions also display abnormal connectivity to other brain areas, such as the prefrontal cortex (PFC; Frith et al. 1995; Benes 2000; Shenton et al. 2001; Fayed et al. 2019), providing evidence that plasticity may be disrupted in schizophrenia.

1.2.1 Dysfunctional plasticity in schizophrenia

Neuroplasticity is a continuous process of short-, medium- and long-term changes in brain connectivity, from the microscopic level of ion channels and synapses to macroscopic phenomena such as brain volume and cortical circuitry (Duffau 2006; Duffau 2016). Numerous processes facilitate plasticity. These include neurogenesis, synaptogenesis, synaptic plasticity, pre- and postsynaptic modifiers, dendrite arborisation, axon modification, and neuronal maturation (Nissant et al. 2009; Gogos et al. 2012). Synaptic plasticity is the process of changing or maintaining both excitatory and inhibitory synaptic connections in cortical networks, and its

disruption is increasingly believed to be a causative factor of schizophrenia (Hughes 1958; Stephan et al. 2006). Historically, there has been vigorous debate as to whether neuroimaging evidence indicates that schizophrenia is a neurodevelopmental (Feinberg 1982) or a neurodegenerative disorder (DeLisi 1997; Saijo et al. 2001; Olabi et al. 2011). Dysfunctional synaptic plasticity is one of the few mechanisms consistent with both hypotheses (Feinberg 1982; DeLisi 1997; Stephan et al. 2006).

Systemic impairment of plasticity may account for both the abnormal neurodevelopment and seemingly progressive changes that occur in the brains of people with schizophrenia (Feinberg 1982; Pantelis et al. 2003; Uhlhaas and Singer 2006; Gogtay 2008; Fayed et al. 2019). Furthermore, deficits in synaptic connectivity may account for the reduced volume of grey matter in the absence of cell loss (Uhlhaas and Singer 2006; Uhlhaas and Singer 2010). Dysfunctional synaptogenesis leads to fewer synapses and reduced volume while the number of neurons in affected areas remain mostly unchanged (Selemon and Goldman-Rakic 1999; Uhlhaas and Singer 2010). Grey matter plasticity, a product of synaptic plasticity and neuronal integrity, is associated with learning and training (Draganski and May 2008; May 2011). N-acetylaspartate (NAA) is a marker for neuronal integrity and it is reduced in several areas in those with schizophrenia. These include the grey matter in the thalamus and the hippocampus, as well as both prefrontal grey and white matter (Deicken et al. 1999; Auer et al. 2001; Steen et al. 2005; Natsubori et al. 2014). Furthermore, the brains of people with schizophrenia tend to have smaller, denser neurons with fewer dendritic spines and reduced arborisation (Domino and Luby 2012). Together, these clustered phenomena are

indicative of synaptic dysregulation, adding to the evidence that grey matter plasticity is disrupted in schizophrenia (Ben-Shachar and Laifenfeld 2004; Stephan et al. 2009).

Synapses are sophisticated, specialised, neuronal junctions whose function are contingent on intricately organised complexes of interacting proteins. The loss or modification of these proteins can affect the properties of the networks in which they are found, causing dysfunction (Fossati et al. 2015). Researchers investigating the pathology of schizophrenia are becoming progressively more interested in compromised neural and synaptic health (Coley and Gao 2018). It is possible to use pre- and postsynaptic proteins as biomarkers to indicate the condition, function and density of synapses (Osimo et al. 2019). These include synaptosome associated protein 25 ($SNAP_{25}$), found predominantly presynaptically, and postsynaptic density protein 95 (PSD_{95}), found in the postsynaptic density (PSD).

The PSD is a protein-dense area of specialisation found in the postsynaptic membrane that is heavily implicated in synaptic plasticity and stabilisation (Hunt et al. 1996; Meyer et al. 2014). It has been described as a scaffold or matrix that holds together various receptors and their accompanying signalling proteins (Cho et al. 1992). Within the PSD, there are a large number of glutamate receptors, including the N-methyl-D-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtypes (Weiler and Greenough 1993; Chen et al. 2000). Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) and therefore plays a critical role in synaptic plasticity (National Academy Medicine 2011; Steeds et al. 2015). Moreover, the glutamate system has

been heavily implicated in the cognitive deficits in schizophrenia (Stephan et al. 2006; Schwartz et al. 2012). NMDA receptors (NMDARs) are prominently involved in the molecular mechanisms of synaptic adaptation (Pérez-Otaño and Ehlers 2005), with functions such as regulating the migration of neurons (Komuro and Rakic 1993), strengthening synapses via long-term potentiation (LTP; Harris, Ganong, and Cotman 1984), and dendritic spine development (Yasumatsu et al. 2008). AMPA receptors are also crucial for LTP and perform critical roles in long-term depression (LTD) and the homeostatic scaling of synapses (Diering and Huganir 2018). Faults or abnormalities in any of these processes have the potential to have a catastrophic impact on neuronal function and adaptation.

PSD_{95} is the most abundant scaffolding protein found in the PSD. It is a member of the membrane-associated guanylate kinase (MAGUK) group (Chen et al. 2011), which play a central role in synaptic plasticity (Kim and Sheng 2004; Funke et al. 2005). It is capable of recruiting and binding both NMDA and AMPA receptors to the postsynaptic membrane and interacts with numerous other proteins in the PSD (Kornau et al. 1995; Nicoll et al. 2006; Chen et al. 2011). The influence that it has over the density of these glutamate receptor subtypes at synapses gives PSD_{95} a central role in the modulation and regulation of synaptogenesis as well as synaptic strength and maturation (Chen et al. 2011; Coley and Gao 2018). PSD_{95} is disrupted in schizophrenia and levels are decreased in the PFC and hippocampus of patients postmortem, with areas of the PFC exhibiting reductions of around 15 - 20% (Ohnuma et al. 2000; Catts et al. 2015). The evidence for this disruption is compelling and these areas are the most significantly affected in the brains of people with the disease (Meyer-Lindenberg et al. 2005). Furthermore, the PFC and

hippocampus are central to learning and memory. PSD_{95}-mediated behavioural irregularities in glutamatergic synapses are associated with the cognitive deficits seen in schizophrenia (Coley and Gao 2018). During neurodevelopment, PSD_{95} is thought to affect dendritic spine densities. Therefore, functional aberrations could interfere with the healthy formation or pruning of spines, causing neuropathology and cognitive impairment (Coley and Gao 2018). The importance of this process during development may explain why cognitive impairments are present before other symptoms in schizophrenia, whereas the positive symptoms of schizophrenia tend to appear in adolescence or early adulthood (Picchioni and Murray 2007).

It has been widely theorised that the developmental elimination, often called 'pruning', of synapses may expose dysfunctional synaptic plasticity during this period of brain maturation (Paolicelli et al. 2011; Uhlhaas 2011; Boksa 2012). Another respected hypothesis posits that those with schizophrenia experience synaptic 'over-pruning' during adolescence (Feinberg 1982). It is argued that this leaves them vulnerable to malfunctions in excitatory and inhibitory neurotransmission and neural circuits, perhaps through disrupted cortical oscillations (Uhlhaas and Singer 2006; Uhlhaas and Singer 2010). As PSD_{95} has such a substantial impact on synaptogenesis and synapse maturation, it is often used as a measure of the structural and functional integrity of excitatory synapses (Chen et al. 2008; Shao et al. 2011). There is robust evidence that PSD_{95} is disrupted in schizophrenia and that it is closely linked to the cognitive deficits in the disease. PSD_{95} has also been shown to be significantly reduced in the subchronic phencyclidine (scPCP) rodent model in the frontal cortex and ventral hippocampus (Gigg et al. 2020).

$SNAP_{25}$ is a target soluble N-ethylmaleimide sensitive factor attachment protein receptor (t-SNARE) pre-synaptic protein that is associated with synaptic regulation and is used as an indicator for synaptic density (Egbujo et al. 2016). It is an integral part of SNARE complexes that regulate the exocytotic release of neurotransmitters at synapses (Tafoya et al. 2006). These complexes play a vital part in action potential (AP)-dependent release in glutamatergic, cholinergic and GABAergic transmission in the central nervous system (CNS; Washbourne et al. 2002; Verderio et al. 2004; Delgado-Martínez et al. 2007). $SNAP_{25}$ also contributes to other processes associated with plasticity, including dendritic spine morphogenesis (Jouroukhin et al. 2016), LTP (Jurado et al. 2013), and postsynaptic receptor trafficking including NMDARs (Matteoli et al. 2009; Lau et al. 2010; Antonucci et al. 2016; Gu and Huganir 2016). Because of its importance for successful synaptic plasticity, the $SNAP_{25}$ gene sequence is highly conserved across species (Catsicas et al. 1991; Risinger et al. 1997). As a marker for synaptic density, it has been proposed as a more sensitive indicator of disrupted connectivity and synaptic pathology than other SNAREs such as synaptophysin (Young et al. 1998). In part, this is because it is restricted to a subset of terminals often found in the limbic system (Oyler et al. 1989). Moreover, synaptophysin has been reported to be unchanged in both patients (Fatemi et al. 2001; Young et al. 1998) and in a neonatal PCP model for schizophrenia (Karlsen et al. 2013).

It is thought that $SNAP_{25}$ plays a role in normal cognition (Gosso et al. 2006) and it is frequently implicated in disorders associated with cognitive impairments (Najera et al. 2019). Genetic evidence has linked $SNAP_{25}$ to several neurological conditions such as intellectual disability (Rohena et al. 2013), Lewy body dementia

(Mukaetova-Ladinska et al. 2009) and schizophrenia (Carroll et al. 2009). Several large genome-associated case-control studies have shown $SNAP_{25}$ single nucleotide polymorphisms (SNPs) are either associated with or are potential risk factors for schizophrenia (Cardno and Gottesman 2000; Sullivan et al. 2003; Carroll et al. 2009). It has also been shown that the *Dde*I polymorphism of $SNAP_{25}$ is significantly associated with cognitive performance in people with schizophrenia (Kirchner et al. 2018). $SNAP_{25}$ has been repeatedly reported to be abnormal in several brain areas postmortem in schizophrenia, including the hippocampus (Young et al. 1998; Thompson, Egbufoama, and Vawter 2003; Fatemi et al. 2001), entorhinal cortex (Young et al. 1998), and prefrontal cortex (Thompson et al. 1998; Karson et al. 1999; Karlsen et al. 2013).

There is extensive evidence of synaptic dysfunction in schizophrenia. Aberrations in the systems that support and maintain plasticity are heavily implicated in the disorder, with many researchers believing these deficits play a causative role (DeLisi 1997; Friston 2002; Ben-Shachar and Laifenfeld 2004; Stephan et al. 2009; Crabtree and Gogos 2014). In the studies described in this thesis, PSD_{95} and $SNAP_{25}$ were measured to indicate the number and health of synapses in brain areas affected in schizophrenia, namely the PFC and hippocampus.

1.3 Exercise as a treatment for cognition in schizophrenia

There are currently no pharmacological treatments for the cognitive impairments experienced by people with schizophrenia. Several causative mechanisms have been proposed, although the drugs that modulate these have had mixed results

(Ahmed and Bhat 2014) and none has made it to the clinic. Cognitive remediation therapy (CRT) has been shown to improve cognition in schizophrenia (Wykes et al. 2011). However, there are considerable variations in content, structure, aims (e.g. 'restorative' or 'compensatory') and delivery of CRT programmes and protocols (Mogami 2018). CRT works best when performed for a long duration (14 weeks or more) and in conjunction with a comprehensive psychiatric rehabilitation programme (Mogami 2018). Known mediators of response to CRT include baseline cognition and clinical stability (Twamley et al. 2011) meaning the more unwell a patient is, the less CRT might help. CRT alone improves cognitive performance with effect sizes in the small-to-medium range (Wykes et al. 2011; Firth et al. 2017; Mogami 2018). Current CRT programmes may ameliorate one third to half of the cognitive deficit seen in schizophrenia (Nuechterlein et al. 2016). Unfortunately, this is a relatively modest improvement, considering the magnitude of the impairment, and the benefit to functional outcome is smaller still (Nuechterlein et al. 2016). The limited success of CRT has prompted further investigation into other non-pharmacological interventions to alleviate the cognitive deficits in schizophrenia.

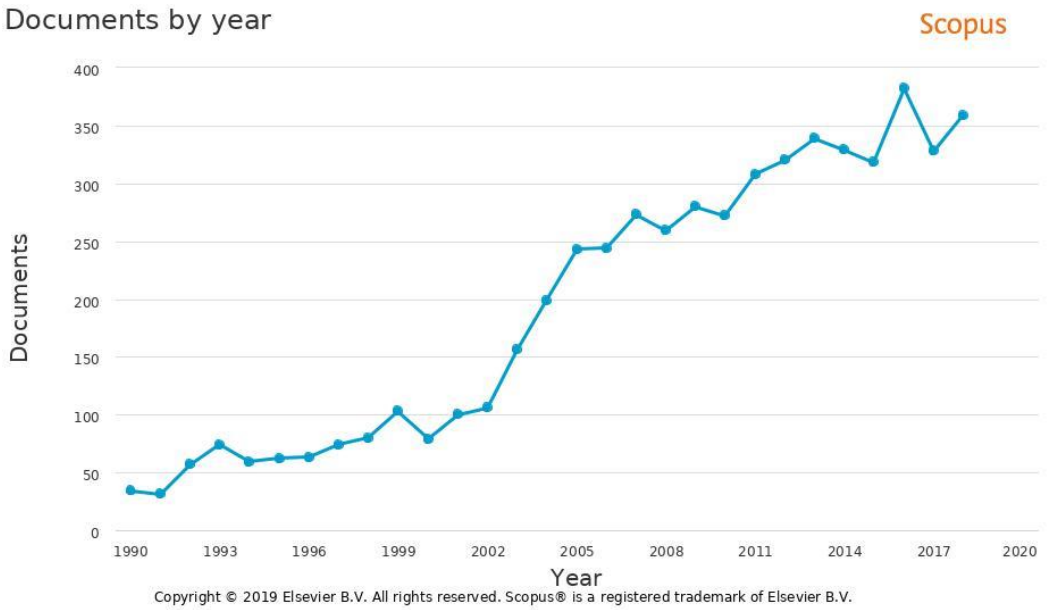


Figure 1.1 The number of published documents returned on Scopus when searching for papers with keywords, titles or abstracts containing schizophrenia and exercise and related terms (Scopus 2019).

Since the 1990s, the interest in exercise as a therapy for mental illness and neurological disorders has increased exponentially. Exercise as a treatment for schizophrenia is no exception (Fox et al. 2000), where the number of published papers continues to rise year on year (see Figure 1.1). In the healthy population, exercise has been repeatedly demonstrated to improve cognition and brain function at every stage of the lifespan (Cotman and Berchtold 2002; Cotman and Berchtold 2007a; Hillman et al. 2008; van Praag 2009). Physical activity has also been shown to reduce the risk, delay the onset, and slow functional decline in Parkinson's, Huntington's and Alzheimer's disease (Podewils 2005; Goodwin et al. 2008; Radak et al. 2010; Harrison et al. 2013; Arcoverde et al. 2014). A considerable literature exists regarding the effects of exercise on depression, including a robust meta-analysis confirming that it substantially reduces clinical symptoms (Schuch et al. 2016). There is also evidence for the amelioration of cognitive deficits in depression, although this remains contentious (Smith et al. 2010; Kvam et al. 2016;

Schuch et al. 2016; Brondino et al. 2017; Sun et al. 2018). In schizophrenia, meta-analyses have demonstrated that exercise can improve positive and negative symptoms, global cognition and social functioning in patients (Firth et al. 2015; Rosenbaum et al. 2015; Dauwan et al. 2016; Firth et al. 2017). It has also been established that the amount and intensity of exercise are predictive of cognitive improvements. Studies have shown that higher 'dosages' (in minutes per week) and more intense exercise correlate with the size of the cognitive effect (Kimhy et al. 2015; Firth, Carney, et al. 2018), although meta-analysis reported this at only the trend level (p = 0.07; Firth et al. 2017). One study found a significant effect of exercise on global cognition ($p < 0.05$) and that increases in cardiorespiratory fitness accounted for one quarter (25.4%, $p < 0.01$) of improvement (Kimhy et al. 2015). Most studies to date in schizophrenia have employed aerobic exercise, although the specific types, duration and frequency of exercise vary considerably (Dauwan et al. 2015; Firth et al. 2015; Rimes et al. 2015; Firth et al. 2017). There has been limited research into the benefits of resistance training in schizophrenia or schizophrenia spectrum disorders, with only a handful of trials examining changes in symptoms (Marzolini et al. 2009; Heggelund et al. 2012; Kim et al. 2014; Leone et al. 2015; Svatkova et al. 2015; Silva et al. 2015). Recently, a small pilot study (n = 15) examined resistance training in people with schizophrenia versus a balance and toning control group and found no difference on symptoms or cognition (Maurus et al. 2020). To date, there have been no randomised control trials solely investigating resistance training and its effect on cognition in schizophrenia (Firth et al. 2017; Keller-Varady et al. 2018), despite evidence that this type of activity is beneficial in other populations (Cassilhas et al. 2007; Liu-Ambrose et al. 2010).

Table 1.1 Summary of exercise studies adapted from Firth et al. (2017).

	Sample Characteristics			**Exercise intervention**				**Study Details**
	Exercise/control (n)	**Mean Age**	**% Male**	**Session Content**	**Supervision**	**Weeks, sessions, time**	**Comparator**	**Cognitive Domains Examined**
Behere et al. (2011)	17/22	32	76	Brisk walking, jogging, standing/sitting exercises.	Yoga instructor	12wk. 4wk supervised, 8wk home	Waitlist (R)	Social cognition
Campos et al. (2015)	13/16	39	73	Interactive physical activity videogame "Move4Health."	Rehabilitation centre staff	8wk, 2, 20min	TAU (NR)	Processing speed
Ho et al. (2016)	51/49	55	50	Stretching, walking, light weights. 50%–60% max O2 consump.	Mental health professionals	12wk, x3, 60min	Waitlist (R)	**Working memory**
Kimhy et al. (2015)	13/13	37	64	Mixed aerobic exercise. 60%–75% VO_2 peak.	Physical trainer	12wk, 3, 60min	TAU (R)	Processing speed **Attention/vigilance** Working memory Verbal learning Visual learning Reasoning **Social cognition**

	Sample Characteristics			Exercise intervention				Study Details
Lin et al. (2015)	31/33	25	0	Treadmill, stationary cycling. 50%–60% VO_2 max.	Yoga instructor	12wk, 3, 45-60min	TAU (R)	Processing speed Attention/vigilance **Working memory** **Verbal learning**
Malchow et al. (2015)	22/21	37	72	Stationary cycling, individually defined intensity	Sports scientist	12wk, 3, 30min	Table football + CR (NR)	Processing speed Working memory Verbal learning Reasoning
Nuechterlein et al. (2016)	7/9	23	73	Aerobic work-out/callisthenics video. 60%–80% of max. heart rate	Physical trainer	10wk, 4, 30-45min	CR (NR)	Processing speed Attention/vigilance Working memory Verbal learning Visual learning Reasoning **Social cognition**
Oertel-Knöchel et al. (2014)	8/11	42	44	Circuit training. 60%–70% of max. heart rate	Physical trainer	4wk, 3, 45min	Relaxation + CR (R)	**Processing speed** **Working memory** Verbal learning **Visual learning**
Pajonk et al. (2010)	8/8	35	100	Stationary cycling, individually defined	Study investigator	12wk, 3, 30min	Table football (R)	Working memory **Verbal learning**

	Sample Characteristics			Exercise intervention				Study Details
Svatkova et al. (2015)	16/17	30	82	Aerobic training 75% of max. heart rate and resistance training	Physical trainer	24wk, 2, 40/20min	Occupational therapy (R)	Global (IQ)

Abbreviations: max/maximum; wk/week;min/minute(s);VO_2/volume of oxygen; CR/cognitive remediation; R/randomised;NR/not randomised. Cognitive domains reported as significant in bold.

In addition to the unknowns around the ideal type of exercise, there is relatively little consensus on the most effective frequency, intensity and duration of physical activity (Firth et al. 2017). However, meta-analyses have identified some requirements and mediators of the effect of aerobic exercise in schizophrenia. Firstly, to produce a measurable psychological improvement of any kind it seems that 90 minutes of moderate- to high-intensity exercise per week is required, with individuals sessions lasting a minimum of 30 minutes (Firth et al. 2015). One meta-analysis found that only exercise programmes led by physical activity professionals (such as certified teachers, physical therapist and exercise physiologists) significantly imporved global cognition $p < 0.001$), but programs led by healthcare or research staff did not ($p = 0.50$). However, when the physical versus non-physical professional groups were compared directly there was not a significant difference between them ($p = 0.19$; Firth et al. 2017). Another meta-analysis investigated the predictors of drop out of the active (versus control) arm of exercise intervention studies in people with schizophrenia. They found that the level of qualification of those supervising the activities was the best predictor of study dropout rate. Regular contact with qualified fitness professionals significantly improved retention rates for people with schizophrenia who were randomised to undertake physical training (Vancampfort et al. 2016). Maintaining exercise adherence in this population is notoriously challenging. Roughly one in four participants assigned to the physical activity group of an experiment fails to complete the programme (Vancampfort et al. 2016). This proportion is considerably higher than in those in other mental health populations. For example, less than 15% of people with depression drop out of the active arm of exercise interventions

(Kvam et al. 2016). It has also been demonstrated that providing access to fitness facilities does not lead to an increase in physical activity in people with schizophrenia (Archie et al. 2003) and sustained supervision is required (Vancampfort et al. 2016; Yung and Firth 2017). It is thought this might be because of more severe negative symptoms in some individuals. However, there is little quantitative evidence for this and the most comprehensive meta-analysis to date on the subject found that no patient characteristic (e.g. age, gender, illness duration or baseline symptomatology) was predictive of dropout prevalence (Vancampfort et al. 2016). Other factors that improve adherence and retention are reward provision, motivational interventions (such a motivational interviewing), and incorporating physical activity video games into interventions (Vancampfort et al. 2016).

People with schizophrenia have poorer health behaviours than the general population, including poor diet, increased incidence of smoking, and physical inactivity (Lambert et al. 2003; Marder et al. 2004). They are more likely to have cardiorespiratory issues and roughly 60% of premature, non-suicide deaths in this population are related to physical comorbidities (Lambert et al. 2003). Antipsychotics increase these problems with side effects such as weight gain (Goff et al. 2005), although long-term antipsychotic treatment does not appear to increase mortality when other factors (e.g. socioeconomic background, antipsychotic dose) are controlled for (Kadra et al. 2018). Improving cardiovascular and cardiorespiratory fitness is a proven way to reduce the risk and prevalence of physical disorders that are highly associated with schizophrenia (Hillman et al. 2008; Goff et al. 2005). These include metabolic syndrome (MetS; Saddichha et al.

2008), coronary heart disease (Hennekens et al. 2005), hypertension (Goff et al. 2005), obesity (Ross and Bradshaw 2009), dyslipidaemia (Hennekens et al. 2005), insulin resistance, and type two diabetes mellitus (Newcomer 2004). Furthermore, there is tentative evidence that improvements in cardiorespiratory fitness are associated with improved cognition (Pajonk et al. 2010; Firth et al. 2017; Firth et al. 2018). Therefore, exercise has the potential to both improve the debilitating cognitive deficits in schizophrenia and to reduce the impact of many of the 'life-limiting' comorbidities prevalent in this population (Goff et al. 2005; Laursen 2011; Hjorthøj et al. 2017).

1.3.1 Exercise and its effects on the brain in schizophrenia

There is accumulating evidence that exercise may improve the persistent and disabling cognitive deficits of schizophrenia. It has been shown to improve overall cognition with particularly notable effects on attention, working memory and social cognition (Firth et al. 2017). However, less is known about the processes by which exercise affects cognition. Much of the uncertainty is caused by the somewhat bewildering number and complexity of systems that respond to exercise. Those mechanisms affecting brain function and cognition are multi-faceted, multi-directional, highly interrelated, and have intricate temporal effects. For example, exercise has been shown to augment brain metabolism via enzyme-linked increases in the availability of electron chain components in the Krebs cycle (Berchtold et al. 2010). Exercise also activates glutamatergic and GABAergic neurotransmitter systems responsible for regulating synaptic excitability (Tong et al. 2001). In addition to this, physical activity upregulates plasticity-related molecules, including

trophic factors such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor (IGF), ventricular endothelial growth factor (VEGF) (Cotman and Berchtold 2007a), as well as synaptic vesicle trafficking molecules such as synapsin 1 (Tong et al. 2001). Exercise produces angiogenesis in several brain areas, increasing neuronal metabolism by increasing the efficiency of the transportation and delivery of oxygen (Berchtold et al. 2010; Kaliman et al. 2011). The countless candidate mechanisms continually interact with one another, making it difficult to tease out those that are the most important.

1.3.1.1 Schizophrenia, inflammation and exercise

It has been demonstrated that there is chronic inflammation in several mental illnesses (Perry 2004; Eisch et al. 2008), including schizophrenia (Reif et al. 2006; Di Nicola et al. 2013). There is a large body of research reporting elevated inflammatory markers in people with schizophrenia, even in first-episode and antipsychotic naïve subpopulations (Doorduin et al. 2009; Meyer et al. 2011; Miller et al. 2011; Na et al. 2014; Noto et al. 2015). Higher levels of inflammatory markers in people with schizophrenia are significantly correlated with neurocognitive dysfunction (tumour necrosis factor α [TNF-α] and interleukin [IL] 12) and poor daily functioning (TNF-α; Kogan et al. 2018). In addition to CNS inflammation being associated with neurocognition, it is also increased in sub-populations of patients with prevalent and persistent negative symptoms (Garcia-Rizo et al. 2012). This association suggests that ameliorating this low-grade chronic inflammation may improve both cognition and the negative symptoms in schizophrenia.

The concept that schizophrenia is linked to immune function is far from new (Wise and Babcock 1896; Buckley 2019). It is well-known that prenatal maternal infection is a risk factor for schizophrenia (Mednick et al. 1988; Khandaker et al. 2013). However, the vulnerability to the inflammatory-mediated risk of psychiatric disorders does not end postnatally (Khandaker et al. 2013). Elevated levels of inflammatory markers in childhood are correlated with a higher risk of developing psychosis and other mental illnesses (Khandaker et al. 2015). Similarly, the risk of developing schizophrenia is almost doubled in adults who survived childhood CNS infections (Khandaker et al. 2012). One of the most significant findings implicating the immune system in schizophrenia came from a genome-wide association study that identified 108 gene loci associated with its development. The data revealed a substantial overlap with genes involved in immune function (Ripke et al. 2014). People with schizophrenia also have a higher incidence of autoimmune disorders, as do their unaffected first-degree relatives (Eaton et al. 2006). Many find the evidence implicating the immune system in schizophrenia compelling and propose inflammation has a central role in the disease (Müller and Schwarz 2006; Fan et al. 2007; Müller 2018).

People with schizophrenia exhibit elevated levels of pro-inflammatory cytokines compared to the general population (Miller et al. 2011). The most consistent findings are for increased levels of IL-1β, IL-6, and TNF-α (Zajkowska and Mondelli 2014). IL-1β and IL-6 are seen in higher concentrations during an acute psychotic relapse decline after antipsychotic treatment and the remission of symptoms (Miller et al. 2011). In contrast, TNF-α remains unchanged by treatment (Miller et al. 2011). There are some caveats to be kept in mind when discussion inflammatory

markers. Firstly, the classification of pro- and anti-inflammatory cytokines (and myokine, in the case of IL-6) as binary is oversimplified. Some have both properties, depending on a variety of factors (Müller 2018). Furthermore, studies rarely control for confounding factors such as BMI, smoking status, sleep, sex, or medication (Miller et al. 2011). Finally, although inflammation is believed to increase the permeability of the blood-brain barrier, facilitating the dispersion of immune components into the brain (Engelhardt and Sorokin 2009), peripheral immune activation does not necessarily reflect the circumstances in the brain (Müller and Bechter 2013).

Cardiometabolic disorders, such as metabolic syndrome (MetS) and insulin sensitivity, are much more prevalent and occur at an earlier age in people with schizophrenia (Hennekens et al. 2005; Vancampfort et al. 2013). These cardiometabolic disorders, and unhealthy behaviours such as smoking and poor diet, result in systemic inflammation. Systemic inflammation increases CNS inflammation, which is associated with a decline in cognitive function (Dik et al. 2007) and is present in at least a subset of people with schizophrenia (Müller 2018). Therapies that reduce systemic inflammation are likely to improve neuroinflammation, and early studies augmenting antipsychotics with anti-inflammatory drugs looked promising (Müller 2018). Unfortunately, the largest multicentre, double-blind, placebo-controlled trial using an anti-inflammatory agent (minocycline) to date reported no effect on peripheral inflammatory markers, positive or negative symptoms, brain imaging, or neurocognition (Deakin et al. 2018; Buckley 2019).

Exercise has been described as 'anti-inflammatory' and activates a cascade of cytokine activity (Petersen and Pedersen 2005; Gleeson et al. 2011). Exercise lowers circulating levels of TNF-α and IL-6, although IL-6 concentration is increased before it is reduced (see Figure 1.2). The IL-6-prompted cytokine cascade is triggered after high-intensity exercise over longer durations but even small amounts of moderate exercise have proven health benefits (Gleeson et al. 2011). A detailed account of every way that exercise affects the body is beyond the scope of this chapter. Nonetheless, exercise is a well-evidenced, potent and effective anti-inflammatory, shown to reduce systemic inflammation significantly (Booth and Lees 2006). Low-grade systemic inflammation, similar to that described in schizophrenia, has neurodegenerative effects (Leonard 2007). Therefore, increasing cardiorespiratory fitness through regular physical activity can reduce systemic inflammation, in turn improving brain health.

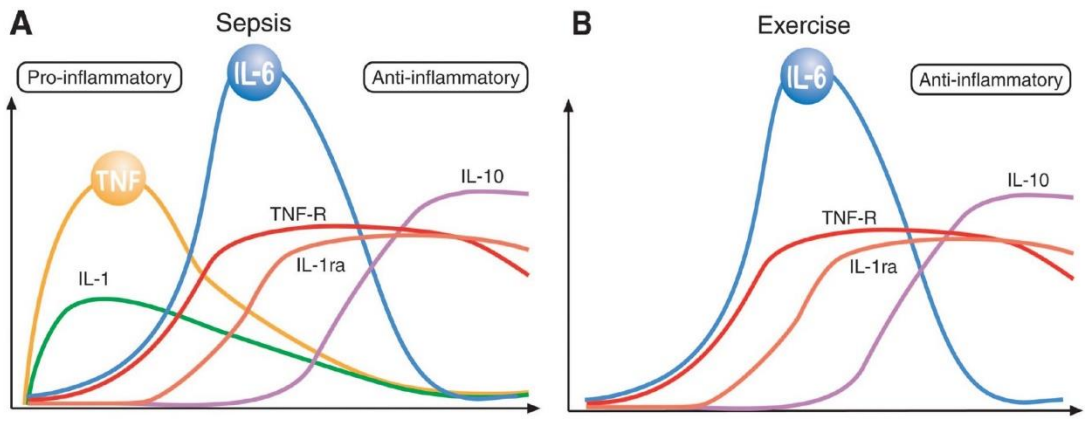


Figure 1.2. Changes in cytokines in sepsis and exercise. In sepsis (A), the cytokine cascade within the first few hours consists of TNF-α, IL-1β, IL-6, IL-lra, TNF-R, and IL-10. The cytokine response to exercise (B) does not include TNF-α and IL-1β but does show a marked increase in IL-6, which is followed by IL-lra, TNF-R, and IL-10. Increased CRP levels do not appear until 8-12 h later. Tumour necrosis factor-α is abbreviated to TNF, interleukin 1 beta to IL-1β, interleukin 6 to IL-6, interleukin 1 receptor agonist to IL-1ra, soluble TNF receptor to TNF-R, interleukin 10 to IL-10, and C-reactive protein to CRP. Adapted from (Petersen and Pedersen 2005).

1.3.1.2 Exercise promotes plasticity

How exercise improves cognition in schizophrenia is still not fully understood. It is believed that exercise can increase plasticity through neurogenesis and synaptogenesis in the brain, which then improves cognition. Exercise has been posited to increase cognitive performance by improving the trophic environment in the brain as a result of its anti-inflammatory properties (Vancampfort et al. 2014). In non-primate animals, there is compelling evidence that exercise induces neurogenesis (van Praag et al. 1999), brain angiogenesis (Isaacs et al. 1992), and can modulate neurotrophic factors such as BDNF (Cotman and Berchtold 2007a). All are processes that support healthy brain plasticity (Cotman and Berchtold 2002). Animal work demonstrates that exercise, BDNF, neurogenesis and cognition are linked (Berchtold et al. 2005; Cotman and Berchtold 2007b; Ding et al. 2011).

However, this association is less robust in primates and humans and the evidence is contentious (Sorrells et al. 2018).

Assuming the human brain does respond to exercise in a similar way to non-primate animals, there are two prominent theories as to how it influences cognition in schizophrenia. The first is that exercise increases brain volume via neurogenesis. It is thought that this can correct volume loss observed in patients, resulting in improved cognition (Firth et al. 2017). The second is that exercise increases levels of BDNF, improving the trophic environment in the brain and improving plasticity. These phenomena are thought to be highly interrelated and appear to have the most significant effects in the hippocampus (Firth et al. 2017), a brain area heavily associated with learning and memory (Heckers et al. 1998; Meyer-Lindenberg et al. 2005).

People with schizophrenia tend to have reduced volume in the temporal lobe with consistent evidence that hippocampal and parahippocampal areas are smaller than controls (Pantelis et al. 2003; Vita et al. 2006; Steen et al. 2014; Fayed et al. 2019). Antipsychotics may be able to partially correct hippocampal volume (Chakos et al. 2005; Panenka et al. 2007), but the evidence is inconsistent (Ho et al. 2011). Nonetheless, even a small improvement in hippocampal volume is correlated with improvement of symptoms (Panenka et al. 2007). The publication of an influential study by Pajonk and colleagues (2010) reported that exercise was able to increase hippocampal volume. In addition to this, the increases correlated with improvements in cognition. In this experiment, people with schizophrenia were assigned to stationary bicycle training or a control condition of table football. The

exercising group showed improvements in short-term memory that were associated with the augmentation of the hippocampus and the authors posited that exercise stimulated neurogenesis in this brain area, enhancing cognition. However, subsequent studies have not had such clear-cut results. While some reported exercise-related increases in volume (Pajonk et al. 2010; Lin et al. 2015; Malchow et al. 2016), cortical thickness (Scheewe et al. 2013) and white-matter tract integrity (Svatkova et al. 2015), others observed no changes (Rosenbaum et al. 2015; Scheewe et al. 2013). A further blow to the neurogenesis hypothesis came when a recent meta-analysis found that exercise was not able to increase total hippocampal volume in humans, although a larger left hippocampus was associated with physical activity (Firth et al. 2018). The authors concluded that exercise might not increase volume per se, but agreed with an earlier review that it could protect neurogenesis, preventing volume losses (Voss et al. 2011; Firth et al. 2018).

It is widely accepted that exercise improves cognition in schizophrenia (Firth et al. 2017). However, the specific ways that it achieves this remain enigmatic. When others were not able to replicate the findings of Pajonk et al. (2010), the conflicting evidence resulted in a shift of focus from grey and white matter volume. It is thought BDNF may mediate the relationship between exercise, neurogenesis and brain volume (Szuhany et al. 2015). Exercise-induced upregulation of BDNF improves brain health and neuroplasticity through multiple pathways, arguably making it more relevant than volume for learning and memory (Kimhy et al. 2015). Animal work shows a clear relationship between exercise, upregulation of BDNF, and improvement in cognition (Vaynman et al. 2004). However, the evidence for BDNF in schizophrenia is also contradictory (Firth et al. 2017). Of the studies

described in the literature, two reported significant increases in circulatory BDNF (Kuo et al. 2013; Kim et al. 2014), two reported non-significant increases (Kimhy et al. 2015; Nuechterlein et al. 2016), and one found no difference at all (Silva et al. 2015). Despite this, increases in BDNF levels were significantly associated with improved cognition in the three studies that examined this relationship. This effect was present even when there was no significant increase in BDNF levels (Kuo et al. 2013; Kim et al. 2014; Kimhy et al. 2015). These data suggest that exercise may cause an increase in peripheral BDNF in those with schizophrenia, improving cognition (Firth et al. 2017). However, the effect size may be small and therefore only observable in larger sample sizes (Firth et al. 2017; Naegelin et al. 2018).

Beyond brain volume and BDNF, exercise can improve or even reverse other brain abnormalities seen in patients with schizophrenia. For example, vascular deficits have been reported in early and chronic stages of the disease (Hanson and Gottesman 2005; Iwashiro et al. 2012; Kanahara et al. 2013; Woodward et al. 2018). People with schizophrenia exhibit reduced blood flow to frontal and temporal brain regions, which may contribute to cognitive deficits (O'Brien et al. 2003; Hanson and Gottesman 2005). Furthermore, vascular abnormalities are linked with cognitive dysfunction in other disorders, such as vascular dementia, and can produce psychotic symptoms (O'Brien et al. 2003; van der Flier et al. 2018). Exercise stimulates angiogenesis in several brain areas including the hippocampus (Lopez-Lopez et al. 2004) and cortex (Kleim et al. 2002). Increased oxygen delivery increases neurotrophin levels and improves neuronal metabolic activity and resilience to stress (Kaliman et al. 2011).

Effective communication between brain areas is required for cognition (Deary et al. 2010), and schizophrenia is associated with both anatomical and functional dysconnectivity (Stephan et al. 2009). For example, white matter tract integrity is compromised in the brains of patients (Kelly et al. 2018) and is linked to deficits in several cognitive domains (Alloza et al. 2016; Knöchel et al. 2016; Hidese et al. 2017). Aerobic exercise not only protects white matter integrity but also improves it in people with schizophrenia (Svatkova et al. 2015). White matter pathology is connected to neuroinflammation even in first-episode psychosis (Najjar and Pearlman 2015). Therefore, exercise may alleviate this pathology through its anti-inflammatory effects (Chaddock-Heyman et al. 2018) and an improved trophic environment (e.g. the upregulation of VEGF and IGF) is thought to enhance cerebrovascular health (Cotman and Berchtold 2007a). Angiogenesis improves oxygen and nutrient delivery to the brain (Black et al. 1990; McDonnell et al. 2013; Chaddock-Heyman et al. 2016) and cardiorespiratory fitness is correlated with white matter volume and integrity (McDonnell et al. 2013). In summary, physical activity promotes several mechanisms that support white matter health and subsequently healthy brain connectivity.

1.4 Modelling schizophrenia

1.4.1 Phencyclidine and schizophrenia-like behaviour

Phencyclidine (PCP), like ketamine, is an *N*-methyl-D-aspartate receptor (NMDAR) antagonist and a dissociative anaesthetic. PCP was used in humans until 1965 when it was discovered that it caused severe side-effects in the form of behavioural disturbances. Ketamine continues to be used as an anaesthetic as the side effects

are less common and comparatively brief in duration (Javitt 2015). Reported PCP-induced adverse reactions include mania, rigidity, catatonia, delusions and disorientation that lasts from a few hours to over a week (Greifenstein et al. 1958). There is robust evidence that a single low dose (0.05 – 0.1 mg/kg) in healthy volunteers can cause schizophrenia-like symptoms, including catatonic posturing, bodily distortion, depersonalisation (Cohen et al. 1960), idiosyncratic and rigid thinking, withdrawal, and ambivalence (Javitt 2015). PCP also causes changes in cognition such as deficits in symbolic thinking, (Cohen et al. 1960), perception (Luby et al. 1959) and attention (Javitt 2015). The cognitive disruptions of PCP have been described as 'strikingly similar' to those of schizophrenia (Javitt 2015; Domino and Luby 2012). If individuals with chronic stable schizophrenia take PCP, their symptoms are exacerbated and they appear to return to the acute phase of the illness (Domino and Luby 2012) and these effects can be observed for a month and more (Luby et al. 1959). In the 1960s, PCP became a common drug of abuse and people frequently arrived in hospitals intoxicated. In North America, this was termed 'street psychosis' (Domino and Luby 2012) and, based purely on the presentation of symptoms, many psychiatrists deemed that PCP-induced behaviours were indistinguishable from schizophrenia (Yesavage and Freman 1978; Erard et al. 1980).

Several research groups replicated schizophrenia-like behavioural and cognitive changes following PCP administration in healthy volunteers (Domino and Luby 2012). Furthermore, people who abuse PCP may also present with stereotypy and hypertension, issues often seen in schizophrenia (Javitt 2015). There has been some dispute in the literature around auditory and visual hallucinations and there is

conflicting evidence on whether PCP can recreate these disturbances. Several reports state that PCP does produce both types of hallucination (Giannini et al. 1984; Jentsch and Roth 1999; Steeds et al. 2015), while others argue that these fundamental aspects of schizophrenia are not reproduced by the drug (Krystal et al. 1994; Domino and Luby 2012). Several authors concluded that lysergic acid diethylamine (LSD) was able to produce a syndrome resembling the positive symptoms of acute schizophrenia, whereas NMDAR antagonists like PCP are only able to simulate some relevant negative behaviours and cognitive deficits (Domino 1964; Krystal et al. 1994; Domino and Luby 2012). Despite this debate around PCP's ability to reproduce the positive symptoms of schizophrenia, it is widely accepted that NMDAR antagonists do generate behaviours analogous to the chronic state of schizophrenia. That is, NMDAR antagonists can simulate schizophrenia-like negative symptoms and cognitive deficits (Domino 1964; Krystal et al. 1994; Krystal et al. 2005; Domino and Luby 2012).

From the initial observations in the clinic and the decades of research that has followed, PCP was identified as a possible pharmaceutical agent to induce schizophrenia-like behaviour in animals. PCP treatment in animals produces a range of behavioural changes similar to abnormalities found in patients (Jentsch and Roth 1999; Jones et al. 2011; Steeds et al. 2015). For example, acute PCP impairs prepulse inhibition (PPI; Wiley 1994) although this effect does not last more than a few days after treatment stops (Egerton et al. 2008). Acute PCP treatment also induces hyperlocomotion in animals, used as an analogue of positive symptoms (Kalinichev et al. 2008; Jones et al. 2011). Subchronic administration of PCP (scPCP) in rodents also reduces social interaction, used to measure social withdrawal

(Seillier and Giuffrida 2017) to investigate behaviour comparable to the negative symptoms of schizophrenia (Jenkins et al. 2008). Moreover, rodent scPCP models induce persistent cognitive deficits that are of relevance to schizophrenia (Jones et al. 2011; Cadinu et al. 2018).

It has been argued that subchronic NMDAR antagonist protocols are the best of the pharmacological models of schizophrenia (Gururajan et al. 2010) as they have good predictive validity (Costall and Naylor 1995). In most part, this is due to their causing schizophrenia-like behaviour in healthy people and exacerbating symptoms of those with schizophrenia (Erard et al. 1980; Balster and Chait 1976; Jones et al. 2011; Javitt 2015). The scPCP rat model for schizophrenia is a widely used and well-validated model that produces schizophrenia-like behavioural and cognitive abnormalities (Neill et al. 2010; Neill et al. 2014; Doostdar et al. 2019). Female animals were used in this study as they exhibit a more robust performance on the novel object recognition (NOR) task compared to males and there is no effect of the oestrus cycle on their performance (Sutcliffe et al. 2007). In addition to this, the national institute of health (NIH) have called for 'balance' in preclinical work, i.e. an end to animal research employing solely male animals (Clayton and Collins 2014). There is evidence that female rats, but not males, have persistent down-regulation of brain BDNF mRNA (Snigdha et al. 2011). This is of relevance because BDNF-mediated neurogenesis is one of the proposed mechanisms by which exercise ameliorates cognitive deficits in schizophrenia (Pajonk et al. 2010).

1.4.2 Phencyclidine and schizophrenia-like brain changes

NMDAR antagonists can induce changes in the brain that are comparable to those seen in schizophrenia and this has been reported in both humans and animals (Neill et al. 2014; Steeds et al. 2015; Doostdar et al. 2019). Chronic users of PCP or ketamine demonstrate various changes in the frontal lobe similar to those in people with schizophrenia (Abdul-Monim et al. 2007; Jenkins et al. 2008; McKibben et al. 2010). For example, D_1 dopamine receptors are upregulated, which is associated with dopaminergic hypofunction, and both blood flow and grey matter volume are reduced in the frontal cortex (Narendran et al. 2005; Liao et al. 2011; Stone et al. 2014; Steeds et al. 2015). The negative symptoms of schizophrenia are thought to involve frontal lobe dysfunction (Wolkin et al. 1992) and the upregulation of dopamine receptors is associated with cognitive impairments (Narendran et al. 2005). Subchronic PCP treatment reduces global brain volume and produces schizophrenia-like structural abnormalities such as ventricular enlargement, decreased hippocampal volume, and cortical thinning (Barnes et al. 2015). PCP also elicits hyperresponsiveness to both amphetamine administration and mild stress in the mesolimbic dopamine pathways, something reported in schizophrenia (Jones et al. 2011; Domino and Luby 2012). Furthermore, first- or second-generation antipsychotics attenuate this PCP-induced sensitisation (Steeds et al. 2015; Phillips et al. 2001).

Glutamatergic pathways play a critical role in synaptic plasticity (Steeds et al. 2015) and the system been heavily implicated in the cognitive deficits in schizophrenia (Stephan et al. 2006; Schwartz et al. 2012). There is decreased glutamate release in

the frontal and temporal lobes in schizophrenia implicating the neurotransmitter in its pathology (Sherman et al. 1991). The NMDA subtype of glutamate receptor is involved in the molecular mechanism of synaptic adaptation (Pérez-Otaño and Ehlers 2005), with functions such as regulating the migration of neurons (Komuro and Rakic 1993), strengthening synapses via long-term potentiation (LTP) (Harris et al. 1984), and dendritic spine development (Yasumatsu et al. 2008). Therefore, NMDARs participate in multiple cellular pathways implicated in the disease (Ahmed and Bhat 2014). Subchronic treatment with the NMDAR antagonist PCP also produces these structural and functional abnormalities comparable to those seen in schizophrenia (Ellison and Sweet 1993; Reynolds et al. 2004; Hajszan et al. 2006; Abdul-Monim et al. 2007; Jenkins et al. 2008; McKibben et al. 2010; Pratt et al. 2008; Dawson et al. 2014). NMDAR antagonists disrupt LTP (Maren 1999), causing abnormalities in synaptic plasticity (Deakin and Simpson 1997). PCP also reduces prefrontal dendritic spine density (Hajszan et al. 2006) and causes the degeneration of neurons in several brain areas (Ellison and Sweet 1993). These changes occur in a way in-keeping with schizophrenia (Keshavan et al. 2008).

In schizophrenia, there is evidence of disrupted y-aminobutyric acid (GABA) interneuron migration (Akbarian et al. 1993), neuronal atrophy (Sweet et al. 2004), and reduced dendritic spines (Sweet et al. 2009). Deficits in parvalbumin (PV) containing GABAergic interneurons occur in schizophrenia and these disruptions in the brain are well documented (Beasley and Reynolds 1997; Danos et al. 1998; Zhang and Reynolds 2002; Nakazawa et al. 2012). Similar changes have also been observed in NMDAR antagonist rodent models (Egerton et al. 2005). For example, repeated treatment with the NMDAR antagonist ketamine produces a reduction in

PV and glutamate acid carboxylase 67 (GAD_{67}) expression in a subpopulation of cortical inhibitory interneurons that are similarly affected in schizophrenia (Volk et al. 2000; Behrens et al. 2007). GABAergic deficits have been identified in several areas in schizophrenia (Beasley et al. 2002; Zhang and Reynolds 2002; Neill et al. 2010), including the PFC (Cochran et al. 2003), hippocampus (Keilhoff et al. 2004; Behrens et al. 2007), and thalamus (Cochran et al. 2003; Jones et al. 2011). GAD_{67} is a GABA-synthesising protein and its decreased expression in the PFC is one of the most robust findings in the brains of people with schizophrenia (Guidotti et al. 2000; Volk et al. 2000; Hashimoto et al. 2003). The mRNA expression of GAD_{67}, GAD_{65} and the pre-synaptic transporter GABA transporter 1 (GAT_1) is diminished after chronic intermittent PCP (Bullock et al. 2009). Once again, these changes corresponded with those observed in the brains of people with schizophrenia (Bullock et al. 2009; Jones et al. 2011).

1.4.3 Measuring cognitive deficits of relevance to schizophrenia

The National Institute of Mental Health Mental Health's Measurement and Treatment Research to Improve Cognition in Schizophrenia (NIHM-MATRICS) conference identified seven domains of cognition affected in people with schizophrenia. There are working memory; attention/vigilance; verbal learning and memory; visual learning and memory; reasoning and problem solving; speed of processing; and social cognition (Green et al. 2004). The PCP animal model for schizophrenia has been shown to reliably produce deficits in tasks corresponding at least five of these (Neill et al. 2010; Jones et al. 2011). Verbal learning and memory are the most profoundly altered of any of the cognitive domains affected in

schizophrenia (Hagan and Jones 2005). Unfortunately, it is not possible to reproduce these uniquely human attributes in an animal model (Powell and Miyakawa 2006). Areas of cognition that have been modelled and are of relevance to schizophrenia include working memory (via the delayed alternation task; Jones et al. 2011), reasoning and problem solving (using tasks such as attentional set-shifting; Idris et al. 2010); and visual learning and memory (using the NOR test; Grayson et al. 2015).

Visual learning and memory is a cognitive domain that is adversely affected by schizophrenia (Keefe and Harvey 2012). A large meta-analysis showed that recognition memory, and in particular visual recognition memory, is significantly impaired in the disease (d = 0.76, Pelletier et al., 2005; Lyon et al. 2012). Deficits in recognition memory were linked to both general and negative symptom severity and disease duration (Pelletier et al. 2005), making visual recognition memory a domain of interest in clinical and preclinical research (Lyon et al. 2012). The NOR behavioural task is widely used as a test of recognition memory in rodents (Grayson et al. 2007). The NOR task (also known as spontaneous object recognition; SOR) is ethologically valid and uses a rat's natural preference for novelty (Ennaceur and Delacour 1988), a preference also seen in humans (Young et al. 2012) and non-human primates (Nemanic et al. 2004). A version of NOR involving preferential looking at novel stimuli is often used to assess visual recognition memory in infants (Overman et al. 1993). The rodent NOR task does not require reward and therefore food restriction is also unnecessary (Grayson et al. 2014). Eliminating this regulated procedure (Home Office [UK] 1986) reduces suffering and improves animal welfare (Tannenbaum and Bennett 2015). Therefore, the NOR task is less stressful than

paradigms that require positive or negative reinforcement (Dere et al. 2007). Animals can perform this test without training meaning it can be done quickly and reasonably easily (Puma et al. 1998). It has been argued that the lack of training required and the low levels of arousal or stress make NOR a better representation of how human recognition memory is measured (Ennaceur and Delacour 1988; Dere et al. 2007). Additionally, the MATRICS Treatment Units for Research and Neurocognition in Schizophrenia (TURNS) subcommittee has endorsed the NOR task as a tool to measure cognitive deficits of relevance in schizophrenia (Young et al. 2006).

The NOR task consists of three stages: acquisition (also known as the sample phase); the intertrial interval (ITI); and retention (also referred to as the choice phase; please see 2.5.2 for a full description of the NOR task, including habituation). The task is used to detect changes in non-spatial memory and intertrial intervals can be adjusted to measure short- versus long-term recall (Grayson et al. 2007; Young et al. 2012). With ITI's of five minutes or less, lesion studies in rodents and non-human primates have shown that the perirhinal cortex and the prefrontal cortex (PFC) are crucial for novel object recognition memory (Hammond et al. 2004; Grayson et al. 2007; Snigdha et al. 2011; Grayson et al. 2014; Young and Geyer 2015; Cadinu et al. 2018). The hippocampus is also implicated in recognition memory although there is evidence that hippocampal-dependent impairments may only be seen with ITI's of three or more hours. A possible explanation for this is that the hippocampus is involved in all object recognition but parahippocampal structures can support short-term recollection (Hammond et al. 2004). The hippocampus is involved in normal memory formation, processing, recognition and

temporal encoding of experience (Antunes and Biala 2012). The hippocampal and parahippocampal structures are heavily implicated in the neuropathology of schizophrenia, with abnormalities in these areas being among the most robust findings from neuroimaging studies (Haijma et al. 2013; van Erp et al. 2016; van Erp et al. 2018; Doostdar et al. 2019). This common neuroarchitecture is further support for the NOR paradigm as a test of relevance for the cognitive deficits seen in schizophrenia (Rajagopal et al. 2010; Redrobe et al. 2010).

1.5 Aims and objectives

Schizophrenia is a disabling mental illness, characterised with persistent and pervasive cognitive deficits. It is a heterogeneous disorder associated with various genetic and environmental risk factors and onset in adolescence or early adulthood. Of the symptoms of schizophrenia, the cognitive deficits are the most detrimental to functioning and impairments are present in several domains, such as verbal and visual memory and attention. There are no drug therapies to improve cognition in schizophrenia and non-drug interventions, such as CRT, are only moderately effective. People with schizophrenia tend to have many comorbidities that contribute to a shortened life span and these include a wide range of cardiometabolic disorders. Exercise is effective at ameliorating cognitive deficits observed in schizophrenia and improving fitness in this population reduces the impact of these associated comorbidities. Despite an understanding of how exercise impacts the brain in other populations, it is unclear how exercise exerts its pro-cognitive effects in schizophrenia.

This thesis aimed to investigate if the cognitive benefits of exercise could be observed in an animals model of relevance to schizophrenia and how this was reflected in the brain. To achieve this, the objectives were as follows:-

- To establish and validate an scPCP exercise paradigm in rats;
- Investigate the effects of voluntary wheel running on cognition in the NOR (visual recognition) test in the scPCP rat model for schizophrenia;
- Ascertain the duration wheel running was able to ameliorate the NOR deficit after exercise cessation;
- Investigate changes in biological markers of neural and synaptic health in active and sedentary scPCP animals and controls.

Chapter 2 General Methods

2.1 Subjects

All subjects were female Lister-hooded rats (Charles River, 250g ±50g at the beginning of the experiment). Female animals were used in this study as they exhibit a more robust performance on the NOR task compared to males, and there is no effect of the oestrus cycle on performance (Sutcliffe et al. 2007). Moreover, analyses of a large number of behavioural datasets revealed that male rodents showed a greater variation in performance than females (Prendergast et al. 2014; Leger et al. 2016). In addition to this, the National Institute of Health (NIH) has called for 'balance' in preclinical work, i.e. an end to animal research employing solely male animals (Clayton and Collins 2014). Regarding activity levels, there is a well-replicated and clear effect of the four-day oestrous cycle on wheel running, with the highest levels on the night of proestrus (Anantharaman-Barr and Decombaz 1989; Kent et al. 1991; Lightfoot et al. 2004; Giles et al. 2010). All experimental groups comprised exclusively of intact female rats of the same breed from the same supplier. Therefore, the effects of the oestrus cycle on activity levels were controlled for during experimental comparisons. Concern about the stages of the oestrous cycle where wheel running is lower is not warranted because, despite fluctuations, female rodents have been shown to run an average of 20% further 38% faster than their male counterparts (Lightfoot et al. 2004).

Rats were housed in groups of five in a temperature (21 ±2°C) and humidity (55 ±5%) controlled environment. Cages were individually ventilated and had two levels

with solid plastic floors (see figure 2.1; Techniplast Group, UK). Rats were housed with environmental enrichment of paper sizzle nest (Datesand Group, UK) and cardboard tunnels (Datesand Group, UK) as standard. Nutritionally complete food (Special Diet Services, Ltd., Essex, England) and purified water were available *ad libitum* in both home and wheel/control cages (see section 2.3 for details).

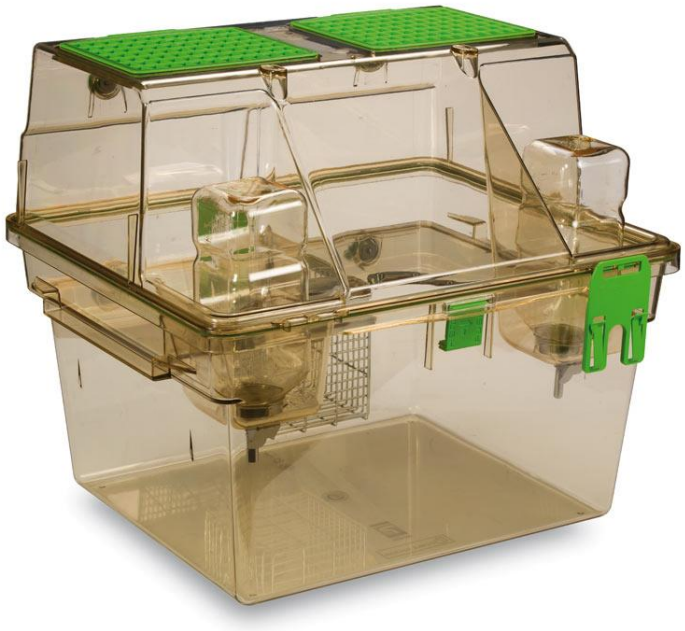


Figure 2.1 The Techniplast™ Double-Decker rat home cage (Techniplast Group, UK)

Animals were housed in a reverse light cycle (lights off at 08:30 and on at 20:30) as rats are nocturnal and therefore most active during their dark cycle. Pilot investigations that compared wheel running behaviour during rats' light and dark cycle confirmed animals ran the furthest and for the longest on wheels during the middle third of their dark cycle (data not shown). Therefore, a reversed light cycle was used in the room in which the rats were housed and exercised. This reversal was for the convenience, health, and safety of the researchers and animal facility

staff. Researchers and technicians used a red-light lamp or red-filtered headlamp if required. To avoid inadvertent light exposure, the door to this room was kept shut, and cages were covered with blankets when being transported or when in rooms with different ambient light patterns. All running and experiments took place in the middle third of the rats' dark cycle (12:30 to 16:30). The order in which rats were transferred to their experimental cages changed each day. Similarly, rats underwent behavioural testing in a rotating order to control for any effects that diurnal rhythm may have. Experiments were designed and conducted to be humane and scientifically rigorous, applying 'the 3Rs' (Prescott and Lidster 2017) and observing Festing and Altman's (2002) guidelines. All animal procedures were carried out in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986 (project number 40/9146, protocol 19b) and were approved by the University of Manchester AWERB (Animal Welfare and Ethical Review Body).

2.2 Subchronic PCP dosing regime

Vehicle (0.9% saline; n = 20) and phencyclidine hydrochloride (PCP; 2mg/kg; Sigma-Aldrich, UK; n = 20) were administered by intraperitoneal (i.p.) injection at a volume of 1mL/kg. This was done once in the morning and once in the afternoon each day for seven days. After seven days of PCP treatment, a seven-day washout period was observed (Figure 2.2). This was to ensure that animals were drug-free and that their behaviour was not influenced by the psychomimetic effects of systemic PCP (such as motor stimulation) or withdrawal from the drug (Jentsch and Roth 1999; Snigdha et al. 2011).

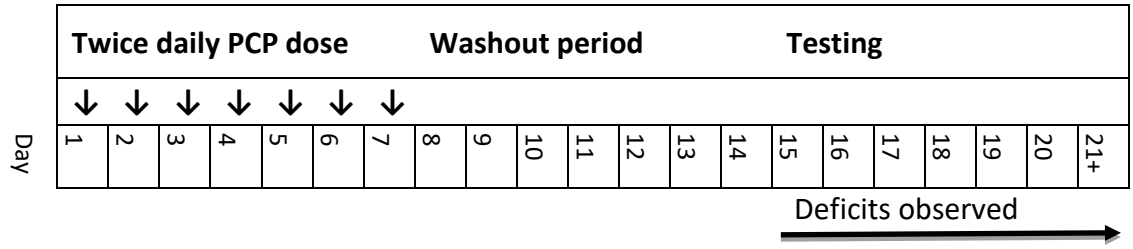


Figure 2.2 Diagram of the subchronic PCP dosing schedule

2.3 Wheel habituation and group randomisation

Animals were pseudo-randomised into four groups of 10 (see Figure 2.3): 10 were treated with vehicle and were in the control condition; 10 were treated with vehicle and were in the exercise condition; 10 were treated with PCP and were in the control condition; ten were treated with PCP and were in the exercise condition. Rats were individually habituated to the 30cm running wheels over three consecutive days. The habituation took place for one hour in the middle third of their dark cycle (12:30 to 16:30).

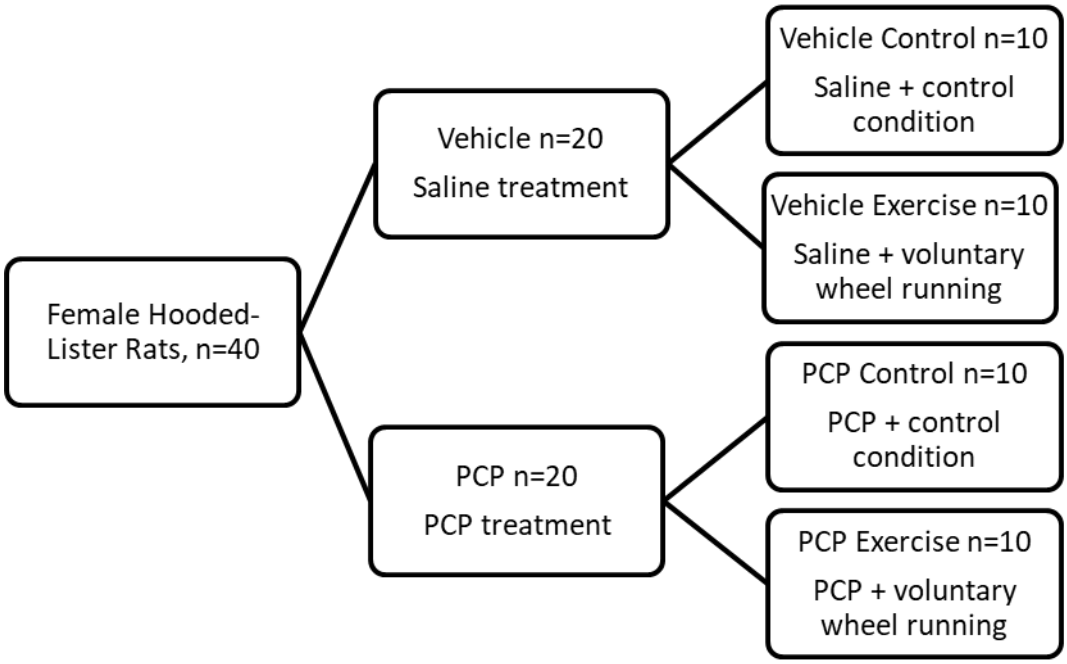


Figure 2.3 Diagram showing treatment, group N numbers and experimental conditions. Subchronic treatment with phencyclidine is abbreviated to PCP.

Rat's total distance and time were measured over the three sessions by a cycle odometer. Means were calculated for each animal and the mean distance run was used to classify each rat as a 'high' or 'low' runner. Rats that had a mean distance run of 50 metres or more were arbitrarily categorised as high runners. Animals with a mean run distance of 50 metres or less were classified low runners, including those who did not run at all. If there were a majority of high runners in one cage (i.e. three or more in a cage of five), that cage would be classified as a 'high' running cage. Correspondingly, if the majority were classified as low runners, the cage would be classified as a 'low' running cage. These categories were then used to ensure that there was one high and one low running cage in each condition (vehicle control; vehicle exercise; scPCP control; and scPCP exercise, see Figure 2.3) to control for the influence of running preference. Two cohorts of rats were habituated to the wheels and used in two experiments (described in detail in Table

2.1). For both studies, a one-way ANOVA was run to compare the mean distances the rats' ran during habituation in metres between the four groups. For the first cohort of rats habituated, there was a significant effect between groups, $F(_{1,39}) = 2.90$, $p < 0.05$. Post-hoc Bonferroni adjusted t-tests revealed a difference in mean distance run between the vehicle control and vehicle exercise groups (*MD* = -11.50, *SE* = 0.04), $p < 0.05$. This difference, while statistically significant, was not deemed problematic as it would not influence the experimental hypothesis. That is, there was no expectation of a cognitive deficit in the vehicle animals, and no hypothesised difference in NOR task performance before and after exercise or control condition. For the second cohort, there were no significant differences in the mean running distance in metres between groups, $F(_{1,39}) = 0.197$, $p = 0.90$). Please see appendix, section 8.1 for habituation data.

Table 2.1 A summary of the four experimental groups/conditions.

Group/Condition Name	**Description of treatment and activity**
Vehicle control group	Group treated with vehicle (saline) according to the procedure outlined in Figure 2.2.
	Group put in individual cages for 1 hour a day, 5 days a week, during the 6-week intervention phase with either: an upturned plastic tunnel (study 1, Figure 2.4); or a locked wheel (study 2, Figure 2.5).
Vehicle exercise group	Group treated with vehicle (saline) according to the procedure outlined in Figure 2.2.
	Group given access to a running wheel for 1 hour a day, 5 days a week, during the intervention phase (Figure 2.5).
scPCP control group	Group treated with PCP according to the procedure outlined in Figure 2.2.
	Group put in individual cages for 1 hour a day, 5 days a week, during the 6-week intervention phase with either: an upturned plastic tunnel (study 1, Figure 2.4); or a locked wheel (study 2, Figure 2.5).

Group/Condition Name	Description of treatment and activity
scPCP exercise group	Group treated with PCP according to the procedure outlined in Figure 2.2.
	Group given access to a running wheel for 1 hour a day, 5 days a week, during the 6-week intervention phase (Figure 2.5).

Subchronic phencyclidine is abbreviated to scPCP.

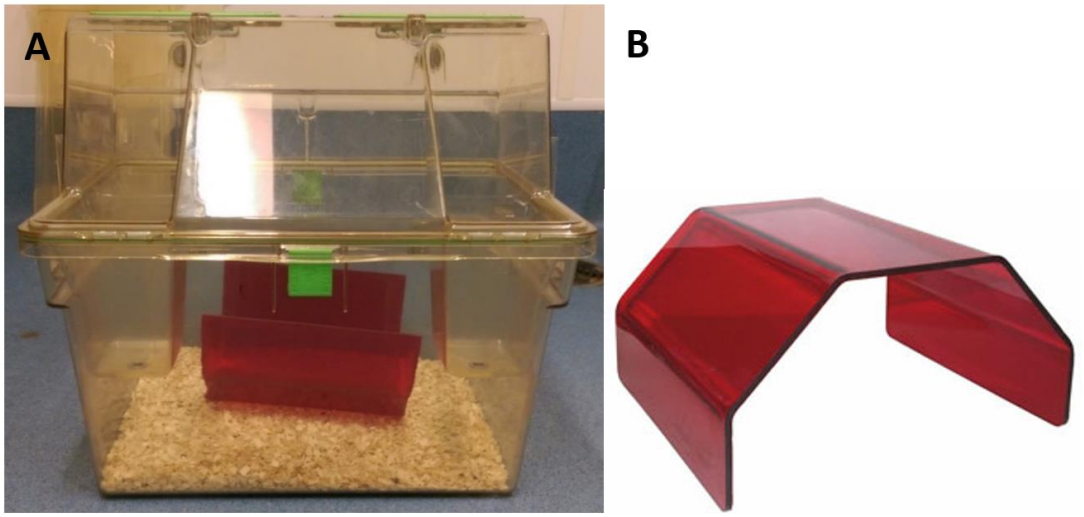


Figure 2.4 Control condition cage set-up with an upturned red plastic tunnel (A) and the Fat Rat Hut (Bio-Serve, USA) plastic tunnel (B).

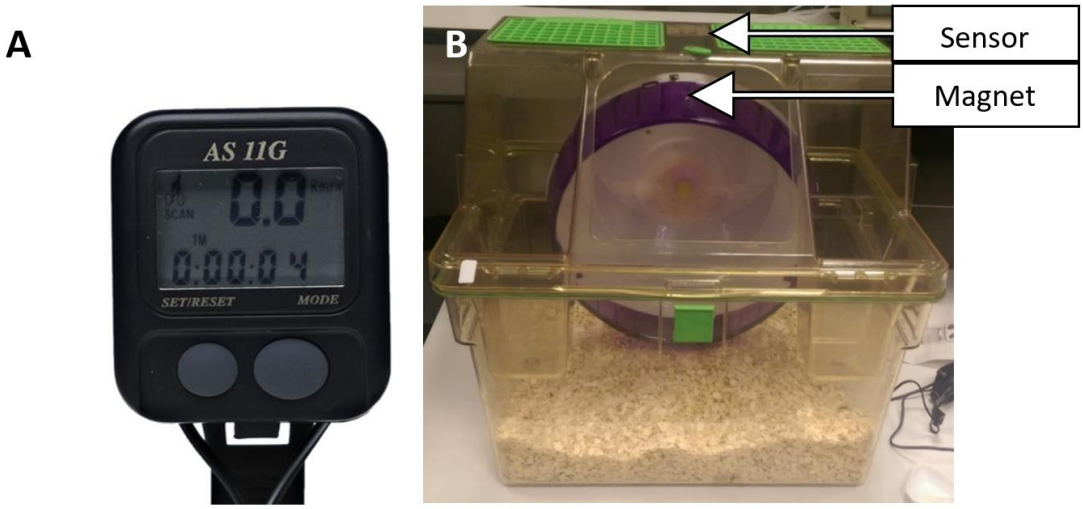


Figure 2.5 Wheel running cage set-up. Odometer (cycle computer) used to measure the distance and time rats spend running on wheels (A) (Wilko, UK). When the magnet mounted on the wheel passed the sensor, the odometer registered one revolution of the wheel. It used the user-set wheel diameter to calculate distance. Wheel cage set-up showing a 30cm diameter running wheel attached to a stable base (below sawdust; B). The wheel was allowed to move freely in both directions in the exercise condition. The wheel was locked in place for the control condition.

2.4 Exercise regime and control condition

To date, there is no published work investigating the effects of exercise in a rat model for schizophrenia. Therefore, it was necessary to create and validate an exercise protocol for use in the scPCP model. To do this, a literature search was

conducted to understand the methodologies employed in exercise studies using rodents. During this search, several important considerations were identified. These were: diurnal activity levels; exercise duration (both for individual training sessions and the length of the exercise protocol); and exercise paradigm (forced exercise or voluntary exercise).

Rats are nocturnal animals and are therefore most active during their dark cycle. Pilot investigations that compared wheel running behaviour during rats' light and dark phases confirmed animals ran the furthest and for the longest on wheels during the middle third of their dark cycle (data not shown). Therefore, a reversed light cycle was used in the room in which the rats were housed and exercised. Wheel running and behavioural experiments were also conducted during the middle third of their dark cycle (see section 2.1, also).

The literature search identified that the length of individual sessions and the number of days or weeks that animals exercise varied markedly between studies. Some animals were housed individually and had continuous access to a wheel, which was particularly common for mouse studies (van Praag et l. 1999a; van Praag et al. 1999b; Kim et al. 2014), and others limited access to wheels to specific periods. Unlike mice, rats are highly social animals and therefore prefer to live in groups. Rats were removed from their home cages and housed in individual cages for as brief a period as possible to minimise their distress. The distance and total time (in minutes) that the animals ran during a one-hour and a four-hour wheel running session were compared using paired sample t-tests. No significant differences were found between the two, indicating rats did the majority of their

running in the first hour of access to wheels (data not shown). This informed the decision to remove rats from their home cage and give them access to wheels (or the control condition) for one hour per day. In regards to the length of the exercise protocol, studies' protocols varied from seven days to eight months (van Praag 1999a; van Praag et al. 1999b; Burghardt et al. 2004; Zheng et al. 2011; Kim et al. 2014). In research using rats, the length of the exercise period was usually between four and twelve weeks, with six or eight weeks being the most common. Therefore, it was decided that rats would undergo six weeks of exercise or the control condition. Interestingly, a subsequent study conducted in the same lab with the exercise protocol described in this thesis tested rats with the NOR task after three weeks of access to running wheels (personal communication). This revealed that scPCP exercise rats continued to demonstrate a cognitive impairment at this time point, suggesting that a longer period of wheel access is required for improvement in cognition

There are two broad categories of exercise employed in animal research, namely forced and voluntary cardiovascular exercise. Forced exercise usually employs treadmill running at a set speed for an allocated time. To ensure the animal runs on the treadmill, there is a footshock pad at the rear of the belt that shocks the animal whenever it touches it. Alternatively, forced exercise can also be achieved by placing animals into motorised wheels with a predetermined resistance and for a specified period (Griesbach et al. 2012; Diederich et al. 2017). Voluntary exercise in rodents is almost exclusively voluntary wheel running (Ke at al. 2011; Griesbach et al. 2012; Diederich et al. 2017). There is good evidence that forced and voluntary exercise has differential effects on the brain and cannot be used interchangeably

(Leasure et al. 2008). For example, forced running has been shown to cause many changes associated with chronic stress, such as adrenal hypertrophy, decreased serum corticosteroid-binding globulin, and suppressed lymphocyte proliferation (Arida et al. 2004; Burghardt et al. 2004; Ploughman et al. 2005; Leasure and Jones 2008). While both forced and voluntary exercise paradigms have been shown to produce neurogenesis (Diederich et al. 2017), voluntary exercise may induce higher hippocampal BDNF (Ke at al. 2011; Griesbach et al. 2012), a protein of interest in this thesis. Moreover, voluntary exercise may result in lower levels of biological and behavioural stress in animals (Griesbach et al. 2012), making it of greater relevance to human exercise conditions. Consequently, voluntary exercise was chosen and the exercise protocol described here has been successfully implemented in several studies by members of the same research group.

Twenty rats (10 treated with vehicle and 10 with PCP, see Figure 2.3) were assigned to the control condition, and 20 were assigned to the exercise condition. Rats in the exercise condition were placed individually into cages with access to a 30cm diameter wheel for one hour a day, five days a week, for six weeks in total. The rats in the control condition were placed in an individual cage with an upturned plastic tunnel (study 1, Figure 2.4) or a wheel that was locked and could not rotate (study 2, Figure 2.5)[1]. This was done to control for possible adverse effects of being housed singularly and any consequences that having a stationary wheel (or upturned tunnel) in the cage might impose on the animals. The order that rats were put into the cages was rotated daily to control for the effects of time of day and any

[1]An upturned plastic tunnel was used as a control condition during study one because the researcher was unable to lock the wheels. During study two, the wheels were locked.

small differences in time spent in the exercise or control cages. An odometer was used to measure the distance and time run each day by each rat (see Figure 2.5).

2.5 Novel Object Recognition Task

The novel object recognition (NOR) task is a widely-used behavioural paradigm to evaluate cognition, specifically recognition memory, in rodents (Grayson et al. 2015). It is an ethologically valid test that utilises animals' instinctive exploratory behaviour and preference for novelty. Because of this, there is no need for external motivation or stress such as reward or punishment (Antunes and Biala 2012). Also, animals undergoing the test require little in the way of training or habituation (Silvers et al. 2007). For the experiments outlined in this thesis, the NOR task was used to measure the scPCP-induced cognitive deficit and to determine if exercise could reverse it.

2.5.1 Apparatus

2.5.1.1 Test environment

The NOR test took place in a four-sided, Plexiglas, open-top box. The walls were black, and the dimensions were 52cm length, 52cm width, and 31cm height. The white floor was divided into nine identical squares by four intersecting black lines that ran between parallel sides of the arena (see Figure 2.6). The box was positioned on a stationary square table approximately 30cm from the ground. There are five identical test boxes to allow one home cage (five rats) to be tested concurrently.

Figure 2.6 NOR test environment with example objects

2.5.1.2 Task objects

Task objects used in NOR tasks included a brown glass bottle with a plastic lid, filled with water (object B), and an unopened 330mL can of Coca Cola (object C). Objects were of approximately equal height (12cm±2cm) and diameter (6cm±1cm) and were heavy enough not to be displaced by the rats (see Figure 2.7).

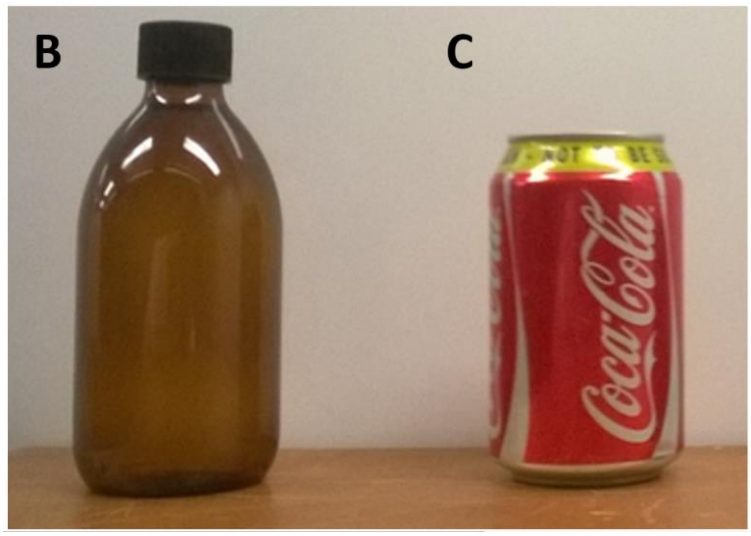


Figure 2.7 Example objects used in NOR. Object B (bottle), a brown glass bottle with plastic lid. Object C (can), red aluminium Coca-Cola can (right).

The combination of objects B and C were changed for each trial group to offset any possible object or location preference. Rats were tested by cage number and this sequence was reversed each day they were tested with the NOR task. Changing the testing order in this way reduced the potential effect of time of day on the rats' behaviour. There are four possible combinations of objects in the acquisition and retention stages of the NOR test. These are: BB, BC; BB, CB; CC, CB; CC, BC (Figure 2.8). Previous work in the same group has shown that animals have no natural preference between these objects (Grayson 2012; Watremez 2016).

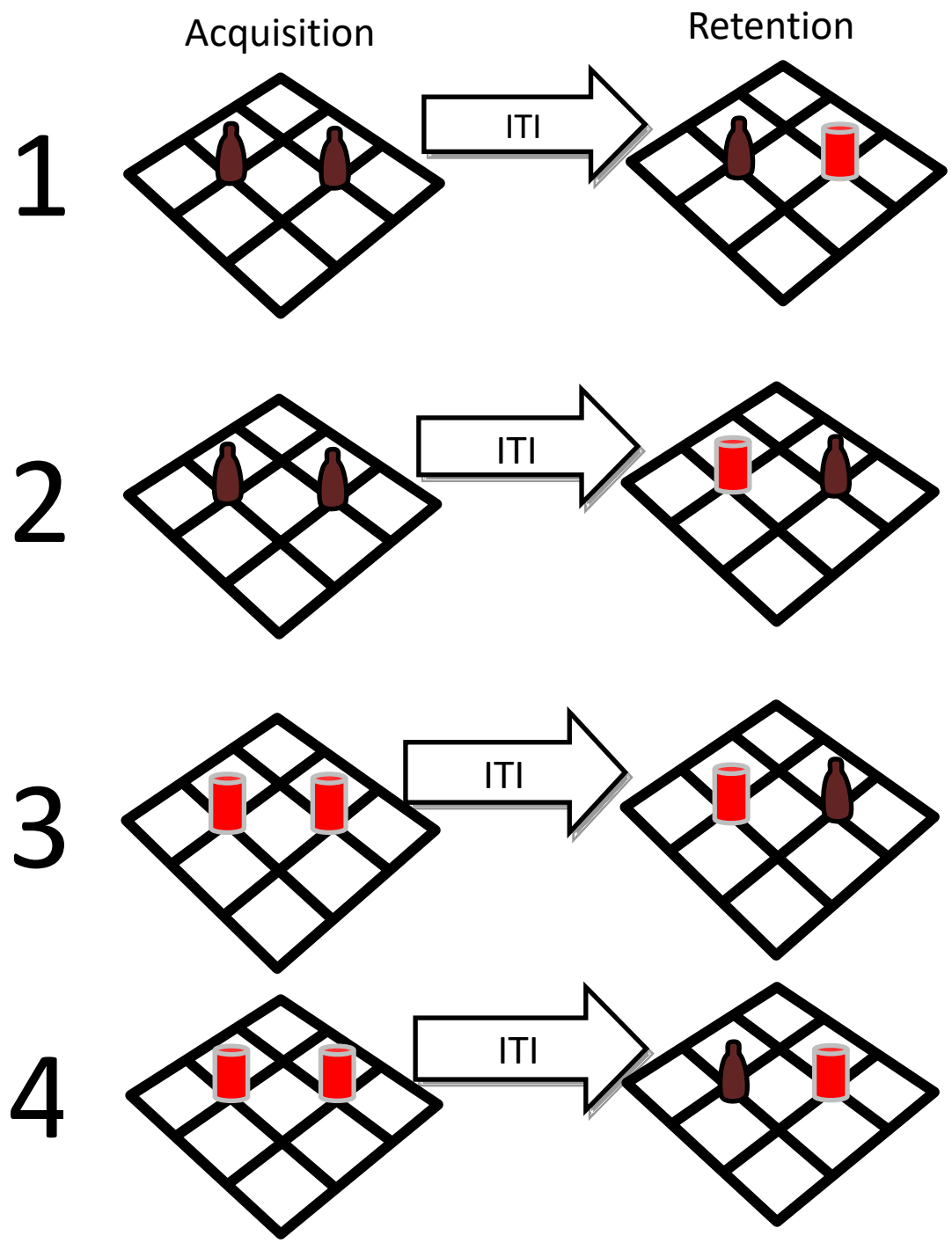


Figure 2.8 Possible combinations of object B (bottle) and object C (can). Inter-trial interval is abbreviated to ITI.

2.5.1.3 Recording equipment and set-up

A CCTV-style camera was placed above each NOR arena (described in 2.5.1.1) to record animal behaviour (Figure 2.9). A stopwatch with an alarm was used to

measure the inter-trial interval. Videos were downloaded from the hard disk of the digital video recorder onto a memory stick and encrypted.

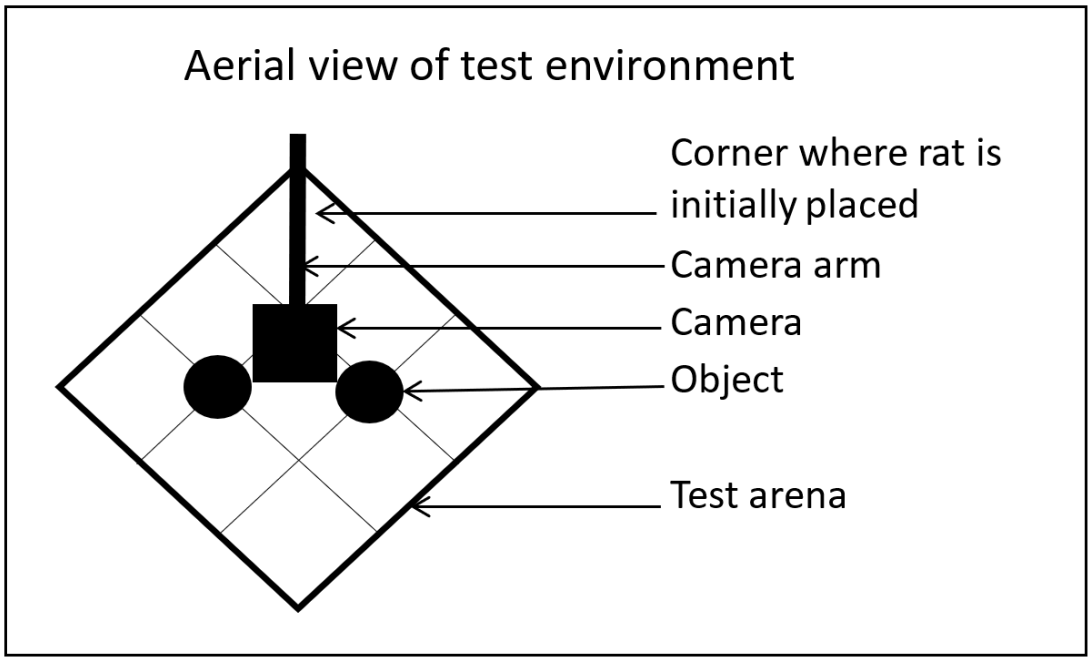


Figure 2.9 Camera set-up

2.5.2 Novel object recognition task protocol

Habituation took place the day before testing. During habituation, all rats from one home cage are allowed to explore the empty arena (described in section 2.5.1.1) for 20 minutes. All animals were tested in the middle third of their dark cycle (12:30 to 16:30) on the same day. They were tested in numerical order, by cage number, and this was reversed each time they repeated a test. The sequence was used to control for the effects of being evaluated at either extreme of the testing period. For example, cages might be tested in order from one to four during the baseline (time point one), and from four to one after the exercise intervention (time point two).

The NOR task was carried out in a quiet environment to minimise the distraction of the animals. The task had three phases: the acquisition phase; the inter-trial interval (ITI); and the retention phase. In the acquisition stage, the animal was

placed in the arena with two identical objects and allowed to explore freely. After three minutes of exploration, the animal was removed from the box for the inter-trial interval (ITI) of one minute. During the ITI, the arena was cleaned with either an animal-safe disinfectant (Anistel) or 70% ethanol. Disposable blue paper roll was used to clean and dry the arena. A novel object and a replica of the familiar object were placed in the arena for the retention phase. Rats were placed in the arena immediately after the ITI ended and could explore the objects and arena for three minutes. A copy of the familiar/seen object was used to avoid olfactory cues interfering with visual recognition. Between animals, both the boxes and objects were cleaned. Objects were used more than once for each animal in the study but only after several weeks when animals did not recollect them. To confirm this is the case, their combined exploration time of the objects should approximate the last trial in which they saw that object. Please see appendix section 8.2 for the NOR task standard operating protocol.

2.5.3 NOR task scoring and statistical analysis

2.5.3.1 NOR task scoring

All scoring of NOR was done from the video recording. The video was played through “H264 Player” software, which was provided with the recording device, and scored using a novel object recognition timer (Rivers-Auty 2015). A sample of videos was scored by at least one independent person who was blind to both treatment groups and object status (novel or familiar). The scores were compared

and a Cronbach's alpha of 0.8 or greater was considered highly-correlated and reliable.

The time that the rat is exploring an object is defined as:

- Sniffing or licking the object;
- Touching and looking at the object;
- Looking at the object from an approximate distance of < 2cm.

Behaviours that are not considered as exploration include:

- Touching the object without looking at it;
- Sitting on the object;
- Staring in the direction of the object from an approximate distance greater than 2cm.

An exploration time of less than one second for either object resulted in a rat's exclusion from the data. At the acquisition phase of the task, the (identical) objects are classified as 'left' and 'right'. For the retention phase of the task, the objects are known as 'novel' for the previously unseen object and 'familiar' for the seen object. Total exploration time is the combined time that a rat explored both objects at either stage of the NOR task, i.e. all object exploration for the acquisition stage and all for the retention stage. Total exploration time of the objects was used to identify any significant differences in general exploratory behaviour between the groups. This comparison safeguarded against group differences in the analyses being due to changes in overall exploratory behaviour. Total exploration time was used as a proxy for locomotor activity (LMA) in the absence of line crossing data..

2.5.3.2 Statistical analysis

Statistical analysis was conducted using SPSS Statistics 23 (IBM Corp. 2015) and graphs were created using GraphPad version 8 (GraphPad Software 2019). All data are presented as mean (*M*) ± standard error of the mean (*SEM*), or mean difference (*MD*) and standard error (*SE*), or mean *(M)* and standard deviation *(SD)*. The following data was being collected and compared:

- Exploration time for 'left' and 'right' objects during NOR acquisition, in seconds;
- Exploration time for 'novel' and 'familiar' objects during NOR retention, in seconds;
- Total exploration time in the acquisition stage of NOR;
- Total exploration time in the retention stage of the task;
- Discrimination indices (DI) for the retention stage of the NOR task, calculated:

$$DI = \frac{novel - familiar}{novel + familiar}$$

The DI score is a ratio and ranges from -1 to +1. The closer a rat's exploration was to -1, the more that rat had explored the familiar object when compared to the novel object. Conversely, the closer to +1 the rat's DI was, the more it favoured exploring the novel object. A score not significantly different to 0 indicates that the exploration of each of the objects was similar, which indicates no preference between them. For groups without a cognitive deficit a positive mean DI that significantly differs from 0 would be expected. For groups demonstrating a cognitive deficit the mean DI might be positive, negative, or 0,

but would not be expected to be significantly different to 0. It can also be beneficial to compare DI between groups to understand if there is a significant difference in their preferences. However, these results should be interpreted with some caution. DI is an indication of preference (or lack thereof) but cannot be used to quantify preference absolutely. The DI will be used to:

- Compare each group to 0;
- Compare DI between groups.

2.5.3.3 Statistical analysis of object exploration time

A two-way ANOVA was performed, using object (left versus right, novel versus familiar) as the within-subject factor and the treatment group as the between-subject factor. If the ANOVA returned a *p*-value of < 0.05, paired-sample student's t-tests with a Bonferroni correction were run to compare the object exploration times in each group. For total exploration time, a one-way ANOVA was run between groups for each phase of the NOR task. A significant interaction would be investigated using post hoc Bonferroni-adjusted t-tests.

2.5.3.4 Statistical analysis of DI

The DI of each group were compared to 0 using a univariate t-test. A one-way ANOVA with post hoc Bonferroni tests was run to examine the pairwise comparison of DI between groups.

2.6 Transcardial perfusion and brain tissue collection

The chemicals used were isoflurane (Abbott), isopentane (Fisher Scientific), paraformaldehyde (PFA; Fisher Scientific), potassium chloride (KCl; Fisher

Scientific), potassium dihydrogen orthophosphate (KH_2PO_4; Sigma-Aldrich), sodium chloride (NaCl; Fisher Scientific), sodium hydroxide (NaOH; Sigma-Aldrich), sodium phosphate dibasic (Na_2HPO_4; Sigma-Aldrich), sucrose (Sigma-Aldrich).

Perfusion or perfusion and fixation of the brain is required before performing neurochemical and neuroanatomical analyses of tissue. Each brain that was taken was split into hemispheres. One hemisphere was placed in a brain box and frozen on dry ice, the other half of the brain was fixed in paraformaldehyde (PFA) but analysis was not possible. The procedure was performed on anaesthetised rats that were humanely killed without regaining consciousness.

2.6.1.1 Solutions

The solutions described here are approximately the volume required for 10 rats. 1g of NaOH was dissolved in 250mL distilled H_2O to make 250mL NaOH solution (0.1M). 80g NaCl, 2g KCl, 14.4g Na_2HPO_4, and 2.4g KH_2PO_4 were dissolved in 800mL distilled H_2O. Distilled water was added to bring the volume to 1L of phosphate-buffered saline (PBS) Ca^{2+}/Mg^{2+} free 10X. NaOH was added to bring the pH to 7.4. This can be stored above 4°C for no more than one month. 200mL PBS 10X was diluted into 1.8L distilled H_2O to create 2L of phosphate-buffered saline (PBS) Ca^{2+}/Mg^{2+} free 1X. This was stored above 4°C.

2.6.1.2 Protocol

2.6.1.3 Perfusion surgery

Surgical instruments were cleaned with 70% ethanol and the rat was placed in an anaesthetic induction chamber. The O_2 flow was set to 2L/min and isoflurane to 5%

until the rat lost consciousness. The rat was then removed from the chamber and laid it on its back on the downflow table. The rat's nose and mouth were in a nose cone connected to the anaesthetic rig to maintain 5% isoflurane. The rat's hind leg was pinched to ensure that there is no withdrawal reflex and to confirm that it was under deep anaesthesia. An incision was made below the xiphoid cartilage and scissors were used to cut the skin and muscles from the incision toward both the left and right forepaw. Care was taken not to damage any of the structures below the skin. The diaphragm was cut from left to right along the length of the ribcage to expose the pleural cavity. The scissors were placed along one side of the ribs and used to move the lungs gently to one side. A cut was made in the rib cage to the collarbone and repeated on the contralateral side. The sternum was lifted and any tissue that connected it to the heart was carefully trimmed. Haemostatic forceps were used to clamp the sternum and were rested above the animal's head to expose the heart

(see

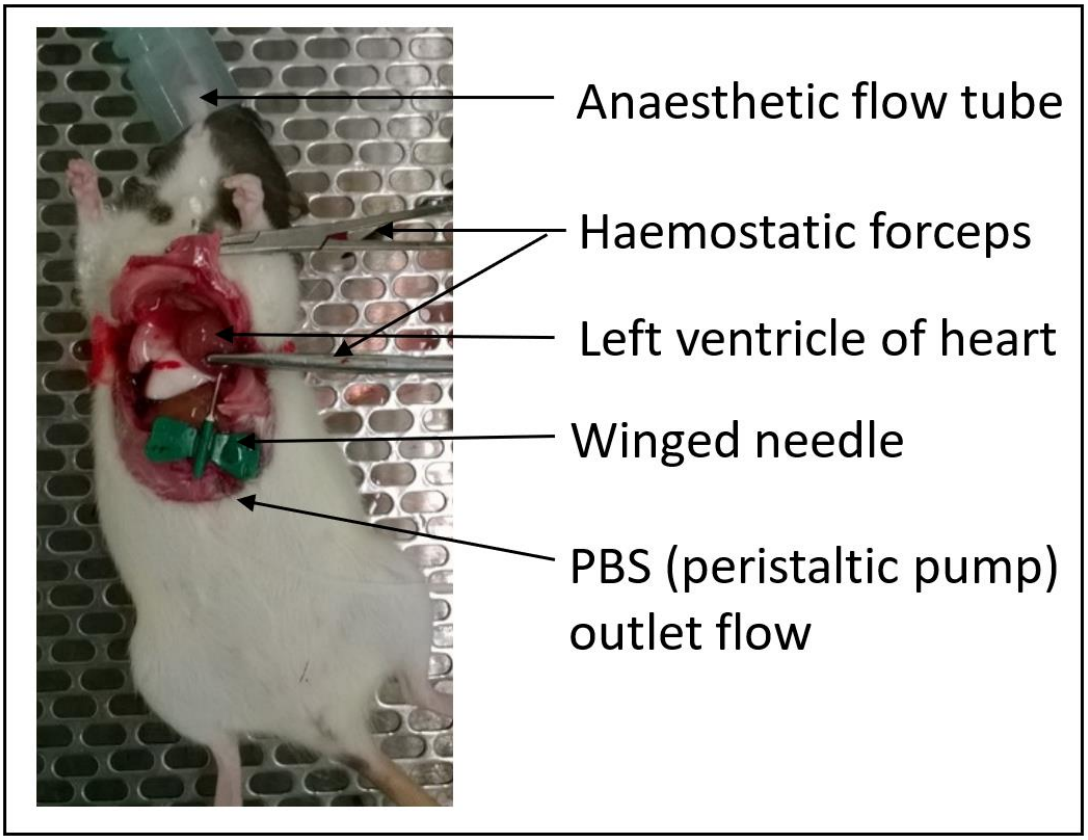


Figure 2.10). An incision was made into the right atrium and a winged needle was inserted into the left ventricle. This was secured with haemostatic forceps (Figure 2.10) while the heart continued to beat.

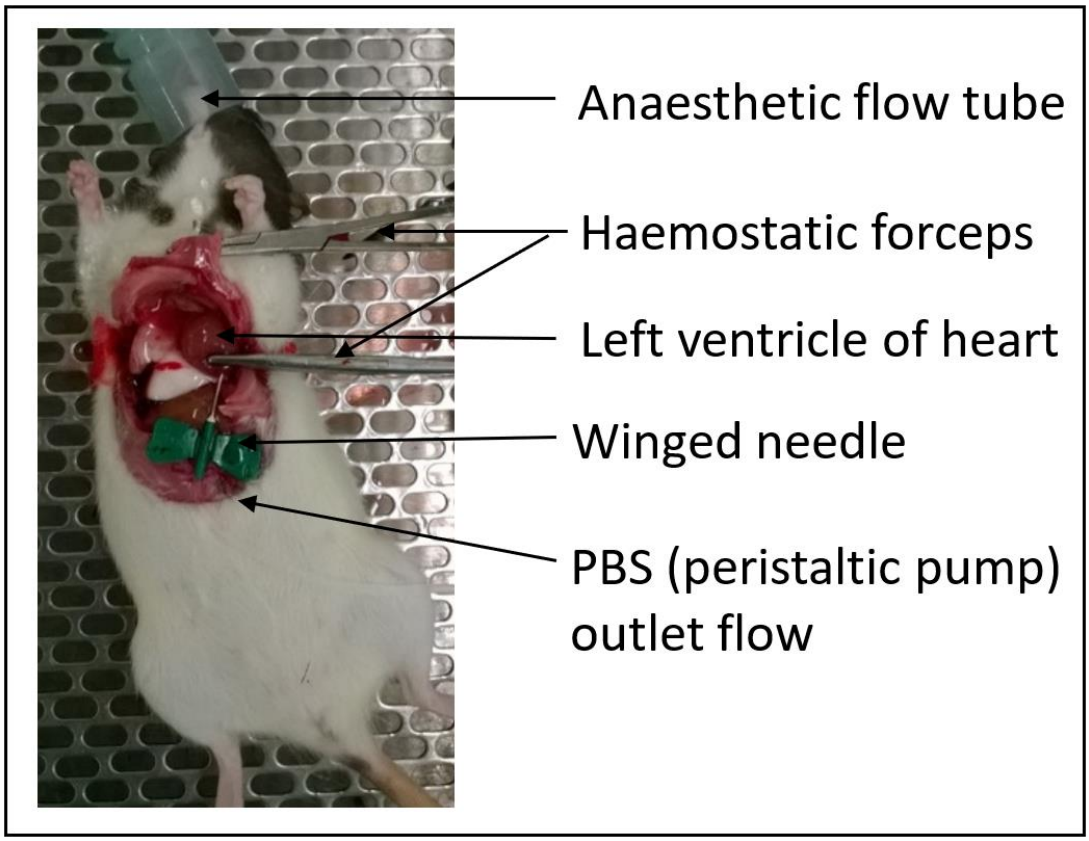


Figure 2.10 Perfusion of an anaesthetised rat.

2.6.1.4 Perfusion and brain collection

The outlet line of the peristaltic pump was attached to the winged needle and the equipment was checked to ensure there were no bubbles in the system. The peristaltic pump was set to 20mL/min (6rpm) and run for eight minutes, or until the blood that ran from the right atrium was clear. The isoflurane flow was stopped and the nose cone removed. Death was confirmed and the head was removed carefully to avoid damage to the skull.

The bone between the eyeballs was broken and scissors were used to break the bone above the foramen magnum. The skull was cut and rongeur forceps were used to carefully remove the skull until the brain was free. Either Iris or curved forceps were used to gently sever the tissue connecting the brain to the skull.

2.6.1.5 Brain processing

The olfactory bulbs and cerebellum were removed from the brain and a scalpel was carefully used to separate the hemispheres in a way that caused minimal damage to the tissue. One hemisphere was placed into a brain box and placed on dry ice and was transferred to a -80°C freezer and the other was submerged in PFA. The treatment (i.e. freezing or submerging in PFA) of hemispheres (right and left) were alternated with each rat. It was not possible to analyse the PFA fixed hemispheres.

2.7 Dissection of frozen brains

2.7.1 Dissection of frozen half brains protocol

The bench area, dissection tray and surgical instruments (forceps, scalpel, etc.) were cleaned with 70% ethanol and microcentrifuge tubes were labelled with the animal identifier and brain region. Brain hemispheres were removed from -80°C storage and kept on dry ice until dissected. A plastic box was filled with a mixture of dry ice and ice and the base of the petri dish was covered with tinfoil and placed upside down onto the mixture of ice and dry ice. The brain hemisphere was placed onto the tinfoil-covered petri dish (single hemispheres were used, as explained in 2.6.1.4). The temperature of the brain tissue was gradually allowed to rise. If the brain was too cold and still completely frozen, it was not possible to slice through it with a razor blade. If the brain became too defrosted the tissue lost its shape and the regions of the brain were not identifiable.

Once the brain had defrosted sufficiently the olfactory bulb was removed. Forceps were used to gently hold the hemisphere in place to prevent the tissue from defrosting with the warmth of fingers. The first area of interest was the frontal

cortex (see Figure 2.11 and Table 2.2 for coordinates in relation to bregma for each brain area of interest). The frontal cortex was removed and transferred into a labelled microcentrifuge tube then immediately placed on dry ice. The prefrontal cortex was removed and transferred to a microcentrifuge tube and placed on dry ice. A hemisphere slice that contained the dorsal hippocampus was cut and curved forceps were used to peel it away from the rest of the tissue. It was then placed in a microcentrifuge tube on dry ice. The steps described to remove the dorsal hippocampus were replicated for the ventral hippocampus. The samples were stored in a -80°C freezer and any unwanted brain tissue was disposed of in accordance with local procedures. The apparatus, tools and bench area were cleaned with 70% ethanol.

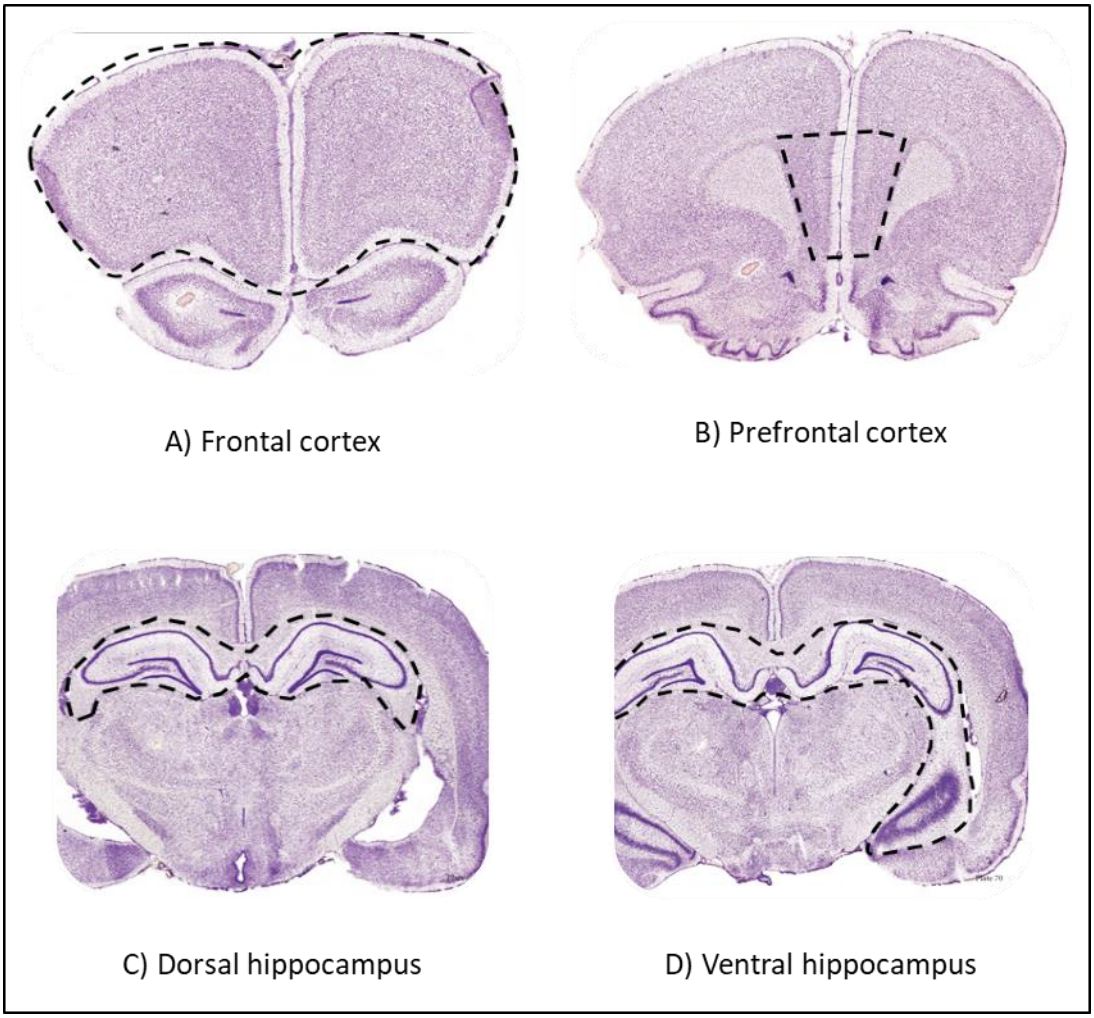


Figure 2.11 Histologically stained areas of interest in whole adult rat brain (A plate 7; B plate 10; C plate 66; D plate 70; Paxinos & Watson, 2006).

Table 2.2 Stereotaxic coordinates of areas of interest (information from Paxinos & Watson, 2006).

Name	**Bregma (mm)**
Frontal cortex (FC)	+4.7 to +3.2
Prefrontal cortex (PFC)	+3 to +1.7
Dorsal hippocampus (DH)	-3.3 to -4.8
Ventral hippocampus (VH)	-4.8 to -6.3

2.8 Homogenisation of brain tissue

2.8.1 Preparation of solutions

The chemicals used for homogenisation were Trizma (Sigma-Aldrich), sucrose (Sigma-Aldrich), ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich), protease inhibitor cocktail tablets cOmplete (Roche), PBS Ca^{2+}/Mg^{2+} free 10X (Sigma-

Aldrich), phenylmethylsulfonyl fluoride (PMSF; Sigma-Aldrich), sodium orthovanadate (Sigma-Aldrich), ethanol absolute (Fisher Chemical). 1mL of PBS Ca^{2+}/Mg^{2+} free 10X was diluted in 9mL of distilled H_2O to produce a volume of PBS Ca^{2+}/Mg^{2+} free 1X sufficient for 40 samples. 87.1mg of PMSF was combined with 5mL of ethanol to create 5mL of PMSF 0.1M stock solution. This is sufficient to homogenise up to 50 batches of up to 15 samples. 91.96mg of sodium orthovanadate was diluted in 5mL of distilled H_2O to produce 5mL of sodium orthovanadate 0.1M stock solution. This is sufficient for up to 50 batches of up to 15 samples. One cOmplete Protease Inhibitor tablet was dissolved in 2mL distilled H_20. If the tablet did not dissolve quickly it was vortexed or left at +15 to +25° for 30–60 minutes. The stock solution was divided into 400µL aliquots to create 2mL of 25X Stock solution of cOmplete™ protease inhibitor and this was sufficient for the homogenisation of up to 5 batches of 15 samples. 242.28mg Trizma base, 21.9mg sucrose, and 148.9mg EDTA were dissolved in 200mL distilled H_2O to produce 200mL of homogenisation buffer, which were then divided into 10mL aliquots. This is enough for 20 batches of 15 samples.

2.8.2 Homogenisation protocol

Between 10 and 15 samples were chosen and processed at any one time to limit the possibility of protein degradation. The homogenised samples were chosen at random and were centrifuged at 3,200rpm within approximately 30 minutes.

Immediately before homogenisation, 10mL of homogenisation buffer was activated by adding 400µL of the prepared 25X Stock solution of cOmplete protease inhibitor, 100µL of PMSF 0.1M stock solution, and 100µL of sodium orthovanadate 0.1M

stock solution. This was done on ice. 400µL of homogenisation buffer was added to each sample and a micro pestle was used to homogenise the tissue. Homogenisation continued until there were no visible pieces of tissue. The sample was left on ice for 1 minute and if any tissue had settled, the micro pestle was used to homogenise the sample again. Micro pestles were reused and were cleaned in ethanol, rinsed in ultrapure water, then dried. The homogenised samples were centrifuged for 15 minutes at 3,200rpm at 4°C. The supernatant (S1) was transferred into a clean, labelled 1.5mL microcentrifuge tube without transferring any of the pellet (P1) and centrifuged for 20 minutes at 12,200rpm at 4°C.

The supernatant (S2) was transferred into a clean and labelled microcentrifuge tube without transferring any of the pellet (P2) and was frozen at -20°C. The pellet (P2) was resuspended with 250µL of the prepared PBS Ca^{2+}/Mg^{2+} free 1X solution, vortexed to mix then frozen at -20°C.

2.9 Bradford assay (modified)

2.9.1 Rationale

A Bradford assay is a colourimetric assay used to determine protein concentration in a solution. This is done by measuring the absorbance of dye in a spectrophotometer at 595nm. The dye reagent used becomes blue when binding with protein and proportionally increases its absorbance to 595nm. A sample's absorbance is compared to a standard curve created from known concentrations. Comparison with this standard curve allows the calculation of a sample's protein concentration.

2.9.2 Preparation of solutions

The substances used for the modified Bradford assay were bovine serum albumin standard (BSA; Bio Rad), protein assay dye reagent concentrate (Bio Rad). Samples were diluted as appropriate for the amount of tissue and the methodology being used. The BSA standards were diluted in the same buffer as the samples (i.e. Ca^{2+}/Mg^{2+} free 1X solution). The standard range was made up as described in Table 2.3.

Table 2.3 Concentrations and volumes of BSA standards for use in the modified Bradford assay.

Standard	Concentration BSA (mg/mL)	Volume BSA	Volume buffer (µL)	Final volume (µL)
F	1	150µL of 2X	150	300
E	0.75	75 µL of F	25	100
D	0.5	50 µL of F	50	100
C	0.2	20 µL of F	80	100
B	0.1	10 µL of F	90	100
A	0	0	100	100

BSA, bovine serum albumin mg (milligrams), mL (millilitres) and µL (microlitres).

2.9.3 Modified Bradford assay protocol

Samples were diluted depending on the brain region. This was because the dissected tissue varied in size and therefore the homogenate varied in protein concentration. All samples were diluted in the same buffer used during homogenisation. The dye reagent concentrate was reduced to 1X solution by diluting it to 1 in 5 using the same buffer as in the samples. Around 600µL of dye reagent 1X was needed per sample (in triplicate). Therefore, for a full 96 well plate 5mL of dye reagent was diluted in 20mL of ultrapure H_2O. 10µL of BSA standard (standards A-F; see Table 2.3) was pipetted in triplicate into separate wells and 10µL of the samples were pipetted in triplicate into the remaining wells. 200µL of

dye reagent 1X was added to each well and the plate was incubated at room temperature (RT) for 5 minutes while being gently homogenised on a shaker. The layout of the plate was documented to allow later identification of samples and an example of a 96 plate layout with standards (please see appendix, section 8.5). The plate was read at 595nm using a plate reader and associated software. The standard curve was used to determine the concentration of each sample employing a linear scale.

2.10 'Wes' automated western blot

The western blot is a widely used analytical technique in molecular biology (Mahmood and Yang 2012). Proteins are separated by their molecular weight, allowing the separation and identification of specific proteins within a cell homogenate. During traditional western blots, separation of proteins by molecular weight and/or other characteristics is achieved by gel electrophoresis. Once proteins are separated, the protein of interest is labelled producing semi-quantitative results. The general procedure for western blotting is relatively simple but protocols can vary considerably and it is notoriously difficult to produce good results. The 'Wes' system (ProteinSimple, UK) uses capillary electrophoresis to separate proteins, and chemiluminescence to label and detect the presence of the protein(s) of interest. Little sample is required (≥ 0.2µg/µl, 3-5µl per well) and results are available in around three hours. The user loads samples and immune reagents onto a plate that is pre-filled with the necessary separation components. Once the plate is inserted into the 'Wes', the process is automatic and produces quantitative results (ProteinSimple 2014).

2.10.1 Preparation of solutions

The substances used for the 'Wes' automated western blot assay were wash buffer (ProteinSimple), 10X sample buffer (ProteinSimple), fluorescent 5X master mix (ProteinSimple), dithiothreitol (DTT; ProteinSimple), streptavidin-HRP (ProteinSimple), antibody diluent 2 (ProteinSimple), luminol-S (ProteinSimple), peroxide (ProteinSimple), biotinylated ladder (ProteinSimple), rabbit secondary antibody (ProteinSimple), mouse secondary antibody (ProteinSimple), PSD_{95} primary antibody (Abcam), $SNAP_{25}$ primary antibody (Abcam), parvalbumin primary antibody (LSBio), GAD_{67} primary antibody (Sigma-Aldrich), and GAPDH primary antibody(LSBio). The volumes below are sufficient to run one 'Wes' plate with a maximum of 24 samples. If a solution formed bubbles it was centrifuged at around 4000rpm for approximately one minute and gently triturated.

44µL of sample buffer 10X was added to 396µL of distilled water, vortexed and stored at room temperature. 200µL of luminol-S and 200µL of peroxide were combined, triturated, and stored at 2-8°C**.** 40µL of distilled water was added to the DTT tube in a ProteinSimple 'EZ Standard Pack' and the solution was gently triturated. 20µL of 10X sample buffer and 20µL of 400mM DTT solution was added to the fluorescence 5X master mix tube in a ProteinSimple 'EZ Standard Pack' and the solution was gently triturated. 20µL of distilled water was added to the biotinylated ladder in a ProteinSimple 'EZ Standard Pack' and the solution was gently triturated.

Primary antibodies for the protein of interest and the housekeeping (control) protein were appropriately diluted with ProteinSimple® antibody diluent two.

These dilutions varied depending on the protein of interest and the tissue used but ranged from 1:50 to 1:200. Mouse or rabbit secondary antibody, or a mixture of both, was used as appropriate for the protein of interest and the housekeeping protein.

2.10.2 'Wes' automated western blot protocol

If appropriate, samples were diluted with 1X sample buffer to desired protein concentration to a total volume of 4µL. If samples did not require dilution, 4µL of sample was aliquoted into a 0.5mL microcentrifuge tube. 1µL of fluorescent 5X master mix was added to each 4µL prepared sample, bringing the total volume to 5µL. Samples were vortexed and denatured in a 95°C water bath for 5 minutes and once denatured, samples were vortexed again and spun in a centrifuge for around 5000rpm for approximately one minute. The samples and reagents were pipetted into a 25 well plate, as shown in Figure 2.12. The wash buffer was not loaded onto the plate at this stage.

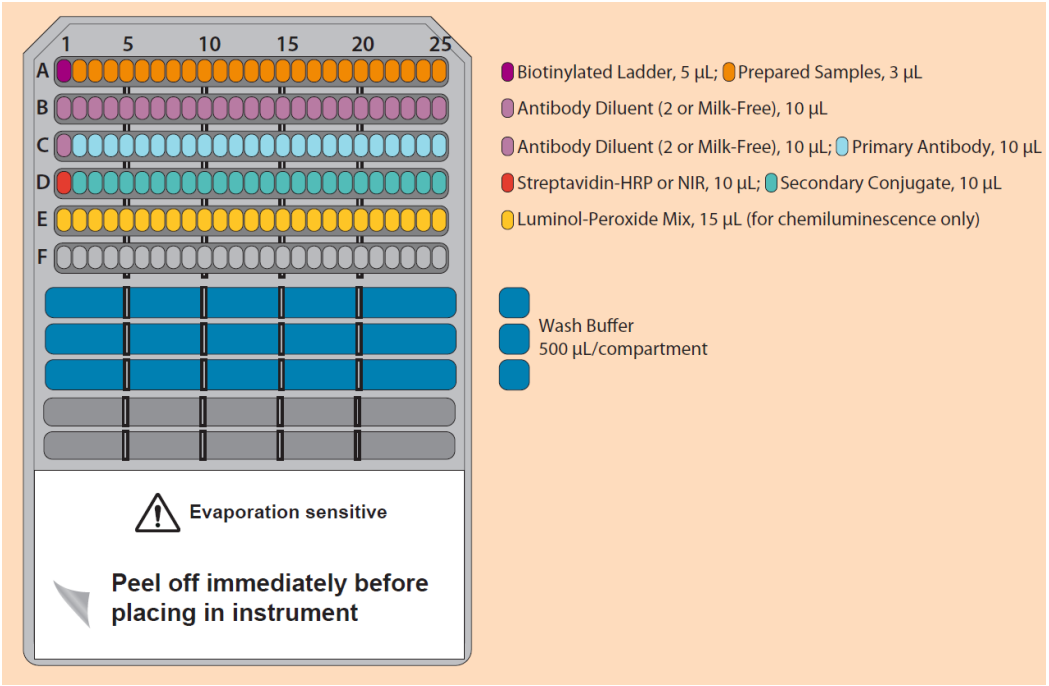


Figure 2.12 Colour-coded Instructions for dispensing reagents into the 'Wes' assay plate. Volume in µL (microliters) (ProteinSimple, 2019).

A plate cover was placed onto the assay plate and the plate was then centrifuged for 2500rpm for five minutes to ensure that the liquid was at the bottom of each well. Wash buffer was added to the compartments as shown in Figure 2.12. A capillary cartridge was loaded into the 'Wes' system and the foil removed from the plate just before putting it into the machine. The assay parameters were selected in the Compass for SW program (ProteinSimple 2018) and the assay run was begun.

2.10.3 'Wes' automated western blot statistical analysis

Data are taken from Compass for SW (ProteinSimple 2018) and statistical analysis was conducted using statistical package for social sciences (SPSS) 25 (IBM Corp. 2017). Graphs were created using GraphPad version 8.2.1 (GraphPad Software 2019). Where there was a discrepancy between the statistical analyses of SPSS and GraphPad Prism the SPSS analysis was used and the GraphPad graph would be altered accordingly. The following data were collected and compared:

- The area of the chemiluminescence peak for the protein of interest, for example, PSD95;
- The area of the chemiluminescence peak for the housekeeping protein GAPDH.

2.10.3.1 Statistical analysis

A one-way ANOVA was performed using the product of the area of the chemiluminescence peak protein of interest, divided by the area of the peak of GAPDH (the housekeeping protein) as the dependent variable. Treatment group was the factor. If the ANOVA reported a *p*-value of < 0.05, paired-sample t-tests with a Bonferroni correction were run to compare the significant interactions.

2.11 Experimental plan

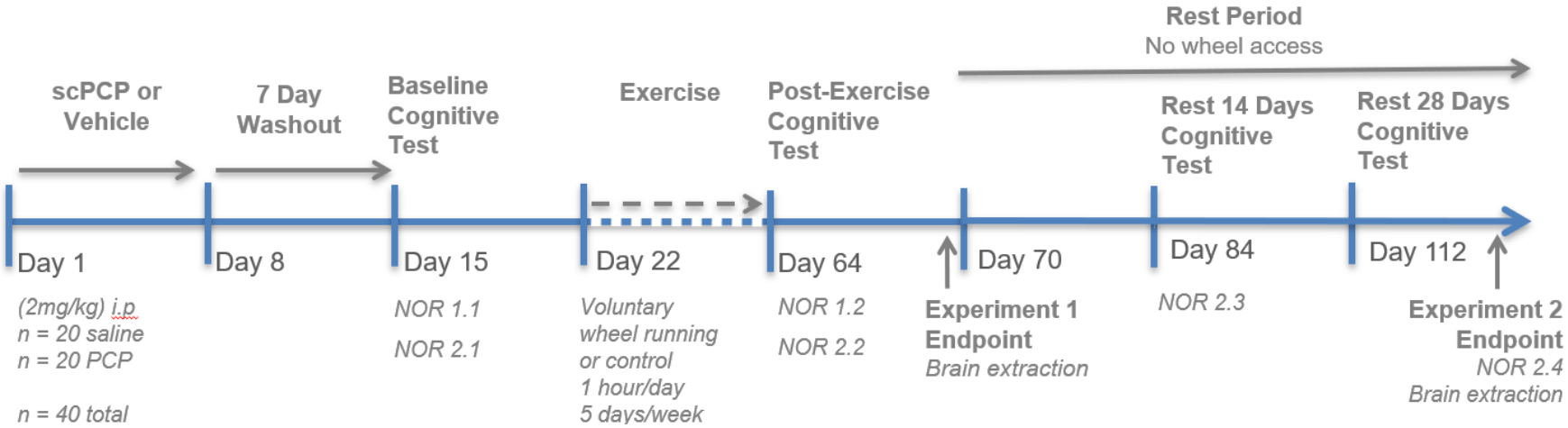


Figure 2.13 Experimental plans and timelines for study one and two. Vehicle is abbreviated to veh, subchronic PCP to scPCP, novel object recognition to NOR, and intraperitoneal to i.p.

2.11.1 Study one: investigating the effects of exercise on cognitive deficits in the subchronic phencyclidine rat model for schizophrenia.

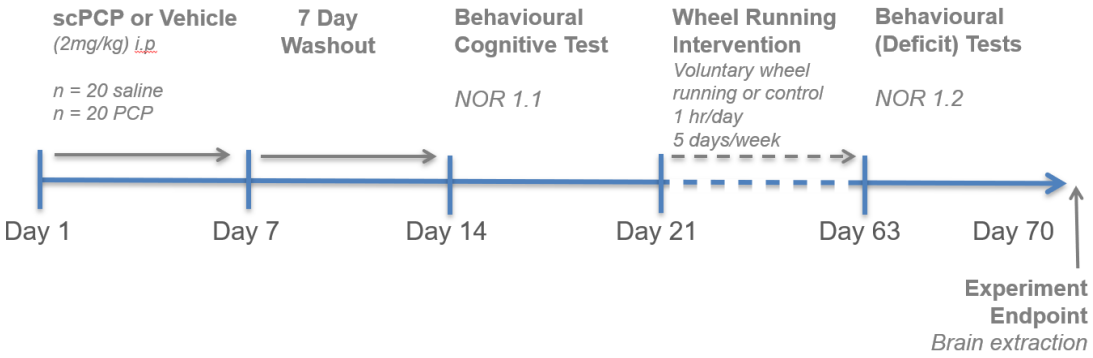


Figure 2.14 Study one experimental timeline. Vehicle is abbreviated to veh, subchronic phencyclidine to scPCP, novel object recognition to NOR, and intraperitoneal to i.p.

Forty rats were habituated in a wheel running cage for one hour per day for three days. They were then pseudo-randomised to one of four groups (described in 2.3). Ten were assigned to the vehicle control condition, 10 to the vehicle exercise condition, 10 to the scPCP control condition, and 10 were to the scPCP exercise condition. Twenty rats (the vehicle control and the vehicle exercise groups) were injected with vehicle, twice a day for seven days. Twenty rats (the scPCP control and scPCP exercise groups) were treated with PCP, twice a day for seven days. All rats were left undisturbed for one week, excluding the usual change of sawdust, food replenishment and health monitoring (such as weighing). This washout period was to ensure that behavioural testing was not affected by active PCP or by withdrawal effects (see Figure 2.2 for the dosing regimen).

After one week all rats were tested with the NOR task to get a baseline measure of cognition. The day after testing, 10 scPCP rats (the scPCP exercise group) and 10 vehicle rats (the vehicle exercise group) were placed in individual cages with

exercise wheels (Figure 2.5) for one hour a day, five days a week, for six weeks. Their total distance, total time running, max speed and average speed were measured using a cycle computer and documented after each exercise session (see Figure 2.5 for apparatus). Ten scPCP rats (the scPCP control group) and 10 vehicle rats (the vehicle control group) were placed in individual cages at the same time (see Figure 2.4 for apparatus). All rats were put in an exercise or control cage during the middle third of their dark cycle (between 12:30 and 16:30). After six weeks, the rats were tested with the NOR cognitive task (section 2.5). They were tested on the next day that they would have been placed in the exercise or control cages. After behavioural testing was complete, all rats were transcardially perfused, and their brains were taken for analysis (see section 2.6).

2.11.2 Study two: exercise and detraining: effects on the NOR cognitive deficit in the subchronic PCP rat model for schizophrenia.

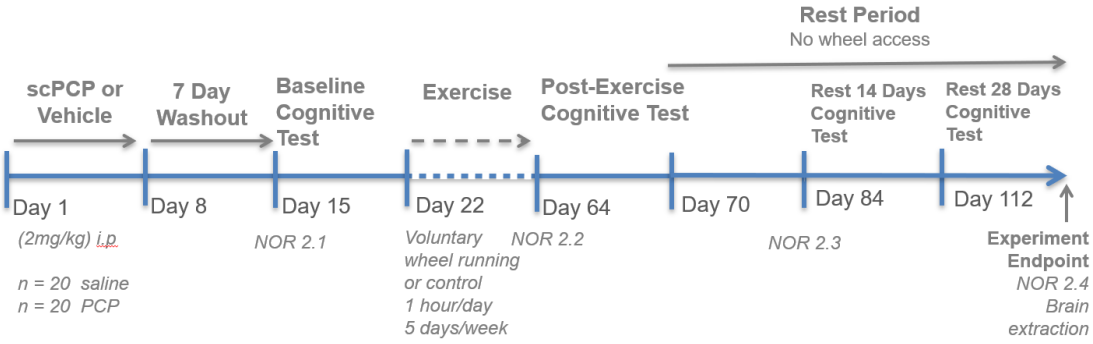


Figure 2.15 Study two experimental timeline. Subchronic phencyclidine is abbreviated to scPCP, phencyclidine to PCP, the novel object recognition task to NOR, and intraperitoneal to i.p.

For the second study, 40 rats were habituated in cages with a running wheels for one hour per day for three days. They were treated with the same protocol described in study one, which is outlined in Figure 2.15. On completion of the exercise intervention, rats were tested with the NOR task. The test took place on the next day that they would have been placed in the cages with wheels. Rats were then returned to their home cages and were not disturbed for two weeks, excluding the usual change of sawdust, food replenishment and health monitoring. After two weeks, rats were tested again with the NOR cognitive test. Once again, rats were returned to their home cages and left undisturbed for two weeks, after which they completed a final NOR test. After behavioural testing was complete, all rats were culled, and their brains were taken for analysis (as above, see section 2.6).

Chapter 3 Investigating the effects of exercise on cognitive deficits in the subchronic phencyclidine rat model for schizophrenia

3.1 Introduction

Schizophrenia is a serious mental illness that is a leading contributor to worldwide disability (van Os and Kapur 2009). Cognitive deficits in schizophrenia are a core component of the disease (Green 2006; Geyer et al. 2012; Cadinu et al. 2018) and are present before the onset of psychotic symptoms (Woodberry et al. 2008). Cognitive impairments best predict both functional outcome (Green 1996) and quality of life (Fujii et al. 2004), yet remain mostly untreated (Young and Geyer 2015). Consequently, treatments to improve cognition in schizophrenia have been declared an unmet clinical need and a research priority (Neill et al. 2010; Jones et al. 2011; Keefe and Harvey 2012). Exercise has become a focus for psychiatric research and there is evidence that it improves symptoms in schizophrenia (Beebe et al. 2005; Alexandratos et al. 2012), including the cognitive deficits (Vancampfort et al. 2014; Nuechterlein et al. 2016; Firth et al. 2017). However, the precise mechanisms by which exercise exerts its effects are unknown (Scheewe et al. 2013). Animal models can help further the understanding of disease (van der Staay 2006; van der Staay et al. 2009) and allow us to examine the behavioural and biological effects of exercise. The subchronic phencyclidine (scPCP) rat model for schizophrenia has been used to investigate schizophrenia-like cognitive deficits and associated brain abnormalities (Neill et al. 2010; Neill et al. 2014; Doostdar et al.

2019). In rodents, pertinent cognitive disturbances can be tested using behavioural protocols, allowing researchers to investigate novel treatments for the cognitive impairments of schizophrenia.

Animal models of disease should mimic the human pathology and aetiology as closely as possible. In mental health research, bridging the 'translational gap' between preclinical and clinical work has been challenging (Hyman and Fenton 2003; Hagan and Jones 2005). In particular, modelling schizophrenia is difficult because it is a complex, heterogeneous and uniquely human disorder (Neill et al. 2010). The cognitive deficits in schizophrenia are a central aspect of the disease yet there is no treatment to improve them. Therefore, there is neither a positive control to compare novel treatments against (Floresco et al. 2005), nor a pharmacology mechanism to investigate and improve upon (Young and Geyer 2015). With so many unknowns and a 'translational bottleneck' (Hyman and Fenton 2003), there is an increasing focus on cross-species validity and clinically relevant outcomes. Visual learning and memory is impaired in schizophrenia (Nuechterlein et al. 2004) and is one of seven core cognitive domains disrupted in the disease (Green et al. 2004). Studies in patients with schizophrenia have reported that exercise can improve visual learning and memory (Oertel-Knöchel et al. 2014; Malchow et al. 2015). Therefore, studying the effect of exercise on visual recognition memory in the scPCP model for schizophrenia is pertinent and clinically relevant.

The novel object recognition (NOR) behavioural task can be used to measure visual recognition memory in rodents and has been identified as a relevant test for cognitive deficits in schizophrenia (Young et al. 2006; Lyon et al. 2012). In this study, rats were treated with vehicle or scPCP and given access to either running wheels or a control condition. They were tested with the NOR task before and after they had access to wheels (or control) to investigate whether exercise rescued the visual recognition impairment in the scPCP model for schizophrenia. After the post-exercise NOR cycle (NOR 1.2) the animals were culled and their brains were taken for protein analysis as described in section 2.6.

3.2 Methods

All animals underwent NOR at baseline and after six weeks of access to wheels or the control condition, full details of which can be found in sections 2.4 and 2.5. The detailed methods of this study may be found in Chapter 2.

3.3 Results

3.3.1 Time point one: baseline NOR task (NOR 1.1)

A novel object recognition test was run at baseline (time point one; NOR 1.1) at the end of the one week washout period. The test aimed to confirm that an scPCP deficit was present in the animals before wheel running/control began. There were four experimental groups: vehicle control; vehicle exercise; scPCP control; and scPCP exercise. Rats were pseudo-randomly assigned to their experimental groups

as described in section 2.3. Statistical analysis was performed as described in section 2.5.3.2.

Acquisition stage (NOR 1.1)

A two-way ANOVA revealed no significant effect of object (left and right) on exploration time at the acquisition stage of the NOR task, $F_{(1,36)} = 1.36$, $p = 0.25$. There was no significant interaction between object and treatment group, $F_{(3,36)} = 0.05$, $p = 0.98$ (Figure 3.1).

Retention (NOR 1.1)

A two-way ANOVA showed a significant effect of object (novel and familiar) on exploration time in the retention phase of the NOR, $F_{(1,36)} = 160.26$, $p < 0.001$. Bonferroni-corrected paired t-tests established that there were significant differences in exploration time between the novel and familiar object in all four groups. In all of the groups, rats explored the novel object more than the familiar object: vehicle control (*MD* = 18.57, *SE* = 1.27), $p < 0.001$; vehicle exercise (*MD* = 19.65, *SE* = 1.27), $p < 0.001$; scPCP control (*MD* = 11.38, *SE* = 1.27), $p < 0.001$; and scPCP exercise (*MD* = 10.53, *SE* = 1.27), $p < 0.001$ (Figure 3.1).

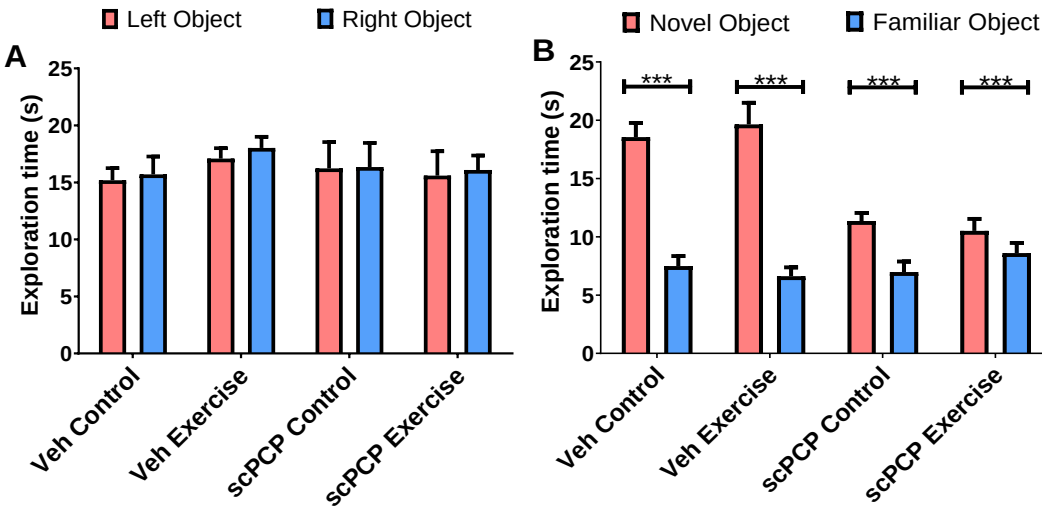


*Figure 3.1 Exploration time in seconds for the left and right objects during the acquisition stage (A), and the novel and familiar objects during the retention phase (B) of NOR 1.1. Data are presented as mean ± SEM (n = 10 per group), ***p < 0.001, paired-sample t-test with Bonferroni correction. Treatment with vehicle is abbreviated to veh, subchronic phencyclidine to scPCP and seconds to s.*

There was also a significant interaction between treatment group and exploration time, $F_{(3,36)} = 19.52$, $p < 0.001$. A one-way ANOVA with post hoc Bonferroni-corrected t-tests was run to compare the novel object exploration and the familiar object exploration between the groups. There was no effect of group for exploration time of the familiar object, $F_{(3,36)} = 1.03$, $p = 0.40$. However, there was an effect of group for the novel object, $F_{(3,36)} = 14.01$, $p < 0.001$. Post hoc Bonferroni-corrected t-tests showed that the vehicle control and vehicle exercise groups did not significantly differ from one another when exploration time of the novel object was compared, (*MD* = -1.09, *SE* = 1.79), $p = 1.00$. Similarly, the scPCP control group did not differ from the scPCP exercise group, (*MD* = 0.85, *SE* = 1.79), $p = 1.00$. However, there were significant differences in novel object exploration time between both vehicle and scPCP groups. The vehicle control group had a significantly higher exploration time from the scPCP control (*MD* = 7.20, *SE* = 1.79),

$p < 0.01$, and the scPCP exercise group ($MD = 8.05$, $SE = 1.79$), $p < 0.001$. The vehicle exercise group also has a significantly higher novel object exploration time than the scPCP control group ($MD = 8.29$, $SE = 1.79$), $p < 0.001$, and the scPCP exercise group ($MD = 9.13$, $SE = 1.79$), $p < 0.001$. This further analysis revealed that despite there being a significant difference between the novel and familiar object exploration at NOR 1.1, both the vehicle groups had a significantly higher novel object exploration time when compared to either of scPCP groups. These differences indicate that, when they are compared to the vehicle groups, there is a reduction in novel object exploration in the scPCP groups. This effect was interpreted as evidence for a deficit in the scPCP control and scPCP exercise groups (Figure 3.2).

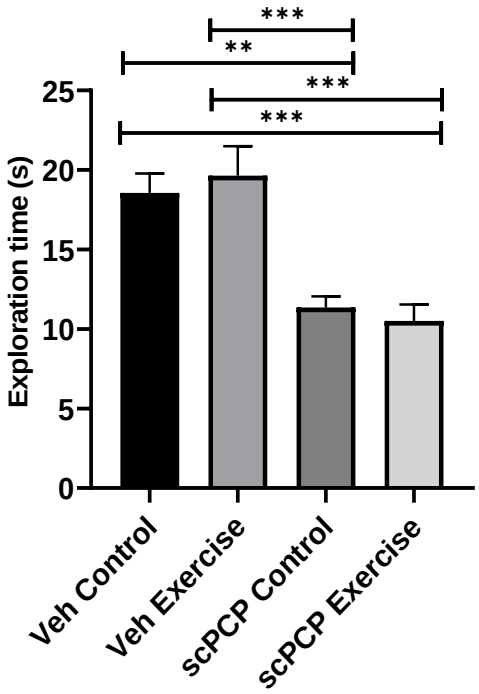


*Figure 3.2 Exploration time in seconds for the novel object during the retention stage of NOR 1.1. Data are presented as mean ± standard error of the mean (n = 10 per group), **p < 0.01, ***p < 0.001, paired-sample t-tests with Bonferroni correction. Treatment with vehicle is abbreviated to veh, subchronic phencyclidine to scPCP and seconds to s.*

Discrimination Indices (NOR 1.1)

One sample t-tests were run to determine if the groups DI were significantly different to zero, where zero indicates no preference between the novel and familiar object. All groups had a DI significantly higher than zero (Figure 3.3): vehicle control group (M = 0.43, SD = 0.15), $t(9) = 9.02$, $p < 0.001$; vehicle exercise group (M = 0.49, SD = 0.15), $t(9) = 10.10$, $p < 0.01$; SCPCP control group (M = 0.27, SD = 0.21), $t(9) = 4.07$, $p < 0.001$; SCPCP exercise group (M = 0.11, SD = 0.08), $t(9) = 4.10$, $p < 0.01$; Figure 3.3).

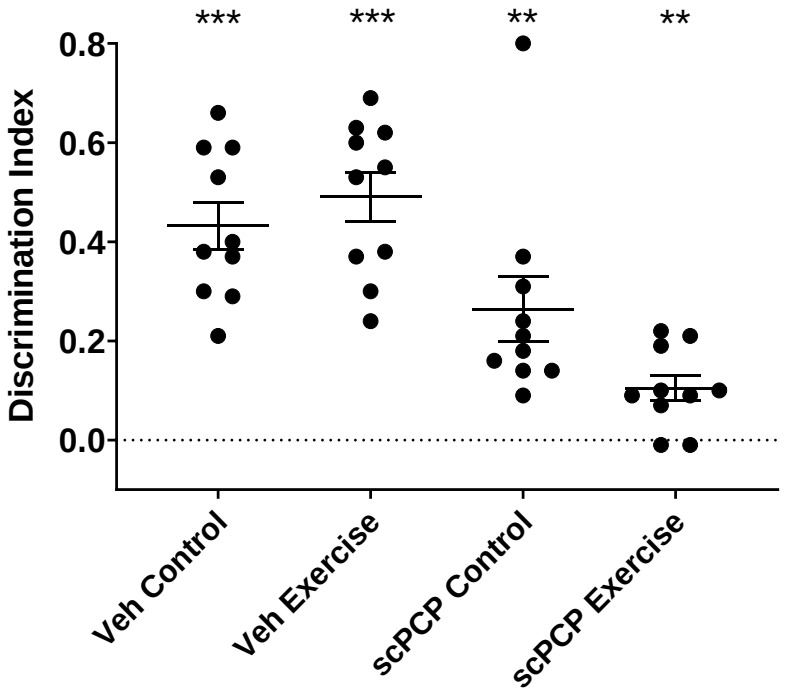


*Figure 3.3 Discrimination indices (DI) compared to 0 for the retention stage of the NOR task at baseline (NOR 1.1). Data are presented as mean ± SEM (n = 10 per group), ** p < 0.01, *** p< 0.001, independent sample t-tests. Vehicle is abbreviated to veh and subchronic PCP to scPCP.*

A one-way ANOVA was run to compare the groups' DI at baseline (NOR 1.1) and a significant effect was found, $F(_{3,36}) = 12.67$, $p < 0.001$. Post hoc Bonferroni-corrected t-tests were run to elucidate the interactions. The DI for the vehicle control group did not differ from the DI for the vehicle exercise group (*MD* = -0.06, *SE* = 0.06, *p* = 1.00). The DI for the vehicle control group was not significantly different than the scPCP control group (*MD* = 0.17, *SE* = 0.07, *p* = 0.13). However, the DI of the vehicle control group was significantly larger than scPCP exercise group (*MD* = 0.33, *SE* = 0.07, *p* < 0.001). The vehicle exercise group also had a significantly higher DI than the scPCP control group (*MD* = 0.23, *SE* = 0.07, *p* < 0.05) and to the scPCP exercise group (*MD* = 0.39, *SE* = 0.07, *p* < 0.001). The scPCP control group did not have a

significantly different DI from the scPCP exercise group (*MD* = 0.16, *SE* = 0.07, *p* = 0.16; Figure 3.4).

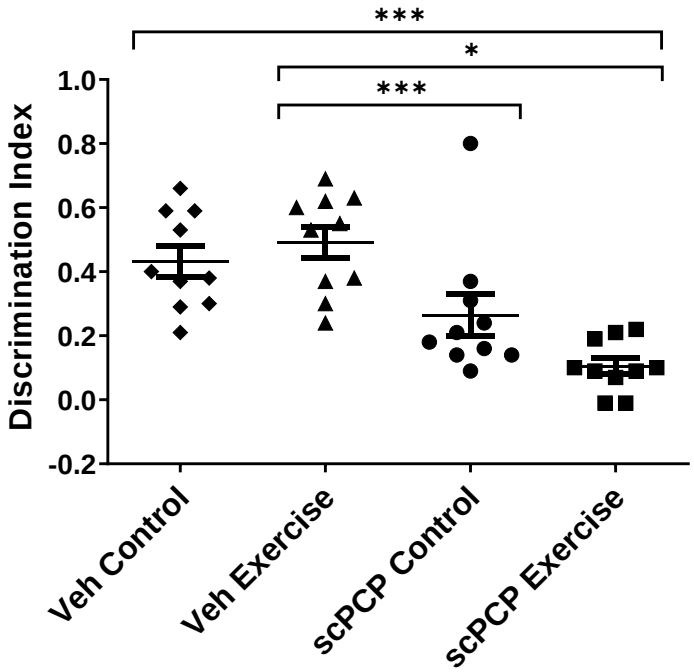


*Figure 3.4 Discrimination indices (DI) for the NOR task at baseline (NOR 1.1). Data are presented as mean ± SEM (n = 10 per group), * p < 0.05, *** p < 0.001, post hoc t-tests with Bonferroni correction. Vehicle is abbreviated to veh and subchronic phencyclidine to scPCP.*

Total exploration time (NOR 1.1)

Total exploration time was compared between groups to ensure that differences in exploratory behaviour were not due to disparities in overall exploration time. This was used as a 'net-best' measure of locomotor activity in the absence of line-crossing data. The total exploration time for the acquisition stage and total exploration time for the retention stage of the NOR task were analysed. No significant differences were found between the groups in the exploration times for the acquisition stage of the NOR, $F(_{3,36}) = 0.39$, p =0.76 (data not shown). However, there was a significant interaction for the total exploration time for the retention

stage of NOR, $F_{(3,36)} = 5.71$, $p < 0.01$. Post hoc Bonferroni-corrected t-tests were run to understand the interactions. The vehicle control group's total retention exploration time was significantly higher to the scPCP exercise group (*MD* = 7.7, $p < 0.05$) but did not differ from the other groups. The vehicle exercise group was significantly greater than both the scPCP control group (*MD* = 7.91, $p < 0.05$) and the scPCP exercise group (*MD* = 7.41, $p < 0.05$), but did not differ from the vehicle control group (Figure 3.5).

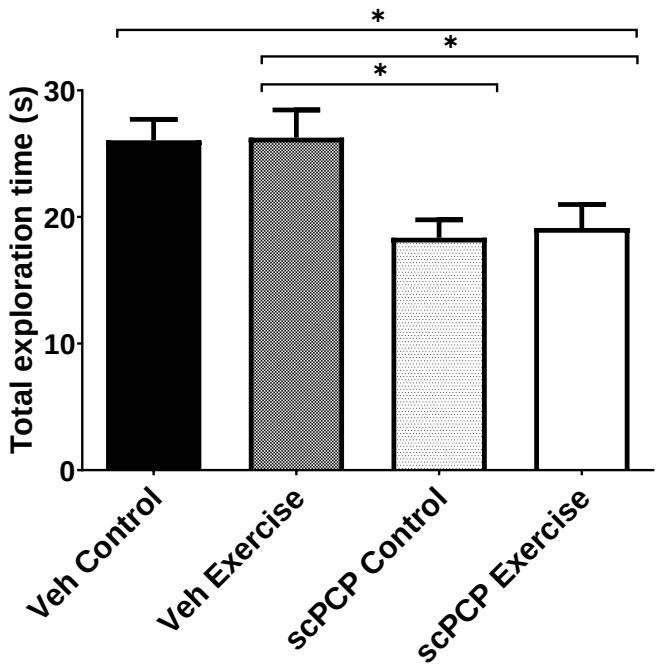


*Figure 3.5 Retention total exploration time in seconds at baseline (NOR 1.1). Data are presented as mean ± SEM (n = 10 per group), *p < 0.05. One-way ANOVA with post hoc Bonferroni-corrected t-tests. Vehicle is abbreviated to veh, subchronic PCP to scPCP and seconds to s*

3.3.2 Time point two: post-exercise NOR task (NOR 1.2)

Acquisition stage (NOR 1.2)

A second NOR was run at time point two (NOR 1.2) after six weeks of exercise or control. A two-way ANOVA indicated no significant effect of object (left and right) at the acquisition stage of the NOR task, $F_{(1,36)} = 1.36$, $p = 0.25$. In addition to this, there was no significant interaction of object and group, $F_{(3,36)} = 0.50$, $p = 0.98$ (Figure 3.6).

Retention stage (NOR 1.2)

A two-way ANOVA indicated a significant interaction between group and object in the retention stage of the NOR (NOR 1.2), $F_{(3,35)} = 7.16$, $p < 0.01$. There was also a

significant effect of object, $F_{(1,35)} = 75.52$, $p < 0.001$. Post hoc Bonferroni-corrected paired sample t-tests showed that the vehicle control group explored the novel object significantly more than the familiar object (*MD* = 9.19, *SE* = 1.69), $p < 0.001$. The vehicle exercise group also demonstrated a significant difference between the exploration of the novel and familiar objects (*MD* = 6.63, *SE* = 1.69), $p < 0.001$. The scPCP control group were not able to discriminate between the novel and familiar objects (*MD* = 1.13, *SE* = 1.60) $p = 0.49$. Like the vehicle groups, the scPCP exercise group were able to differentiate between the novel and familiar objects (*MD* = 10.60., *SE* = 1.60), $p < 0.001$ (Figure 3.6).

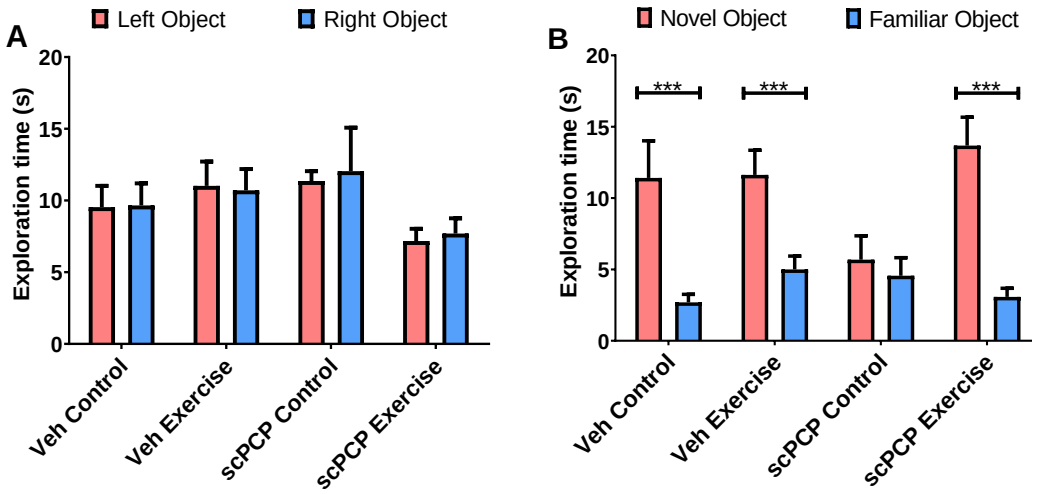


*Figure 3.6 Exploration time in seconds for the left and right objects during the acquisition stage (A), and the novel and familiar objects during the retention phase (B) for NOR 1.2. Data are presented as mean ± SEM (n = 9-10 per group), ***p < 0.001, two-way ANOVA with Bonferroni-corrected p values. Vehicle is abbreviated to veh, subchronic PCP to scPCP, and seconds to s.*

Discrimination Indices (NOR 1.2)

A one sample t-test was run to determine if groups favoured the novel or familiar objects by comparing DI to 0. The vehicle control group displayed discrimination

between objects and favoured the novel object. This was shown by a positive DI significantly different from zero (M = 0.62, SD = 0.22), $t_{(8)}$ = 8.58, p < 0.001. The vehicle exercise group were also observed to distinguish the novel and familiar object (M = 0.40, SD = 0.20), $t_{(9)}$ = 6.36, p < 0.001. The PCP control group did not have a DI significantly different to zero (M = 0.08, SD = 0.21), $t_{(8)}$ = 1.27, p = 0.24. The PCP exercise group did have a DI significantly different to zero (M = 0.63, SD = 0.17), $t_{(9)}$ = 11.70, p < 0.001; Figure 3.7).

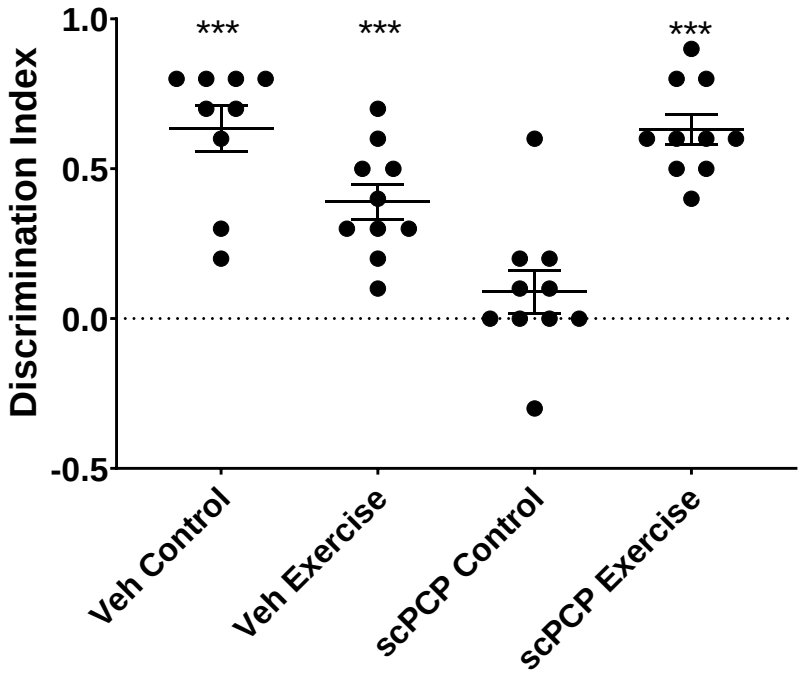


*Figure 3.7 Discrimination indices (DI) compared to 0 for the retention stage of the NOR task after 6 weeks of exercise (NOR 1.2). Data are presented as mean ± SEM (n = 9-10 per group) *** p < 0.001, independent sample t-tests. Vehicle is abbreviated to veh, and subchronic PCP to scPCP.*

A one-way ANOVA was run to compare the groups' DI after six weeks of exercise or control (NOR 1.2) and a significant effect was found, $F_{(3,35)}$ = 16.10, p < 0.001. Post hoc Bonferroni-corrected t-tests were run to elucidate the interactions (Figure 3.4). The DI for the vehicle control group did not differ from the DI for the vehicle

exercise group (*MD* = 0.22, *SE* = 0.09, *p* = 0.13). The DI for the vehicle control group was significantly higher than the scPCP control group (*MD* = 0.54, *SE* = 0.09, *p* < 0.001) but was not significantly larger than scPCP exercise group (*MD* = 0.00, *SE* = 0.09, *p* = 1.00). The vehicle exercise group also had a significantly higher DI than the scPCP control group (*MD* = 0.32, SE = 0.09, p < 0.01) but no significant difference was found with the scPCP exercise group (MD = 0.39, *SE* = 0.07, *p* < 0.001). The scPCP control group did not have a significantly different DI from the scPCP exercise group (*MD* = 0.16, *SE* = 0.07, *p* = 0.16; Figure 3.8).

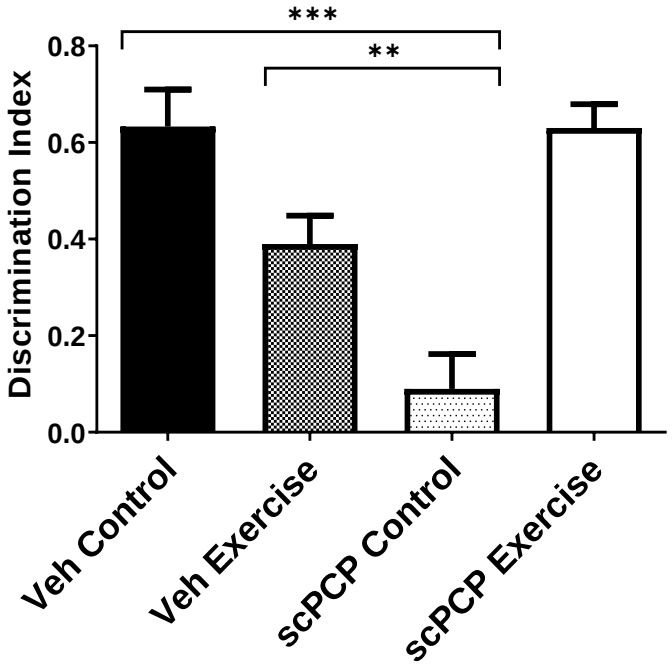


*Figure 3.8 Discrimination indices (DI) for the NOR task after 6 weeks of exercise or the control condition (NOR 1.2). Data are presented as mean ± SEM (n = 9-10 per group), ** p < 0.01, *** p < 0.001, post hoc t-tests with Bonferroni correction. Vehicle is abbreviated to veh and subchronic phencyclidine to scPCP.*

Total exploration time (NOR 1.2)

To assess if differences in object exploration were influenced by general exploratory behaviour, the total exploration at the acquisition stage and the retention stage of

the NOR were compared. A one-way ANOVA reported no significant effect in total exploration time in the NOR test: acquisition total time, $F(_{3,36}) = 1.10$, $p = 0.36$; retention total time, $F(_{3,35}) = 1.37$, $p = 0.27$ (data not shown). The exploration time for all objects at this time point was lower than for NOR 1.1. This may be explained by the rats' overfamiliarity with their environment as other behavioural tests (not presented in this thesis) were performed in the same arena on consecutive days. While the NOR deficit is stable in scPCP female rats, this repetition and exposure to the arena may have reduced exploration time.

3.3.3 Baseline and post-exercise discrimination indices comparison (NOR 1.1 versus NOR 1.2)

A two-way ANOVA was run comparing DI from baseline (NOR 1.1) and after six weeks of exercise or control condition (NOR 1.2). This analysis included planned post hoc paired t-tests with Bonferroni adjustment. There was a significant effect of DI between the two time points, $F(_{1,34}) = 9.73$, $p < 0.01$, and of DI and group treatment, $F(_{3,34}) = 14.37$, $p < 0.001$. Paired sample t-tests with Bonferroni corrections indicated a difference in DI between the two time points for vehicle control animals ($MD = 0.18$, $SE = 0.08$), $p < 0.05$. There was no significant difference in DI for the vehicle exercise animals ($MD = -0.09$, $SE = 0.07$), $p = 0.25$. There was also no significant difference in the DI between the two time points for the scPCP control group ($MD = 0.11$, $SE = 0.08$), $p = 0.17$, but there was a significant increase in the scPCP exercise DI from baseline to post-exercise ($MD = 0.52$, $SE = 0.08$), $p < 0.001$; Figure 3.9).

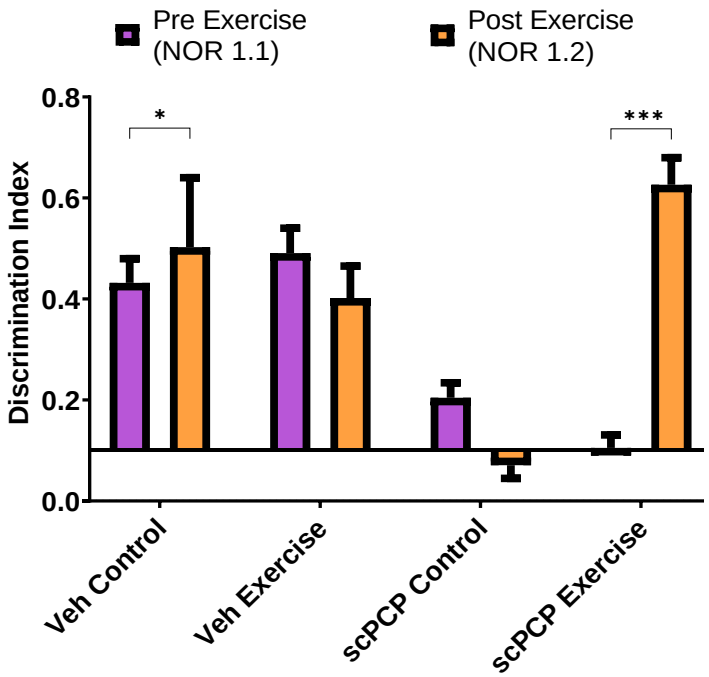


*Figure 3.9 A comparison of discrimination indices before and after the exercise intervention (NOR 1.1 versus NOR 1.2). Data are presented as mean ± SEM (n = 9-10 per group), * p < 0.05, *** p < 0.001, post hoc paired sample t-tests with Bonferroni correction. Vehicle is abbreviated to veh, and subchronic PCP to scPCP.*

3.4 Discussion

The results of this study suggest that voluntary exercise can potentially ameliorate the NOR cognitive deficit observed in the scPCP rodent model for schizophrenia. This was indicated by a preference for the novel object in the NOR task for scPCP-treated animals who were given access to freely turning wheels. This preference was not present in scPCP-treated animals in the control condition. In addition to this, there was a significant increase in the DI of scPCP exercise group from baseline (NOR 1.1) to post-exercise (NOR 1.2), suggesting a cognitive improvement. These effects were not deemed be due to changes in locomotor activity and general exploratory behaviour as there were no significant differences between the groups.

Total exploration time was used as a proxy measure of locomotor activity because line crossing data was not available.

The scPCP model for schizophrenia is known to produce cognitive deficits of relevance to schizophrenia (Jentsch and Roth 1999; Neill et al. 2014) during the NOR task (Grayson et al. 2007; Rajagopal et al. 2010; Grayson et al. 2015). However, in the baseline NOR (NOR 1.1) the scPCP groups appeared to explore the novel object significantly more than the familiar, suggesting an ability to discriminate. Further analysis provided evidence that the scPCP control and scPCP exercise groups were impaired when they were compared with the vehicle control and vehicle exercise groups. After access to running wheels (or the control condition), the rats underwent a second NOR test (NOR 1.2). These results showed a deficit in only the scPCP control group. There were unexpected significant differences in both scPCP groups' object exploration in the baseline (NOR 1.1) data. The ANOVA reported no significant effect for the familiar object but did reveal a significant effect for the novel object. The vehicle control and vehicle exercise group showed significantly longer exploration of the novel object than both the scPCP control and scPCP exercise group. The vehicle groups did not differ from one another, nor did the scPCP groups. These results suggest a disparity in novel object exploration between vehicle and scPCP animals, with the scPCP rats spending less time exploring the novel object. These differences could have affected the detection of the NOR task deficit that is usually reported (by a two-way ANOVA) in rats treated with the scPCP protocol. Therefore, the DI from before and after the experimental

condition (NOR 1.1 and NOR 1.2) were compared for all groups. There was a significant increase in the DI for the vehicle control group, but no significant change in DI for the vehicle exercise group or the scPCP control group. There was a significant increase in DI for the scPCP exercise group, providing evidence that voluntary exercise in running wheels may have improved the cognitive deficit seen in scPCP treated rats during the NOR task.

The results from this study correspond with human research that has demonstrated that exercise improves both positive and negative symptoms and cognitive impairments experienced by patients (Pajonk et al. 2010; Vancampfort et al. 2012; Firth et al. 2015). Exercise positively affects several cognitive processes including planning, working memory, multitasking, and resolving ambiguity (Hillman et al. 2008). Its most substantial effects are reported in tasks involving executive function (Hillman et al. 2008), a cognitive domain incontrovertibly impaired in schizophrenia (Green et al. 2004; Nuechterlein et al. 2004; Nuechterlein et al. 2005; Fond et al. 2013; Orellana and Slachevsky 2013; Knowles et al. 2015; Gold et al. 2017). There is a growing body of literature demonstrating that exercise improves cognition in schizophrenia (Pajonk et al. 2010; Nuechterlein et al. 2016) and recent meta-analyses confirm this (Vancampfort et al. 2014; Firth et al. 2017). However, how exercise alleviates deficits is not known, and researchers acknowledge that the specific mechanisms are ‘under-researched’ and remain enigmatic (Scheewe et al. 2013; Firth et al. 2017). There have been a small number of studies investigating the mechanisms by which exercise improves cognition in schizophrenia (Firth et al.

2017). For example, brain imaging has shown increases in grey matter volume in numerous brain areas (Lin et al. 2015; Malchow et al. 2015) but the results are inconsistent. Some studies report an increase in hippocampal volume (Pajonk et al. 2010; Lin et al. 2015), while others report increases in the anterior lobe (Malchow et al. 2016) or a general increase in cerebral volume and cortical thickening (Scheewe et al. 2013). Biomarker studies are also limited and peripheral brain-derived neurotrophic factor (BDNF) is the most studied. Once again, the research is equivocal with some studies observing significantly higher levels of BDNF after a variety of exercise interventions (Kuo et al. 2013; Kim et al. 2014; Nuechterlein et al. 2016) and some reporting non-significant increases (Kimhy et al. 2015; Silva et al. 2015).

The current evidence indicates that exercise does exert pro-cognitive effects on the brain in people with schizophrenia. However, it is difficult to identify the subtle and interconnected processes involved (Vancampfort et al. 2014). Furthermore, studies report poor adherence to exercise regimes (Scheewe et al. 2013; Firth et al. 2016; Stubbs et al. 2016; Vancampfort et al. 2016), with a third or more of participants not reaching the weekly minimum duration of activity (Scheewe et al. 2013). Confounding variables are also present, including antipsychotic treatment (Vancampfort et al. 2014), duration of illness (Chakos et al. 2005), and baseline symptoms (Vancampfort et al. 2012; Scheewe et al. 2013). Animal research allows more control over experimental conditions and the ability to perform invasive investigation of molecular and structural changes in the brain (Vancampfort et al.

2014). Animal models of schizophrenia allow expeditious monitoring of disease progression and the testing of novel treatments in a way that is not possible in humans (Jones et al. 2011). The scPCP rat model for schizophrenia is a well-validated and widely utilised tool for studying schizophrenia-like abnormalities in both brain and behaviour (Abdul-Monim et al. 2007; Neill et al. 2010; Neill et al. 2014; Grayson et al. 2015; Doostdar et al. 2019). The resultgs from this experiment indicate that exercise could improve cognitive deficits seen in scPCP treated animals, mirroring the positive effects of exercise in schizophrenia. These data offer an encouraging insight supporting exercise as an effective treatment for the cognitive symptoms of schizophrenia. Furthermore, being able to produce relevant behavioural changes in an animal model offers an exciting opportunity to understand how exercise exerts its influence on the brain in schizophrenia.

Chapter 4 The effect of exercise on synaptic and GABAergic markers in the subchronic PCP rat model for schizophrenia

4.1 Introduction

Schizophrenia is a severe mental disorder associated with various neurobiological changes (Vancampfort et al. 2014). Among them is grey matter volume loss (Haijma et al. 2013), decreased white matter integrity (Lim et al. 1999), and increased ventricular volume (Saijo et al. 2001). The brains of people with schizophrenia exhibit abnormal neurogenesis, plasticity and cerebral blood flow (Selemon and Goldman-Rakic 1999; Stephan et al. 2009), as well as disturbances in monoamine and trace amine systems (Berry 2008; Howes and Kapur 2009; Crabtree and Gogos 2014). Subchronic treatment with the NMDAR antagonist phencyclidine (scPCP) produces structural and functional abnormalities comparable to those seen in schizophrenia (Ellison and Sweet 1993; Reynolds et al. 2004; Hajszan et al. 2006; Abdul-Monim et al. 2007; Jenkins et al. 2008; Pratt et al. 2008; McKibben et al. 2010; Dawson et al. 2014).

Disturbances in glutamatergic, dopaminergic and y-aminobutyric acid (GABA)ergic systems suggest that schizophrenia pathology has a synaptic component (Rapoport et al. 2012; Karlsen et al. 2013). In patients, the most pronounced synaptopathology is apparent in the prefrontal cortex (PFC) and hippocampus, areas involved in cognition (Heckers et al. 1998; Meyer-Lindenberg et al. 2005). Neuronal and synaptic plasticity are the cellular mechanisms that underlie learning and memory and dysfunction in these processes can lead to cognitive deficits. Synaptic markers are proteins found in structures adjacent to the synaptic cleft and

their measurement provides an estimate of the number of synapses in a piece of tissue. Lower levels of synaptic markers can suggest a loss of synapses, therefore indicating the poor health of the neural networks they are associated with.

Glutamate is the major excitatory neurotransmitter in the human brain and plays a critical role in synaptic plasticity (Steeds et al. 2015). Glutamate release is decreased in the frontal and temporal lobes of people with schizophrenia (Sherman et al. 1991) and is associated with cognitive impairments (Howes et al. 2015). The N-methyl-D-aspartate (NMDA) subtype of glutamate receptor is involved in the molecular mechanism of synaptic adaptation (Pérez-Otaño and Ehlers 2005), with functions such as regulating the migration of neurons (Komuro and Rakic 1993), strengthening synapses via long-term potentiation (LTP; Harris et al. 1984), and dendritic spine development (Yasumatsu et al. 2008). These processes are critical for healthy learning and memory and are also disrupted in schizophrenia. Therefore, NMDA receptors (NMDARs) participate in multiple cellular pathways of relevance to the disease (Ahmed and Bhat 2014). Postsynaptic density protein 95 (PSD_{95}) is a scaffolding protein found postsynaptically in glutamatergic pathways (Chen et al. 2008). Functions of PSD_{95} include the mediation of the density of NMDARs in the postsynaptic neuron, thereby influencing glutamatergic transmission (Coley and Gao 2018). Measures of PSD_{95} are thought to indicate glutamatergic synaptic density with lower concentrations suggesting a loss of postsynaptic terminals. Synaptosomal-associated protein of 25 kDa ($SNAP_{25}$) is concentrated on presynaptic glutamatergic terminals and is essential for exocytotic neurotransmission (Gu and Huganir 2016). Similarly to PSD_{95}, $SNAP_{25}$ can be measured to evaluate glutamatergic synaptic density. Reduced expression of

$SNAP_{25}$ and PSD_{95} reflect a loss of pre- and postsynaptic sites and dysfunctional synaptic processes. Furthermore, both $SNAP_{25}$ and PSD_{95} expression are reduced in schizophrenia and are associated with cognitive deficits in the disease (Carroll et al. 2009; Coley and Gao 2018).

Synaptic plasticity and maintenance is a process of balancing excitatory and inhibitory activity in the brain. GABA is the primary inhibitory neurotransmitter and is therefore crucial for healthy synaptic function. The GABAergic system exhibits abnormal behaviour in schizophrenia (Cross et al. 1979; Neill et al. 2010; Nakazawa et al. 2012), leading to disordered interneuron migration (Akbarian et al. 1993), neuronal atrophy (Sweet et al. 2004), and the reduction of dendritic spines in populations of neurons (Sweet et al. 2009). Deficits in parvalbumin-containing GABAergic interneurons have been reported in the hippocampus (Benes and Berretta 2001; Zhang and Reynolds 2002) and the PFC (Beasley and Reynolds 1997; Benes 1997; Beasley et al. 2002) of people with schizophrenia. Additionally, the expression of glutamate decarboxylase of 67 kDa (GAD_{67}), a GABA-synthesising enzyme specific to GABAergic parvalbumin-containing interneurons (Hashimoto et al. 2003), is also decreased in these cells in the PFC of people with schizophrenia (Volk et al. 2000). This evidence suggests that both GABA synthesis and reuptake is disrupted in these specific subpopulations of PFC neurons (Hashimoto et al. 2003). These interneurons also receive excitatory input through NMDARs making them sensitive to changes in the glutamatergic system (Uhlhaas 2011). The use of NMDA antagonists not only leads to schizophrenia-like behaviour (Domino and Luby 2012) but causes changes in GABA transmission (Neill et al. 2010). Furthermore, NMDA antagonist such as PCP and ketamine cause reductions in the power of gamma-

frequency oscillations (20-80 Herz), a phenomenon that has been linked to the cognitive deficits in schizophrenia (Uhlhaas and Singer 2010). This interaction between glutamatergic and GABAergic pathways may explain why PCP treated rats exhibit similar GABAergic abnormalities in corresponding brain areas to patients (Cochran et al. 2003; Abdul-Monim et al. 2007; Jenkins et al. 2008).

Fast-spiking parvalbumin-containing interneurons are known to synapse with pyramidal projection neurons and exert inhibitory influence over them (Mascagni et al. 2008). In turn, pyramidal cells regulate the oscillations of inhibitory neurons, resulting in synchronised behaviour of neuronal populations (Uhlhaas 2011). Synchrony within and between large populations of cells, particularly in fast-spiking parvalbumin-containing interneurons, is necessary for healthy cognition. People with schizophrenia demonstrate dysfunctional neural oscillations (Uhlhaas and Singer 2006), particularly in gamma-band oscillations that play a role in numerous cognitive processes (Uhlhaas and Singer 2010). Therefore, deficits in the GABAergic, parvalbumin-containing interneurons can reduce coordinated activity in neural networks and have the potential to disrupt cognition (Crabtree and Gogos 2014). This likely contributes to PFC dysfunction and negatively impacts learning and memory in those with schizophrenia (Neill et al. 2010).

The mechanisms by which exercise improves cognition in schizophrenia are not known (Firth et al. 2017). Physical activity benefits the brain in many ways and promotes angiogenesis, neurogenesis, and the upregulation of trophic factors (Cotman and Berchtold 2007a; Hillman et al. 2008). These processes support plasticity in the brain, a phenomenon that underlies learning and memory (Ganguly

and Poo 2013) and is compromised in schizophrenia. Therefore, exercise may improve cognition in this population by improving synaptic health (Scheewe et al. 2013; Firth et al. 2017).

In the previous chapter, it was demonstrated that wheel running may be able to rescue the novel object recognition task (NOR) deficit in the scPCP rat model for schizophrenia. Cognitive deficits in scPCP rodent models for schizophrenia are also associated with glutamatergic and GABAergic transmission in the dorsal hippocampus (Gigg et al. 2020). In addition to this, the temporal lobe is involved in the cognitive processes involved in the NOR task (Hammond et al. 2004). This study used brain samples from these animals to investigate the effect of scPCP treatment on markers of glutamatergic and GABAergic function. The protein analysis was performed in the PFC and dorsal hippocampus. The study also investigated if voluntary wheel running modified these markers when active rats were compared with sedentary controls.

4.2 Methods

All animals underwent NOR at baseline and after six weeks of access to wheels or the control condition, full details of which can be found in sections 2.4 and 2.5. The detailed methods of this study may be found in Chapter 2. Modified Bradford assays were completed for the hippocampal tissue to estimate protein concentration for analysis using the 'Wes' automated capillary electrophoresis western blot system (see section 2.9). Bradford assays were not run for the PFC tissue as the concentrations were expected to be too low to be accurately detected.

The 'Wes' automated western blotting system (ProteinSimple, UK) is described in full in section 2.10. Briefly, the system employs capillary electrophoresis to separate proteins of different molecular weight, and chemiluminescence to label and measure protein(s) of interest. A 'Wes' assay requires only 3µl of sample per well, with a minimum protein concentration of 0.2µg/µl, and produces quantitative results in around three hours. The user loads samples and the specific antibodies required onto the assay plate, and the plate is pre-filled with the separation entities required. A full protocol can be found in section 2.10.2.

Four proteins of interest were investigated in each region. These were the synaptic density markers PSD_{95} and $SNAP_{25}$, and the GABAergic markers parvalbumin and GAD_{67}. Before analysis studies were carried out for each protein to optimise total protein loaded and primary antibody concentrations for both the PFC and dorsal hippocampus. The experimental groups were: vehicle control; vehicle exercise; PCP control; and PCP exercise.

4.2.1 Statistical analysis

The area of the chemiluminescence peak for the protein of interest and for the housekeeping protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was collected from Compass for SW (ProteinSimple 2018). One-way ANOVAs were run (as described in detail in section 2.10.3.1). If an ANOVA returned a p-value of < 0.05, paired-sample t-tests with a Bonferroni correction were run to examine the significant interactions, as described in section 2.10.3.1.

4.3 Results

4.3.1 Prefrontal cortex

Levels of PSD_{95}, $SNAP_{25}$, parvalbumin and GAD_{67} in PFC tissue were analysed using the Wes system. All four proteins were measured in the supernatant (S2) fraction produced by the homogenisation protocol described in section 2.8.2.

4.3.1.1 PSD_{95}

A one-way ANOVA indicated that there were no significant differences between the groups in the ratio of the protein of interest, PSD_{95}, to the housekeeping protein GAPDH, $F(_{3,23}) = 0.55$, $p = 0.66$ (Figure 4.1).

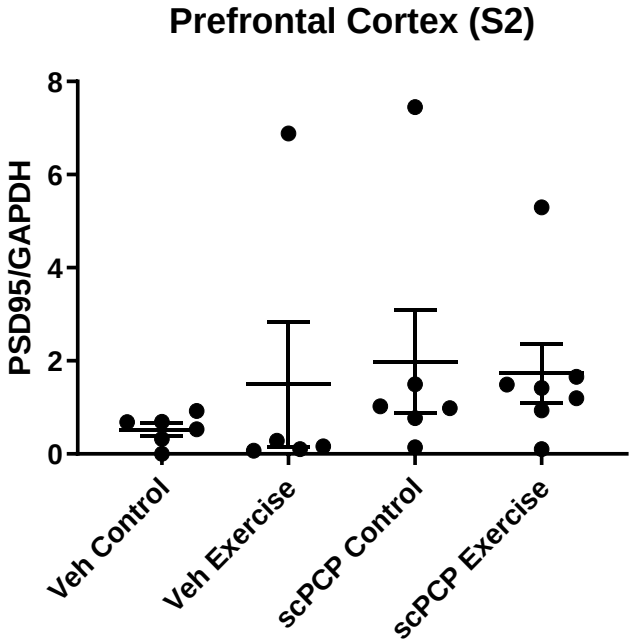


Figure 4.1 Ratio of the synaptic protein PSD_{95} to the housekeeping protein GAPDH in S2 of the prefrontal cortex. Data are presented as mean ± SEM (n = 6 per group), one-way ANOVA with Bonferroni-corrected t-tests. Postsynaptic density protein 95 is abbreviated to PSD_{95}, glyceraldehyde 3-phosphate dehydrogenase to GAPDH, and supernatant two to S2.

4.3.1.2 $SNAP_{25}$

A one-way ANOVA showed no significant differences between the groups in the ratio of $SNAP_{25}$ to the housekeeping protein GAPDH, $F(_{3,23}) = 1.56$, $p = 0.23$ (Figure 4.2).

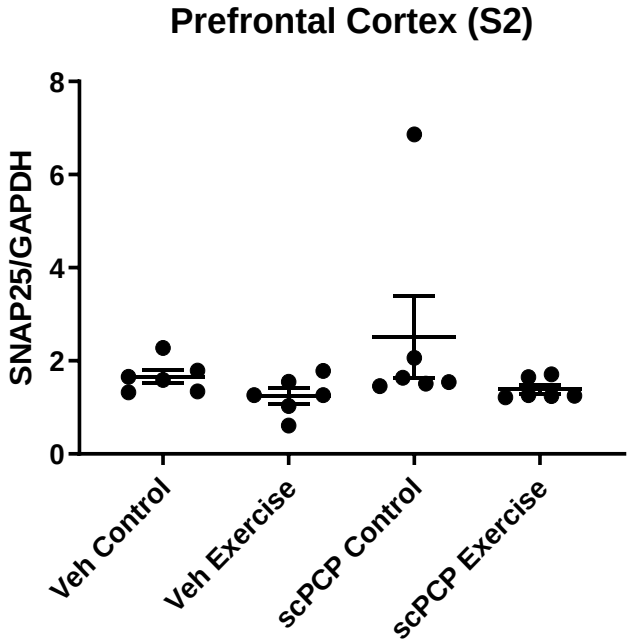


Figure 4.2 Ratio of the synaptic protein $SNAP_{25}$ to the housekeeping protein GAPDH in S2 of the prefrontal cortex. Data are presented as mean ± SEM (n = 6 per group), one-way ANOVA with Bonferroni-corrected t-tests. Synaptosome associated protein 25 is abbreviated to $SNAP_{25}$, glyceraldehyde 3-phosphate dehydrogenase to GAPDH, and supernatant two to S2.

4.3.1.3 Parvalbumin

A one-way ANOVA demonstrated no significant differences between the groups in the ratio of parvalbumin to the housekeeping protein GAPDH, $F(_{3,23}) = 0.33$, $p = 0.80$ (Figure 4.3).

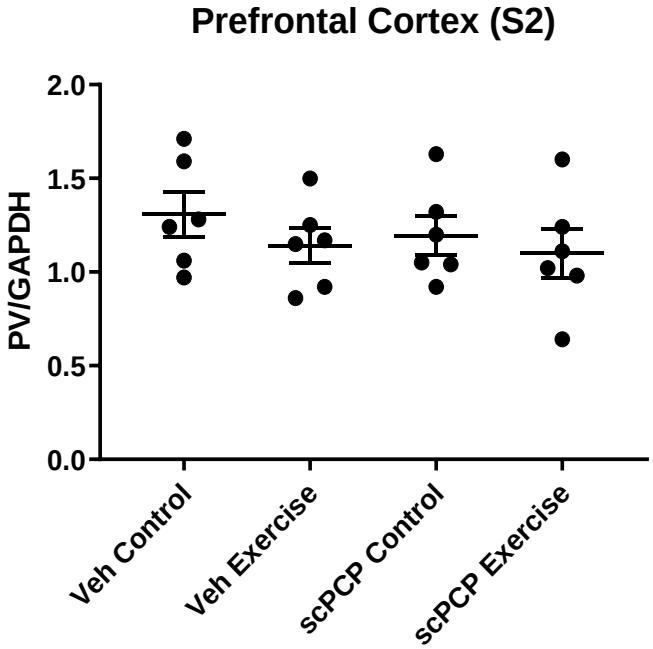


Figure 4.3 Ratio of PV to the housekeeping protein GAPDH in S2 of the prefrontal cortex. Data are presented as mean ± SEM (n = 6 per group), one-way ANOVA with Bonferroni-corrected t-tests. Parvalbumin is abbreviated to PV, glyceraldehyde 3-phosphate dehydrogenase to GAPDH, and supernatant two to S2.

4.3.1.4 GAD_{67}

A one-way ANOVA returned a significant difference between the groups for the ratio of GAD_{67} to GAPDH, $F(_{3,23}) = 10.00$, $p < 0.001$. Post hoc Bonferroni-corrected t-tests reported that the vehicle control group had a significantly lower ratio of GAD_{67}/GAPDH when compared to the PCP exercise group (*MD* = -0.63, *SE* = 0.13), $p < 0.01$. This was also true for the vehicle exercise group (*MD* = -0.63, *SE* = 0.13), $p <$

0.01. The PCP control group also demonstrated a significantly lower GAD_{67}/GAPDH ratio to the PCP exercise group (*MD* = -0.43, *SE* = 0.13), $p < 0.05$; Figure 4.4).

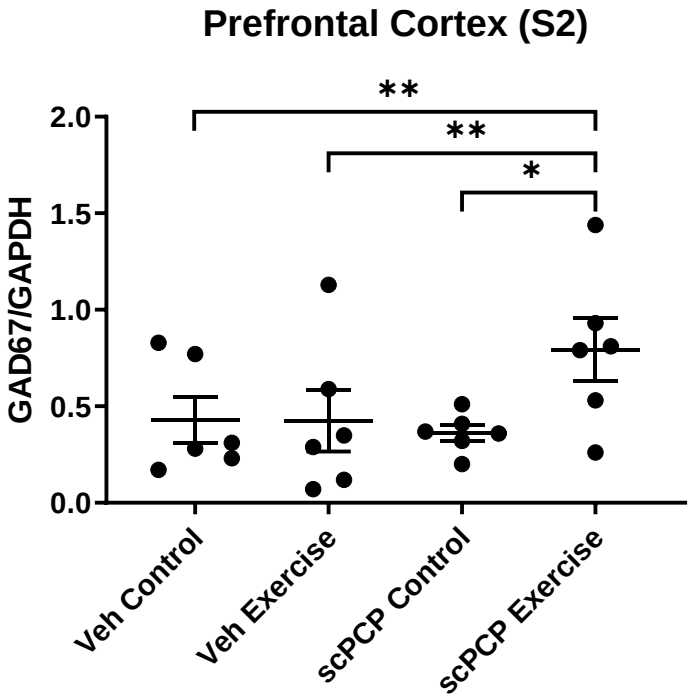


*Figure 4.4 Ratio of GAD_{67} to the housekeeping protein GAPDH in S2 of the prefrontal cortex. Data are presented as mean ± SEM (n = 6 per group), * p < 0.05, ** p < 0.001, one-way ANOVA with Bonferroni-corrected t-tests. Glutamate decarboxylase 67 is abbreviated to GAD_{67}, glyceraldehyde 3-phosphate dehydrogenase to GAPDH, and supernatant two to S2.*

4.3.2 Dorsal hippocampus

Levels of PSD_{95}, $SNAP_{25}$, parvalbumin and GAD_{67} in dorsal hippocampal tissue were analysed using the 'Wes' system. As with the PFC tissue, all four proteins were measured in the S2 fraction produced by the homogenisation protocol described in section 2.8.2.

4.3.2.1 PSD_{95}

A one-way ANOVA indicated no significant difference between the groups regarding the amount of PSD_{95} (the protein of interest) compared to GAPDH (the housekeeping protein), $F(_{3,19}) = 1.37$, $p = 0.29$ (Figure 4.5).

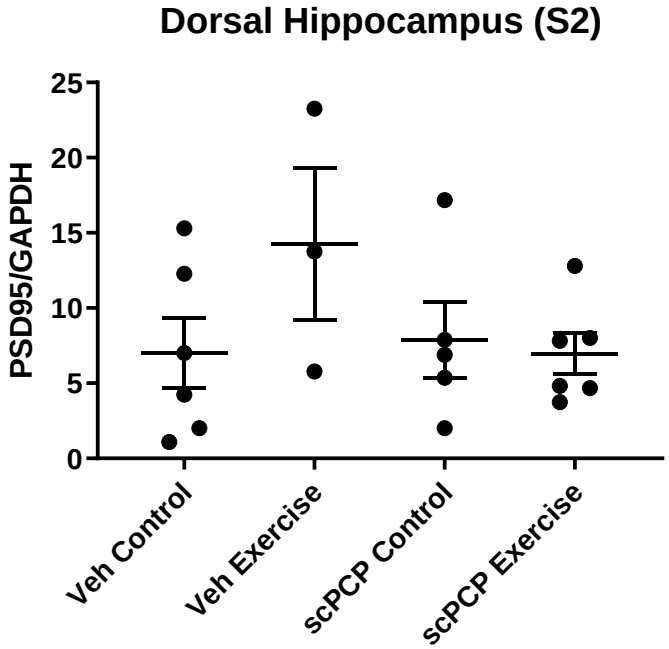


Figure 4.5 Ratio of PSD_{95} to the housekeeping protein GAPDH in S2 of the dorsal hippocampus. Data are presented as mean ± SEM (n = 3–6 per group), one-way ANOVA with post hoc Bonferroni-corrected t-tests. Postsynaptic density protein 95 is abbreviated to PSD_{95}, glyceraldehyde 3-phosphate dehydrogenase to GAPDH, and supernatant two to S2.

4.3.2.2 $SNAP_{25}$

A one-way ANOVA indicated that there was no significant difference between the groups when the amount of $SNAP_{25}$ detected was compared to the housekeeping protein GAPDH, $F(_{3,19}) = 0.66$, $p = 0.98$ (Figure 4.6).

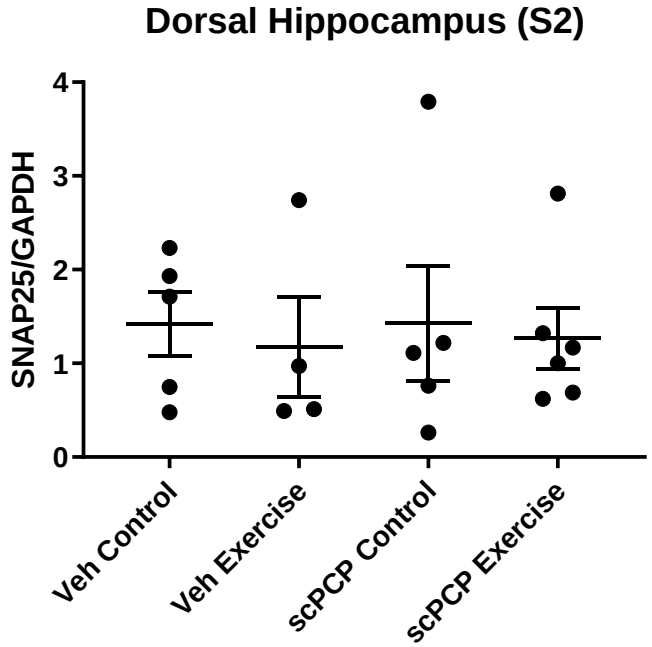


Figure 4.6 Ratio of $SNAP_{25}$ and housekeeping protein GAPDH in S2 of the dorsal hippocampus. Data are presented as mean ± SEM (n = 4–6 per group), one-way ANOVA with post hoc Bonferroni-corrected t-tests. Synaptosome associated protein 25 is abbreviated to $SNAP_{25}$, glyceraldehyde 3-phosphate dehydrogenase to GAPDH, and supernatant two to S2.

4.3.2.3

4.3.2.4 Parvalbumin

A one-way ANOVA revealed a significant difference between the groups regarding the amount of the protein parvalbumin in comparison to the housekeeping protein GAPDH, $F(_{3,18}) = 3.63$, $p < 0.05$. However, there were no significant differences between the groups when they were compared using post hoc Bonferroni-corrected t-tests (Figure 4.7).

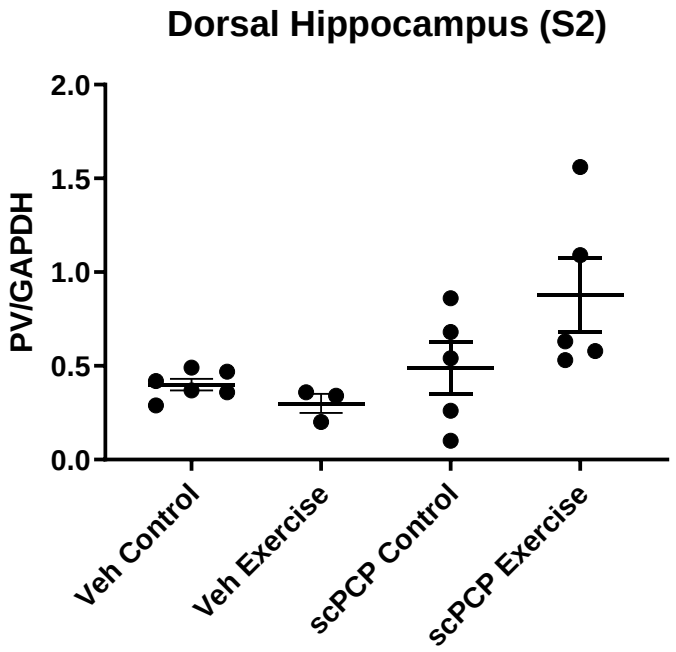


Figure 4.7 Ratio of PV and housekeeping protein GAPDH in S2 of the dorsal hippocampus. Data are presented as mean ± SEM (n = 3–6 per group), one-way ANOVA with post hoc Bonferroni-corrected t-tests. Parvalbumin is abbreviated to PV, glyceraldehyde 3-phosphate dehydrogenase to GAPDH, and supernatant two to S2.

4.3.2.5 GAD_{67}

A one-way ANOVA did not identify any significant differences between the groups for the protein GAD_{67} versus the housekeeping protein GAPDH, $F(_{3,18}) = 1.12$, $p = 0.34$ (Figure 4.8).

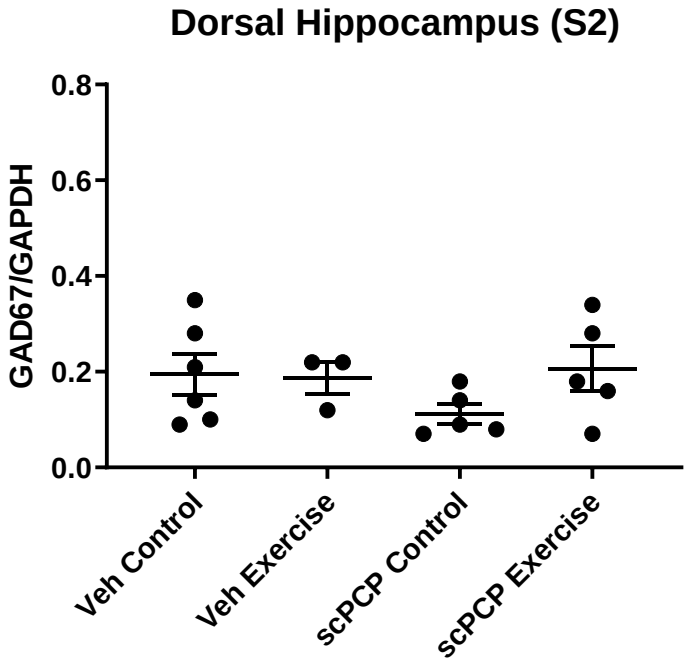


Figure 4.8 Ratio of GAD_{67} and housekeeping protein GAPDH in S2 of the dorsal hippocampus. Data are presented as mean ± SEM (n = 3–6 per group), one-way ANOVA with post hoc Bonferroni-corrected t-tests. Glutamate decarboxylase is abbreviated to GAD_{67}, glyceraldehyde 3-phosphate dehydrogenase to GAPDH, supernatant two to S2.

4.4 Discussion

This study examined synaptic and GABAergic markers in the PFC and hippocampus in the scPCP rat model for schizophrenia. It also sought to investigate the effect of voluntary wheel running on these markers. The PFC and dorsal hippocampus of four groups of rats were dissected and homogenised then compared using the 'Wes' automated western blotting system. The experimental groups were vehicle control,

vehicle exercise, scPCP control, and scPCP exercise. In the PFC, no significant effects were present between the four groups for PSD_{95}, $SNAP_{25}$, or parvalbumin expression. For GAD_{67}, a one-way ANOVA returned a significant effect of group for GAD_{67}/GAPDH levels. Post hoc Bonferroni-corrected paired t-tests reported higher GAD_{67} levels in the scPCP exercise group compared to the other groups. In the dorsal hippocampus, one-way ANOVA returned no significant group effects for PSD_{95}, $SNAP_{25}$, or GAD_{67} expression. For parvalbumin levels, the ANOVA revealed a significant effect of group but post hoc Bonferroni-corrected t-tests reported no significant differences between the groups. This and the failure to find significant differences in the levels of PSD_{95}, $SNAP_{25}$, or GAD_{67} may be explained by the low n (the lowest n was 3) of available hippocampus samples. Several of the samples were sent to the University of Nottingham and underwent mRNA analysis to investigate the expression of several proteins of interest. The student that carried out the experiments found no significant differences between the groups of animals for any protein.

Deficits in PSD_{95}, $SNAP_{25}$ and GABAergic pathways (measured by parvalbumin and GAD_{67}) are present in schizophrenia in the PFC and hippocampus (Ohnuma et al. 2000; Keefe and Harvey 2012; Coley and Gao 2018). This study did not observe these differences, assessed using western analysis, in the scPCP treated rats when compared to vehicle-treated animals. In the PFC, an ANOVA returned a significant effect of treatment group on GAD_{67} expression, with the scPCP exercise rats exhibiting significantly higher levels of this protein than the other groups. There

were no significant differences found in the vehicle control or exercise group when they were compared to the scPCP control group. Neither were there significant differences between the vehicle control or exercise groups. Because of this, the interpretation of the significant increase in the scPCP exercise group is challenging. The differences in this group did not appear to be due to correction of GAD_{67} in the scPCP model as the scPCP control group did not display a deficit. There was also no evidence that exercise increased GAD_{67} expression in the PFC of the rats in general. If this were the case, the vehicle exercise group would also have demonstrated increased expression.

GAD_{67} is one of two isoforms of GAD and GAD_{65} is the other. GAD_{67} is thought to reflect basal levels of GABA-mediated inhibitory activity as its ablation results in a 90% reduction of GABA in the brain (Pinal and Tobin 1998). GAD_{67} synthesises GABA for neuron activity that is not directly related to neurotransmission, such as synaptogenesis. Findings from immunohistochemistry confirm that the specificity of GAD_{67} distribution in the rat brain makes this protein it a useful marker for GABAergic cells and terminal processes (Fong et al. 2005). Unlike GAD_{67}, the function of GAD_{65} is limited to the synthesis of GABA for release into the synaptic cleft (Pinal and Tobin 1998). A study in male rats demonstrated that four weeks of wheel running caused increases in GAD_{67}, although in the hippocampus rather than the PFC (Salas-Vega et al. 2011). However, another reported that wheel running did not affect the expression of GAD_{67} but did increase levels of GAD_{65} (Ferreira-Junior et al. 2019). GAD_{65} may have been upregulated in these samples but was not

measured. The inconsistent evidence for exercise-mediated changes of the two isoforms in rats indicates the relationship may not be straightforward and requires further characterisation.

An alternative explanation for the significant difference in GAD_{67} expression observed in the PFC of scPCP exercise rats is the heterogeneity of variance among the groups. Levene's test did not return a significant result for any of the markers, including GAD_{67}. However, each group had a small n of six because several of the homogenised samples contained too little protein to analyse effectively. Normality testing with small sample sizes (< 20) is argued to be underpowered and unable to identify distribution accurately or reliably (Zylstra 1994; Fay and Proschan 2010). Therefore, the result could be explained as a statistical artefact that is the result of small sample sizes and a lack of homogeneity of variance between the treatment groups.

A significant effect of treatment group was observed in parvalbumin levels in the dorsal hippocampus, although post hoc analysis did not report significant differences between the groups. Disturbances in parvalbumin-containing interneuron are strongly associated with schizophrenia and are thought to contribute to cognitive impairment (Neill et al. 2010). However, the interpretation of this result is also challenging because the nature of the effect is unclear. The lack of significant differences could be due to insufficient power to detect the effect in group parvalbumin levels using western analysis. While a sample size of six was possible for the PFC analysis, sample numbers were as low as three when

comparing the hippocampal tissue. Larger sample sizes providing more power are likely required to understand the effect of exercise on parvalbumin expression in the scPCP rat model for schizophrenia.

In this study, the density of excitatory synapses was determined using pre- and postsynaptic markers $SNAP_{25}$ and PSD_{95}, respectively. These proteins play key roles in synaptic plasticity and maturation and are found in glutamatergic synapses (Gigg et al. 2020). Also, $SNAP_{25}$ and PSD_{95} expression have both been shown to be decreased in the brains of people with schizophrenia (Ohnuma et al. 2000; Gu and Huganir 2016). The density of inhibitory synapses was investigated by measuring parvalbumin and GAD_{67} levels. GAD_{67} is a GABA-synthesising enzyme that is also present in parvalbumin-containing interneurons, a subset of GABAergic cells. GABA is the major inhibitory neurotransmitter in the human brain and disruptions in PFC parvalbumin-containing interneurons are linked with cognitive deficits in schizophrenia (Nakazawa et al. 2012).

These data did not suggest a change of excitatory or inhibitory synaptic density in the scPCP model for schizophrenia. These results contradict previous animal work demonstrating a reduction in markers of GABAergic function, such as decreased parvalbumin mRNA in the PFC (Cochran et al. 2003; Pratt et al. 2008), and density of immunohistochemically-stained parvalbumin-containing interneurons in the prefrontal cortex and hippocampus (Abdul-Monim et al. 2007; Jenkins et al. 2008; McKibben et al. 2010). ScPCP treatment also produces a decline in GAD_{67} mRNA, although this was demonstrated in the cerebellum, an area not analysed in this

study (Bullock et al. 2009). There was also no indication of an effect of voluntary wheel running on glutamatergic or GABAergic function. Vehicle treated rats with access to wheels did not differ from sedentary controls on any synaptic marker. A significant group effect was revealed in GAD_{67} expression in the PFC and for parvalbumin expression in the dorsal hippocampus. However, the evidence was insufficient for speculation on the neuropathology underlying the behavioural changes reported in Chapter 3.

There are several limitations to this study, some of which have been alluded to above. For example, there were as few as three samples per group available for analysis in the dorsal hippocampus. There was also sizeable variation between samples, and the analysis may have been underpowered to detect effects in this tissue. For the PFC, an n of six was possible for each group. However, half of the dorsal hippocampal samples were sent for mRNA analysis, reducing the number available for Wes processing. In regards to the PFC samples, while there were ten rats per group it was not possible to dissect the PFC in some brains because of the way that the tissue has frozen within the brain boxes. In addition to this, the protein concentration in the samples was low due to the limited amount of PFC tissue in one rat hemisphere. Consequently, not all the samples could be processed with the Wes system, despite the low concentration required for successful detection.

There is evidence from both clinical and preclinical work that the changes in neurotransmitters associated with schizophrenia are highly spatially specific. For

instance, changes in parvalbumin-containing, fast-spiking interneuron expression is found in a specific subset of cells in the PFC of patients (Benes et al. 1991; Beasley et al. 2002). Similarly, selective abnormalities have been replicated using chronic-intermittent treatment with PCP (Cochran et al. 2003). In this study, tissue from each brain area in each animal was dissected and homogenised. Therefore, it was not possible to discern individual subregions or subpopulations of cells from this analysis. One hemisphere from each animal was perfused and fixed in paraformaldehyde (PFA) for analysis with immunohistochemistry. Slices from the PFC and hippocampus were stained for markers of interest, including parvalbumin, but the samples were destroyed and analysis was not possible. This methodology would allow for a better spatial acuity and may have revealed changes in immunolabelled cells in functionally distinct subregions in the PFC and hippocampus.

Exercise has been shown to improve symptoms and cognitive deficits in schizophrenia. While the pro-cognitive effects of physical activity are widely recognised, the mechanisms that mediate them are not known (Firth et al. 2017a; Firth et al. 2017b). Upregulation of BDNF and increases in brain volume are the best-investigated phenomena in patients (Firth et al. 2017). In the experiment described in section 2.11.2, peripheral (plasma) BDNF was compared between the four experimental groups after exercise or the control condition. No significant differences were found and there was considerable variance between animals (Gonzalez et al. unpublished). An enzyme-linked immunosorbent assay (ELISA) was

run to measure BDNF in the brain. However, the samples had been prepared for the 'Wes' system and the protein concentrations were inadequate for accurate measurement. Still, BDNF levels cannot account for the improvement in cognition alone (Kimhy et al. 2015), suggesting that other mechanisms are involved. Previous work has shown that scPCP treated rats display deficits in glutamatergic and GABAergic pathways (Cochran et al. 2003; Jenkins et al. 2008; McKibben et al. 2010). The data presented here using western analysis do not provide evidence for changes in glutamatergic or GABAergic synaptic transmission in the scPCP rat model for schizophrenia.

Chapter 5 Exercise and detraining: effects on the NOR cognitive deficit in the subchronic PCP rat model for schizophrenia

5.1 Introduction

Schizophrenia is a disabling mental health issue that is costly not only to those who live with it, but to their family, carers and the global economy (Chong et al. 2016; Fasseeh et al. 2018). The cognitive deficits seen in schizophrenia are pervasive and persistent aspects of the disease and are the best predictors of quality of life and global functioning (Fujii et al. 2004; Milev et al. 2005; Green 2006; Agid et al. 2013). Despite this, there is currently no treatment for them (Young and Geyer 2015). The development of novel therapeutics for schizophrenia has been challenging with many of the front-line therapies discovered with some serendipity decades ago (Pieper and Baraban 2017). More recently came the development of second-generation antipsychotic (SGA) drugs. However, they generally function via 'me too' mechanisms and have not lived up to their promise of improved cognition (Conn et al. 2008; Swartz et al. 2008; Leucht et al. 2013). Schizophrenia is a particularly challenging disease to understand and treat, not least because it is multifaceted, complex and heterogeneous. While animal models can be valuable for understanding the behavioural and biological aspects of a disease (van der Staay et al. 2009) it is challenging to replicate a mental illness like schizophrenia. Schizophrenia-spectrum disorders are often described as the 'price' that humans pay for language and the abstract conceptualisation it allows (Crow 2000). It is therefore perhaps unsurprising that many of the core symptoms of schizophrenia revolve around abnormalities in thinking and speech (American Psychiatric

Association 2013), many of which are either difficult or impossible to measure in non-human animals (Powell and Miyakawa 2006). However, there are aspects of schizophrenia that can be modelled. For example, the development of the subchronic phencyclidine (scPCP) rat model for schizophrenia began when schizophrenia-like behaviours were seen in people who had ingested phencyclidine (PCP; Domino 1964; Neill et al. 2014). The model mimics aspects of the cognitive, anatomical and molecular abnormalities seen in schizophrenia (Ripke et al. 2014; Doostdar et al. 2019) and it has become a useful tool to understand these aspects of the disease. It can be used to investigate abnormalities of relevance to schizophrenia and to test potential therapies (Neill et al. 2010). It is possible to measure cognitive disturbances of relevance to schizophrenia in animals using carefully designed behaviour paradigms (Young et al. 2006; Young et al. 2012). For example, visual recognition memory is a cognitive domain disrupted in schizophrenia (Nuechterlein et al. 2005) and can be measured in rodents using the novel object recognition (NOR) task (Neill et al. 2010; Redrobe et al. 2010). The scPCP rat model for schizophrenia produces a reliable visual recognition deficit that is evident in the NOR paradigm (Grayson et al. 2007).

Evidence is accumulating that exercise can ameliorate some of the treatment-resistant symptoms for people with schizophrenia (Dauwan et al. 2016), including the quality-of-life-limiting (Fujii et al. 2004) cognitive deficits (Firth et al. 2017). Exercise has also been studied extensively in animals and improves performance on a variety of cognitive tasks (Berchtold et al. 2010). However, there has been little exploration of exercise in animal models for schizophrenia, with evidence for the effects on cognition in these models scarcer still. To date, there is no published

work on the effects of cognition in the scPCP rat model, or any rat model, for schizophrenia. There has also been no investigation of how long exercise influences cognition in an animal model of schizophrenia after detraining. Likewise, there is scant evidence for how long the pro-cognitive effects of exercise endure in people with schizophrenia (Su et al. 2016). Understanding the timeline by which exercise exerts its effect in schizophrenia may provide insights into both the mechanisms at play and the optimal 'dosage' and frequency (Berchtold et al. 2010). This study investigates the duration for which positive effects of exercise can be observed in the scPCP rat model for schizophrenia once exercise has ceased.

5.2 Methods

All animals underwent NOR before and after six weeks of access to freely turning or locked wheels (the control condition). Rats were then tested with the NOR paradigm after two weeks of rest (no access to wheels) and four weeks of rest. Full details of the methodology can be found in section 2.4.

5.3 Results

5.3.1 Time point one: baseline NOR task (NOR 2.1)

A novel object recognition test was run at baseline, i.e. time point one (NOR 2.1), at the end of the one week washout period. There were four experimental groups: vehicle control; vehicle exercise; scPCP control; or scPCP exercise (all n = 10). Rats were pseudo-randomly assigned to their experimental groups as described in section 2.3. Planned statistical analyses were performed as described in section 2.5.3.2.

Acquisition (NOR 2.1)

A two-way ANOVA revealed no interaction between the exploration of the objects (left and right) at the acquisition stage of the NOR test, $F_{(1,35)} = 0.34$, $p = 0.56$, and no interaction between object exploration and treatment group, $F_{(3,35)} = 1.29$, $p = 0.29$ (Figure 5.1).

Retention (NOR 2.1)

A two-way ANOVA revealed a significant difference between the objects in the retention phase of NOR, $F_{(1,35)} = 22.22$, $p < 0.001$, and no significant difference

between the groups, $F_{(3,35)} = 1.19$, $p = 0.33$. Post hoc Bonferroni-corrected paired sample t-tests were run. The vehicle control group explored the novel object significantly more than the familiar object (*MD* = 5.00, *SE* = 2.12), $p < 0.05$. The vehicle exercise group also favoured the novel object (*MD* = 7.70, *SE* = 2.00), $p < 0.001$. Neither the scPCP control group (*MD* = 3.8, *SE* = 2.00), $p = 0.07$, nor the scPCP exercise group (*MD* = 2.60, *SE* = 2.00), $p = 0.20$, differentiated between the novel and familiar objects (Figure 5.1).

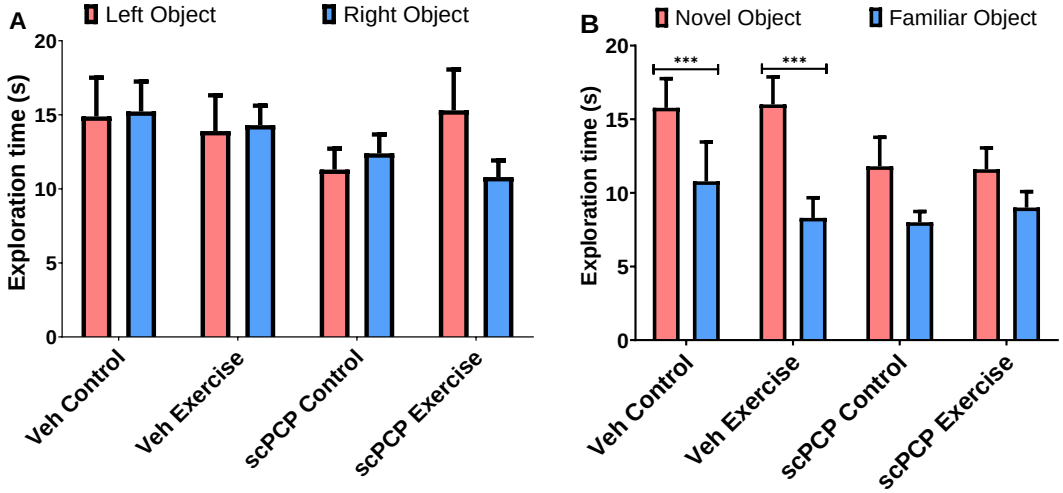


*Figure 5.1 Exploration time in seconds for the left and right objects during the acquisition stage (A), and the novel and familiar objects during the retention phase (B) of NOR 2.1. Data are presented as mean ± SEM (n = 9-10 per group), ***p < 0.001, two-way ANOVA and post hoc paired-sample t-test with Bonferroni correction. Vehicle is abbreviated to veh, subchronic phencyclidine to scPCP, and seconds to s.*

Discrimination Indices (NOR 2.1)

One-sample t-tests were run to understand whether the groups had a DI different from zero where zero indicated no preference between the novel and familiar object. The vehicle control (*M* = 0.23, *SD* = 0.20), $t_{(8)} = 3.48$, $p < 0.01$, and vehicle exercise (*MD* = 0.32, *SD* = 0.22) groups, $t_{(9)} = 4.54$, $p < 0.01$, both had a DI significantly greater than 0. This indicated a preference for the novel object. Neither the scPCP control group (*M* = 0.14, SD = 0.30), $t_{(9)} = 1.51$, $p = 0.17$, nor the scPCP

exercise group (*M* = 0.12, SD = 0.23), $t_{(9)}$ = 1.72, *p* = 0.12, had a DI that varied significantly from 0 (Figure 5.2).

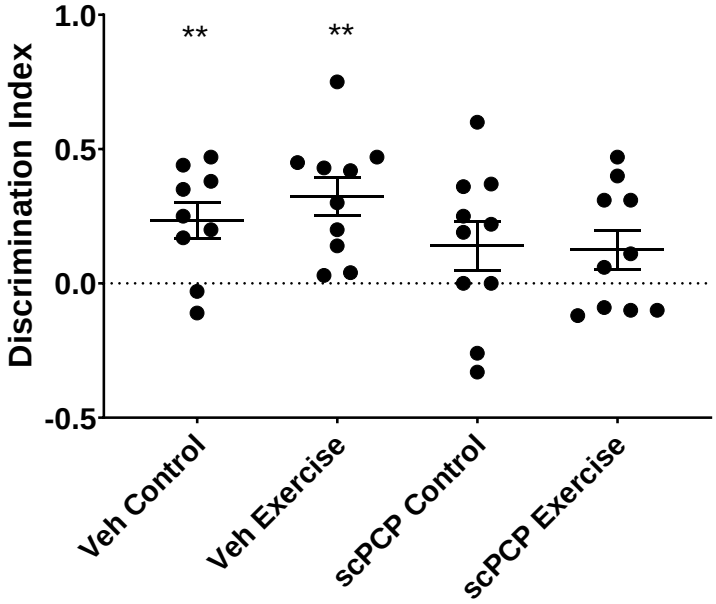


*Figure 5.2 Discrimination indices (DI) compared to 0 for the retention stage of NOR 2.1. Data are presented as mean ± SEM (n = 9-10 per group), **p < 0.01, independent sample t-tests. Vehicle is abbreviated to veh and subchronic phencyclidine to scPCP.*

A one-way ANOVA established that there were no significant differences between the groups' DI scores, $F_{(3,35)}$ = 1.48, *p* = 0.24 (data not shown).

Total exploration time (NOR 2.1)

Total exploration times for each stage of the NOR task were compared to investigate if there were significant differences between the groups. These measures were used to assess whether general exploratory behaviour was affecting the differences seen between object exploration in the NOR test. A one-way ANOVA indicated that there were no significant differences in total exploration time for the acquisition stage of NOR task, $F_{(3,35)}$ = 0.75, *p* = 0.53 (data not shown). Similarly, no effect was found in the retention part of the NOR test, $F_{(3,35)}$ = 1.56, *p* = 0.22 (data not shown).

5.3.2 Time point two: post-exercise NOR task (NOR 2.2)

Acquisition (NOR 2.2)

A two-way ANOVA showed no difference in the exploration of the objects (left and right) during the acquisition of the NOR task, $F_{(1,35)} = 0.004$, $p = 0.95$, as well as no interaction between object exploration and treatment group, $F_{(3,35)} = 0.67$, $p = 0.58$ (Figure 5.3).

Retention (NOR 2.2)

A two-way ANOVA revealed a significant effect of object in the retention stage of the NOR task, $F_{(1,35)} = 24.21$, $p < 0.001$, and no interaction between object and group, $F_{(3,35)} = 1.01$, $p = 0.40$. Post hoc t-tests with Bonferroni correction revealed that the vehicle control group spent significantly more time exploring the novel object than the familiar object, (*MD* = 3.40, *SE* = 1.47), $p < 0.05$. Similarly, the vehicle exercise group preferred to explore the novel more than the familiar object, (*MD* = 4.00, *SD* = 1.47), $p < 0.05$. The scPCP control group did not appear to differentiate between the novel and familiar objects (*MD* = 1.80, *SE* = 1.47), $p = 0.23$, whereas the scPCP exercise group spent longer exploring the novel versus the familiar object (*MD* = 5.44, *SD* = 1.55), $p < 0.01$ (Figure 5.3).

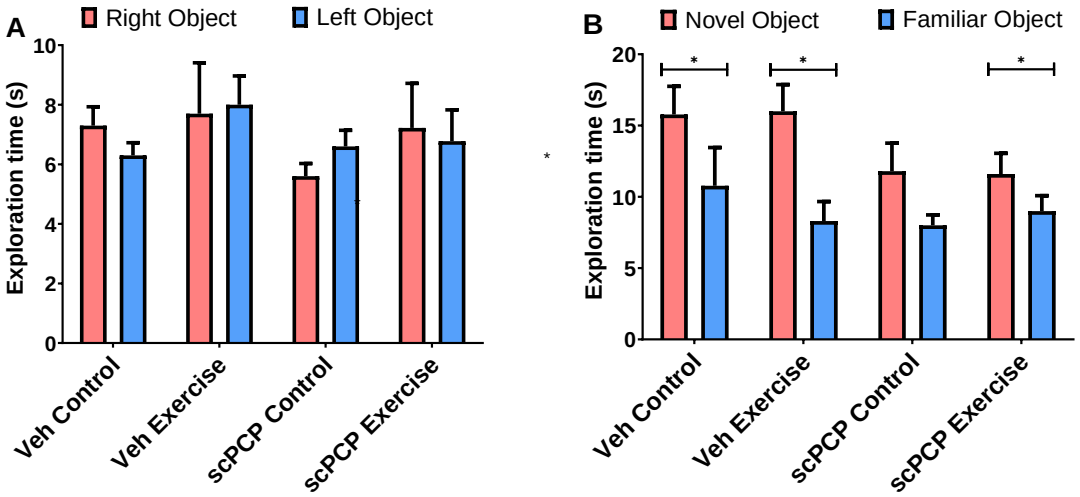


*Figure 5.3 Exploration time in seconds for the left and right objects during the acquisition stage (A), and the novel and familiar objects during the retention phase (B), of NOR 2.2. Data are presented as mean ± SEM (n = 9-10 per group), * p < 0.05, two-way ANOVA and paired-sample t-test with Bonferroni corrections. Vehicle is abbreviated to veh, subchronic phencyclidine to scPCP, and seconds to s.*

Discrimination Indices (NOR 2.2)

A one sample t-test compared the DI of the groups to 0. The vehicle control group had a significantly positive DI (M = 0.24, SD = 0.24), $t(9) = 3.06$, $p < 0.05$. The vehicle exercise group also had a statistically significant positive DI (M = 0.26, SD = 0.35), $t(9) = 4.54$, $p < 0.01$. The scPCP control group DI did not differ from zero (M = 0.15, SD = 0.22), $t(9) = 2.24$, $p = 0.52$, and the scPCP exercise group did (M = 0.30, SD = 0.23), $t(9) = 3.92$, $p < 0.01$ (Figure 5.4).

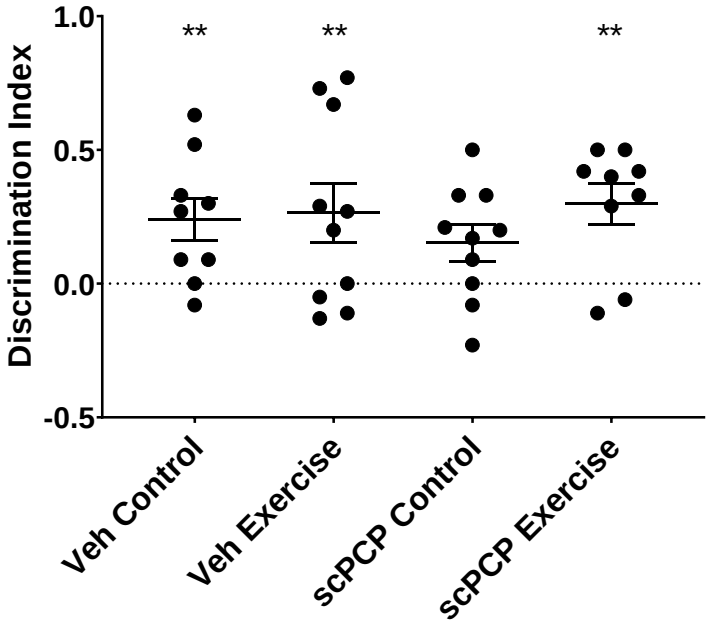


*Figure 5.4 Discrimination indices (DI) compared to 0 for the retention stage of the NOR task post-exercise (NOR 2.2). Data are presented as mean ± SEM (n = 8-10 per group), **p < 0.01, independent sample t-tests. Vehicle is abbreviated to veh and subchronic phencyclidine to scPCP.*

There were no significant differences between the DI scores of the groups, as determined by a one-way ANOVA, $F_{(3,37)} = 0.53$, p = 0.66 (data not shown).

Total exploration time (NOR 2.2)

The total exploration times for both stages of the NOR test were compared to rule out an effect of general exploration. Total exploration was used as a proxy for locomotor activity, and there were no differences between the groups at the acquisition phase, $F_{(3,35)} = 1.55$, $p = 0.22$, or the retention phase, $F_{(3,35)} = 0.49$, $p = 0.70$ (data not shown).

5.3.3 Time point three: NOR task after two weeks of exercise cessation (NOR 2.3)

Acquisition (NOR 2.3)

No significant effects were found between exploration times of left and right object in the acquisition phase of the NOR test, as determined by a two-way ANOVA, $F_{(1,32)} = 0.02$, $p = 0.90$. There was also no interaction between object exploration and treatment group, $F_{(3,32)} = 6.53$, $p = 2.54$, $p = 0.07$ (Figure 5.5).

Retention (NOR 2.3)

A two-way ANOVA identified a significant interaction for object exploration, $F_{(1,31)} = 36.92$, $p < 0.001$ but there was no group x object interaction, $F_{(3,31)} = 0.80$, $p = 0.50$. Post-hoc Bonferroni-corrected t-tests established that the vehicle control group explored the novel more than the familiar object (*MD* = 8.78, *SE* = 2.74), $p < 0.01$. The vehicle exercise group also preferred the novel object over the familiar (*MD* = 10.66, *SE* = 2.89), $p < 0.01$. The scPCP control group displayed an object recognition deficit and showed no significant difference in their exploration of the novel and familiar objects (*MD* = 5.16, *SE* = 3.07), $p = 0.10$. The scPCP exercise group did not have this deficit and explored the novel object for significantly longer than the familiar object (*MD* = 11.17, *SE* = 3.07), $p < 0.01$ (Figure 5.5).

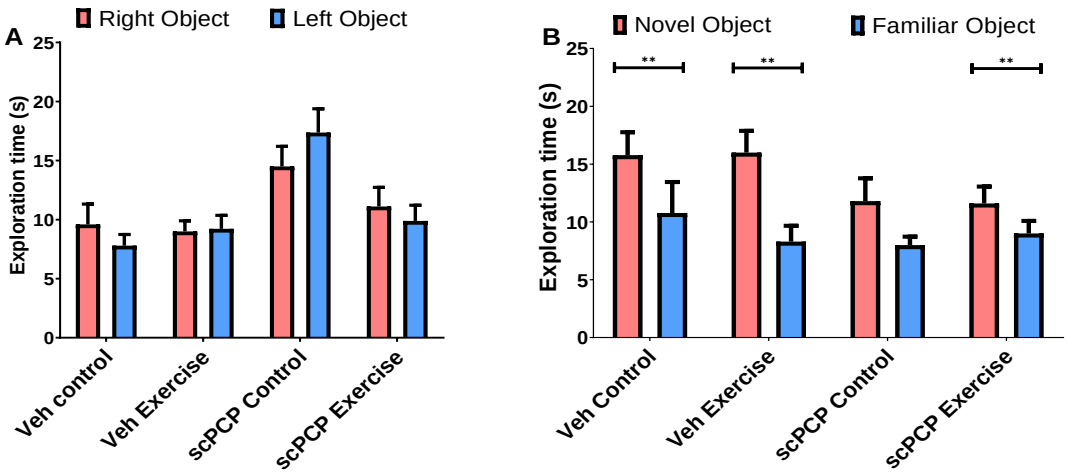


*Figure 5.5 Exploration time in seconds for the left and right objects during the acquisition stage (A), and the novel and familiar objects during the retention phase (B) of NOR 2.3. Data are presented as mean ± SEM (n = 8-10 per group), **p < 0.01, two-way ANOVA and paired-sample t-test with Bonferroni correction. Vehicle has been abbreviated to veh, subchronic phencyclidine to scPCP, and seconds to s.*

Discrimination Indices (NOR 2.3)

One sample t-tests were run to determine if the DI scores for each group differed significantly from zero. The vehicle control group differed significantly from zero (M = 0.49, SD = 0.24), $t(9) = 6.50$, $p < 0.001$. The vehicle exercise group also had a DI significantly higher than zero (M = 0.42, SD = 0.33), $t(8) = 3.87$, $p < 0.01$. The scPCP control group's DI was not significantly different to zero (M = 0.22, SD = 0.31), $t(7) = 2.01$, $p = 0.08$. The scPCP exercise group had a DI significantly different to zero (M = 0.49, SD = 0.22), $t(7) = 6.22$, $p < 0.001$ (Figure 5.5).

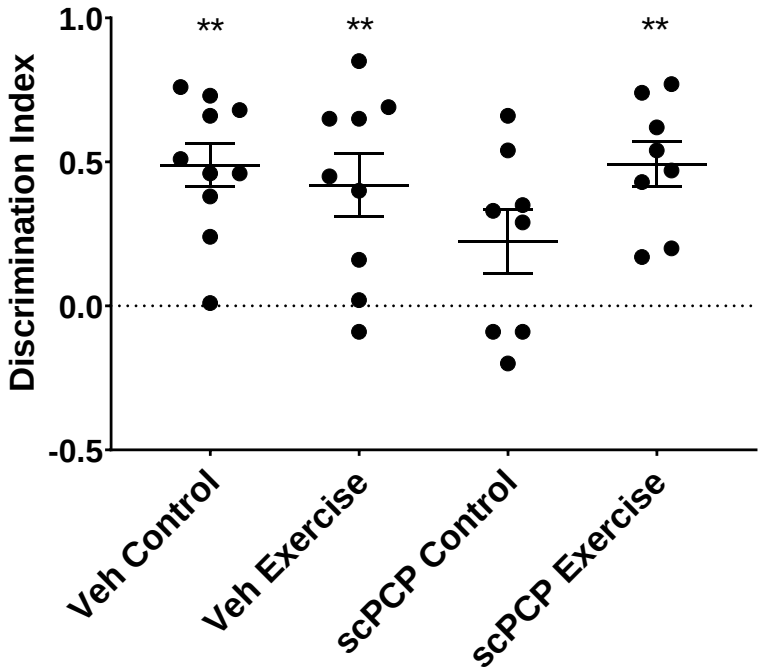


*Figure 5.6 Discrimination indices (DI) compared to 0 for the retention stage of NOR 2.3 task. Data are presented as mean ± SEM (n = 8-10 per group), **p < 0.01, independent sample t-tests. Vehicle is abbreviated to veh and subchronic phencyclidine to scPCP.*

A two-tailed one-way ANOVA revealed no significant differences in DI between the groups, $F(_{3,34}) = 1.67$, $p = 0.66$ (data not shown).

Total Exploration Time (NOR 2.3)

Total exploration time was compared between the groups to reveal if differences were due to abnormal exploratory behaviour. Total exploration time for the acquisition and retention stages of the NOR task were used to approximate locomotor activity in the absence of line-crossing data. A one-way ANOVA indicated significant differences in total exploration time for the acquisition phase of after two weeks of inactivity (NOR 2.3), $F(_{3,32}) = 8.40$, $p < 0.001$. The scPCP control group's total acquisition exploration time was significantly higher than each of the other groups as demonstrated by post hoc t-tests with Bonferroni corrections (Figure 5.7): vehicle control (*MD* = 14.64, *SE* = 3.47, $p < 0.05$); vehicle exercise (*MD* =

13.55, *SE* = 3.55, $p < 0.05$); scPCP exercise (*MD* = 15.64, *SE* = 3.55, $p < 0.05$; Figure 5.7). There were no significant differences between the groups at the retention stage of the NOR task, $F_{(3,34)} = 0.53$, $p = 0.67$ (data not shown).

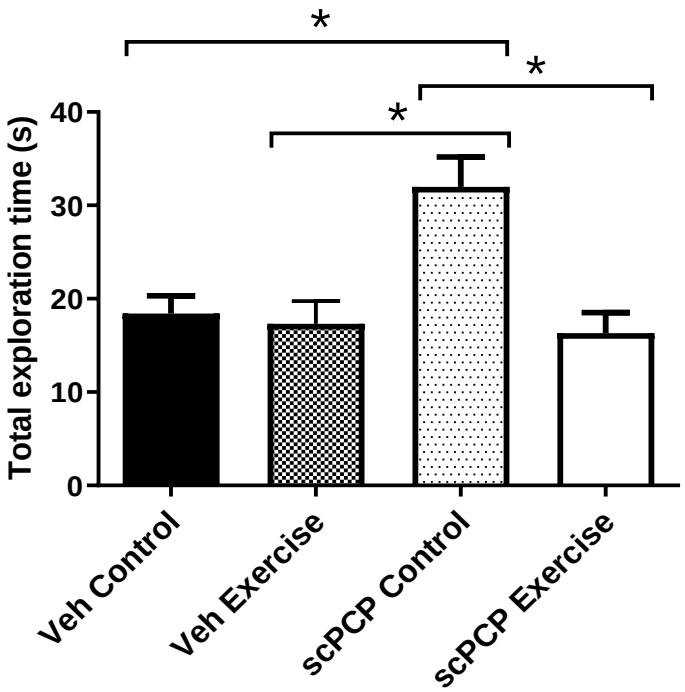


*Figure 5.7 NOR total exploration time in seconds during the acquisition stage (A), and the novel and familiar objects during the retention phase (B). Data are presented as mean ± SEM (n = 8-10 per group), *p < 0.05, two-way ANOVA and paired-sample t-test with Bonferroni correction. Vehicle is abbreviated to veh, subchronic phencyclidine to scPCP, and seconds to s.*

5.3.4 Time point four: NOR task after four weeks of exercise cessation (NOR 2.4)

Acquisition (NOR 2.4)

A two-way ANOVA indicated that there were no statistically significant differences between the exploration of the left and right objects during acquisition, $F_{(1,36)} = 0.01$, $p = 0.91$ (Figure 5.8). In addition to this, there was no significant interaction between object exploration and group membership, $F_{(3,36)} = 0.07$, $p = 0.79$ (data not shown).

Retention (NOR 2.4)

Statistically significant differences were found between the novel and familiar objects in the retention portion of the NOR task, $F_{(1,35)} = 12.19$, $p < 0.001$. There was a significant interaction of object exploration and group, $F_{(1,35)} = 8.72$, $p < 0.01$. Paired sample t-tests were run to establish the differences and a Bonferroni correction was applied. The vehicle control group demonstrated a difference in exploration between the novel and familiar object (*MD* = 9.78, *SE* = 2.61), $p < 0.01$. Similarly, the vehicle exercise group favoured the novel over the familiar object (*MD* = 6.20, *SE* = 2.48), $p < 0.05$. The scPCP control group showed no significant differentiation between the objects (*MD* = -2.80, *SE* = 2.48), $p = 0.27$. The scPCP exercise group also failed to discriminate between the novel and familiar objects (*MD* = 2.6, *SE* = 2.48), $p = 0.30$ (Figure 5.8). Exploration time appeared lower at this time point, which may be due to repeated behavioural testing of the animals.

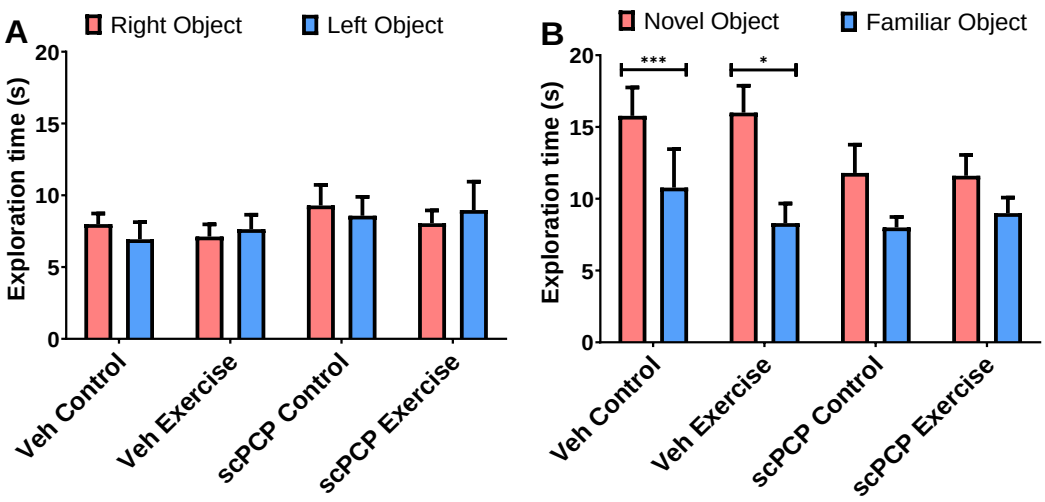


*Figure 5.8 Exploration time in seconds for the left and right objects during the acquisition stage (A), and the novel and familiar objects during the retention phase (B) of NOR 2.4. Data are presented as mean ± SEM (n = 9-10 per group), *p < 0.05, ***p < 0.001, two-way ANOVA and paired-sample t-test with Bonferroni correction. Vehicle is abbreviated to veh, subchronic phencyclidine to scPCP, the novel object recognition task to NOR, and seconds to s.*

Discrimination Indices (NOR 2.4)

The vehicle control group had a mean DI that significantly differed to zero (M = 0.48, SD = 0.31), $t(_8) = 4.68$, $p < 0.001$, as determined by a single sample t-test. This was also the case for the vehicle exercise group (M = 0.28, SD = 0.22), $t(_9) = 3.94$, $p <$ 0.01. The scPCP control group had a mean DI that was not significantly different to zero (M = -0.10, SD = 0.32), $t(_9) = -0.95$, $p = 0.37$. The scPCP exercise group also failed to show a significant difference in DI when compared to zero (M = 0.73, SD = 0.30), $t(_9) = 0.73$, $p = 0.37$ (Figure 5.9).

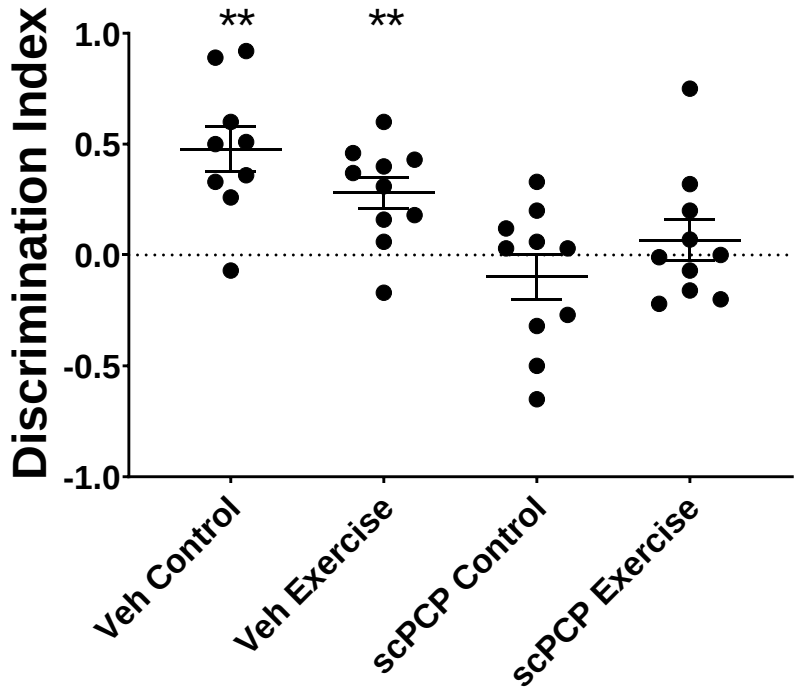


*Figure 5.9 Discrimination indices (DI) compared to 0 for the retention stage of the NOR task. Data are presented as mean ± SEM (n = 9-10 per group), **p < 0.01, independent sample t-tests. Vehicle is abbreviated to veh and subchronic phencyclidine to scPCP.*

A one-way ANOVA indicated a difference between the groups' DI, $F_{(3,35)} = 7.18$, $p < 0.01$. The vehicle groups did not differ from one another (*MD* = 0.20, *SE* = 0.13), $p = 0.84$. The vehicle control group differed from the scPCP control group (*MD* = 0.58, *SE* = 0.13), $p < 0.01$, and the scPCP exercise group (*MD* = 0.41, *SE* = 0.13), $p < 0.05$. The vehicle exercise group had significantly higher DI than the scPCP control group (*MD* = 0.38, *SE* = 0.13), $p < 0.05$, but not the scPCP exercise group (*MD* = 0.21, *SE* = 0.13), $p = 0.66$ (Figure 5.10).

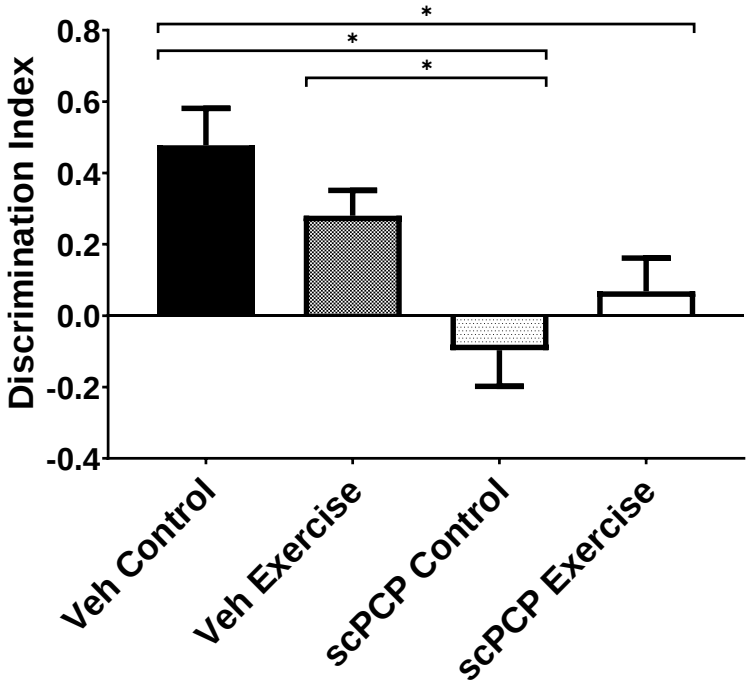


*Figure 5.10 Discrimination indices (DI) for the NOR 2.4. Data are presented as mean ± SEM (n = 9-10 per group), *p < 0.05, post hoc paired sample t-tests with Bonferroni correction. Vehicle is abbreviated to veh and subchronic phencyclidine to scPCP.*

Total Exploration Time (NOR 2.4)

Total exploration time was analysed as a proxy for locomotor activity in the absence of line-crossing data. They were examined to understand if the differences in object exploration were due to changes in general exploratory behaviour. There was no significant difference between the total acquisition exploration in the NOR test as demonstrated by a one-way two-tailed ANOVA, $F(_{3,36}) = 0.95$, $p = 0.66$ (data not shown). The same was true for the retention portion of the task, $F(_{3,35}) = 0.95$, $p = 0.43$ (data not shown).

5.3.5 Comparison of Discrimination Indices Across All Time points (NOR 2.1 to 2.4)

A two-way ANOVA was run to compare the DI scores between the time points (NOR 2.1 to 2.4). This analysis included planned post hoc paired sample t-tests with

Bonferroni adjustment. There was a significant effect of DI between the four time points, $F(_{3,78}) = 5.98$, $p < 0.01$. There was also a significant interaction of DI and group treatment, $F(_{9,78}) = 2.78$, $p < 0.05$. Post hoc paired sample t-tests with Bonferroni correction indicated no difference in DI between any of the time points for vehicle control animals. There was also no difference between any of the time points for the scPCP control group. There were no significant differences for the scPCP exercise group for NOR 2.1, 2.2 or 2.3. Table 5.1 summarises the results of the statistical tests for the vehicle control, vehicle exercise, and scPCP control group. However, there was a significant decrease in DI between NOR 2.3 (two weeks of rest) and 2.4 (four weeks of rest), for the scPCP exercise group (MD = 0.51, SE 0.14), $p < 0.05$ (Figure 5.11).

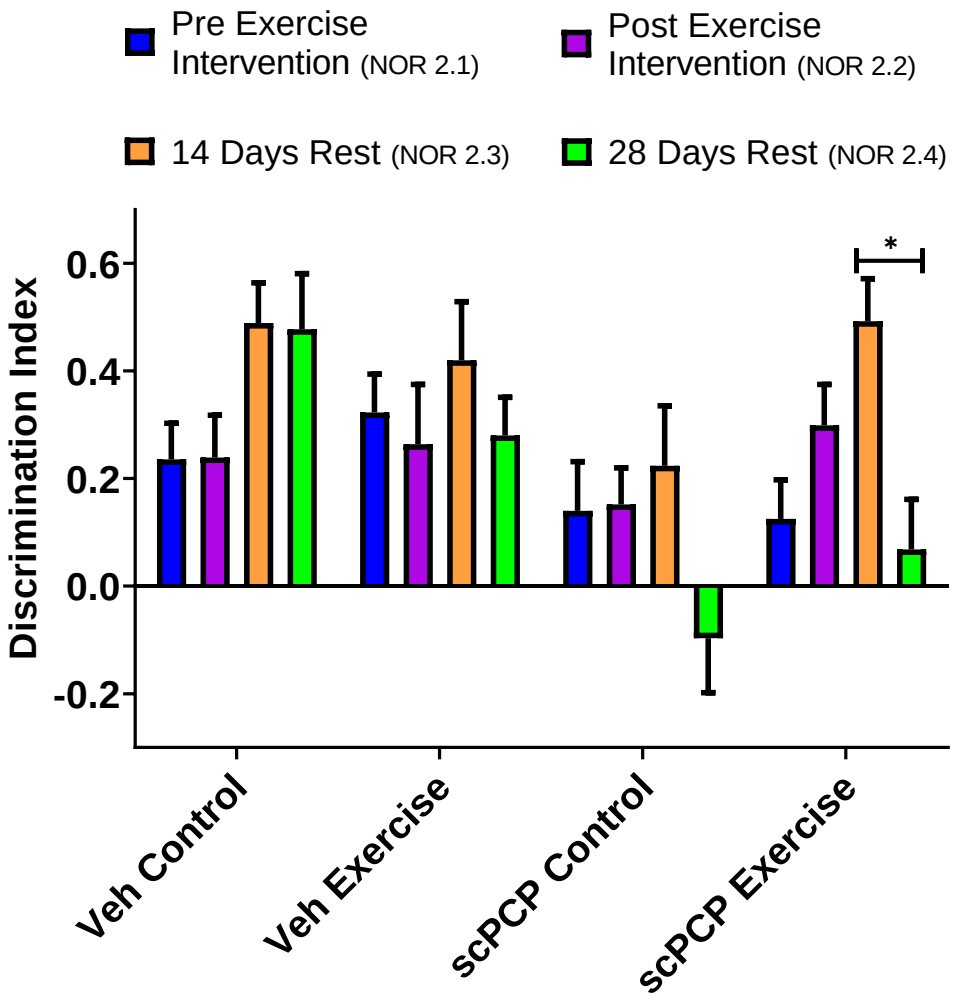


*Figure 5.11 A comparison of discrimination indices (DI) at each time point of the experiment (NOR 2.1 to NOR 2.4). Data are presented as mean ± SEM (n = 8-10 per group), *p < 0.05 paired sample t-tests with Bonferroni adjustment. Vehicle is abbreviated to veh, subchronic phencyclidine to PCP, and novel object recognition task to NOR.*

Table 5.1 Mean difference, standard error and p-value from Bonferroni corrected post hoc tests comparing discrimination indices for novel object recognition tasks 2.1, 2.2, 2.3 and 2.4.

		Baseline (NOR 2.1)			Post-Exercise (NOR 2.2)			14 Days Rest (NOR 2.3)			28 Days Rest (NOR 2.4)		
Group		MD	SE	p	MD	SE	p	MD	SE	p	MD	SE	p
Vehicle Control	Baseline (NOR 2.1)	-	-	-	-0.01	0.13	=1.00	0.26	0.12	=0.27	0.29	0.10	=0.06
	Post-Exercise (NOR 2.2)	0.01	0.13	=1.00	-	-	-	-0.28	0.11	=0.09	0.30	0.11	=0.07
	14 Days Rest (NOR 2.3)	-0.26	0.12	=0.27	-0.28	0.11	=0.09	-	-	-	-0.03	0.13	=1.00
	28 Days Rest (NOR 4.4)	-0.29	0.10	=0.06	-0.30	0.10	=0.07	-0.03	0.13	=1.00	-	-	-
Vehicle Exercise	Baseline (NOR 2.1)	-	-	-	-0.14	0.11	=1.00	0.07	0.11	=1.00	-0.06	0.09	=1.00
	Post-Exercise (NOR 2.2)	0.14	0.11	=1.00	-	-	-	0.20	0.09	0.24	0.07	0.10	=1.00
	14 Days Rest (NOR 2.3)	-0.07	0.11	=1.00	-0.20	0.09	=0.24	-	-	-	-0.13	0.12	=1.00
	28 Days Rest (NOR 4.4)	0.06	0.09	=1.00	-0.07	0.10	=1.00	0.13	0.12	=1.00	-	-	-

		Baseline (NOR 2.1)			Post-Exercise (NOR 2.2)			14 Days Rest (NOR 2.3)			28 Days Rest (NOR 2.4)		
Group		MD	SE	p	MD	SE	p	MD	SE	p	MD	SE	p
scPCP Control	Baseline (NOR 2.1)	-	-	-	0.05	0.12	=1.00	0.06	0.12	=1.00	-0.22	0.10	=0.17
	Post-Exercise (NOR 2.2)	-0.05	0.12	=1.00	-	-	-	-0.16	0.10	=1.00	-0.27	0.10	=0.09
	14 Days Rest (NOR 2.3)	-0.06	0.12	=1.00	-0.16	0.10	=1.00	-	-	-	0.29	0.12	0.16
	28 Days Rest (NOR 4.4)	0.22	0.10	0.17	0.27	0.10	=0.09	0.29	0.12	=0.16	-	-	-
scPCP Exercise	Baseline (NOR 2.1)	-	-	-	0.08	0.14	=1.00	0.30	0.13	=0.20	-0.21	0.11	=0.43
	Post-Exercise (NOR 2.2)	-0.84	0.14	=1.00	-	-	-	0.22	0.11	=0.40	0.30	0.12	=0.13
	14 Days Rest (NOR 2.3)	-0.30	0.13	=0.20	-0.22	0.11	=0.40	-	-	-	-0.51	0.14	<0.05
	28 Days Rest (NOR 4.4)	0.21	0.11	0.43	0.30	0.12	=0.13	0.51	0.14	<0.05	-	-	-

Mean difference is abbreviated to MD, standard error to SE, discrimination indices to DI, and novel object recognition test to NOR.

5.4 Discussion

This study extends the results of the first behavioural experiment, confirming that voluntary wheel running reverses the visual memory deficit in the scPCP rat model for schizophrenia. These results correspond with clinical research demonstrating that exercise is beneficial for cognition in schizophrenia (Firth et al. 2017) and further endorses the scPCP model's relevance for the disease. In addition to this, exercise continued to exert its pro-cognitive effects for at least two weeks after detraining, but by four weeks the NOR deficit had returned. This result is consistent with previous work demonstrating that cognition in normal rats (measured by the radial arm water maze) is improved for around two weeks after wheel running but returns to baseline at three to four weeks (Berchtold et al. 2010). The authors also demonstrated that hippocampal brain-derived neurotrophic factor (BDNF) was positively correlated with cognitive performance and continued to be upregulated in the brain for two weeks after exercise. Levels then dropped to those comparable to sedentary animals by four weeks of rest (Berchtold et al. 2010). As these behavioural results follow a similar temporal pattern BDNF may be the mechanism by which visual recognition memory is improved by exercise in the scPCP model for schizophrenia.

The scPCP rat model for schizophrenia is a useful tool to understand schizophrenia-like disturbances in brain and behaviour (Neill et al. 2014). The NOR task can be used to assess cognitive deficits of relevance to schizophrenia and our previous study demonstrated a positive effect of exercise on visual recognition memory. In this study, both vehicle groups spent significantly longer exploring the novel object

than the familiar object during the retention portion of the test at any time point, suggesting in-tact memory of the familiar object. The scPCP-treated rats showed a cognitive deficit in the NOR task demonstrated by no significant difference between the exploration of the novel and familiar object for either group. After access to freely turning or locked wheels, the vehicle groups continued to exhibit a preference for the novel object in the NOR task. The scPCP control group (that had access to only locked wheels) continued to display a deficit and showed no preference for the novel object. However, the scPCP exercise group (that had access to freely turning wheels) began to display a preference for the novel over the familiar object, spending significantly more time exploring it. This data suggests that voluntary wheel running can reverse the NOR cognitive deficit in the scPCP rat model for schizophrenia and corroborates the findings from the first study.

In this experiment, the investigation was extended to determine how long the pro-cognitive effects of exercise would last after exercise cessation. Rats were kept in their home cage with no access to wheels (locked or otherwise) to rest/detrain for 14 days. After this, they were tested with the NOR task again (NOR 2.3). The scPCP control (locked wheel) group continued to show no preference between the objects. The scPCP exercise (wheel running) group spent significantly longer exploring the novel object, suggesting they did not have a cognitive deficit in the NOR paradigm. The rats were then left to rest for another 14 days and underwent NOR 2.4. During the NOR task, the scPCP control group did not explore the novel significantly more than the familiar object, suggesting a cognitive deficit. The scPCP exercise group, that had now had no access to wheels for 28 days, failed to show a preference for the novel object in the retention portion of the NOR test. The results

of this experiment suggest that the visual recognition deficit in the scPCP rat model for schizophrenia can be rescued by voluntary exercise and that this effect continues for two weeks after access to wheels is removed. By four weeks of detraining, the positive effects of exercise on cognition were no longer present. These results have important implications for both clinical practice and research into exercise and schizophrenia. They suggest that future studies examining exercise and cognition in people with schizophrenia should include follow-up points. At follow-up, investigators should document the time since the participants' last session of exercise and whether and for how long the effects of exercise appear to last.

The cognitive deficits seen in schizophrenia are present before the onset of illness and do not respond to antipsychotic treatment (Agid et al. 2013). Exercise appears to improve cognition in schizophrenia but there is still a question as to how (Firth et al. 2017). It is also not known how long the pro-cognitive effects of exercise last, something that is relevant to both research and clinical practice (Firth et al. 2017). Like a prescribed medication that must be taken regularly, the positive effects of exercise appear to be mostly transient (Berchtold et al. 2005). Some headway has been made into understanding the time course of the effects of exercise on the brains of animals, but there is still a good deal of work to be done (Hopkins et al. 2011). From human studies, two prominent interrelated theories are described in the literature. These are that exercise stimulates neurogenesis, particularly in the hippocampus, and upregulates BDNF (Firth et al. 2017). It is hypothesised that one or both phenomena lead to an improvement in cognition. The evidence for exercise increasing either of these in schizophrenia is inconsistent, including some studies

demonstrating improvements in cognition in the absence of change in brain volume or increased BDNF (Firth et al. 2017).

Pragmatically, how exercise affects the brain and cognition in people with schizophrenia is unlikely to be as straight forward as modulation of one or two biological mechanisms. Exercise has a global effect on the body and brain and therefore influences a wide range of systems. It stimulates angiogenesis in several brain areas, including the hippocampus, and improves the efficiency of oxygen delivery to neurons, increasing their metabolic activity (Berchtold et al. 2010; Kaliman et al. 2011). Physical activity also influences the trophic environment in the brain, causing increases in BDNF, insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF; Cotman and Berchtold 2007a). These are mechanisms that promote healthy neuroplasticity, which is proposed to improve cognition in people with schizophrenia.

An ELISA was used to analyse BDNF in plasma taken from rats at each NOR time point. The results showed no significant differences between the groups of animals in BDNF in plasma (Gonzalez et al. unpublished). In addition to this, an ELISA was used to investigate BDNF in the brain. Unfortunately, the samples were homogenised for use in the 'Wes' automatic western blot system and were subsequently too dilute to quantify protein levels accurately. Numerous methodological issues can affect the sampling and analysis of BDNF, so much so that several papers have addressed many of them (Trajkovska et al. 2007; Elfving et al. 2010; Polacchini et al. 2015; Naegelin et al. 2018). The difficulty of accurately and consistently measuring BDNF in the periphery is well-known and there is also

debate on whether brain and circulatory BDNF are linked (Karege et al. 2002; Kyeremanteng et al. 2012). In this study, several methodological issues could have produced a false negative, i.e. no observed difference between groups of animals. The most likely include the purity of the plasma fraction, as this was variable but generally quite poor and the colour of plasma was not uniform. Furthermore, the blood samples were stored at 4 - 5°C before being centrifuged and, while this was part of a published protocol, it can have a substantial effect on the release of BDNF from platelets. It is also likely that the analysis was underpowered (each group n = 4 - 5) and evidence from humans suggests groups of 60 or more are necessary to detect moderate changes in peripheral BDNF in humans (Naegelin et al. 2018). In addition to these methodological issues, there is evidence that sex mediates the BDNF-exercise relationship. Females have a significantly lower increase in BDNF after exercise (Szuhany et al. 2015) as well as lower baseline levels (Kight and McCarthy 2017). Therefore, sex could be a covariate in the exercise-BDNF connection and examining exercise-related BDNF levels in female animals may add a layer of complexity not present in male animals. Future work should include both male and female animals to control for this.

This study demonstrates that voluntary wheel running reverses the NOR visual recognition task deficit in the scPCP rat model for schizophrenia. It supports the results reported in Chapter 3 that suggested that six weeks of exercise improves cognition in an animal model of relevance to schizophrenia. These data parallel research in patients that has established that exercise is effective at ameliorating cognitive deficits in schizophrenia (Firth et al. 2017). In addition to this, the positive effect lasted for at least 14 days, but not 28 days after scPCP rats had access to

wheels. The scPCP rat model for schizophrenia is well-validated and widely used (Cadinu et al. 2018). Moreover, PCP induces behaviours in humans that bear a striking resemblance to symptoms and deficits present in those with schizophrenia (Javitt 2015), conferring good predictive validity for animal models using PCP and testing behaviour (Jones et al. 2011). Therefore, evidence that exercise improves cognition in this model, and can do so for at least two weeks but returns to baseline by four, has important implications for psychiatric clinical practice.

Chapter 6 General discussion

6.1 Main findings and conclusions

This project investigated the effect of exercise on cognitive deficits in the subchronic phencyclidine (scPCP) rat model for schizophrenia. The studies described within this thesis represent the first investigation of the behavioural and neurophysiological consequences of exercise in a rat model for schizophrenia.

Rats were tested using the novel object recognition (NOR) paradigm before and after being given access to running wheels for six weeks. The brains of these animals were collected and used to investigate several synaptic and GABAergic markers of relevance to the pathology of schizophrenia. Tissue from the prefrontal cortex (PFC) and dorsal hippocampus was analysed using automated western blotting. In each brain area, two markers of synaptic and two markers of GABAergic function were investigated. Postsynaptic density protein 95 (PDS_{95}) and synapse-associated protein of 25 kDa ($SNAP_{25}$) were used as indicators of the density of glutamatergic synapses, and parvalbumin and glutamate decarboxylase 67 (GAD_{67}) were used to measure GABAergic interneurons.

A second behavioural study examined the temporal characteristics of exercise's influence on the NOR task deficit. It used the same exercise protocol in the same model. As in the previous experiment, exercise reversed the NOR task deficit in scPCP treated animals. After the initial six weeks, rats were not given access to wheels for the duration of the experiment. During this time they remained in their

home cages. Animals were then tested using the NOR paradigm at two weeks and four weeks of 'rest'. Blood was collected at each of the NOR time points, and brain-derived neurotrophic factor (BDNF) levels were analysed using an enzyme-linked immunoassay (ELISA).

The experiment described in chapter three indicates that exercise may improve cognitive deficits in the scPCP rat model for schizophrenia. More specifically, that wheel running nay be able to improve visual recognition memory in the NOR task. The improvement was suggested by a significant increase in discrimination index (DI) in the scPCP exercise group and no significant increase in the control group. On completion of the behavioural tasks, the brains of the animals were removed and analysed using western blotting and these data are presented in chapter four. No differences were found between treatment groups in the PFC for PSD_{95}, $SNAP_{25,}$ or parvalbumin. A significant effect of group was reported in levels of GAD_{67}, and further analysis revealed that the scPCP exercise group (animals treated with scPCP then given access to running wheels) had increased levels of GAD_{67} in the PFC compared to the other groups. In the dorsal hippocampus, PSD_{95}, $SNAP_{25}$, and GAD_{67} levels did not differ between the groups. An ANOVA returned a significant effect of treatment group for parvalbumin levels, but on comparing groups with corrected paired-sample t-tests, no significant differences were found. An ELISA was used to measure BDNF in PFC and dorsal hippocampal tissue but the protein in the samples was too dilute for an accurate reading.

In the first behavioural experiment, the data suggested that exercise may improve cognition in animals treated with scPCP and given access to wheels. The second

behavioural study provided further evidence that this was the case and sought to understand how long this effect lasted. An NOR test was run after two and four weeks of detraining. The scPCP exercise group were able to discriminate between the novel and the familiar object, with a preference for the novel, after two weeks. When retested at four weeks, the scPCP group failed to discriminate between the objects. These results established that the pro-cognitive effects of wheel running lasted for at least two weeks but were not evident after four weeks of sedentary behaviour.

To the author's knowledge, this is the first research into the effect of voluntary wheel running on cognition in the scPCP rat model for schizophrenia. Furthermore, it is the first time that exercise of any type has been explored in any rat model for schizophrenia. This is true for both behavioural and physiological investigations. There is one paper published regarding the effects of exercise on male mice treated subchronically with the N-methyl-D-aspartate receptor (NMDAR) antagonist MK-801 (dizocilpine) once daily for two weeks. Kim et al. (2014) compared four groups (n = 10), namely a control group of animals, an MK-801 treated group, an MK-801 group treated given access to running wheels, and an MK-801 group treated with aripiprazole as a positive control (see Table 6.1)**Error! Reference source not found.**. Each mouse was housed individually and wheels were in the home cage. The results of this study are summarised in Table 6.2. The authors concluded that MK-801 treatment-induced schizophrenia-like behaviour, cognitive deficits, and brain abnormalities, and that voluntary wheel running reduced them.

Table 6.1 Description of treatment group and experimental condition in Kim et al. (2014)

Group	**Treatment**
Control	Not reported
MK-801	Subchronic treatment with MK-801 for 2 weeks
MK-801 + wheel running	Subchronic treatment with MK-801 for 2 weeks, then 2 weeks running wheel in home cage
MK-801 + aripiprazole (positive control)	Subchronic treatment with MK-801 for 2 weeks, then treatment with aripiprazole (schedule not reported)

Table 6.2 A summary of results from Kim et al. (2014)

	'Schizophrenia-like' behaviour	**Cognition**	**NMDAR-positive cells**	**BDNF expression**
MK-801 (vs. control)	↑	↓	↓PFC/Hippo	↓
MK-801 + exercise (vs. MK-801)	↑	↑	↑PFC/Hippo	↑
MK-801 + aripiprazole (vs. MK-801)	↑	↑	↑PFC/Hippo	↑

'Schizophrenia-like behaviour' measure by locomotor activity in the open field test. 'Cognition' measured by time spent in the probe quadrant of the Morris water maze. NMDA receptor-positive cells were measured by immunofluorescence in the PFC and CA2-3 in the hippocampus. BDNF expression was measure using western blotting in the hippocampus. N-methyl-D-aspartate receptor is abbreviated to NMDAR, brain-derived neurotrophic factor to BDNF, prefrontal cortex to PFC, and hippocampus to hippo. A comparative increase is indicated by an upwards facing arrow and a comparative decrease by a downward pointing arrow.

There is an extensive body of work into the effects of exercise on the brains of both normal animals and preclinical models of disease including other neuropsychiatric disorders (van Praag et al. 1999a; van Praag et al. 1999b; Berchtold et al. 2001; Cotman et al. 2002; Kleim et al. 2002; Ding 2004; Berchtold et al. 2005; van Praag et al. 2005; Adlard et al. 2005; Cotman and Berchtold 2007a; Cotman and Berchtold 2007b; van Praag 2009; Ding et al. 2011; Harrison et al. 2013; Svensson et al. 2015). However, this is not the case for schizophrenia. Despite mounting evidence from clinical studies and meta-analyses that physical activity improves cognition in

people with schizophrenia (e.g. Firth et al. 2017a), the literature in animal models for schizophrenia is sparse at best (Wolf et al. 2011; Chung et al. 2014; Kim et al. 2014; Park et al. 2014),

The evidence for exercise as a treatment for cognitive deficits in schizophrenia continues to grow (Knöchel et al. 2012; Dauwan et al. 2016; Firth et al. 2017). Nevertheless, the mechanisms of the effects are unclear, something frequently highlighted in papers on the subject (Takahashi et al. 2012; Firth et al. 2017a; Firth et al. 2017b; Kim et al. 2019; Takahashi et al. 2019). Arguably, clinical research into exercise and cognition in schizophrenia is at an impasse due mainly to the contradictory evidence for increases in volume and BDNF levels (Vancampfort et al. 2014; Firth et al. 2017b). Numerous factors may contribute to the inconsistent evidence for the neurobiological effects of physical activity in schizophrenia. Issues include heterogeneity in both illness and patient characteristics (from baseline symptoms and disease duration, to medication and sex) and diverse exercise regimes (spanning treadmill running to Tai-Chi), may confound the effects exercise has in schizophrenia. Meta-analyses have identified multiple other factors that influence the effects of exercise in this population, such as the level and type of qualifications held by the instructor or supervisor of the activity (Vancampfort et al. 2016; Firth et al. 2017a). Besides these issues, there is the challenge that around one in three participants is non-compliant to exercise regimes (Seaton et al. 2001; Chakos et al. 2005; Miller et al. 2011 Clementz et al. 2016; Vancampfort et al. 2016; Firth et al. 2017a; Misiak et al. 2018; Maurus et al. 2019).

Animal models are useful tools for the investigation of disease (van der Staay 2006; van der Staay et al. 2009). Preclinical research affords greater control over experimental conditions and the ability to perform invasive investigation of molecular and structural changes in the brain (Vancampfort et al. 2014). Animal models of schizophrenia, such as the scPCP rat model, allow expedient and expeditious monitoring of disease progression. Moreover, these models facilitate the testing of novel treatments in a way not possible in humans (Jones et al. 2011).

The behavioural work in this thesis and subsequent research performed in the same lab provides evidence that exercise can improve cognition in the scPCP rat model for schizophrenia. In schizophrenia research, interventions shown to be efficacious for cognitive deficits in both humans and animals are rare. It is possible that this lack of commonality may contribute to the 'translational bottleneck' for cognitive treatments, as described in the literature (Hyman and Fenton 2003; Jones et al. 2011; Young and Geyer 2015). Therefore, the evidence that voluntary wheel running in the scPCP rat model for schizophrenia emulates exercise-mediated cognitive improvement in people with schizophrenia is an exciting development.

Using PCP to model for schizophrenia arose from observations that abuse of the drug produced schizophrenia-like behavioural and cognitive disturbances in healthy people, and exacerbated symptoms in patients. Consequently, this model has good predictive validity for the behaviours linked to the disorder. This work demonstrates that the scPCP rat model and the NOR cognitive test have predictive validity for treatment response to exercise. This novel finding provides an opportunity to investigate the procognitive mechanisms of exercise in a way that

reduces confounding factors and may accelerate discovery. Animal research will prove particularly useful for defining dosage and intensity of exercise required to ameliorate schizophrenia-like cognitive deficits. Furthermore, the ability to look at the effects in the brains of the animals could assist with the diminishing progress in human research. Novel candidate mechanisms could be investigated and identified faster, providing evidence-driven hypotheses that can be tested in a clinical sample.

6.2 Limitations and future work

Whilst there are benefits for studying psychiatric illness in animal models there are also inherent limitations. Modelling schizophrenia is challenging because it is a complex, heterogeneous, and arguably a uniquely human disorder (Neill et al. 2010). The scPCP model has good predictive validity for behaviour in humans (Yesavage and Freman 1978). However, it does not reproduce the genetic or neurodevelopmental aspects of schizophrenia. Future work should test wheel running in other models for schizophrenia such as gestational maternal immune activation, neonatal stress, and genetic knockouts (Jones et al. 2011).

Despite an effect in behaviour, no links to pathology were found. Synaptic and GABAergic markers were measured using the 'Wes' western blotting system. It was hypothesised that these measures would be lower in scPCP control animals, signifying synaptic dysfunction. Levels were not significantly lower and the variation between samples was considerable. However, demonstrating and replicating that wheel running improves cognition in the scPCP rat model allows for future work to include different methods of analysis such as immunohistochemistry or enzyme-

linked immunoassay (ELISA). Other markers could also be investigated such as tropomyosin receptor kinase B (TrkB), the receptor for BDNF (Soppet et al. 1991).

It was hypothesised that peripheral BDNF would be lower in sedentary animals, but no significant differences were found between the groups. Once again, there was substantial variation in individual animals and no effect was found. The difficulty of accurately measuring BDNF in the periphery is well known and there is debate as to whether levels in the blood reflect levels in the brain (Karege et al. 2002; Kyeremanteng et al. 2012). For example, even small changes in sample collection, storage and analysis drastically affect BDNF levels (Trajkovska et al. 2007) and concentrations reported in papers varies, depending on the timings and storage temperatures of different protocols (Polacchini et al. 2015). Furthermore, standardised commercially available ELISA kits are not consistent when measuring BDNF, even when other variables are carefully controlled (Polacchini et al. 2015). Measuring BDNF in a meaningful way is made more difficult by its transience (Sirianni et al. 2010). It has been shown that its half-life in plasma is less than 10 minutes (Pardridge et al. 1997), although it may be around an hour in the brain (Pan et al. 1998). Measuring upregulation of TrkB receptors, rather than BDNF itself, may overcome this issue as receptor expression is more stable. Effect sizes are also small. In humans, it is estimated that a sample size of 60 would be required to detect a 20% change in peripheral BDNF. The analysis in this project had an n of 4, meaning it was likely underpowered (Naegelin et al. 2018).

Finally, although there are several unknowns about the effect of sex on exercise-mediated BDNF changes, there is emerging evidence that human females have

significantly lower increases in BDNF after exercise (Szuhany et al. 2015) and lower baseline levels (Kight and McCarthy 2017). Animal work also suggests that exercise-induced BDNF increases may be dependent on oestrogen (Berchtold et al. 2001). However, this is not exclusively in females and the relationship is not well understood (Scharfman and MacLusky 2006). Nonetheless, this evidence suggests that sex could be a covariate in the exercise-BDNF connection. Therefore, examining exercise-related BDNF levels in female animals may have added an unknown variable and prospective studies should use both male and female animals to explore this relationship in greater detail.

In summary, demonstrating the procognitive effects of exercise in the scPCP rat model for schizophrenia signals an exciting new direction for preclinical research. Furthermore, subsequent studies in this lab have shown that exercise is neuroprotective, reducing the insult caused by scPCP treatment (unpublished data). Future work should examine wheel running in other animal models for schizophrenia such as gestational immune activation models. Experiments should also examine the relationship of sex, exercise and BDNF expression. As the literature grows, different types, durations, intensities of exercise can be compared, providing valuable evidence for both clinical trials and practice.

Chapter 7 References

Abdul-Monim, Z. et al. (2007). Sub-chronic psychotomimetic phencyclidine induces deficits in reversal learning and alterations in parvalbumin-immunoreactive expression in the rat. *Journal of Psychopharmacology*, 21(2), pp.198–205.

Adlard, P.A. et al. (2005). Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. *J. Neurosci.*, 25, pp.4217–4221 Available from: http://dx.doi.org/10.1523/JNEUROSCI.0496-05.2005.

Agid, O. et al. (2013). Early prediction of clinical and functional outcome in schizophrenia. *European Neuropsychopharmacology*, 23(8), pp.842–851. Available from: http://dx.doi.org/10.1016/j.euroneuro.2012.10.005.

Ahmed, A.O. and Bhat, I.A. (2014). Psychopharmacological treatment of neurocognitive deficits in people with schizophrenia: a review of old and new targets. *CNS Drugs*, 28(4), pp.301–318.

Akbarian, S. et al. (1993). Altered Distribution of Nicotinamide-Adenine Dinucleotide Phosphate—Diaphorase Cells in Frontal Lobe of Schizophrenics Implies Disturbances of Cortical Development. *Archives of General Psychiatry*, 50(3), pp.169–177.

Albert, N. et al. (2011). Predictors of recovery from psychosis: Analyses of clinical and social factors associated with recovery among patients with first-episode psychosis after 5years. *Schizophrenia Research*, 125(2), pp.257–266. Available from: https://doi.org/10.1016/j.schres.2010.10.013.

Alexandratos, K. et al. (2012). The impact of exercise on the mental health and quality of life of people with severe mental illness: a critical review. *British Journal of Occupational Therapy*, 75(2), pp.48–60. Available from: https://doi.org/10.4276/030802212X13286281650956.

Alloza, C. et al. (2016). Information processing speed mediates the relationship between white matter and general intelligence in schizophrenia. *Psychiatry research. Neuroimaging*, 254, pp.26–33.

American Psychiatric Association. (2013). Schizophrenia Spectrum and Other Psychotic Disorders. In *Diagnostic and Statistical Manual of Mental Disorders*. DSM Library. Washington, D.C.: American Psychiatric Association. Available from: https://doi.org/10.1176/appi.books.9780890425596.dsm02.

Anantharaman-Barr H.G. and Decombaz J. (1989). The effect of wheel running and the estrous cycle on energy expenditure in female rats. *Physiology and Behavior*, 46(2): pp.259-263. doi:10.1016/0031-9384(89)90265-5

Antonucci, F. et al. (2016). SNAP-25, a known presynaptic protein with emerging postsynaptic functions. *Frontiers in synaptic neuroscience*, 8(24), p.7.

Antunes, M. and Biala, G. (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cognitive Processing*, 13(2), pp.93–110. Available from: https://doi.org/10.1007/s10339-011-0430-z.

Archie, S. et al. (2003). Pilot Study: Access to Fitness Facility and Exercise Levels in Olanzapine-Treated Patients. *Canadian Journal of Psychiatry*, 48(9), pp.628–632. Available from: https://doi.org/10.1177/070674370304800910.

Arcoverde, C. et al. (2014). Treadmill training as an augmentation treatment for Alzheimer's disease: a pilot randomized controlled study. *Arquivos de Neuro-Psiquiatria*, 72(3), pp.190–196.

Arida, R.M. et al. (2004). Differential effects of spontaneous versus forced exercise in rats on the staining of parvalbumin-positive neurons in the hippocampal formation. *Neuroscience Letters*, 364(3): pp.135-138.

Auer, D.P. et al. (2001). Reduced NAA in the thalamus and altered membrane and glial metabolism in schizophrenic patients detected by 1H-MRS and tissue segmentation. *Schizophrenia Research*, 52(1–2), pp.87–99. Available from: https://doi.org/10.1016/S0920-9964(01)00155-4.

Balster, R.L. and Chait, L.D. (1976). The behavioral pharmacology of phencyclidine. *Clin Toxicol*, 9, pp.513–528.

Barnes, S.A. et al. (2015). Impaired limbic Cortico-striatal structure and sustained visual attention in a rodent model of schizophrenia. *International Journal of Neuropsychopharmacology*, 18(2), pp.1–12.

Barnett, J.H. et al. (2010). Assessing cognitive function in clinical trials of schizophrenia. *Neuroscience and Biobehavioral Reviews*, 34(8), pp.1161–1177. Available from: https://doi.org/10.1016/j.neubiorev.2010.01.012.

Beasley, C.L. et al. (2002). Selective deficits in prefrontal cortical gabaergic neurons in schizophrenia defined by the presence of calcium-binding proteins. *Biological Psychiatry*, 52(7), pp.708–715. Available from: https://doi.org/10.1016/S0006-3223(02)01360-4.

Beasley, C.L. and Reynolds, G.P. (1997). Parvalbumin-immunoreactive neurons are reduced in the prefrontal cortex of schizophrenics. *Schizophrenia Research*, 24(3), pp.349–355.

Beebe, L.H. et al. (2005). Effects of exercise on mental and physical health parameters of persons with schizophrenia. *Issues in Mental Health Nursing*, 26(6), pp.661–676. Available from: http://www.tandfonline.com/doi/abs/10.1080/01612840590959551.

Behere, R. V. et al. (2011). Effect of yoga therapy on facial emotion recognition deficits, symptoms and functioning in patients with schizophrenia. *Acta Psychiatrica Scandinavica*, 123(2), pp.147–153. Available from: http://dx.doi.org/10.1111/j.1600-0447.2010.01605.x.

Behrens, M.M. et al. (2007). Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. *Science (New York, N.Y.)*, 318(5856), pp.1645–1647.

Ben-Shachar, D. and Laifenfeld, D. (2004). Mitochondria, synaptic plasticity, and schizophrenia. *International Review of Neurobiology*, 59, pp.273–296.

Benes, F.M. et al. (1991). Deficits in small interneurons in prefrontal and cingulate

cortices of schizophrenic and schizoaffective patients. *Archives of general psychiatry*, 48(11), pp.996–1001.

Benes, F.M. (2000). Emerging principles of altered neural circuitry in schizophrenia. *Brain Research Reviews*, 31(2–3), pp.251–269. Available from: https://doi.org/10.1016/S0165-0173(99)00041-7.

Benes, F.M. (1997). Is there evidence for neuronal loss in schizophrenia? *International Review of Psychiatry*, 9(4), pp.429–436.

Benes, F.M. and Berretta, S. (2001). Gabaergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 25(1), pp.1–27.

Berchtold, N.C. et al. (2001). Estrogen and exercise interact to regulate brain-derived neurotrophic factor mRNA and protein expression in the hippocampus. *The European Journal of Neuroscience*, 14(12), pp.1992–2002. Available from: https://www.ncbi.nlm.nih.gov/pubmed/11860494.

Berchtold, N.C. et al. (2005). Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. *Neuroscience*, 133, pp.853–861. Available from: http://dx.doi.org/10.1016/j.neuroscience.2005.03.026.

Berchtold, N.C. et al. (2010). Exercise and time-dependent benefits to learning and memory. *Neuroscience*, 167(3), pp.588–597.

Berry, M. (2008). The potential of trace amines and their receptors for treating neurological and psychiatric diseases. *Reviews on Recent Clinical Trials*, 2(1), pp.3–19.

Bilder, R.M. et al. (2000). Neuropsychology of first-episode schizophrenia: initial characterization and clinical correlates. *American Journal of Psychiatry*, 157(4), pp.549–559.

Bio-Serve. (2018). Fat Rat Hut. Available from: http://www.wffisher.com/?Page_id=845 [Accessed 12th November 2018].

Black, J.E. et al. (1990). Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proceedings of the National Academy of Sciences of the United States of America*, 87(14), pp.5568–5572. Available from: http://dx.doi.org/10.1073/pnas.87.14.5568.

Boksa, P. (2012). Abnormal synaptic pruning in schizophrenia: Urban myth or reality? *Journal of Psychiatry and Neuroscience*, 37(2), pp.75–77.

Brondino, N. et al. (2017). A systematic review of cognitive effects of exercise in depression. *Acta Psychiatrica Scandinavica*, 135(4), pp.285–295.

Brugger, S.P. and Howes, O.D. (2017). Heterogeneity and homogeneity of regional brain structure in schizophrenia: A meta-analysis. *JAMA Psychiatry*, 74(11), pp.1104–1111.

Buckley, P.F. (2019). Neuroinflammation and schizophrenia. *Current Psychiatry Reports*, 21(8), p.72.

Bullock, W.M. et al. (2009). Schizophrenia-like gabaergic gene expression deficits in cerebellar Golgi cells from rats chronically exposed to low-dose phencyclidine. *Neurochemistry International*, 55(8), pp.775–782.

Burghardt, P.R. et al. (2004). The effects of chronic treadmill and wheel running on behavior in rats. Brain Research, 1019(1–2): pp.84-96.

Cadinu, D. et al. (2018). NMDA receptor antagonist rodent models for cognition in schizophrenia and identification of novel drug treatments, an update. *Neuropharmacology*, 142, pp.41–62.

Campos, C. et al. (2015). Feasibility and acceptability of an exergame intervention for schizophrenia. *Psychology of Sport and Exercise*, 19, pp.50–58.

Cardno, A.G. and Gottesman, I.I. (2000). Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. *American journal of medical genetics*, 97(1), pp.12–17. Available from: https://pubmed.ncbi.nlm.nih.gov/10813800.

Carroll, L.S. et al. (2009). Evidence that putative ADHD low risk alleles at SNAP25 may increase the risk of schizophrenia. *American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics*, 150(7), pp.893–899.

Cassilhas, R.C. et al. (2007). The impact of resistance exercise on the cognitive function of the elderly. *Medicine and science in sports and exercise*, 39(8), pp.1401–1407. Available from: https://pubmed.ncbi.nlm.nih.gov/17762374.

Catsicas, S. et al. (1991). Expression of a conserved cell-type-specific protein in nerve terminals coincides with synaptogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, 88(3), pp.785–789.

Catts, V.S. et al. (2015). Postsynaptic density levels of the NMDA receptor NR1 subunit and PSD-95 protein in prefrontal cortex from people with schizophrenia. *NPJ Schizophrenia*, 1(1), pp.1–8.

Chaddock-Heyman, L. et al. (2016). Aerobic fitness is associated with greater hippocampal cerebral blood flow in children. *Developmental Cognitive Neuroscience*, 20, pp.52–58.

Chaddock-Heyman, L. et al. (2018). Physical activity increases white matter microstructure in children. *Frontiers in Neuroscience*, 12, p.950. Available from: https://www.frontiersin.org/article/10.3389/fnins.2018.00950.

Chakos, M.H. et al. (2005). Duration of illness and treatment effects on hippocampal volume in male patients with schizophrenia. *British Journal of Psychiatry*, 186(1), pp.26–31. Available from: https://doi.org/10.1192/bjp.186.1.26.

Chen, Q.S. et al. (2000). Impairment of hippocampal long-term potentiation by Alzheimer amyloid β-peptides. *Journal of Neuroscience Research*, 60(1), pp.65–72. Available from: https://doi.org/10.1002/(SICI)1097-4547(20000401)60:1%3C65::AID-JNR7%3E3.0.CO;2-Q.

Chen, X. et al. (2008). Organization of the core structure of the postsynaptic density. *Proceedings of the National Academy of Sciences of the United States of America*, 105(11), pp.4453–4458.

Chen, X. et al. (2011). PSD-95 is required to sustain the molecular organization of the postsynaptic density. *Journal of Neuroscience*, 31(17), pp.6329–6338.

Cho, K.O., Hunt, C.A. and Kennedy, M.B. (1992). The rat brain postsynaptic density fraction contains a homolog of the drosophila discs-large tumor suppressor protein. *Neuron*, 9(5), pp.929–942.

Chong, H.Y. et al. (2016). Global economic burden of schizophrenia: a systematic review. *Neuropsychiatric Disease and Treatment*, 12, pp.357–373. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26937191.

Chung, J.W. et al. (2014). Treadmill exercise inhibits hippocampal apoptosis through enhancing N-methyl-D-aspartate receptor expression in the MK-801-induced schizophrenic mice. *Journal of Exercise Rehabilitation*, 10(4), pp.218–224. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25210696.

Clayton, J.A. and Collins, F.S. (2014). NIH to balance sex in cell and animal studies. *Nature*, 509(7500), pp.282–283.

Clementz, B.A. et al. (2016). Identification of distinct psychosis biotypes using brain-based biomarkers. *American Journal of Psychiatry*, 173(4), pp.373–384.

Cochran, S.M. et al. (2003). Induction of metabolic hypofunction and neurochemical deficits after chronic intermittent exposure to phencyclidine: Differential modulation by antipsychotic drugs. *Neuropsychopharmacology*, 28(2), pp.265–275.

Cohen, B.D. et al. (1960). Combined sernyl and sensory deprivation. *Comprehensive Psychiatry*, 1, pp.345–348.

Coley, A.A. and Gao, W.-J. (2018). PSD95: A synaptic protein implicated in schizophrenia or autism? *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 82, pp.187–194. Available from: https://doi.org/10.1016/j.pnpbp.2017.11.016.

Conn, P.J. et al. (2008). Schizophrenia: moving beyond monoamine antagonists. *Molecular interventions*, 8(2), pp.99–107.

Costall, B. and Naylor, R.J. (1995). Animal neuropharmacology and its prediction of clinical response. *Schizophrenia. Oxford: Blackwell Science*, 4, pp.401–424.

Cotman C.W. and Engesser-Cesar, C. Exercise enhances and protects brain function. *Exercise and Sport Science Reviews*, 30(2):75-79. doi:10.1097/00003677-200204000-00006.

Cotman, C.W. and Berchtold, N.C. (2002). Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends Neurosci.*, 25, pp.295–301. Available from: http://dx.doi.org/10.1016/S0166-2236(02)02143-4.

Cotman, C.W. and Berchtold, N.C. (2007a). Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci.*, 30, pp.464–472. Available from: http://dx.doi.org/10.1016/j.tins.2007.06.011.

Cotman, C.W. and Berchtold, N.C. (2007b). Physical activity and the maintenance of cognition: Learning from animal models. *Alzheimer's and Dementia*, 3(2 suppl.), pp.30–37.

Crabtree, G. and Gogos, J.A. (2014). Synaptic plasticity, neural circuits and the emerging role of altered short-term information processing in schizophrenia. *Frontiers in synaptic neuroscience*, 6(Oct), p.28. Available from: https://www.frontiersin.org/article/10.3389/fnsyn.2014.00028.

Cross, A.J et al. (1979). Gamma-aminobutyric acid in the brain in schizophrenia. *The Lancet*, 313(8115), pp.560–561. Available from: https://doi.org/10.1016/S0140-6736(79)90991-7.

Crow T. J. (2000). Schizophrenia as the price that homo sapiens pays for language: a resolution of the central paradox in the origin of the species. *Brain research. Brain research reviews*, 31(2-3), 118–129. https://doi.org/10.1016/s0165-0173(99)00029-6.

Danos, P. et al. (1998). Schizophrenia and anteroventral thalamic nucleus: Selective decrease of parvalbumin-immunoreactive thalamocortical projection neurons. *Psychiatry research - neuroimaging*, 82(1), pp.1–10.

Datesand Group UK. (2019). Play Tunnel. Available from: http://datesand.com/index.php/product/mouse-play-tunnel/ [Accessed 11th January 2019].

Datesand Group UK. (2016). Sizzle Nest. Available from: http://datesand.com/index.php/product/sizzle-nest/ [Accessed 11th January 2019].

Dauwan, M. et al. (2016). Exercise improves clinical symptoms, quality of life, global functioning, and depression in schizophrenia: A systematic review and meta-analysis. *Schizophrenia Bulletin*, 42(3), pp.588–599. Available from: https://doi.org/10.1093/schbul/sbv164.

Davies, L.M. et al. (2008). A randomized controlled trial of the cost-utility of second-generation antipsychotics in people with psychosis and eligible for clozapine. *Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research*, 11(4), pp.549–562.

Dawson, N. et al. (2014). Sustained NMDA receptor hypofunction induces compromised neural systems integration and schizophrenia-like alterations in functional brain networks. *Cerebral cortex*, 24(2), pp.452–464.

Deakin, B. et al. (2018). The benefit of minocycline on negative symptoms of schizophrenia in patients with recent-onset psychosis (benemin): a randomised, double-blind, placebo-controlled trial. *The Lancet Psychiatry*, 5(11), pp.885–894.

Deakin, J.F.W. and Simpson, M.D.C. (1997). A two-process theory of schizophrenia: Evidence from studies in post-mortem brain. *Journal of psychiatric research*, 31(2), pp.277–295.

Deary, I.J., Penke, L. and Johnson, W. (2010). The neuroscience of human intelligence differences. *Nature reviews neuroscience*, 11(3), pp.201–211.

Deicken, R.F., Pegues, M. and Amend, D. (1999). Reduced hippocampal N-acetylaspartate without volume loss in schizophrenia. *Schizophrenia Research*, 37(3), pp.217–223. Available from: https://doi.org/10.1016/S0920-9964(98)00173-X.

Delgado-Martínez, I., Nehring, R.B. and Sørensen, J.B. (2007). Differential abilities of SNAP-25 homologs to support neuronal function. *Journal of Neuroscience*, 27(35), pp.9380–9391.

Delisi, L.E. (1997). Is schizophrenia a lifetime disorder of brain plasticity, growth and aging? *Schizophrenia Research*, 23(2), pp.119–129. Available from: http://dx.doi.org/10.1016/S0920-9964(96)00079-5.

Dere, E., Huston, J.P. and De Souza Silva, M.A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neuroscience and biobehavioral reviews*, 31(1), pp.673–704.

Dickson, H. et al. (2012). Meta-analyses of cognitive and motor function in youth aged 16 years and younger who subsequently develop schizophrenia. *Psychological medicine*, 42(4), pp.743–755.

Diering, G.H. and Huganir, R.L. (2018). The AMPA receptor code of synaptic plasticity. *Neuron*, 100(2), pp.314–329. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30359599.

Dik, M.G. et al. (2007). Contribution of metabolic syndrome components to cognition in older individuals. *Diabetes care*, 30(10), pp.2655–2660.

Ding, Q., Ying, Z. and Gómez-Pinilla, F. (2011). Exercise influences hippocampal plasticity by modulating brain-derived neurotrophic factor processing. *Neuroscience*, 192, pp.773–780.

Ding, Y. (2004). Exercise pre-conditioning reduces brain damage in ischemic rats that may be associated with regional angiogenesis and cellular overexpression of neurotrophin. *Neurosci.*, 124, pp.583–591. Available from: http://dx.doi.org/10.1016/j.neuroscience.2003.12.029.

Domino, E.F. (1964). Neurobiology of phencyclidine (Sernyl), a drug with an unusual spectrum of pharmacological activity. *International review of neurobiology*, 6(C), pp.303–347.

Domino, E.F. and Luby, E.D. (2012). Phencyclidine/schizophrenia: One view toward the past, the other to the future. *Schizophrenia Bulletin*, 38(5), pp.914–919.

Doorduin, J. et al. (2009). Neuroinflammation in schizophrenia-related Psychosis: A PET study. *Journal of nuclear medicine*, 50(11), pp.1801–1807. Available from: http://jnm.snmjournals.org/content/50/11/1801.

Doostdar, N. et al. (2019). Global brain volume reductions in a sub-chronic phencyclidine animal model for schizophrenia and their relationship to recognition memory. *Journal of psychopharmacology*, 33(10), pp.1274–1287.

Draganski, B. and May, A. (2008). Training-induced structural changes in the adult human brain. *Behavioural brain research*, 192(1), pp.137–142.

Duffau, H. (2006). Brain plasticity: from pathophysiological mechanisms to therapeutic applications. *Journal of Clinical Neuroscience*, 13(9), pp.885–897.

Duffau, H. (2016). Brain Plasticity and Reorganization Before, During, and After Glioma Resection. In *Glioblastoma*. Elsevier, pp. 225–226. Available from: http://www.sciencedirect.com/science/article/pii/B9780323476607000185.

Eaton, W.W. et al. (2006). Association of schizophrenia and autoimmune diseases: linkage of Danish national registers. *American Journal of Psychiatry*, 163(3), pp.521–528.

Egan, M.F. et al. (2001). Relative risk for cognitive impairments in siblings of patients with schizophrenia. *Biological Psychiatry*, 50(2), pp.98–107. Available from: http://www.sciencedirect.com/science/article/pii/S0006322301011337.

Egbujo, C.N., Sinclair, D. and Hahn, C.G. (2016). Dysregulations of Synaptic Vesicle Trafficking in Schizophrenia. *Current Psychiatry Reports*, 18(8).

Egerton, A. et al. (2005). Impairment in perceptual attentional set-shifting following PCP administration: a rodent model of set-shifting deficits in schizophrenia. *Psychopharmacology*, 179(1), pp.77–84. Available from: https://doi.org/10.1007/s00213-004-2109-y.

Egerton, A. et al. (2008). Subchronic and chronic PCP treatment produces temporally distinct deficits in attentional set shifting and prepulse inhibition in rats. *Psychopharmacology*, 198(1), p.37.

Eisch, A.J. et al. (2008). Adult Neurogenesis, Mental Health, and Mental Illness: Hope or Hype? *Journal of Neuroscience*, 28(46), pp.11785–11791.

Elfving, B. et al. (2010). Detection of brain-derived neurotrophic factor (BDNF) in rat blood and brain preparations using ELISA: pitfalls and solutions. *Journal of Neuroscience Methods*, 187(1), pp.73–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20043947.

Ellison, G. and Sweet, R.A. (1993). Dissimilar patterns of degeneration in brain following four different addictive stimulants. *Neuroreport*, 5, pp.17–20. Available from: http://dx.doi.org/10.1097/00001756-199310000-00004.

Engelhardt, B. and Sorokin, L. (2009). The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction. *Seminars in Immunopathology*, 31(4), pp.497–511.

Ennaceur, A. and Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research*, 31(1), pp.47–59. Available from: http://www.sciencedirect.com/science/article/pii/016643288890157X.

Erard, R. et al. (1980). The PCP psychosis: Prolonged intoxication or drug-precipitated functional illness? *J Psychedelic Drugs*, 12, pp.235–245.

Van Erp, T.G.M. et al. (2018). Cortical Brain Abnormalities in 4474 Individuals With Schizophrenia and 5098 Control Subjects via the Enhancing Neuro Imaging Genetics Through Meta-Analysis (ENIGMA) Consortium. *Biological Psychiatry*, 84(9), pp.644–654.

Van Erp, T.G.M. et al. (2016). Subcortical brain volume abnormalities in 2028 individuals with schizophrenia and 2540 healthy controls via the ENIGMA consortium. *Molecular Psychiatry*, 21(4), pp.547–553.

Fan, X. et al. (2007). Inflammation and schizophrenia. *Expert Review of Neurotherapeutics*, 7(7), pp.789–796.

Fasseeh, A. et al. (2018). A systematic review of the indirect costs of schizophrenia in Europe. *European Journal of Public Health*, 28(6), pp.1043–1049. Available from: https://doi.org/10.1093/eurpub/cky231.

Fatemi, S.H. et al. (2001). Altered levels of the synaptosomal associated protein SNAP-25 in hippocampus of subjects with mood disorders and schizophrenia. *Neuroreport*, 12(15), pp.239–243. Available from: https://journals.lww.com/neuroreport/Fulltext/2001/10290/Altered_levels_of_the_synaptosomal_associated.23.aspx.

Fay, M.P. and Proschan, M.A. (2010). Wilcoxon-Mann-Whitney or t-test? On assumptions for hypothesis tests and multiple interpretations of decision rules. *Statistics surveys*, 4, pp.1–39. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20414472.

Fayed, N. et al. (2019). Neuroimaging Studies in Psychotic Disorders. In P. Á. Gargiulo & H. L. Mesones Arroyo, eds. *Psychiatry and Neuroscience Update*. Cham: Springer International Publishing, pp. 371–378. Available from: https://doi.org/10.1007/978-3-319-95360-1_30.

Feinberg, I. (1982). Schizophrenia: Caused by a fault in programmed synaptic elimination during adolescence? *Journal of Psychiatric Research*, 17(4), pp.319–334.

Ferreira-Junior, N.C. et al. (2019). Exercise training increases GAD65 expression, restores the depressed GABA(A) receptor function within the PVN and reduces sympathetic modulation in hypertension. *Physiological reports*, 7(13), pp.e14107–e14107. Available from: https://pubmed.ncbi.nlm.nih.gov/31264387.

Festing, M.F.W. and Altman, D.G. (2002). Guidelines for the design and statistical analysis of experiments using laboratory animals. *ILAR Journal*, 43(4), pp.244–258.

Firth, J. et al. (2015). A systematic review and meta-analysis of exercise interventions in schizophrenia patients. *Psychological Medicine*, 45(7), pp.1343–1361. Available from: https://www.cambridge.org/core/product/identifier/S0033291714003110/type/journal_article.

Firth, J. et al. (2016). The effects and determinants of exercise participation in first-episode psychosis: a qualitative study. *BMC Psychiatry*, 16(1), pp.1–9. Available from: http://dx.doi.org/10.1186/s12888-016-0751-7.

Firth, J. et al. (2017a). Aerobic exercise improves cognitive functioning in people with schizophrenia: a systematic review and meta-analysis. *Schizophrenia Bulletin*, 43(3), pp.546–556. Available from: http://dx.doi.org/10.1093/schbul/sbw115.

Firth, J. et al. (2017b). The pro-cognitive mechanisms of physical exercise in people with schizophrenia. *British Journal of Pharmacology*, 174(19), pp.3161–3172.

Available from: https://doi.org/10.1111/bph.13772.

Firth, J. et al. (2018a). Effect of aerobic exercise on hippocampal volume in humans: a systematic review and meta-analysis. *Neuroimage*, 166, pp.230–238.

Firth, J. et al. (2018b). Exercise as an intervention for first-episode psychosis: a feasibility study. *Early Intervention in Psychiatry*, 12(3), pp.307–315.

Van der Flier, W.M. et al. (2018). Vascular cognitive impairment. *Nature reviews. Disease primers*, 4, p.18003.

Floresco, S.B. et al. (2005). Developing predictive animal models and establishing a preclinical trials network for assessing treatment effects on cognition in schizophrenia. *Schizophrenia Bulletin*, 31(4), pp.888–894.

Fond, G. et al. (2013). A further evaluation of decision-making under risk and under ambiguity in schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience*, 263(3), pp.249–257. Available from: http://dx.doi.org/10.1007/s00406-012-0330-y.

Fong, A.Y. et al. (2005). Immunohistochemical localization of GAD67-expressing neurons and processes in the rat brainstem: subregional distribution in the nucleus tractus solitarius. *The Journal of comparative neurology*, 493(2), pp.274–290.

Fossati, G. et al. (2015). Reduced SNAP-25 increases PSD-95 mobility and impairs spine morphogenesis. *Cell Death and Differentiation*, 22(9), pp.1425–1436.

Fox, K.R. et al. (2000). The case for exercise in the promotion of mental health and psychological well-being. In S. Biddle, ed. *Physical Activity and Psychological Well-Being*. London: Routledge, pp. 205.

Friston, K.J. (2002). Dysfunctional connectivity in schizophrenia. *World psychiatry : official journal of the World Psychiatric Association (WPA)*, 1(2), pp.66–71. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1489874/pdf/wpa010066.pdf.

Frith, C.D. et al. (1995). Regional brain activity in chronic schizophrenic patients during the performance of a verbal fluency task. *British Journal of Psychiatry*, 167(Sept.), pp.343–349. Available from: https://www.cambridge.org/core/product/identifier/S0007125000064953/type/journal_article.

Fucetola, R. et al. (2000). Age and neuropsychologic function in schizophrenia: a decline in executive abilities beyond that observed in healthy volunteers. *Biological psychiatry*, 48(2), pp.137–146.

Fujii, D.E. et al. (2004). Neurocognition and long-term prediction of quality of life in outpatients with severe and persistent mental illness. *Schizophrenia Research*, 69(1), pp.67–73.

Funke, L. et al. (2005). Membrane-associated guanylate kinases regulate adhesion and plasticity at cell junctions. *Annual Review of Biochemistry*, 74(1), pp.219–245.

Ganguly, K. and Poo, M-M. (2013). Perspective Activity-Dependent Neural Plasticity from Bench to Bedside. *Neuron*, 80, pp.729–741. Available from:

http://dx.doi.org/10.1016/j.neuron.2013.10.028.

Garcia-Rizo, C. et al. (2012). Inflammatory markers in antipsychotic-naïve patients with nonaffective psychosis and deficit vs. Nondeficit features. *Psychiatry Research*, 198(2), pp.212–215.

Geyer, M.A. et al. (2012). From antipsychotic to anti-schizophrenia drugs: role of animal models. *Trends in Pharmacological Sciences*, 33(10), pp.515–521.

Giannini, A.J. et al. (1984). Comparison of Haloperidol and Chlorpromazine in the Treatment of Phencyclidine Psychosis. *The Journal of Clinical Pharmacology*, 24(4), pp.202–204.

Gigg, J. et al. (2020). Synaptic biomarker reduction and impaired cognition in the sub-chronic PCP mouse model for schizophrenia. *Journal of Psychopharmacology*, 34(1), pp.115–124. Available from: https://pubmed.ncbi.nlm.nih.gov/31580184.

Giles, E. D. et al. (2010). Effect of the estrous cycle and surgical ovariectomy on energy balance, fuel utilization, and physical activity in lean and obese female rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 299(6): R1634–R1642. https://doi.org/10.1152/ajpregu.00219.2010

Gleeson, M. et al. (2011). The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol*, 11(9), pp.607–615. Available from: http://dx.doi.org/10.1038/nri3041.

Goff, D.C. et al. (2005). Medical morbidity and mortality in schizophrenia: guidelines for psychiatrists. *The Journal of clinical psychiatry*, 66(2), pp.183–274. Available from: https://pubmed.ncbi.nlm.nih.gov/15705003.

Gogos, A. et al. (2012). The role of estrogen and testosterone in female rats in behavioral models of relevance to schizophrenia. *Psychopharmacology*, 219(1), pp.213–224. Available from: http://www.scopus.com/inward/record.url?Eid=2-s2.0-84856677505&partnerid=40&md5=4a321e4368aa8b3b7d700916354e554f.

Gogtay, N. (2008). Cortical Brain Development in Schizophrenia: Insights From Neuroimaging Studies in Childhood-Onset Schizophrenia. *Schizophrenia Bulletin*, 34(1), pp.30–36. Available from: https://doi.org/10.1093/schbul/sbm103.

Gold, J.M. et al. (2017). Selective attention, working memory, and executive function as potential independent sources of cognitive dysfunction in schizophrenia. *Schizophrenia Bulletin*, 44(6), pp.1227–1234. Available from: https://doi.org/10.1093/schbul/sbx155.

Gonzalez, A.J. et al. (2017). Brain-Derived Neurotrophic Factor and Exercise-Induced Reversal of Cognitive Deficit Symptoms of Relevance to Schizophrenia [Poster]. *From the London BPS summer meeting 2017: proceedings of the British Pharmacological Society at*. Available from: http://www.pa2online.org/abstracts/vol18issue1abst196p.pdf.

Goodwin, V.A. et al. (2008). The effectiveness of exercise interventions for people with Parkinson's disease: A systematic review and meta-analysis. *Movement Disorders*, 23(5), pp.631–640.

Gosso, M.F. et al. (2006). The SNAP-25 gene is associated with cognitive ability:

Evidence from a family-based study in two independent Dutch cohorts. *Molecular Psychiatry*, 11(9), pp.878–886.

Graphpad Software. (2019). Graphpad Prism version 8.2.1 for Windows. Available from: www.graphpad.com.

Grayson, B. et al. (2015). Assessment of disease-related cognitive impairments using the novel object recognition (NOR) task in rodents. *Behavioural Brain Research*, 285, pp.176–193. Available from: https://www.sciencedirect.com/science/article/pii/S0166432814006901.

Grayson, B. et al. (2014). The involvement of distraction in memory deficits induced by NMDAR antagonism: Relevance to cognitive deficits in schizophrenia. *Behavioural Brain Research*, 266, pp.188–192. Available from: http://dx.doi.org/10.1016/j.bbr.2014.03.011.

Grayson, B. (2012). Validation of an animal model of cognitive dysfunction associated with schizophrenia. Development and validation of the novel object recognition task using behavioural manipulations and psychotomimetic dosing regimens to induce cognitive deficits of relevance to schizophrenia. *University of Bradford*. Available from: http://hdl.handle.net/10454/5481.

Grayson, B. et al. (2007). Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. *Behavioural Brain Research*, 184(1), pp.31–38. Available from: http://www.sciencedirect.com/science/article/pii/S0166432807003208.

Green, M.F. et al. (2004a). Approaching a consensus cognitive battery for clinical trials in schizophrenia: The NIMH-MATRICS conference to select cognitive domains and test criteria. *Biological Psychiatry*, 56(5), pp.301–307. Available from: https://www.sciencedirect.com/science/article/pii/S0006322304007012.

Green, M.F. (2006). Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. *Journal of Clinical Psychiatry*, 67(SUPPL. 9), pp.3–8.

Green, M.F. (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *American Journal of Psychiatry*, 153(3), pp.321–330. Available from: https://www.scopus.com/inward/record.uri?Eid=2-s2.0-0029918481&partnerid=40&md5=8337f7535f349aa99ff923746f77b2ed.

Green, M.F. et al. (2004). Longitudinal studies of cognition and functional outcome in schizophrenia: Implications for MATRICS. *Schizophrenia Research*, 72(1), pp.41–51. Available from: https://www.sciencedirect.com/science/article/pii/S0920996404003445?Via%3Dihub.

Greifenstein, F.E. et al. (1958). A study of a 1-arylcyclohexylamine for anesthesia. *Anesthesia & Analgesia*, 37(5), pp.283–294. Available from: https://journals.lww.com/anesthesia-analgesia/Fulltext/1958/09000/A_STUDY_OF_A_1_ARYL_CYCLO_HEXYL_AMINE_FOR.7.aspx.

Griesbach et al. (2012). Differential effects of voluntary and forced exercise on stress responses after traumatic brain injury. *Journal of Neurotrauma*, 29, pp.1426-1433. Available from: http://dx.doi.org/10.1089/neu.2011.2229

Gu, Y. and Huganir, R.L. (2016). Identification of the SNARE complex mediating the exocytosis of NMDA receptors. *Proceedings of the National Academy of Sciences of the United States of America*, 113(43), pp.12280–12285.

Guidotti, A. et al. (2000). Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: A postmortem brain study. *Archives of General Psychiatry*, 57(11), pp.1061–1069.

Gururajan, A. et al. (2010). Current pharmacological models of social withdrawal in rats: Relevance to schizophrenia. *Behavioural Pharmacology*, 21(8), pp.690–709. Available from: http://www.scopus.com/inward/record.url?Eid=2-s2.0-78651439497&partnerid=40&md5=a9894785c78ed2fd2adead3e12d030e5.

Hagan, J.J. and Jones, D.N.C. (2005). Predicting drug efficacy for cognitive deficits in schizophrenia. *Schizophrenia Bulletin*, 31(4), pp.830–853. Available from: https://doi.org/10.1093/schbul/sbi058.

Haijma, S. V. et al. (2013). Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. *Schizophrenia Bulletin*, 39(5), pp.1129–1138.

Hajszan, T. et al. (2006). Subchronic phencyclidine treatment decreases the number of dendritic spine synapses in the rat prefrontal cortex. *Biol Psychiatry*, 60, pp.639–644. Available from: http://dx.doi.org/10.1016/j.biopsych.2006.03.015.

Hammond, R.S.et al. (2004). On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiology of Learning and Memory*, 82(1), pp.26–34.

Hanson, D.R. and Gottesman, I.I. (2005). Theories of schizophrenia: a genetic-inflammatory-vascular synthesis. *BMC Medical Genetics*, 6(1), p.7. Available from: https://doi.org/10.1186/1471-2350-6-7.

Harris, E.W. et al. (1984). Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. *Brain Research*, 323(1), pp.132–137.

Harrison, D.J. et al. (2013). Exercise attenuates neuropathology and has greater benefit on cognitive than motor deficits in the R6/1 Huntington's disease mouse model. *Experimental Neurology*, 248, pp.457–469.

Harvey, P.D. et al. (1998). Symptoms, Cognitive Functioning, and Adaptive Skills in Geriatric Patients With Lifelong Schizophrenia: A Comparison Across Treatment Sites. *American Journal of Psychiatry*, 155(8), pp.1080–1086. Available from: http://psychiatryonline.org/doi/abs/10.1176/ajp.155.8.1080.

Harvey, P.D. and Keefe, R.S.E. (2001). Studies of Cognitive Change in Patients With

Schizophrenia Following Novel Antipsychotic Treatment. *American Journal of Psychiatry*, 158(2), pp.176–184. Available from: http://psychiatryonline.org/doi/abs/10.1176/appi.ajp.158.2.176.

Hashimoto, T. et al. (2003). Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 23(15), pp.6315–6326.

Heaton, R.K. et al. (2001). Stability and course of neuropsychological deficits in schizophrenia. *Archives of general psychiatry*, 58(1), pp.24–32.

Heckers, S. et al. (1998). Impaired recruitment of the hippocampus during conscious recollection in schizophrenia. *Nature Neuroscience*, 1(4), pp.318–323.

Heggelund, J. et al. (2012). Therapeutic effects of maximal strength training on walking efficiency in patients with schizophrenia - A pilot study. *BMC Research Notes*, 5(1), p.1. Available from: BMC Research Notes.

Hennekens, C.H. et al. (2005). Schizophrenia and increased risks of cardiovascular disease. *American Heart Journal*, 150(6), pp.1115–1121.

Hidese, S. et al. (2017). Association between the scores of the Japanese version of the Brief Assessment of Cognition in Schizophrenia and whole-brain structure in patients with chronic schizophrenia: A voxel-based morphometry and diffusion tensor imaging study. *Psychiatry and clinical neurosciences*, 71(12), pp.826–835.

Hillman, C.H. et al. (2008). Be smart, exercise your heart: Exercise effects on brain and cognition. *Nature Reviews Neuroscience*, 9(1), pp.58–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18094706.

Hjorthøj, C. et al. (2017). Years of potential life lost and life expectancy in schizophrenia: a systematic review and meta-analysis. *The lancet. Psychiatry*, 4(4), pp.295–301. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28237639.

Ho, B.-C. et al. (2011). Long-term Antipsychotic Treatment and Brain Volumes. *Archives of General Psychiatry*, 68(2), p.128.

Ho, R.T.H. et al. (2016). A randomized controlled trial on the psychophysiological effects of physical exercise and Tai-chi in patients with chronic schizophrenia. *Schizophrenia Research*.

Hofer, A. et al. (2005). Patient outcomes in schizophrenia II: the impact of cognition. *European Psychiatry*, 20(5–6), pp.395–402. Available from: http://www.sciencedirect.com/science/article/pii/S0924933805000520.

Hoff, A.L. et al. (2005). Ten year longitudinal study of neuropsychological functioning subsequent to a first episode of schizophrenia. *Schizophrenia Research*, 78(1), pp.27–34. Available from: http://www.sciencedirect.com/science/article/pii/S0920996405002033.

Home Office (UK). (1986). *Animals (Scientific Procedures) Act 1986*. UK: The National Archives, UK. Available from: https://www.legislation.gov.uk/ukpga/1986/14 [Accessed 18th June 2019].

Hopkins, M.E. et al. (2011). Physical exercise during adolescence versus adulthood:

Differential effects on object recognition memory and brain-derived neurotrophic factor levels. *Neuroscience*, 194, pp.84–94.

Howes, O. et al. (2015). Glutamate and dopamine in schizophrenia: An update for the 21st century. *Journal of Psychopharmacology*, 29(2), pp.97–115. Available from: https://doi.org/10.1177/0269881114563634.

Howes, O.D. and Kapur, S. (2009). The dopamine hypothesis of schizophrenia: Version III - The final common pathway. *Schizophrenia Bulletin*, 35(3), pp.549–562.

Hubbard, L. et al. (2015). Evidence of common genetic overlap between schizophrenia and cognition. *Schizophrenia Bulletin*, 42(3), pp.832–842. Available from: https://doi.org/10.1093/schbul/sbv168.

Hughes, J.R. (1958). Post-tetanic potentiation. *Physiological Reviews*, 38(1), pp.91–113.

Hunt, C.A. (1996). PSD-95 is associated with the postsynaptic density and not with the presynaptic membrane at forebrain synapses. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 16(4), pp.1380–1388. Available from: https://pubmed.ncbi.nlm.nih.gov/8778289.

Hunter, R. and Barry, S. (2012). Negative symptoms and psychosocial functioning in schizophrenia: Neglected but important targets for treatment. *European Psychiatry*, 27(6), pp.432–436. Available from: http://www.sciencedirect.com/science/article/pii/S0924933811000459.

Hyman, S.E. and Fenton, W.S. (2003). What are the right targets for psychopharmacology? *Science*, 299(5605), pp.350 LP – 351. Available from: http://science.sciencemag.org/content/299/5605/350.abstract.

IBM Corp. (2015). IBM SPSS Statistics for Windows. Available from: https://www.ibm.com/support/pages/ibm-spss-statistics-25-documentation.

IBM Corp. (2017). IBM SPSS Statistics for Windows. Available from: https://www.ibm.com/support/pages/ibm-spss-statistics-25-documentation.

Idris, N. et al. (2010). Sertindole improves sub-chronic PCP-induced reversal learning and episodic memory deficits in rodents: involvement of 5-HT 6 and 5-HT 2A receptor mechanisms. *Psychopharmacology*, 208(1), p.23.

Isaacs, K.R. et al. (1992). Exercise and the Brain: Angiogenesis in the Adult Rat Cerebellum after Vigorous Physical Activity and Motor Skill Learning. *Journal of Cerebral Blood Flow & Metabolism*, 12(1), pp.110–119. Available from: https://doi.org/10.1038/jcbfm.1992.14.

Iwashiro, N. et al. (2012). Localized gray matter volume reductions in the pars triangularis of the inferior frontal gyrus in individuals at clinical high-risk for psychosis and first episode for schizophrenia. *Schizophrenia Research*, 137(1–3), pp.124–131.

Javitt, D.C. (2015). Substance Abuse: Phencyclidine Use Disorders. In A. Tasman et al., eds. *Psychiatry, Volume 2*. John Wiley & Sons, pp. 1470–1478. Available from: https://ebookcentral.proquest.com/lib/manchester/reader.action?Docid=1895577&ppg=1520.

Jenkins, T.A. et al. (2008). Disturbances in social interaction occur along with pathophysiological deficits following sub-chronic phencyclidine administration in the rat. *Behavioural Brain Research*, 194(2), pp.230–235. Available from: http://www.scopus.com/inward/record.url?Eid=2-s2.0-50549102891&partnerid=40&md5=5b63fda5015961e56f6432c8cf4df60c.

Jentsch, J.D. and Roth, R.H. (1999). The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology*, 20(3), pp.201–225.

Jones, C.A. (2011). Animal models of schizophrenia. *British journal of pharmacology*, 164(4), pp.1162–1194. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21449915.

Jouroukhin, Y. et al. (2016). Modeling gene-environment Interaction in Schizophrenia. In M. V. Pletnikov & J. Waddington, eds. *Handbook of Behavioral Neuroscience*. Academic Press, pp. 345–360. Available from: https://www.sciencedirect.com/handbook/handbook-of-behavioral-neuroscience/vol/23/suppl/C.

Jurado, S. et al. (2013). LTP Requires a Unique Postsynaptic SNARE Fusion Machinery. *Neuron*, 77(3), pp.542–558.

Kadra, G. et al. (2018). Long-term antipsychotic polypharmacy prescribing in secondary mental health care and the risk of mortality. *Acta Psychiatrica Scandinavica*, 138(2), pp.123–132. Available from: https://pubmed.ncbi.nlm.nih.gov/29845597.

Kaliman, P. et al. (2011). Neurophysiological and epigenetic effects of physical exercise on the aging process. *Ageing Research Reviews*, 10(4), pp.475–486.

Kalinichev, M. et al. (2008). Comparison between intraperitoneal and subcutaneous phencyclidine administration in Sprague-Dawley rats: A locomotor activity and gene induction study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 32(2), pp.414–422.

Kanahara, N. et al. (2013). Onset Pattern and Long-Term Prognosis in Schizophrenia: 10-Year Longitudinal Follow-Up Study. *PloS one*, 8(6), p.e67273.

Kaneko, K. (2018). Negative Symptoms and Cognitive Impairments in Schizophrenia: Two Key Symptoms Negatively Influencing Social Functioning. *Yonago Acta Medica*, 61(2), pp.91–102. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29946215.

Karege, F., Schwald, M. and Cisse, M. (2002). Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neuroscience Letters*, 328(3), pp.261–264.

Karlsen, A.S. et al. (2013). Expression of presynaptic markers in a neurodevelopmental animal model with relevance to schizophrenia. *Neuroreport*, 24(16), pp.928–933.

Karson, C.N. et al. (1999). Alterations in synaptic proteins and their encoding mRNAs in prefrontal cortex in schizophrenia: A possible neurochemical basis for

'hypofrontality'. *Molecular Psychiatry*, 4(1), pp.39–45.

Ke Z. et al. (2011). The effects of voluntary, involuntary, and forced exercises on motor recovery in a stroke rat model. *Conf Proc IEEE Annual International Conference of the IEEE Engineering in Medicine and Biology Society*. pp.8223-8226. doi:10.1109/IEMBS.2011.6092028

Keefe, R.S.E. et al. (2007). Neurocognitive effects of antipsychotic medications in patients with chronic schizophrenia in the CATIE trial. *Archives of General Psychiatry*, 64(6), pp.633–647. Available from: https://dx.doi.org/10.1001/archpsyc.64.6.633.

Keefe, R.S.E. (2008). Should cognitive impairment be included in the diagnostic criteria for schizophrenia? *World psychiatry : official journal of the World Psychiatric Association (WPA)*, 7(1), pp.22–28. Available from: https://www.ncbi.nlm.nih.gov/pubmed/18458774.

Keefe, R.S.E. and Harvey, P.D. (2012). Cognitive impairment in schizophrenia. In M. A. Geyer & G. Gross, eds. *Novel Antischizophrenia Treatments*. Berlin: Springer Berlin Heidelberg, pp. 11–37. Available from: https://link.springer.com/chapter/10.1007%2F978-3-642-25758-2_2.

Keilhoff, G. et al. (2004). Repeated application of ketamine to rats induces changes in the hippocampal expression of parvalbumin, neuronal nitric oxide synthase and CFOS similar to those found in human schizophrenia. *Neuroscience*, 126(3), pp.591–598.

Keller-Varady, K. et al. (2018). A systematic review of trials investigating strength training in schizophrenia spectrum disorders. *Schizophrenia Research*, 192, pp.64–68.

Kelly, S. et al. (2018). Widespread white matter microstructural differences in schizophrenia across 4322 individuals: results from the ENIGMA Schizophrenia DTI Working Group. *Molecular psychiatry*, 23(5), pp.1261–1269.

Kent, S. et al. (1991). Interactions between body temperature and wheel running over the estrous cycle in rats. *Physiology and Behavior*, 49(6): pp.1079-84. doi:10.1016/0031-9384(91)90334-k

Keshavan, M.S. et al. (2008). Schizophrenia, `just the facts': what we know in 2008 Part 3: neurobiology. *Schizophr Res*, 106, pp.89–107. Available from: http://dx.doi.org/10.1016/j.schres.2008.07.020.

Khandaker, G.M. et al. (2012). Childhood infection and adult schizophrenia: A meta-analysis of population-based studies. *Schizophrenia Research*, 139(1–3), pp.161–168.

Khandaker, G.M. et al. (2015). Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. *The Lancet Psychiatryancet. Psychiatry*, 2(3), pp.258–270.

Khandaker, G.M. et al. (2013). Prenatal maternal infection, neurodevelopment and adult schizophrenia: A systematic review of population-based studies. *Psychological Medicine*, 43(2), pp.239–257.

Kight, K.E. and McCarthy, M.M. (2017). Sex differences and estrogen regulation of BDNF gene expression, but not propeptide content, in the developing hippocampus. *Journal of neuroscience research*, 95(1–2), pp.345–354. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27870444.

Kim, E. and Sheng, M. (2004). PDZ domain proteins of synapses. *Nature Reviews Neuroscience*, 5(10), pp.771–781.

Kim, H.J. et al. (2014). Increase of circulating BDNF levels and its relation to improvement of physical fitness following 12 weeks of combined exercise in chronic patients with schizophrenia: a pilot study. *Psychiatry Research*, 220(3), pp.792–796.

Kim, J. et al. (2019). Physical performance is more strongly associated with cognition in schizophrenia than psychiatric symptoms. *European Psychiatry*, 61, pp.72–78. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0924933819301087.

Kim, T.-W. et al. (2014). Voluntary wheel running ameliorates symptoms of MK-801-induced schizophrenia in mice. *Molecular Medicine Reports*, 10(6), pp.2924–2930.

Kimhy, D. et al. (2015). The impact of aerobic exercise on brain-derived neurotrophic factor and neurocognition in individuals with schizophrenia: A single-blind, randomized clinical trial. *Schizophrenia Bulletin*, 41(4), pp.859–868.

Kirchner, S.K. et al. (2018). Polygenic analysis suggests the involvement of calcium signaling in executive function in schizophrenia patients. *European Archives of Psychiatry and Clinical Neuroscience*, pp.1–7.

Kirschner, M., Aleman, A. and Kaiser, S. (2017). Secondary negative symptoms — A review of mechanisms, assessment and treatment. *Schizophrenia Research*, 186, pp.29–38. Available from: https://www.sciencedirect.com/science/article/pii/S0920996416302249.

Kitchen, H. et al. (2012). Cognitive Impairment Associated with Schizophrenia: A Review of the Humanistic Burden. *Advances in Therapy*, 29(2), pp.148–162. Available from: https://doi.org/10.1007/s12325-012-0001-4.

Kleim, J.A., Cooper, N.R. and Vandenberg, P.M. (2002). Exercise induces angiogenesis but does not alter movement representations within rat motor cortex. *Brain Res.*, 934, pp.1–6. Available from: http://dx.doi.org/10.1016/S0006-8993(02)02239-4.

Knöchel, C. et al. (2012). Cognitive and behavioural effects of physical exercise in psychiatric patients. *Progress in Neurobiology*, 96(1), pp.46–68.

Knöchel, C. et al. (2016). White matter abnormalities in the fornix are linked to cognitive performance in SZ but not in BD disorder: An exploratory analysis with DTI deterministic tractography. *Journal of affective disorders*, 201, pp.64–78.

Knowles, E.E.M. et al. (2015). The puzzle of processing speed, memory, and executive function impairments in schizophrenia: fitting the pieces together. *Biological Psychiatry*, 78(11), pp.786–793.

Ko, S.Y. et al. (2003). Correlation between performance on the Continuous Performance Test and economic costs in patients with schizophrenia. *Psychiatry*

and Clinical Neurosciences, 57(4), pp.373–377. Available from: https://doi.org/10.1046/j.1440-1819.2003.01134.x.

Kogan, S. et al. (2018). Inflammation in individuals with schizophrenia: implications for neurocognition and daily function. *Brain, Behavior, and Immunity*, 74, pp.296–299.

Komuro, H. and Rakic, P. (1993). Modulation of neuronal migration by NMDA receptors. *Science*, 260(5104), pp.95–97.

Konick, L.C. and Friedman, L. (2001). Meta-analysis of thalamic size in schizophrenia. *Biological Psychiatry*, 49(1), pp.28–38.

Kornau, H.C. et al. (1995). Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science*, 269(5231), pp.1737–1740.

Krystal, J.H. et al. (2005). Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: Implications for glutamatergic and dopaminergic model psychoses and cognitive function. *Archives of General Psychiatry*, 62(9), p.985.

Krystal, J.H. et al. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Archives of general psychiatry*, 51(3), pp.199–214.

Kuo, F.C. et al. (2013). Lifestyle modification and behavior therapy effectively reduce body weight and increase serum level of brain-derived neurotrophic factor in obese non-diabetic patients with schizophrenia. *Psychiatry Research*, 209(2), pp.150–154.

Kvam, S. et al. (2016). Exercise as a treatment for depression: A meta-analysis. *Journal of Affective Disorders*, 202(67), p.86.

Kyeremanteng, C. et al. (2012). A study of brain and serum brain-derived neurotrophic factor protein in Wistar and Wistar-Kyoto rat strains after electroconvulsive stimulus. *Pharmacopsychiatry*, 45(6), pp.244–249. Available from: https://www.ncbi.nlm.nih.gov/pubmed/22454252.

Lally, J. and Maccabe, J.H. (2015). Antipsychotic medication in schizophrenia: A review. *British Medical Bulletin*, 114(1), pp.169–179. Available from: https://academic.oup.com/bmb/article-lookup/doi/10.1093/bmb/ldv017.

Lambert, T.J.R. et al. (2003). Medical comorbidity in schizophrenia. *The Medical Journal of Australia*, 178(S9), pp.S67–S70. Available from: https://pubmed.ncbi.nlm.nih.gov/12720526.

Lau, C.G. et al. (2010). SNAP-25 is a target of protein kinase C phosphorylation critical to NMDA receptor trafficking. *Journal of Neuroscience*, 30(1), pp.242–254.

Laursen, T.M. (2011). Life expectancy among persons with schizophrenia or bipolar affective disorder. *Schizophrenia Research*, 131(1–3), pp.101–104.

Leasure, J.L. and M. Jones (2008). Forced and voluntary exercise differentially affect brain and behavior. *Neuroscience*, 156(3): pp.456-465.

Leger M. and Neill J.C. (2016). A systematic review comparing sex differences in

cognitive function in schizophrenia and in rodent models for schizophrenia, implications for improved therapeutic strategies. *Neuroscience and Biobehavioral Reviews*, 68: pp. 979-1000. doi:10.1016/j.neubiorev.2016.06.029

Leonard, B.E. (2007). Inflammation, depression and dementia: are they connected? *Neurochemical Research*, 32(10), pp.1749–1756.

Leone, M. et al. (2015). Impact of an exercise program on the physiologic, biologic and psychologic profiles in patients with schizophrenia. *Schizophrenia Research*, 164(1–3), pp.270–272.

Leucht, S. et al. (2013). Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: A multiple-treatments meta-analysis. *The Lancet*, 382(9896), pp.951–962. Available from: http://www.sciencedirect.com/science/article/pii/S0140673613607333.

Leucht, S. et al. (2009). Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. *The Lancet*, 373(9657), pp.31–41. Available from: http://www.sciencedirect.com/science/article/pii/S014067360861764X.

Leucht, S. et al. (2017). Sixty years of placebo-controlled antipsychotic drug trials in acute schizophrenia: Systematic review, Bayesian meta-analysis, and meta-regression of efficacy predictors. *American Journal of Psychiatry*, 174(10), pp.927–942.

Liao, Y. et al. (2011). Reduced dorsal prefrontal gray matter after chronic ketamine use. *Biological Psychiatry*, 69(1), pp.42–48.

Lieberman, J.A. et al. (2005). Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *New England Journal of Medicine*, 353(12), pp.1209–1223.

Liemburg, E. et al. (2013). Two subdomains of negative symptoms in psychotic disorders: Established and confirmed in two large cohorts. *Journal of Psychiatric Research*, 47(6), pp.718–725. Available from: http://www.scopus.com/inward/record.url?Eid=2-s2.0-84875908782&partnerid=40&md5=3a22e5a2fa583dfe6a28a4d4ba0585e1.

Lightfoot, J. T. et al. (2004). Genetic influence on daily wheel running activity level. *Physiological Genomics*, 19(3): pp.270–276. https://doi.org/10.1152/physiolgenomics.00125.2004

Lim, K.O. et al. (1999). Compromised White Matter Tract Integrity in Schizophrenia Inferred From Diffusion Tensor Imaging. *Archives of General Psychiatry*, 56(4), pp.367–374. Available from: https://doi.org/10.1001/archpsyc.56.4.367.

Lin, J. et al. (2015). Aerobic exercise and yoga improve neurocognitive function in women with early psychosis. *NPJ Schizophrenia*, 1(1), p.15047. Available from: https://doi.org/10.1038/npjschz.2015.47.

Liu-Ambrose, T. et al. (2010). Resistance training and executive functions: a 12-month randomized controlled trial. *Archives of internal medicine*, 170(2), pp.170–178. Available from: https://pubmed.ncbi.nlm.nih.gov/20101012.

Lopez-Lopez, C. et al (2004). Insulin-like growth factor I is required for vessel

remodeling in the adult brain. *Proc. Natl Acad. Sci. USA*, 101, pp.9833–9838. Available from: http://dx.doi.org/10.1073/pnas.0400337101.

Luby, E.D. et al. (1959). Study of a New Schizophrenomimetic Drug—Sernyl. *Archives of Neurology and Psychiatry*, 81(3), pp.363–369.

Lyon, L. et al. (2012). Spontaneous object recognition and its relevance to schizophrenia: a review of findings from pharmacological, genetic, lesion and developmental rodent models. *Psychopharmacology*, 220(4), pp.647–672. Available from: https://doi.org/10.1007/s00213-011-2536-5.

Mahmood, T. and Yang, P.-C. (2012). Western blot: technique, theory, and troubleshooting. *North American Journal of Medical Sciences*, 4(9), pp.429–434. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23050259.

Malchow, B. et al. (2015). Effects of endurance training combined with cognitive remediation on everyday functioning, symptoms, and cognition in multi-episode schizophrenia patients. *Schizophrenia Bulletin*, 41(4), pp.847–858.

Malchow, B. et al. (2016). Effects of endurance training on brain structures in chronic schizophrenia patients and healthy controls. *Schizophrenia Research*, 173(3), pp.182–191.

Marder, S.R. et al. (2004). Physical health monitoring of patients with schizophrenia. *The American journal of psychiatry*, 161(8), pp.1334–1349. Available from: https://pubmed.ncbi.nlm.nih.gov/15285957.

Maren, S. (1999). Long-term potentiation in the amygdala: a mechanism for emotional learning and memory. *Trends in Neurosciences*, 22(12), pp.561–567. Available from: http://www.sciencedirect.com/science/article/pii/S0166223699014654.

Marzolini, S. et al. (2009). Feasibility and effects of a group-based resistance and aerobic exercise program for individuals with severe schizophrenia: A multidisciplinary approach. *Mental Health and Physical Activity*, 2(1), pp.29–36. Available from: http://www.sciencedirect.com/science/article/pii/S1755296608000410.

Mascagni, F. et al. (2009). Immunohistochemical characterization of parvalbumin-containing interneurons in the monkey basolateral amygdala. *Neuroscience*, 158(4), pp.1541–1550. https://doi.org/10.1016/j.neuroscience.2008.11.017

Matteoli, M. et al. (2009). The synaptic split of SNAP-25: Different roles in glutamatergic and gabaergic neurons? *Neuroscience*, 158(1), pp.223–230.

Maurus, I. et al. (2019). Neurobiological effects of aerobic exercise, with a focus on patients with schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience*, 269(5), pp.499–515. Available from: https://doi.org/10.1007/s00406-019-01025-w.

Maurus, I. et al. (2020). Resistance training in patients with schizophrenia: Concept and proof of principle trial. *Journal of Psychiatric Research*, 120, pp.72–82.

May, A. (2011). Experience-dependent structural plasticity in the adult human brain. *Trends in Cognitive Sciences*, 15(10), pp.475–482.

Mcdonnell, B.J. et al. (2013). Habitual Exercise and Blood Pressure: Age Dependency and Underlying Mechanisms. *American Journal of Hypertension*, 26(3), pp.334–341. Available from: https://doi.org/10.1093/ajh/hps055.

Mckibben, C.E. et al. (2010). Effect of pretreatment with risperidone on phencyclidine-induced disruptions in object recognition memory and prefrontal cortex parvalbumin immunoreactivity in the rat. *Behavioural Brain Research*, 208(1), pp.132–136. Available from: http://www.scopus.com/inward/record.url?Eid=2-s2.0-77951883850&partnerid=40&md5=a08aad197491df9aed9d3136162bc039.

Mednick, S.A. et al. (1988). Adult schizophrenia following prenatal exposure to an influenza epidemic. *Archives of general psychiatry*, 45(2), pp.189–192.

Meier, M.H. et al. (2014). Neuropsychological Decline in Schizophrenia From the Premorbid to the Postonset Period: Evidence From a Population-Representative Longitudinal Study. *American Journal of Psychiatry*, 171(1), pp.91–101. Available from: http://psychiatryonline.org/doi/abs/10.1176/appi.ajp.2013.12111438.

Mesholam-Gately, R.I. et al. (2009). Neurocognition in first-episode schizophrenia: a meta-analytic review. *Neuropsychology*, 23(3), p.315.

Meyer-Lindenberg, A.S. et al. (2005). Regionally specific disturbance of dorsolateral prefrontal-hippocampal functional connectivity in schizophrenia. *Archives of General Psychiatry*, 62(4), pp.379–386.

Meyer, D., Bonhoeffer, T. and Scheuss, V. (2014). Balance and stability of synaptic structures during synaptic plasticity. *Neuron*, 82(2), pp.430–443.

Meyer, U., Schwarz, M.J. and Müller, N. (2011). Inflammatory processes in schizophrenia: A promising neuroimmunological target for the treatment of negative/cognitive symptoms and beyond. *Pharmacology and Therapeutics*, 132(1), pp.96–110. Available from: http://dx.doi.org/10.1016/j.pharmthera.2011.06.003.

Milev, P. et al. (2005). Predictive values of neurocognition and negative symptoms on functional outcome in schizophrenia: a longitudinal first-episode study with 7-year follow-up. *The American journal of psychiatry*, 162(3), pp.495–506.

Miller, B.J. et al. (2011). Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biological psychiatry*, 70(7), pp.663–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21641581%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?Artid=PMC4071300.

Misiak, B. et al. (2018). Vascular endothelial growth factor in patients with schizophrenia: A systematic review and meta-analysis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 86, pp. 24-29. Available from: https://doi.org/10.1016/j.pnpbp.2018.05.005.

Miyamoto S et al. Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Molecular Psychiatry,* 10(1): pp.79-104 doi:10.1038/sj.mp.4001556

Mogami, T. (2018). Cognitive Remediation for Schizophrenia with Focus on NEAR.

Frontiers in psychiatry, 8, p.304. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29387019.

Mukaetova-Ladinska, E.B. et al. (2009). Lewy body variant of Alzheimer's disease: Selective neocortical loss of t-SNARE proteins and loss of MAP2 and α-Synuclein in medial temporal lobe. *The Scientific World Journal*, 9, pp.1463–1475.

Müller, N. (2018). Inflammation in schizophrenia: pathogenetic aspects and therapeutic considerations. *Schizophrenia Bulletin*, 44(5), pp.973–982. Available from: https://doi.org/10.1093/schbul/sby024.

Müller, N. and Bechter, K. (2013). The mild encephalitis concept for psychiatric disorders revisited in the light of current psychoneuroimmunological findings. *Neurology Psychiatry and Brain Research*, 19(3), pp.87–101.

Müller, N. and Schwarz, M. (2006). Schizophrenia as an Inflammation-Mediated Dysbalance of Glutamatergic Neurotransmission. , 10(2), pp.131–148.

Na, K.S. et al. (2014). The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 48, pp.277–286.

Naegelin, Y. et al. (2018). Measuring and Validating the Levels of Brain-Derived Neurotrophic Factor in Human Serum. *Eneuro*, 5(2), p.ENEURO.0419-17.2018. Available from: http://www.eneuro.org/content/5/2/ENEURO.0419-17.2018.abstract.

Najera, K. et al. (2019). SNAP-25 in Major Psychiatric Disorders: A Review. *Neuroscience*, 420, pp.79–85.

Najjar, S. and Pearlman, D.M. (2015). Neuroinflammation and white matter pathology in schizophrenia: Systematic review. *Schizophrenia Research*, 161(1), pp.102–112.

Nakazawa, K. et al. (2012). Gabaergic interneuron origin of schizophrenia pathophysiology. *Neuropharmacology*, 62(3), pp.1574–1583. Available from: http://www.sciencedirect.com/science/article/pii/S0028390811000256.

Narendran, R. et al. (2005). Altered prefrontal dopaminergic function in chronic recreational ketamine users. *American Journal of Psychiatry*, 162(12), pp.2352–2359.

National Academy Medicine Forum on Neuroscience and Nervous System Disorders (2011). Overview of the Glutamatergic System. In *Glutamate-Related Biomarkers in Drug Development for Disorders of the Nervous System: Workshop Summary*. Washington (DC): National Academies Press (US). Available from: https://www.ncbi.nlm.nih.gov/books/NBK62187/ [Accessed 20th February 2020].

National Institute for Health and Care Excellence. (2014). *Psychosis and schizophrenia in adults*. NICE. Available from: https://www.nice.org.uk/guidance/cg178 [Accessed 19th March 2019].

Natsubori, T. et al. (2014). Reduced frontal glutamate + glutamine and N-acetylaspartate levels in patients with chronic schizophrenia but not in those at clinical high risk for psychosis or with first-episode schizophrenia. *Schizophrenia*

Bulletin, 40(5), pp.1128–1139. Available from: http://www.scopus.com/inward/record.url?Eid=2-s2.0-84907375396&partnerid=40&md5=7be48dc50925c591f097dfbd77e50d99.

Neill, J.C. et al. (2014). Acute and chronic effects of NMDA receptor antagonists in rodents, relevance to negative symptoms of schizophrenia: a translational link to humans. *European Neuropsychopharmacology*, 24(5), pp.822–835. Available from: http://dx.doi.org/10.1016/j.euroneuro.2013.09.011.

Neill, J.C. et al. (2010). Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. *Pharmacology and Therapeutics*, 128(3), pp.419–432. Available from: http://dx.doi.org/10.1016/j.pharmthera.2010.07.004.

Nemanic, S. et al. (2004). The Hippocampal/Parahippocampal Regions and Recognition Memory: Insights from Visual Paired Comparison versus Object-Delayed Nonmatching in Monkeys. *The Journal of Neuroscience*, 24(8), pp.2013 LP – 2026. Available from: http://www.jneurosci.org/content/24/8/2013.abstract.

Newcomer, J.W. (2004). Metabolic risk during antipsychotic treatment. *Clinical Therapeutics*, 26(12), pp.1936–1946.

Di Nicola, M. et al. (2013). Serum and gene expression profile of cytokines in first-episode psychosis. *Brain, Behavior, and Immunity*, 31, pp.90–95. Available from: http://dx.doi.org/10.1016/j.bbi.2012.06.010.

Nicoll, R.A., Tomita, S. and Bredt, D.S. (2006). Auxiliary subunits assist AMPA-type glutamate receptors. *Science*, 311(5765), pp.1253–1256.

Nissant, A. et al. (2009). Adult neurogenesis promotes synaptic plasticity in the olfactory bulb. *Nat Neurosci*, 12(6), pp.728–730. Available from: http://dx.doi.org/10.1038/nn.2298.

Noto, C. et al. (2015). High predictive value of immune-inflammatory biomarkers for schizophrenia diagnosis and association with treatment resistance. *World Journal of Biological Psychiatry*, 16(6), pp.422–429.

Nuechterlein, K.H. et al. (2016). Enhancing cognitive training through aerobic exercise after a first schizophrenia episode: theoretical conception and pilot study. *Schizophrenia Bulletin*, 42(suppl_1), pp.S44–S52. Available from: https://doi.org/10.1093/schbul/sbw007.

Nuechterlein, K.H. et al. (2004). Identification of separable cognitive factors in schizophrenia. *Schizophrenia Research*, 72(1), pp.29–39.

Nuechterlein, K.H., Robbins, T.W. and Einat, H. (2005). Distinguishing separable domains of cognition in human and animal studies: what separations are optimal for targeting interventions? A summary of recommendations from breakout group 2 at the measurement and treatment research to improve cognition in schizophrenia. *Schizophrenia Bulletin*, 31(4), pp.870–874.

O'Brien, J.T. et al. (2003). Vascular cognitive impairment. *The Lancet. Neurology*, 2(2), pp.89–98.

O'Leary, D.S. et al. (2000). Cognitive correlates of the negative, disorganized, and

psychotic symptom dimensions of schizophrenia. *The Journal of neuropsychiatry and clinical neurosciences*, 12(1), pp.4–15.

Oertel-Knöchel, V. et al. (2014). Effects of aerobic exercise on cognitive performance and individual psychopathology in depressive and schizophrenia patients. *European Archives of Psychiatry and Clinical Neuroscience*, 264(7), pp.589–604.

Ohnuma, T. et al. (2000). Gene expression of PSD95 in prefrontal cortex and hippocampus in schizophrenia. *Neuroreport*, 11(14), pp.3133–3137. Available from: https://pubmed.ncbi.nlm.nih.gov/11043537.

Olabi, B. et al. (2011). Are there progressive brain changes in schizophrenia? A meta-analysis of structural magnetic resonance imaging studies. *Biological psychiatry*, 70(1), pp.88–96.

Orellana, G. and Slachevsky, A. (2013). Executive functioning in schizophrenia. *Frontiers in Psychiatry*, 4, p.35. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3690455/.

Van Os, J. and Kapur, S. (2009). Schizophrenia. *The Lancet*, 374(9690), pp.635–645. Available from: https://www.sciencedirect.com/science/article/pii/S0140673609609958?Via%3Dihub.

Osimo, E.F. et al. (2019). Synaptic loss in schizophrenia: a meta-analysis and systematic review of synaptic protein and mRNA measures. *Molecular Psychiatry*, 24(4), pp.549–561.

Overman, W.H. et al. (1993). A comparison of children's performance on two recognition memory tasks: Delayed nonmatch-to-sample versus visual paired-comparison. *Developmental Psychobiology*, 26(6), pp.345–357. Available from: https://doi.org/10.1002/dev.420260605.

Oyler, G.A. et al. (1989). The identification of a novel synaptosomal-associated protein, SNAP-25, differentially expressed by neuronal subpopulations. *Journal of Cell Biology*, 109(6 pt1), pp.3039–3052.

Pajonk, F.-G.G. et al. (2010). Hippocampal plasticity in response to exercise in schizophrenia. *Archives of General Psychiatry*, 67(2), pp.133–143. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20124113.

Pan, W. et al. (1998). Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology*, 37(12), pp.1553–1561.

Panenka, W.J. et al. (2007). A longitudinal study on the effects of typical versus atypical antipsychotic drugs on hippocampal volume in schizophrenia. *Schizophrenia Research*, 94(1–3), pp.288–292.

Pantelis, C. et al. (2003). Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. *The Lancet*, 361(9354), pp.281–288. Available from: https://www.sciencedirect.com/science/article/pii/S0140673603123239.

Paolicelli, R.C. et al. (2011). Synaptic pruning by microglia is necessary for normal

brain development. *Science*, 333(6048), pp.1456–1458.

Pardridge, W.M. et al. (1997). Combined Use of Carboxyl-Directed Protein Pegylation and Vector-Mediated Blood-Brain Barrier Drug Delivery System Optimizes Brain Uptake of Brain-Derived Neurotrophic Factor Following Intravenous Administration. *Pharmaceutical Research*, 15(4), pp.576–582. Available from: https://doi.org/10.1023/A:1011981927620.

Park, J.-K. et al. (2014). Treadmill exercise enhances NMDA receptor expression in schizophrenia mice. *J Exerc Rehabil*, 10(1), pp.15–21. Available from: https://doi.org/10.12965/jer.140088.

Paxinos, G. and Watson, C. (2007). *The Rat Brain in Stereotaxic Coordinates*. 6th ed. Academic Press.

Pelletier, M. et al. (2005). Cognitive and clinical moderators of recognition memory in schizophrenia: a meta-analysis. *Schizophrenia Research*, 74(2–3), pp.233–252. Available from: https://www.sciencedirect.com/science/article/pii/S0920996404003044.

Pérez-Otaño, I. and Ehlers, M.D. (2005). Homeostatic plasticity and NMDA receptor trafficking. *Trends in Neurosciences*, 28(5), pp.229–238.

Perry, V.H. (2004). The influence of systemic inflammation on inflammation in the brain: Implications for chronic neurodegenerative disease. *Brain, Behavior, and Immunity*, 18(5), pp.407–413.

Petersen, A.M.W. and Pedersen, B.K. (2005). The anti-inflammatory effect of exercise. *Journal of Applied Physiology*, 98(4), pp.1154–1162. Available from: http://www.physiology.org/doi/10.1152/japplphysiol.00164.2004.

Phillips, M. et al. (2001). Pharmacological Characterization of Locomotor Sensitization Induced by Chronic Phencyclidine Administration. *Journal of Pharmacology and Experimental Therapeutics*, 296(3), pp.905 LP – 913. Available from: http://jpet.aspetjournals.org/content/296/3/905.abstract.

Picchioni, M.M. and Murray, R.M. (2007). Schizophrenia. *BMJ : British Medical Journal*, 335(7610), pp.91–95. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1914490/.

Pieper, A.A. and Baraban, J.M. (2017). Moving Beyond Serendipity to Mechanism-Driven Psychiatric Therapeutics. *Neurotherapeutics*, 14(3), pp.533–536. Available from: https://doi.org/10.1007/s13311-017-0547-6.

Pinal, C.S. and Tobin, A.J. (1998). Uniqueness and redundancy in GABA production. *Perspectives on developmental neurobiology*, 5(2–3), pp.109–118.

Ploughman, M. et al. (2005). Endurance exercise regimens induce differential effects on brain-derived neurotrophic factor, synapsin-I and insulin-like growth factor I after focal ischemia. *Neuroscience*, 136(4): pp. 991-1001.

Podewils, L.J. (2005). Physical activity, APOE genotype, and dementia risk: findings from the cardiovascular health cognition study. *Am. J. Epi.*, 161, pp.639–651. Available from: http://dx.doi.org/10.1093/aje/kwi092.

Polacchini, A. et al. (2015). A method for reproducible measurements of serum BDNF: comparison of the performance of six commercial assays. *Scientific Reports*, 5(1), p.17989. Available from: https://doi.org/10.1038/srep17989.

Powell, C.M. and Miyakawa, T. (2006). Schizophrenia-Relevant Behavioral Testing in Rodent Models: A Uniquely Human Disorder? *Biological Psychiatry*, 59(12), pp.1198–1207. Available from: http://www.sciencedirect.com/science/article/pii/S0006322306005920.

Van Praag, H. (2009). Exercise and the brain: something to chew on. *Trends in Neurosciences*, 32(5), pp.283–290.

Van Praag, H. et al. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *J. Neurosci.*, 25, pp.8680–8685. Available from: http://dx.doi.org/10.1523/JNEUROSCI.1731-05.2005.

Van Praag, H. et al. (1999a). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc. Natl Acad. Sci. USA*, 96, pp.13427–13431. Available from: http://dx.doi.org/10.1073/pnas.96.23.13427.

Van Praag, H. et al. (1999b). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neurosci.*, 2, pp.266–270.

Pratt, J.A. et al. (2008). Modelling prefrontal cortex deficits in schizophrenia: implications for treatment. *British Journal of Pharmacology*, 153(S1), pp.S465–S470. Available from: http://dx.doi.org/10.1038/bjp.2008.24.

Prendergast B.J. et al. Female mice liberated for inclusion in neuroscience and biomedical research. *Neuroscience and Biobehavioral Reviews*, 40, pp.1-5. doi:10.1016/j.neubiorev.2014.01.001

Prescott, M.J. and Lidster, K. (2017). Improving quality of science through better animal welfare: the NC3Rs strategy. *Lab Animal*, 46(4), p.152.

Proteinsimple. (2018). Compass for Simple Western. Available from: https://www.proteinsimple.com/compass/downloads/.

Proteinsimple. (2019). Jess and Wes product insert. Available from: https://www.proteinsimple.com/documents/PL3-0005_revb_Jess-Wes_Separation_Module_(SM-W001_to_SM-W012).pdf [Accessed 1st October 2019].

Proteinsimple. (2014). *Wes Data Sheet*. Available from: https://www.proteinsimple.com/documents/Wes_datasheet_revf.PDF [Accessed 8th November 2019].

Puma, C., Baudoin, C. and Bizot, J.C. (1998). Effects of intraseptal infusions of N-methyl-D-aspartate receptor ligands on memory in an object recognition task in rats. *Neuroscience Letters*, 244(2), pp.97–100.

Radak, Z. et al. (2010). Exercise plays a preventive role against Alzheimer's disease. *Journal of Alzheimer's disease*, 20(3), pp.777–783.

Rajagopal, L. et al. (2010). The novel object recognition test in rodents in relation to cognitive impairment in schizophrenia. *Current Pharmaceutical Design*, 20(31),

pp.5104–5114. Available from: https://www.ingentaconnect.com/content/ben/cpd/2014/00000020/00000031/art00017.

Rapoport, J.L. et al. (2012). Neurodevelopmental model of schizophrenia: Update 2012. *Molecular Psychiatry*, 17(12), pp.1228–1238.

Redrobe, J. et al. (2010). Translational Aspects of the Novel Object Recognition Task in Rats Abstinent Following Sub-Chronic Treatment with Phencyclidine (PCP): Effects of Modafinil and Relevance to Cognitive Deficits in Schizophrenia. *Frontiers in Psychiatry*, 1, p.146. Available from: https://www.frontiersin.org/article/10.3389/fpsyt.2010.00146.

Reichenberg, A. and Harvey, P.D. (2007). Neuropsychological impairments in schizophrenia: integration of performance-based and brain imaging findings. *Psychological Bulletin*, 133(5), p.833.

Reif, A. et al. (2006). Neural stem cell proliferation is decreased in schizophrenia, but not in depression. *Mol Psychiatry*, 11(5), pp.514–522. Available from: http://dx.doi.org/10.1038/sj.mp.4001791.

Reynolds, G.P. et al. (2004). Calcium-binding protein markers of GABA deficits in schizophrenia - Post mortem studies and animal models. *Neurotoxicity Research*, 6(1), pp.57–61.

Ribeiro, E.L.A. et al. (2018). Efficacy and safety of aripiprazole for the treatment of schizophrenia: an overview of systematic reviews. *European Journal of Clinical Pharmacology*, 74(10), pp.1215–1233. Available from: http://link.springer.com/10.1007/s00228-018-2498-1.

Rimes, R. et al. (2015). Effects of Exercise on Physical and Mental Health, and Cognitive and Brain Functions in Schizophrenia: Clinical and Experimental Evidence. *CNS & Neurological Disorders - Drug Targets*, 14(10), pp.1244–1254.

Ripke, S. et al. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature*, 511(7510), pp.421–427.

Risinger, C. et al. (1997). Complex gene organization of synaptic protein SNAP-25 in Drosophila melanogaster. *Gene*, 194(2), 169–177. Available at: https://doi.org/10.1016/s0378-1119(97)00106-6

Rivers-Auty, J. (2015). Novel Object Recognition Task Timer | Jack R Auty. Available from: https://jackauty.com/program/ [Accessed 30th November 2018].

Rohena, L. et al. (2013). Mutation in SNAP25 as a novel genetic cause of epilepsy and intellectual disability. *Rare diseases (Austin, Tex.)*, 1, pp.e26314–e26314. Available from: https://pubmed.ncbi.nlm.nih.gov/25003006.

Rosenbaum, S. et al. (2015). Aerobic exercise intervention in young people with schizophrenia spectrum disorders; improved fitness with no change in hippocampal volume. *Psychiatry Research: Neuroimaging*, 232(2), pp.200–201. Available from: https://www.sciencedirect.com/science/article/pii/S0925492715000396 [Accessed January 8, 2020].

Ross, R. and Bradshaw, A.J. (2009). The future of obesity reduction: beyond weight

loss. *Nature reviews. Endocrinology*, 5(6), pp.319–325.

Saddichha, S. et al. (2008). Metabolic syndrome in first-episode schizophrenia - A randomized double-blind controlled, short-term prospective study. *Schizophrenia Research*, 101(1–3), pp.266–272.

Saijo, T. et al. (2001). Ten-year progressive ventricular enlargement in schizophrenia: An MRI morphometrical study. *Psychiatry and Clinical Neurosciences*, 55(1), pp.41–47.

Salas-Vega, S. et al. (2011). Running coordinately upregulates hippocampal BDNF and GAD67 expression in Long-Evans rats. *The FASEB Journal*, 25(1_supplement), pp.677.11-677.11. Available from: https://www.fasebj.org/doi/abs/10.1096/fasebj.25.1_supplement.677.11.

Samara, M.T. et al. (2019). How Many Patients With Schizophrenia Do Not Respond to Antipsychotic Drugs in the Short Term? An Analysis Based on Individual Patient Data From Randomized Controlled Trials. *Schizophrenia Bulletin*, 45(3), pp.639–646.

Scharfman, H.E. and MacLusky, N.J. (2006). Estrogen and brain-derived neurotrophic factor (BDNF) in hippocampus: complexity of steroid hormone-growth factor interactions in the adult CNS. *Frontiers in neuroendocrinology*, 27(4), pp.415–435. Available from: https://www.ncbi.nlm.nih.gov/pubmed/17055560.

Scheewe, T.W. et al. (2013a). Exercise therapy, cardiorespiratory fitness and their effect on brain volumes: a randomised controlled trial in patients with schizophrenia and healthy controls. *European Neuropsychopharmacology*, 23(7), pp. 675-685.

Scheewe, T.W. et al. (2013b). Exercise therapy improves mental and physical health in schizophrenia: a randomised controlled trial. *Acta Psychiatrica Scandinavica*, 127(6), pp.464–473.

Schuch, F.B. et al. (2016). Exercise as a treatment for depression: A meta-analysis adjusting for publication bias. *Journal of Psychiatric Research*, 77, pp.42–51. Available from: https://www.sciencedirect.com/science/article/pii/S0022395616300383.

Schwartz, T.L. et al. (2012). Glutamate Neurocircuitry: Theoretical Underpinnings in Schizophrenia. *Frontiers in Pharmacology*, 3, p.195. Available from: http://journal.frontiersin.org/article/10.3389/fphar.2012.00195/abstract.

Scopus. (2019). Scopus - Analyze search results. *Elsevier: Scopus*. Available from: https://www.scopus.com/term/analyzer.uri?Sid=47ea3adc276b049088431ebae981086d&origin=resultslist&src=s&s=%28%28TITLE-ABS-KEY%28schizophrenia%29+OR+TITLE-ABS-KEY%28psychosis%29+OR+TITLE-ABS-KEY%28schiophren*%29+OR+TITLE-ABS-KEY%28psychotic%29%29%29+AND+%28 [Accessed 9th July 9 2019].

Seaton, B.E. et al. (2001). Sources of Heterogeneity in Schizophrenia: The Role of Neuropsychological Functioning. *Neuropsychology Review*, 11(1), pp.45–67. Available from: https://doi.org/10.1023/A:1009013718684.

Seillier, A. and Giuffrida, A. (2017). Anxiety does not contribute to social withdrawal

in the subchronic phencyclidine rat model of schizophrenia. *Behavioural Pharmacology*, 28(7). Available from: https://journals.lww.com/behaviouralpharm/Fulltext/2017/10000/Anxiety_does_not_contribute_to_social_withdrawal.2.aspx.

Selemon, L.D. and Goldman-Rakic, P.S. (1999). The reduced neuropil hypothesis: A circuit based model of schizophrenia. *Biological Psychiatry*, 45(1), pp.17–25.

Sevy, S. and Davidson, M. (1995). The cost of cognitive impairment in schizophrenia. *Schizophrenia Research*, 17(1), pp.1–3. Available from: https://www.sciencedirect.com/science/article/pii/092099649500025H?Via%3Dihub.

Shao, C.Y. et al. (2011). Postsynaptic degeneration as revealed by PSD-95 reduction occurs after advanced Aβ and tau pathology in transgenic mouse models of Alzheimer's disease. *Acta Neuropathologica*, 122(3), pp.285–292.

Shenton, M.E. et al. (2001). A review of MRI findings in schizophrenia. *Schizophrenia Research*, 49(1–2), pp.1–52. Available from: https://www.sciencedirect.com/science/article/pii/S0920996401001633.

Sherman, A.D. et al. (1991). Evidence of glutamatergic deficiency in schizophrenia. *Neuroscience Letters*, 121(1–2), pp.77–80. Available from: http://dx.doi.org/10.1016/0304-3940(91)90653-B.

Silva, B.A. et al. (2015). A 20-week program of resistance or concurrent exercise improves symptoms of schizophrenia: results of a blind, randomized controlled trial. *Brazilian Journal of Psychiatry*, 37, pp.271–279.

Silvers, J.M. et al. (2007). Automation of the novel object recognition task for use in adolescent rats. *Journal of Neuroscience Methods*, 166(1), pp.99–103. Available from: https://www.sciencedirect.com/science/article/pii/S0165027007003330.

Sirianni, R.W. et al. (2010). The behavioral and biochemical effects of BDNF containing polymers implanted in the hippocampus of rats. *Brain Research*, 1321, pp.40–50. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20096671.

Sitskoorn, M.M. et al. (2004). Cognitive deficits in relatives of patients with schizophrenia: a meta-analysis. *Schizophrenia Research*, 71(2), pp.285–295. Available from: http://www.sciencedirect.com/science/article/pii/S0920996404001033.

Smith, P.J. et al. (2010). Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. *Psychosomatic medicine*, 72(3), p.239.

Snigdha, S. et al. (2011). Phencyclidine (PCP)-Induced Disruption in Cognitive Performance is Gender-Specific and Associated with a Reduction in Brain-Derived Neurotrophic Factor (BDNF) in Specific Regions of the Female Rat Brain. *Journal of Molecular Neuroscience*, 43(3), pp.337–345. Available from: https://doi.org/10.1007/s12031-010-9447-5.

Snitz, B.E., Macdonald, A.W.I. and Carter, C.S. (2006). Cognitive deficits in unaffected first-degree relatives of schizophrenia patients: a meta-analytic review

of putative endophenotypes. *Schizophrenia Bulletin*, 32(1), pp.179–194. Available from: https://www.ncbi.nlm.nih.gov/pubmed/16166612.

Soppet, D. et al. (1991). The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the TrkB tyrosine kinase receptor. *Cell*, 65(5), pp.895–903. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1645620.

Sorrells, S.F. et al. (2018). Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. *Nature*, 555(7696), pp.377–381. Available from: https://doi.org/10.1038/nature25975.

Van der Staay, F.J. (2006). Animal models of behavioral dysfunctions: basic concepts and classifications, and an evaluation strategy. *Brain Research Reviews*, 52(1), pp.131–159.

Van der Staay, F.J. et al. (2009). Evaluation of animal models of neurobehavioral disorders. *Behavioral and Brain Functions*, 5(1), p.11. Available from: https://doi.org/10.1186/1744-9081-5-11.

Steeds, H. et al. (2015). Drug models of schizophrenia. *Therapeutic Advances in Psychopharmacology*, 5(1), pp.43–58.

Steen, R.G. et al. (2014). Brain volume in first-episode schizophrenia : Systematic review and meta-analysis of magnetic resonance imaging studies. *British Journal of Psychiatry*, i(JUNE), pp.510–518.

Steen, R.G. et al. (2005). Measurement of brain metabolites by 1H magnetic resonance spectroscopy in patients with schizophrenia: A systematic review and meta-analysis. *Neuropsychopharmacology*, 30(11), pp.1949–1962. Available from: http://www.scopus.com/inward/record.url?Eid=2-s2.0-30744443492&partnerid=40&md5=263d323a5c46d1a04a26938db8fa7e7e.

Stephan, K.E. et al. (2006). Synaptic Plasticity and Dysconnection in Schizophrenia. *Biological Psychiatry*, 59(10), pp.929–939.

Stephan, K.E. et al. (2009). Dysconnection in schizophrenia: from abnormal synaptic plasticity to failures of self-monitoring. *Schizophrenia Bulletin*, 35(3), pp.509–527.

Stone, J.M. et al. (2014). Glutamate, N-acetyl aspartate and psychotic symptoms in chronic ketamine users. *Psychopharmacology*, 231(10), pp.2107–2116.

Stubbs, B. et al. (2016). Dropout from exercise randomized controlled trials among people with depression: A meta-analysis and meta-regression. *Journal of Affective Disorders*, 190, pp.457–466. Available from: https://www.sciencedirect.com/science/article/abs/pii/S016503271530882X.

Su, C.-Y. et al. (2016). The effects of aerobic exercise on cognition in schizophrenia: A 3-month follow-up study. *Psychiatry Research*, 244, pp.394–402.

Sullivan, P.F. et al. (2003). Schizophrenia as a Complex Trait: Evidence from a Meta-analysis of Twin Studies. *Archives of General Psychiatry*, 60(12), pp.1187–1192.

Sun, M. et al. (2018). Exercise for Cognitive Symptoms in Depression: A Systematic Review of Interventional Studies. *Canadian Journal of Psychiatry*, 63(2), pp.115–128.

Sutcliffe, J.S. et al. (2007). Influence of gender on working and spatial memory in the novel object recognition task in the rat. *Behavioural Brain Research*, 177(1), pp.117–125. Available from: https://www.sciencedirect.com/science/article/pii/S0166432806005894.

Svatkova, A. et al. (2015). Physical Exercise Keeps the Brain Connected: Biking Increases White Matter Integrity in Patients with Schizophrenia and Healthy Controls. *Schizophrenia Bulletin*, 41(4), pp.869–878.

Svensson, M. et al. (2015). Effects of Physical Exercise on Neuroinflammation, Neuroplasticity, Neurodegeneration, and Behavior. *Neurorehabilitation and Neural Repair*, 29(6), pp.577–589.

Swartz, M.S. et al. (2008). What CATIE Found: results from the schizophrenia trial hhs public access author manuscript. *Psychiatr Serv*, 59(5), pp.500–506. Available from: https://www-ncbi-nlm-nih-gov.proxy.bc.edu/pmc/articles/PMC5033643/pdf/nihms816833.pdf.

Sweet, R.A. et al. (2004). Pyramidal cell size reduction in schizophrenia: Evidence for involvement of auditory feedforward circuits. *Biological Psychiatry*, 55(12), pp.1128–1137.

Sweet, R.A. et al. (2009). Reduced dendritic spine density in auditory cortex of subjects with schizophrenia. *Neuropsychopharmacology*, 34(2), pp.374–389.

Szuhany, K.L. et al. (2015). A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *Journal of Psychiatric Research*, 60(56–64).

Tafoya, L.C.R. et al. (2006). Expression and function of SNAP-25 as a universal SNARE component in gabaergic neurons. *Journal of Neuroscience*, 26(30), pp.7826–7838.

Takahashi, H. et al. (2012). Effects of sports participation on psychiatric symptoms and brain activations during sports observation in schizophrenia. *Translational Psychiatry*, 2(3), pp.e96–e96. Available from: https://doi.org/10.1038/tp.2012.22.

Takahashi, S. et al. (2019). Effect of aerobic exercise combined with cognitive remediation on cortical thickness and prediction of social adaptation in patients with schizophrenia. *Schizophrenia Research*, 216(397–407).

Tannenbaum, J. and Bennett, B.T. (2015). Russell and Burch's 3Rs Then and Now: The Need for Clarity in Definition and Purpose. *Journal of the American Association for Laboratory Animal Science : JAALAS*, 54(2), pp.120–132. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4382615/.

Techniplast Group UK. (2017). GR100 Double-Decker for Rats. Available from: http://www.tecniplast.it/en/product/double-decker.html [Accessed 2nd November 2018].

Thompson, P.M. et al. (2003). SNAP-25 reduction in the hippocampus of patients with schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 27(3), pp.411–417.

Thompson, P.M. et al. (1998). Altered levels of the synaptosomal associated protein SNAP-25 in schizophrenia. *Biological Psychiatry*, 43(4), pp.239–243.

Tong, L. et al. (2001). Effects of exercise on gene-expression profile in the rat hippocampus. *Neurobiology of Disease*, 8(6), pp.1046–1056.

Trajkovska, V. et al. (2007). Measurements of brain-derived neurotrophic factor: Methodological aspects and demographical data. *Brain Research Bulletin*, 73(1–3), pp.143–149.

Twamley, E.W.et al. (2011). Compensatory cognitive training for psychosis: who benefits? Who stays in treatment? *Schizophrenia Bulletin*, 37(suppl_2), pp.S55–S62.

Uhlhaas, P.J. (2011). The adolescent brain: Implications for the understanding, pathophysiology and treatment of schizophrenia. *Schizophrenia Bulletin*, 37(3), pp.480–483.

Uhlhaas, P.J. and Singer, W. (2010). Abnormal neural oscillations and synchrony in schizophrenia. *Nature Reviews Neuroscience*, 11(2), pp.100–113. Available from: http://dx.doi.org/10.1038/nrn2774.

Uhlhaas, P.J. and Singer, W. (2006). Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. *Neuron*, 52(1), pp.155–168.

Vancampfort, D. et al. (2013). A meta-analysis of cardio-metabolic abnormalities in drug naïve, first-episode and multi-episode patients with schizophrenia versus general population controls. *World Psychiatry*, 12(3), pp.240–250.

Vancampfort, D. et al. (2012). A systematic review of correlates of physical activity in patients with schizophrenia. *Acta Psychiatrica Scandinavica*, 125(5), pp.352–362.

Vancampfort, D. et al. (2014). Neurobiological effects of physical exercise in schizophrenia: a systematic review. *Disability and Rehabilitation*, 36(21), pp.1749–1754.

Vancampfort, D. et al. (2016). Prevalence and predictors of treatment dropout from physical activity interventions in schizophrenia: a meta-analysis. *General Hospital Psychiatry*, 39, pp.15–23.

Vaynman, S., Ying, Z. and Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *Eur. J. Neurosci.*, 20, pp.1030–1034. Available from: http://dx.doi.org/10.1111/j.1460-9568.2004.03720.x.

Verderio, C. et al. (2004). SNAP-25 Modulation of Calcium Dynamics Underlies Differences in gabaergic and Glutamatergic Responsiveness to Depolarization. *Neuron*, 41(4), pp.599–610.

Vierling-Claassen, D. et al. (2008). Modeling GABA alterations in schizophrenia: a link between impaired inhibition and altered gamma and beta range auditory entrainment. *Journal of Neurophysiology*, 99(5), pp.2656–2671.

Vita, A. et al. (2006). Brain morphology in first-episode schizophrenia: A meta-analysis of quantitative magnetic resonance imaging studies. *Schizophrenia Research*, 82(1), pp.75–88.

Volk, D.W. et al. (2000). Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical γ-aminobutyric acid neurons in subjects

with schizophrenia. *Archives of General Psychiatry*, 57(3), pp.237–245.

Voss, M.W. et al. (2011). Exercise, brain and cognition across the life span. *Journal of Applied Physiology*, 111(5), pp.1505–1513. Available from: https://doi.org/10.1152/japplphysiol.00210.2011.

Washbourne, P. et al. (2002). Genetic ablation of the t-SNARE SNAP-25 distinguishes mechanisms of neuroexocytosis. *Nature Neuroscience*, 5(1), pp.19–26.

Watremez, W. (2016). *Investigating cognition and neuropathological markers following administration of amyloid-β oligomers : Developing a rat model for Alzheimer's disease research*. Available from: https://www.research.manchester.ac.uk/portal/en/theses/investigating-cognition-and-neuropathological-markers-following-administration-of-amyloid-oligomersdeveloping-a-rat-model-for-alzheimers-disease-research(6e2009f6-4a76-451b-8469-471c21964add).html.

Weiler, I.J. and Greenough, W.T. (1993). Metabotropic glutamate receptors trigger postsynaptic protein synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, 90(15), pp.7168–7171.

Wiley, J.L. (1994). Clozapine's effects on phencyclidine-induced disruption of prepulse inhibition of the acoustic startle response. *Pharmacology, Biochemistry and Behavior*, 49(4), pp.1025–1028.

Wilko. (2018). Wilko 12 Function Cycle Computer. Available from: https://www.wilko.com/en-uk/wilko-12-function-cycle-computer/p/0128451 [Accessed 13th November 2018].

Wise, P.M. and Babcock, W. (1896). Is insanity due to a microbe. *Sci Am*, 75, p.303.

Wolf, S.A. et al. (2011). Physical exercise increases adult neurogenesis and telomerase activity, and improves behavioral deficits in a mouse model of schizophrenia. *Brain, Behavior and Immunity*, 25(5), pp.971–980.

Wolkin, A. et al. (1992). Negative symptoms and hypofrontality in chronic schizophrenia. *Archives of general psychiatry*, 49(12), pp.959–965.

Woodberry, K.A. et al. (2008). *Premorbid IQ in schizophrenia: a meta-analytic review*. American Psychiatric Association. Available from: http://psychiatryonline.org/doi/abs/10.1176/appi.ajp.2008.07081242.

Woodward, M.L. et al. (2018). Hippocampal volume and vasculature before and after exercise in treatment-resistant schizophrenia. *Schizophrenia Research*, 202, pp.158–165.

Wright, I.C. et al. (2000). Meta-Analysis of Regional Brain Volumes in Schizophrenia. *American Journal of Psychiatry*, 157(1), pp.16–25. Available from: http://www.scopus.com/inward/record.url?Eid=2-s2.0-0033961355&partnerid=40&md5=e405e1a39e1a196d28b92a2c2ab64de3.

Wykes, T. et al. (2011). A meta-analysis of cognitive remediation for schizophrenia: methodology and effect sizes. *American Journal of Psychiatry*, 168(5), pp.472–485.

Yasumatsu, N. et al. (2008). Principles of long-term dynamics of dendritic spines.

Journal of Neuroscience, 28(50), pp.13592–13608.

Yesavage, J.A. and Freman, A.M. (1978). Acute phencyclidine (PCP) intoxication: Psychopathology and prognosis. *Journal of Clinical Psychiatry*, 39(8), pp.664–666.

Young, C.E. et al. (1998). SNAP-25 deficit and hippocampal connectivity in schizophrenia. *Cerebral Cortex*, 8(3), pp.261–268. Available from: https://doi.org/10.1093/cercor/8.3.261.

Young, J.W. et al. (2012). Behavioral animal models to assess pro-cognitive treatments for schizophrenia. In M. A. Geyer & G. Gross, eds. *Novel Antischizophrenia Treatments*. Berlin: Springer, Berlin, Heidelberg, pp. 39–79. Available from: http://link.springer.com/10.1007/978-3-642-25758-2_3.

Young, J.W. and Geyer, M.A. (2015). Developing treatments for cognitive deficits in schizophrenia: the challenge of translation. *Journal of psychopharmacology*, 29(2), pp.178–196.

Young, J.W. and the TURNS preclinical Subcommitee. (2006). TURNS Preclinical Subcommittee Cognitive Task List and Preclinical Task Survey. In *TURNS Preclinical Subcommittee Cognitive Task List and Preclinical Task Survey*. Available from: http://www.gtpti.ucla.edu/turns/preclinical-TURNS-report-2006b.pdf%0A.

Yung, A.R. and Firth, J. (2017). How should physical exercise be used in schizophrenia treatment. *Expert review of neurotherapeutics*, 17(3), pp.213–214.

Zajkowska, Z. and Mondelli, V. (2014). First-Episode Psychosis: An Inflammatory State? *Neuroimmunomodulation*, 21(2–3), pp.102–108. Available from: https://www.karger.com/DOI/10.1159/000356536.

Zhang, Z.J. and Reynolds, G.P. (2002). A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. *Schizophrenia Research*, 55(1–2), pp.1–10.

Zylstra, R.R. (1994). Normality tests for small sample sizes. *Quality Engineering*, 7(1), pp.45–58. Available from: https://doi.org/10.1080/08982119408918766.

Chapter 8 Appendices

8.1 Tables of habituation data

8.1.1 Study one habituation data

Please see next page.

	Rat	Average Distance Run (m)	Classification high/low runner (mean ≥50m, high)	Cage Classification	Group
Cage 4	1	80*	High	Low Average 50m	PCP Control
	2	150	High		
	3	30*	Low		
	4	20*	Low		
	5	50*	Low		
Cage 5	1	110*	High	High Average 100m	PCP Exercise
	2	75*	High		
	3	20*	Low		
	4	215	High		
	5	70	High		
Cage 10	1	103	High	High Average 90m	PCP Control
	2	123	High		
	3	40*	High		
	4	215*	High		
	5	60*	High		
Cage 11	1	100	High	High Average 170m	Vehicle Exercise
	2	100*	High		
	3	55*	High		
	4	546	High		
	5	40*	Low		
Cage 12	1	20*	Low	Low Average 60m	Vehicle Control
	2	100	High		
	3	90*	Low		
	4	10	Low		
	5	125	High		
Cage 13	1	70	High	High Average 110m	PCP Exercise
	2	166	High		
	3	150	High		
	4	10	High		
	5	125	High		
Cage 14	1	60*	High	Low Average 10m	Vehicle Control
	2	30*	High		
	3	30*	Low		
	4	0*	Low		
	5	0*	Low		
Cage 15	1	143	High	High Average 130m	Vehicle Exercise
	2	310*	High		
	3	167	High		
	4	60*	High		
	5	55	High		

*Fault experienced with odometer set up in at least one habituation session. This included the sensor dislodging or wires being chewed by animals. Mean was calculated from sessions where data was available. Odometer displayed to 0.01 km level.

Table 8.1 Study one animal habituation. Includes high/low animal classification, mean distance run and experimental group assignment. Accurate to approximately 10m.

8.1.2 Study two habituation data

Table 8.2 Study two animal habituation Includes mean distance run and experimental group assignment. Accurate to approximately 10m

	Rat	**Animal Average Distance Run (m)**	**Home Cage -Average Distance Run (m)**	**Group**
Cage 1	1	170	50m	PCP Control
	2	60		
	3	10		
	4	20		
	5	10		
Cage 2	1	90	70m	PCP Exercise
	2	90		
	3	80		
	4	30		
	5	50		
Cage 3	1	50	80m	PCP Control
	2	10		
	3	90		
	4	110		
	5	10		
Cage 4	1	50	80m	Vehicle Exercise
	2	10		
	3	210		
	4	10		
	5	40		
Cage 5	1	210	80m	PCP Exercise
	2	30		
	3	20		
	4	30		
	5	90		
Cage 6	1	240	80m	Vehicle Control
	2	50		
	3	70		
	4	30		
	5	0		
Cage 7	1	140	50m	Vehicle Control
	2	30		
	3	30		
	4	20		
	5	0		
Cage 9	1	10	30m	Vehicle Exercise
	2	30		
	3	50		
	4	50		
	5	10		

8.2 NOR standard operating procedure

Set-up

- Clean and dry the test area and objects with surface disinfectant or 70% alcohol.
- Switch on the recording system.
- Transport rats to the room in small groups to minimise time in the test room. Cover home cages with blankets when rats are housed in them in the testing room.

Habituation

- The day before testing, individually place each home cage (up to 5 rats) into an empty test environment and allow them to explore freely for 20 minutes.

Acquisition phase

- Place two matching objects in the top left and bottom right corner of the arena, where the vertical and horizontal lines intercept, 6cm away from the test environment walls).
- Begin recording and start the stopwatch.
- Carefully place a rat into the top right square of the test arena, with its nose facing the corner, allowing it to freely explore the test environment for three minutes, undisturbed.
- After three minutes have passed, stop recording.
- Place the rat in an ITI holding cage, away from the test environment.
- Start the stopwatch again.

Inter-trial interval (ITI)

- ITI can vary, depending on the design of the experiment. The ITI commences when the rat is removed from the test environment, and concludes when the rat is returned to it. In this experimental design, the ITI is one minute.
- Remove the objects and any urine or faeces from the test environment and clean it with a disinfectant or 70% ethanol solution.
- Place the retention phase objects into the test arena. The objects will be an identical copy of the object that is familiar to the rat, and a previously unseen object.

Retention phase

- After one minute ITI, restart the stopwatch and start recording.
- Return the rat to the test environment in the same way as in the acquisition stage of the test, allowing the rat to explore freely for three minutes.
- After three minutes, stop recording and return the rat to its home cage.
- Remove the objects from the arena, spray disinfectant or 70% ethanol solution into the test arena and onto the objects, and dry with blue roll.

Video retrieval

- Select the videos to upload from the recording system and download them onto a memory stick.
- Transfer the files onto an encrypted hard drive or memory stick before leaving the test room.

8.3 Protocol for half brain PFA fixation

8.3.1 Solutions

Paraformaldehyde (PFA) 4% solution – 2L *(for fixation)*

1L PBS 1X was heated to over 60°C in a fume cupboard, on a stirring plate.

2.3mL of NaOH solution was added to increase pH.

80g PFA was dissolved into the solution.

PBS 1X was added to bring up the volume to 2L.

NaOH was added to bring the pH to 7.4.

Stored above 4°C.

30% Sucrose solution – 150mL *(for fixation)*

45g sucrose was dissolved in 150mL PBS 1X.

8.4 Brain processing

Brains were split into two hemispheres. One hemisphere was placed into a brain box and placed on dry ice. It was transferred to a -80°C freezer. The other hemisphere was placed into a sample tube of PFA at a minimum of 4°C for 24 hours, or until the brain descended to the bottom of its container. The treatment (freezing or submerging in PFA) of hemispheres (right and left) were alternated with each rat. Unfortunately, due to a freezer fault this tissue was unusable and immunohistochemistry was not possible.

8.5 Example of 96 well plate for the modified Bradford assay

Sample Well
EMPTY/Control well
Control Wells

96 well

	1	2	3	4	5	6	7	8	9	10	11	12
A	**A**	**A**	**A**	S3	S3	S3	S11	S11	S11	S19	S19	S19
B	**B**	**B**	**B**	S4	S4	S4	S12	S12	S12	S20	S20	S20
C	**C**	**C**	**C**	S5	S5	S5	S13	S13	S13	S21	S21	S21
D	**D**	**D**	**D**	S6	S6	S6	S14	S14	S14	S22	S22	S22
E	**E**	**E**	**E**	S7	S7	S7	S15	S15	S15	S23	S23	S23
F	**F**	**F**	**F**	S8	S8	S8	S16	S16	S16	S24	S24	S24
G	S1	S1	S1	S9	S9	S9	S17	S17	S17	S25	S25	S25
H	S2	S2	S2	S10	S10	S10	S18	S18	S18	S26	S26	S26

8.6 Protocols created for Protein Simple The Simple Western system

The following protocols were created as a training resource to support people using the *Wes* system, to enable them to learn to run plates with minimal instruction . The *Wes* system is owned by the School of Pharmacy and Optometry, The University of Manchester and resides in our laboratory. As there is a high volume of researchers and students using the system, I saw an opportunity to support their self-directed learning. The protocols cover processes from homogenisation to data processing and contain the knowledge I have accrued, through using the system and training others to use it. They are now used within and outside our group and are provided for anyone wishing to use the The Simple Western™ system. The creation of these training materials was submitted as a case study on a successful application for Fellowship of the Higher Education Authority.

Analysing Protein with Wes

Lisa Heaney 2019

This document offers guidance on how to prepare and run samples for Wes. It includes how to prepare your tissue and ascertain sample protein concentrations. For how to run a Wes plate, go straight to '3) Wes'.

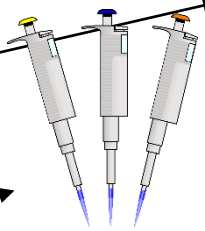
1) Homogenisation
2) Modified Bradford Protein Assay
3) Wes

1) Homogenisation

The following instructions are supplementary to "Synaptosomal preparation from rat brain tissue", SOP number 2015001.

<u>You will need:</u>

- Ice
- Slim permanent marker
- Twice as many Eppendorf tubes (1.5ml) as you have samples to homogenise
- Eppendorf micropestles
- Trizma base (Sigma-Aldrich; room temperature)
- Sucrose (Sigma-Aldrich; room temperature)
- EDTA (Sigma-Aldrich; room temperature)
- PMSF (Sigma-Aldrich; room temperature)
- Sodium orthovanadate (Sigma-Aldrich; room temperature)
- Ethanol (Fisher Chemical, room temperature)
- PBS CA^{2+}/Mg^{2+} 10X to dilute (or premixed 1X if it is pre-mixed)
- cOmplete Protease inhibitor cocktail tablets (Roche; refrigerated). We create a 25X stock solution (see 1)iv)
- A bottle to hold PBS (we often use screw top reagent bottles, like these)
- A bottle for the homogenisation buffer (same as above)
- Samples (kept on ice)
- Vortex, for mixing
- Calibrated pipettes: 200µL, 1mL and 5mL



Synaptosomal preparation is obtained from frozen rat brain tissue after perfusion of the brain, extraction and dissection of the selected area. Synaptosomal preparation is used to investigate biomarkers in synaptic vesicles, synaptic membrane, and mitochondria from brain tissue.

<u>This procedure is for producing pellet 2 (P2) and supernatant 2 (S2).</u>

1) Prepare the solutions you will need. This can be done in advance as the solutions can be refrigerated or frozen until needed. Volumes are given for the preparation of approximately <u>300 samples</u>. We often make up large amounts of the stock solutions and create one aliquot of active solution (i.e. with protease inhibitor cocktail in it).
    i. PBS Ca^{2+}/Mg^{2+} 1X (for 35ml)[1]

i. Dilute 3.5ml PBS Ca^{2+}/Mg^{2+} 10X in 31.5ml of distilled H_20.

ii. SOP says to store at 2-8°C. PBS should remain stable for a few months.

ii. PMSF 0.1M, (for 5 ml)

i. 87.1mg of PMSF and 5 ml of ethanol

ii. Can be stored for 9+ months at 2 to 8°C

iii. In the Standard Operating Protocol, Synaptosomal preparation from rat brain tissue v 1.1, this is said to be stored for at 2-8°C for 3 months. If you have access to the shared drive, clicking on the hyperlink will take you to the document.

iii. Sodium orthovanadate 0.1M, (for 5ml)

i. Dilute 91.96mg of sodium orthovanadate in 5ml of distilled H_20.

ii. SOP says to store at 2-8°C. Sigma-Aldrich website says it can be stored at room temperature for several months, or frozen at -20°C.

iv. 25X Stock solution of cOmplete™ protease inhibitor stock solution

i. Dissolve one cOmplete™ Protease Inhibitor tablet in 2 ml dist. water or in 2 ml 100 mM phosphate buffer, pH 7.0. The stock solution can also be prepared in 10 mM Tris buffer, pH 7.2 to 7.5 (protocol from here). If the tablet does not dissolve easily, vortex or leave at +15 to +25° for 30 – 60 minutes.

ii. Aliquot the stock solution as appropriate for your needs and refrigerate or freeze as appropriate.

iii. The stock solution can be stored at 2 to 8 °C for 1 to 2 weeks, or at least 12 weeks at -15 to -25 °C.

v. Homogenisation buffer (200ml)

i. Dissolve the following in 200 ml distilled H_20:

1. 242.28mg Trizma base
2. 21.9mg sucrose
3. 148.9mg EDTA

ii. This can be divided into 10ml aliquots and stored at -20°C.

iii. To **activate 10ml of homogenisation buffer** (enough to process up to 15/16 samples)

1. Immediately before (see 7) homogenising the samples
    a. Add 0.4ml of 25X cOmplete™ stock solution per 10ml.
    b. Add 100µl of PMSF.
    c. Add 100µl of sodium orthovanadate.

Table 8.3 The proportions of substances sufficient to homogenise a particular number of samples.

Volume (ml) of homogenisation buffer (see "Synaptosomal preparation from rat brain tissue", SOP #2015001)	Volume (µl) of 25X stock solution (total 2ml per tablet)	Volume (µl) sodium orthovanadate and PMSF (each)	Max no. of samples that can be processed (600µl per sample + 100µl spare)
6	240	60	8
7	280	70	10
8	320	80	12
9	360	90	14
10	400	100	16
11	440	110	17

PMSF is abbreviated to phenylmethylsulfonyl fluoride.

2) Get a receptacle (usually a polystyrene box works well) and get ice from the cold room on the 2nd floor.
3) Get samples you would like to homogenise.
4) Put the samples on ice to defrost gradually. This can take some time, so it is best to do this before setting up the rest of the experiment.
5) Turn on the larger centrifuge, set it to 4°C and close the top to allow it to get to temperature. By 'larger' centrifuge, I mean the one usually on the right on the backbench of furthest lab bay from the write-up area. It's the Centrifuge 5402 – Rotor F-45-18-11 – Radius 7.2cm by Eppendorf. **It takes a while to get down to temperature.** It's worth regularly checking that the centrifuge doesn't warm up, as it tends to do this. The best way to prevent this is to ensure the lid is properly shut.
6) Label enough Eppendorf tubes for P2 and S2 for each of your samples.
7) Immediately before homogenising the samples, activate 10ml of homogenisation buffer (enough to process up to 15 samples) by adding:
    a. 0.4ml of 25X cOmplete™ stock solution per 10ml.
    b. 100µl of PMSF.
    c. 100µl of sodium orthovanadate.

    Ideally, you should complete homogenising your samples within 30 minutes of activating the homogenisation buffer. Because of this, we often homogenisation samples in batches of 10.
8) **ON ICE**, add 400µl[2] of the homogenisation buffer to the sample and gently homogenise (a.k.a. 'smoosh' or 'mush') the sample by moving and twisting the micropestle in the sample's Eppendorf tube. Continue to do this until no visible tissue remains and the solution is cloudy. Leave on ice for 1 minute and then use a micropestle to 'mush' the tissue again, using a combination of grinding and mixing to ensure that the tissue has been broken down, with no lumps.

IMPORTANT – it is possible to use micropestles more than once, however they must be dipped in 70% ethanol solution, then ultrapure water and then dried or allowed to dry. I usually have a small beaker with 70% EtOH (ethanol) and one of deionised or ultrapure water, near or on some blue roll.

9) After you have mushed a few samples, use the centrifuge [see 5) above] to spin at for 15 minutes at around 3200rpm (800g) and 4°C. There is a maximum of 15 spaces in the centrifuge. When using the centrifuge, be sure to put the samples opposite each other and add an empty Eppendorf tube to any empty slot that has a sample opposite. This is called balancing the centrifuge.

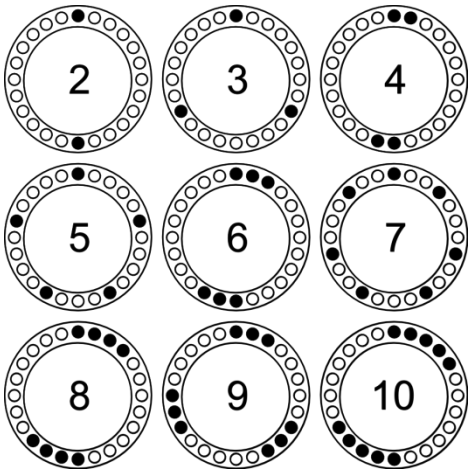
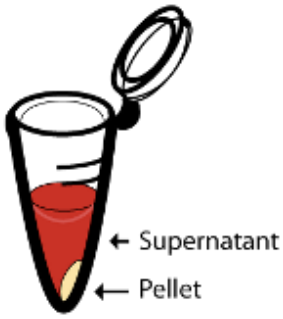


Diagram of some examples of balancing (this is on a larger, 24 space centrifuge). Accessed 14/08/2017 from http://www.openwetware.org/wiki/Griffitts:Culture_tips.



10) Pipette out the supernatant (S1) from the tube and transfer it into the (clean) Eppendorf that you have labelled with the sample name/number and "P2" and discard the remaining pellet (P1). DO NOT get any of the pellet material in your clean tube. If you do you *might* be able to get away with pipetting everything back into the original tube and spinning it again.

11) Centrifuge S1 for 20 minutes and 12200rpm (12000g) at 4°C.
    i. N.B. The synaptosomes are in the pellet (P2)

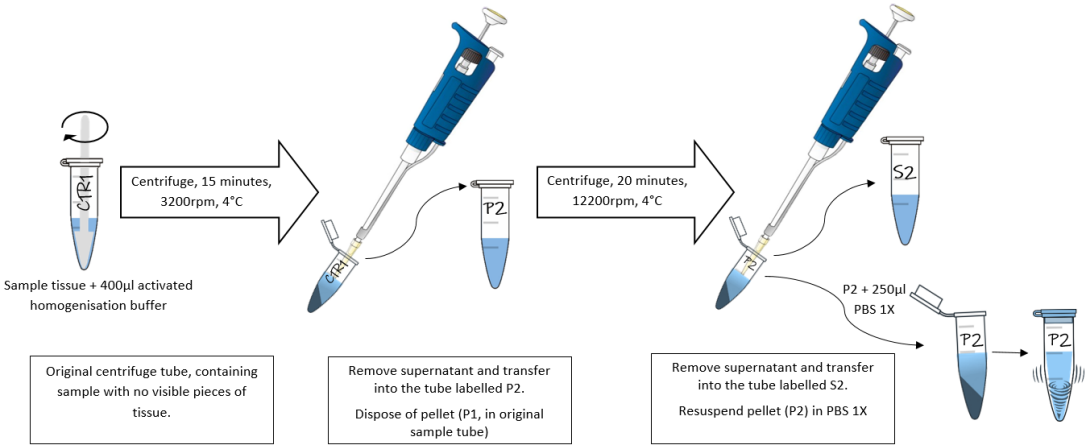
12) Transfer the supernatant (S2) into the (clean) Eppendorf tube labelled with your sample name/number and "S2". You are done with S2 for the meantime.

13) Now you need to re-suspend the pellet, P2. Use 250 µL PBS 1X and vortex the mixture thoroughly.

    i. The model we currently have in our lab is the Fisher WhirliMixer and the settings should be as in the picture opposite.

    ii. Use the vortex by placing the Eppendorf onto the black foam pad at the top of the machine and pressing down (see below picture). It feels very tingly and makes your fingers go numb after a while.

14) Freeze the homogenised samples on dry ice and store them at -20°C or colder.



-

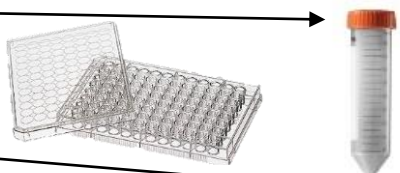
Simplified Diagram of the Homogenisation Process

- Modified Bradford Protein Assay

The following instructions are supplementary to “Protein assay – Modified Bradford assay”, SOP number 2015005.

<u>You will need</u>

- Ice
- Eppendorf centrifuge tubes (1.5ml) (as in part 1)
- Bovine Serum Albumin standard BSA (2mg/ml) 2X
- Protein assay dye reagent. Our lab usually uses Bio-Rad Protein Assay Dye Reagent Concentrate, 450 ml #5000006 (in a fridge in the lab).
- Like-for-like buffer. If the samples have been homogenised with PBS, use PBS. Likewise, if the samples have been homogenised with a specific homogenisation buffer, use that homogenisation buffer.
- Falcon tube (50ml)
- 96-well plate
- Platform rocker/shaker
- Plate reader (BioTek µQuant microplate spectrophotometer)
- Plate reader software
- Samples
- Optional: multichannel pipette
- Optional: plastic pipetting reservoir
-
-
-



Prepare the samples. This varies for experiments but this guide is for Wes, so you will have followed the homogenising step in part 1.

a) Get a receptacle (usually a polystyrene box works well) and get ice from near the cold room on the 2nd floor.
b) Get samples you would like to use.
c) Put the samples on ice to defrost. If you have a large volume of sample this can take some time, so it is best to do this <u>before</u> setting up the rest of the experiment.

d) The Bradford protein assay is a spectroscopic (related to light) procedure that allows us to measure the content of a protein in a solution. The assay is a colorimetric assay, i.e. it measures absorbance of a dye in acid conditions. The denser the protein the bluer it will look to the naked eye.

e) Standards are used to plot a curve on your protein assay so that the absorbance of your sample concentrations can be compared to the 'known' concentrations of the standard(s). Bovine serum albumin (BSA) is used in this protein assay, ELISAs (enzyme-linked immunosorbent assay), immunoblots and immunohistochemistry. BSA is often used because it is stable, small and is not very reactive.

f) Use the same buffer as you did with the samples (i.e. if the samples are in PBS the standards should be in PBS, but if a specific homogenisation buffer was used in the samples, use that in the standards). Dilute the buffer you are using at the same dilution as it was prepared in the samples (e.g. 1 in 10 or 0.1X). Check the amount of buffer that you will need beforehand and make enough for your standards and samples.

g) Each of the standards should have 100µl in it. Depending on the concentration of the BSA standard means varying amounts of ultrapure water is added to the standard.

h) Make up your standards as below:

Table 8.4 Details of standards for modified Bradford assay.

Standard	*Concentration BSA [mg/mL]*	*Volume BSA*	*Volume buffer [µL]*	*Final volume [µL]*
F	*1*	*150µL of 2X*	*150*	*300*
E	*0.75*	*75 µL of F*	*25*	*100*
D	*0.5*	*50 µL of F*	*50*	*100*
C	*0.2*	*20 µL of F*	*80*	*100*
B	*0.1*	*10 µL of F*	*90*	*100*
A	*0*	*0*	*100*	*100*

i) Dilute the dye reagent to 1X by diluting it to 1:5. For a full 96 well plate, dilute 5ml dye reagent in 20ml of ultrapure water in a 50ml falcon tube.

j) Be sure to make a note of the layout of your plate at some point in this process. You need to know which samples are which when you are analysing the results. See here for a template in the shared drive you can fill in and print, or vice versa. See below for an example of a completed 96 well template that can be accessed in the Neill-Harte/Wes/96-plate-template.xls and at the end of this document. Please note that the **standards are often put into the plate from F to A, rather than A to F** as shown below.

96 Well - Single Pt Templates

96well - All Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	A	S3	S3	S3	S11	S11	S11	S19	S19	S19
B	B	B	B	S4	S4	S4	S12	S12	S12	S20	S20	S20
C	C	C	C	S5	S5	S5	S13	S13	S13	S21	S21	S21
D	D	D	D	S6	S6	S6	S14	S14	S14	S22	S22	S22
E	E	E	E	S7	S7	S7	S15	S15	S15	S23	S23	S23
F	F	F	F	S8	S8	S8	S16	S16	S16	S24	S24	S24
G	S1	S1	S1	S9	S9	S9	S17	S17	S17	S25	S25	S25
H	S2	S2	S2	S10	S10	S10	S18	S18	S18	S26	S26	S26

Template Name: 96 Well All Assay Wells (Type:Expt)

-
-

k) Ensure that the lid is off the 96 well plate. Pipette 10µl of each of the BSA standards (A-F) in triplicate, putting 10µL into three wells for each standard.

l) In the remaining wells, pipette 10µL of your diluted samples. Once again doing each sample in triplicate.

m) Add 200µL of dye reagent to each well. The most efficient way of doing this is to use a multichannel pipette and a pipette reservoir. Using a tip box, line up the multichannel pipette with the number of pipette tips you need (8, if you are doing one column at a time). Be sure to change your pipette tips each time you add the dye reagent to the wells.

n) Replace the plastic lid on the plate and incubate it at room temperature (i.e. leave at room temperature) while gently mixing on a rocker/shaker.

o) Take the plate (carefully) to room 3.234 on the 3rd floor of the Stopford Building. Turn left after getting into the lab space, the spectrophotometer is on a bench and is attached to a computer. You may need to get access to this room if you do not already have it. The computer is not on a network (i.e. LAN or Wi-Fi) so you will need to take a USB drive to bring back the data. Important: the computer is quite old. Although (newer) USB 3.0 drives *should* be backwards compatible and therefore should work, in my experience they do not. I would recommend taking an older/cheaper USB drive, or more than one drive to try. You may also be able to use the plate reader in Annalisa Tirella's lab (where the -80°C we store samples in is located), but I don't know the manufacturer of it and have only used it once myself.

p) Turn on the plate reader (spectrophotometer).

q) On the computer, open the program Gen5. Select: *New item* → *New Experiment* → *Josh Protocol*. To the left of the screen, click *Procedure*. Click *Read* and check that the absorbance reads 595. This is 595nm.

r) Open the door to the spectrophotometer. Remove the plastic lid on the plate and put it into the plate reader. Column/row A/1 should be in the top left-hand corner of the space in the machine. Close the door.

s) In the program Gen5, select Read plate → Date reduction. Remove Transformation. Select Curve analysis and Plate layout.

t) The plate runs quickly (in less than a minute) and you can save the results onto the computer next to the plate reader and transfer it to a USB drive, or simple save it on the drive.

u) To analyse the data, use the standard curve to determine the concentration of each sample. You should use a linear scale. The protein concentrations can be used to ascertain the dilution of your proteins for other techniques, such as Wes.

•

2) Wes

<u>You will need:</u>

- Ice
- A small amount of dry ice (optional)**
- As many 0.5ml Eppendorf's/microcentrifuge tubes as you have samples (this is 24 if you are running a full plate).

- A permanent marker for labelling.
- Samples.
- ProteinSimple sample buffer 10X (1:10; 440µl), diluted with distilled or ultra-pure water to 1X (1:100). This is needed if you are diluting your samples rather than running them 'pure'. Store at room temperature.

- ProteinSimple wash buffer (4ml). Store at room temperature.
- Water bath (set to 95°C).

- Antibodies[3], store on ice:
    - Primary antibodies. Common ones we have used so far in our lab are PSD95, SNAP25, parvalbumin (PV), GAD67 and Kv3.4. Volume depends on the antibody concentrations you will use (e.g. 1:100, 1:200 etc.)
    - Secondary antibodies (we tend to use "anti-rabbit" or "anti-mouse").

- Streptavidin (10 µl).
- Antibody diluent (min 260µl)
- Luminol-S (min 200µl)
- Peroxide (min 200µl)

- ProteinSimple EZ standard pack, containing DTT, biotinylated ladder and fluorescence. Store at 4°C. The standard packs are small and green-blue and you need one per run.

- Capillary cartridge. Store at room temperature in packaging for a minimum of 24 hours before using.
- ProteinSimple pre-filled Wes plate at the correct molecular weight range, and a lid for it. Store at room temperature for a minimum of 24 hours before running the plate.

- A spare Wes plate (a used one is fine).

- A centrifuge with the ability to spin plates.
- Pipettes and suitable tips[4]:
    - P2
    - P10
    - P100
    - P200
    - P500/P1000

-

a) Get the samples out of the freezer so they thaw.

b) Turn on the water bath, setting it to 95°C.

c) Draw out your plate. There are templates on the shared drive and in the appendices of these notes.

d) Label 0.5ml Eppendorf/microcentrifuge tubes (if you are running a full plate you will need 24 microcentrifuge tubes).

e) Unless you are running your samples pure (not diluted), make 100µl (or 150µl[5]) sample buffer to 1X by mixing sample buffer by mixing:

    i. 1µl (or 1.5µl) 10X sample buffer

    ii. 99µl (or 148.5µl) deionised water

f) Dilute samples as appropriate with 1X sample buffer to get a total of 4µl[6]. If you have done a Bradford (or modified Bradford) assay, you can use these results to ascertain appropriate sample dilutions. There is a samples calculator to help with this. Ask around to see if someone can send you a copy of the spreadsheet. If that isn't possible or successful, I have included the formulae below, so you can make your own spreadsheet in Excel.

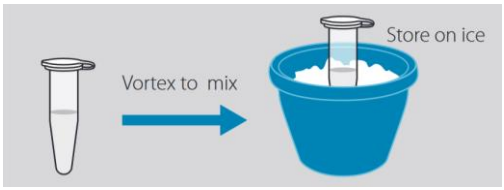
	A	B		C	D	E
3						
4		**Desired Volume (µl):**		5		
5		**Desired Protein Concentration (mg/mL)**		0.02		
6						
7	**Sample**	**Conc (µg/µl)**	**Sample Vol (µl)**	**Sample Buffer(µl)**	**Fluorescent Mix**	**Total Volume**
8			=($D$5/B8)*$D$4	=($D$4-[@[Sample Vol (µl)]])-[@[Fluorescent Mix]]	=$D$4/5	=[@[Fluorescent Mix]]+[@[Sample Buffer(µl)]]+[@[Sample Vol (µl)]]

From Wes training insert (a copy comes in each box of consumables):

A few things you should know

- Warm microplates up to room temperature for at least 24 hours before you start the first assay.
- Capillaries are moisture- and light-sensitive.
- Store unopened cartridge packages and plates at room temperature and do not remove the seals until ready to use.
- The first capillary in the cartridge has been optimized for running the ready-to-use biotinylated ladder. Pipette the biotinylated ladder and samples only as shown in Step 3.
- Plate well evaporation dramatically affects experimental results. To prevent evaporation, keep the lid on the assay plate and do not remove the evaporation seal until you're ready to put the assay plate into the instrument. Keep the lid on between reagent additions and post-preparation.
- An optional System Control Primary Antibody (PN 042-196 or 042-191) can be mixed with your primary antibody in the assay to calculate inter-assay and inter-instrument variability (for chemiluminescence only).

g) The following contains the instructions from the Wes training insert but modified to reflect the order/way in which we do the steps in the lab. The main difference is that we prepare our samples first, and then our sample pack solutions.

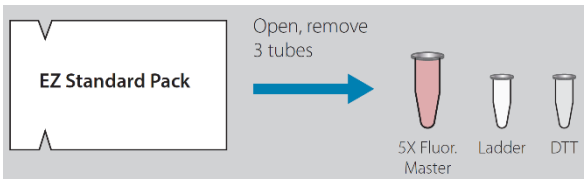


i. Dilute your samples. Unfortunately, I can't include what dilution/ratio you should aim for. It varies a lot depending on the protein of interest and the concentration of it in your sample. If you have run a Bradford assay on your samples, you will hopefully have input the information into the sample dilution calculator and know your sample-to-sample-buffer volumes.

ii. Mix Luminol-S and Peroxide

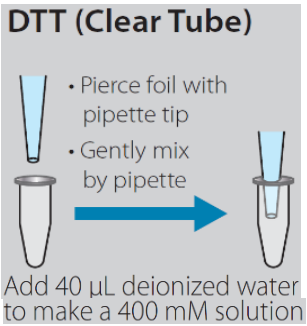
   i. Mix 200µl Luminol-S and 200µl peroxide in a microcentrifuge tube (15µl per well, 25 wells)

   ii. Vortex and store on ice



h) Prepare standard pack reagents.

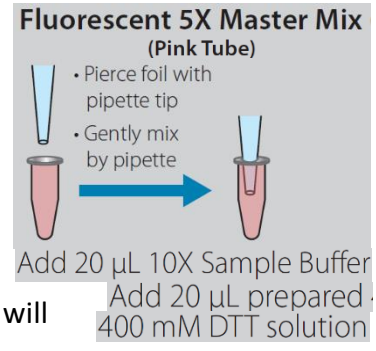
a. **DTT** - Pierce foil of clear (with white powder) DTT[7] tube with a pipette tip and add **40µl ionised water** to make a 400mM solution



   i. Gently triturate solution (i.e. mix using the pipette).

   ii. If the solution gets too aerated (i.e. there are bubbles), spin the solution. Put the DTT tube inside a 0.5ml Eppendorf/microcentrifuge tube and put that in a 1.5ml microcentrifuge tube. Spin this in the smaller/desktop centrifuge at around 4000 for around 1 minute. The 0.5ml and 1.5ml microcentrifuge tubes are to prevent the DTT tube from getting stuck in the centrifuge. Balance the centrifuge as described in part 1 of this guide (homogenisation).

b. Fluorescent 5X master mix

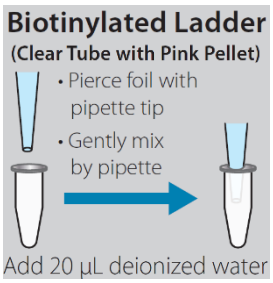


   i. Pierce foil of the fluorescent master mix tube (bright pink powder) and add **20µl 10X sample buffer** and **20µl** prepared 400mM **DTT solution**

   ii. No matter how careful you try to be, the fluorescence will

probably get bubbles in it. This is because the sample buffer is bubbly. As above, put the tube into a 0.5ml inside a 1.5ml Eppendorf/microcentrifuge tube and spin it in the smaller/desktop centrifuge at around 4000 for around 1 minute.

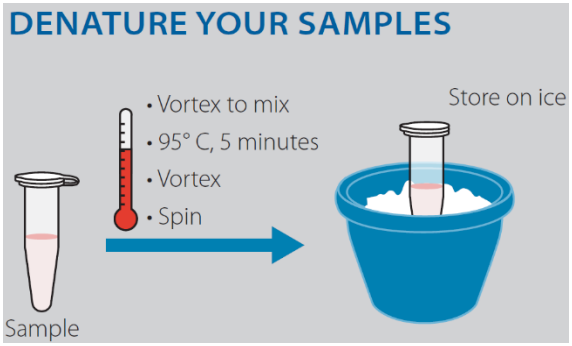
c. Biotinylated ladder (clear tube with pale pink powder)



   i. Pierce the foil of the biotinylated ladder with a pipette tip and add 20µl deionised water

   ii. As with the fluorescence, centrifuge at around 4000 for 1 minute

i) Add 1µl of fluorescence to each sample, bringing the total volume to 5µl.



j) Denature your samples in a water bath at 95°C for 5 minutes. Make VERY sure that the lids are very firmly closed. The microcentrifuge tubes are prone to popping open in the water bath.

   i. Vortex each sample, then spin in the centrifuge. A minute or two at 4k or 5k is sufficient.

k) Prepare your antibodies. You will need a primary antibody (e.g. PSD95, SNAP25, parvalbumin/PV, GAD67, Kv3.4) and a secondary antibody (proteinsimple's mouse, rabbit or both).

   i. The primary antibodies will be the protein of interest and a 'housekeeping' protein. For example, parvalbumin might be our protein of interest and GAPDH is the housekeeping protein we compare it against. The housekeeping protein should theoretically remain stable between our experimental groups and can, therefore, be used as a comparison to our protein of interest.

   ii. The secondary antibody should have the same IgG as the primary. I.e., if the primary is raised in mouse, then the secondary antibody should be mouse. If it's rabbit, then the secondary should be in rabbit. If one of the primary antibodies is mouse (say PSD95), and one is rabbit (say GAPDH), then you need to mix half and half, mouse and rabbit secondary antibody. This could be 1:1 mouse/rabbit, for example. We often have 20X rabbit secondary antibody, so you can either put the correct, smaller amount into the mouse antibody, or you may prefer/need to dilute it to 1X.

iii. If someone has completed Wes runs on your protein of interest, they are likely to know the dilutions that work well. Some 'tried and tested' dilution examples are included in the appendix, but please be sure to check what is most appropriate for your protein and samples. If there is no information available for your protein, you may need to run some Wes plates to 'optimise' for that protein. I've included some training slides below. Housekeeping proteins[8] we tend to use are GAPDH or β-actin.

2. Wes Assay Optimization.pdf

-

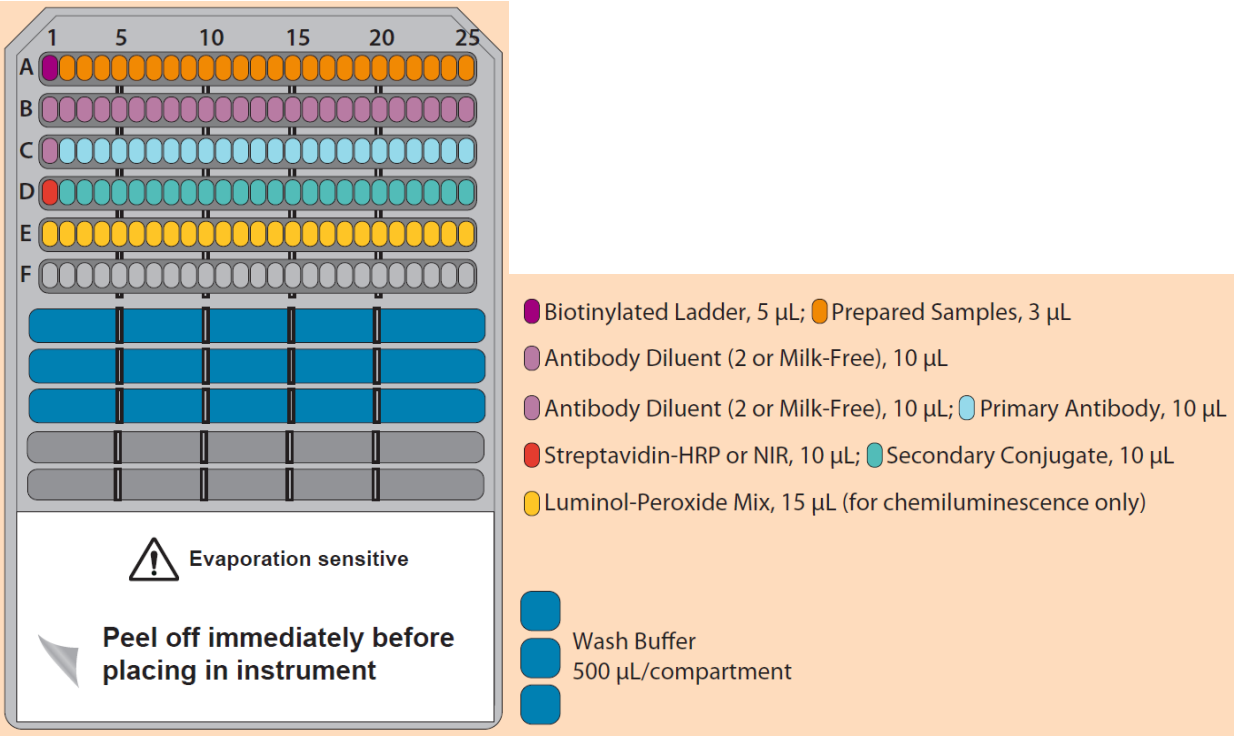
l) Select the correct size of plate, there are usually two options in the lab, but there may be more. These options might be either 4-10kDa or 20-230kDa. Select the one that is most appropriate for the size of the protein you are looking at. For example, parvalbumin is around 14kDa, so you would select the 4-20kDa plate. Remember your housekeeping protein also needs to be within the range of the plate. β-actin has a molecular weight of 42kDa and GAPDH has a molecular weight of 37kDa.

m) Start filling the Wes plate. The order and volume of each substance is included in the Wes instructions in the Wes product insert (diagram below). The one modification is that we pipette 4µl of sample into each well, rather than 3µl. To achieve the best result when pipetting:

i. For rows A-D, use a p10 pipette and make sure that the tip is right in the bottom of the well before expelling the liquid

ii. For row E, 15µl fills the well to the brim. Rest the pipette against a side of the well, near the top. Do not put the pipette tip to the bottom as this will cause the luminol-peroxide to overflow.

iii. DO NOT pipette the wash buffer until the plate has been spun.



n) Put the lid on the plate that you have been filling.

o) Spin the plate to ensure that liquid is at the bottom of the wells and bubbles have been removed. Put on a lid for the plate you have partially filled and use an empty plate to balance it in the small plate centrifuge in the lab. I've had good results with this piece of apparatus. You run it for about 20 seconds at a time for around 5 minutes. You cannot run it continuously. If are having problems with bubbles and the smaller spinner is in not working for you, Mike may have to show you where the large plate centrifuge is and how to use it. It is another group's equipment, so if you use it you must fill out the sign-in sheet and be respectful of the centrifuge and the lab. If you use this centrifuge, spin the plate for 2500 rpm for 5 minutes at room temperature (although most temperatures are fine and won't make much difference).

p) Remove the plate from the spinner and add 500µl wash buffer to each of the wells, as indicated on the plate fill instruction above.

q) Turn on the Wes machine (the switch is at the back) and the computer next to it.

r) Open the door the Wes. Do this by touching the silver rectangle on top of the machine's door.

s) On the computer, open 'Compass for SW'. In the program, click *Instrument > Connect > Wes WS2526, Direct connection > Connect.* The computer and Wes should connect so you are able to see the plate layout etc.

t) Check on the 'layout' tab that the correct plate size is selected (e.g. 12-230kDa). There is a representation of a plate near the bottom of the screen. Fill it in with your plate layout, concentrations etc. Double click and type to change the content of cells.

u) Take the cartridge out from its packaging and slide it into the slot near the orange light. This inserting and removing capillary cartridges only requires a very gentle amount of pressure. When the cartridge is in the correct place, the orange light will change to blue.

v) Peel off the silver evaporation seal at the bottom of the plate, being careful to keep the plate steady, and place in the machine. The slot is shaped in such a way that it is possible to tell which way is the 'right way' up. (Hint: row A should be furthest away from you and it is the same 'way-up' as the diagram of a plate, above).

w) Gently shut the Wes door. It will secure itself without much pressure.

x) Click the green 'start' button in the Compass Program and save the run in a convenient place. Runs are saved in the 'Runs' folder, usually under your name. Give the file a suitable label so that you are able to identify at a later date. Click start once again. The run will take roughly 3 hours to complete and you can see the time the run will end on the screen.

y) It is a good idea to write down the batch/identification numbers for the chemicals, plate, capillary cartridge and anti-bodies that you use. There are two reasons for this: if there are differences between plates you can check these numbers; or if the manufacturer recalls anything you will know if your results are affected.

z) When the run is complete, the file will be open on the computer if it was the last run. If it is not visible, open the file in Compass. Go through the following steps:

   i. Check the standards are all in the right place. There should be three peaks. Check the introductory PowerPoint for where they should be. If any of the samples' standards are in the wrong place you can force them to be in the right place by right-clicking on the peak where the standard should be, then selecting 'force standard'. Equally, you can remove standards in this way.

   ii. Go through each sample and check that your antibody has picked up the protein that you want. Look for a peak (bump) around the molecular weight of your protein of interest.

   iii. If you have no protein (i.e. no peak), concentrate your samples further[9]. Try not to change the antibody concentration - see Wes manual for details as to why. You are looking for an average y-axis reading to be preferably between 10,000 and 15,000 (although, provided it is not saturated[10], there isn't an upper limit, but you don't want it below 5000 really).

aa) The computer in the lab is not connected to a network i.e. by LAN or Wi-Fi. Once the Wes has run, put the data into a spreadsheet and process in GraphPad (or your statistical software of choice). A guide to using the Compass for SW software can be found here.

Appendix

Blank 96 Well Bradford Protein Assay Template

Key:	Sample Well
	EMPTY/Control well
	Control Wells

# Of Samples	96
# of Empty/Ctrl Wells	6
Volume Ranges (nL)	
Volume Ranges (uL)	
Duplicates/Plate	3

96well - All Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	**A**	**A**	**A**									
B	**B**	**B**	**B**									
C	**C**	**C**	**C**									
D	**D**	**D**	**D**									
E	**E**	**E**	**E**									
F	**F**	**F**	**F**									
G												
H												

Template Name: 96 Well All Assay Wells (Type:Expt)

Wes Plate Template

Created by Lisa Heaney, 2017

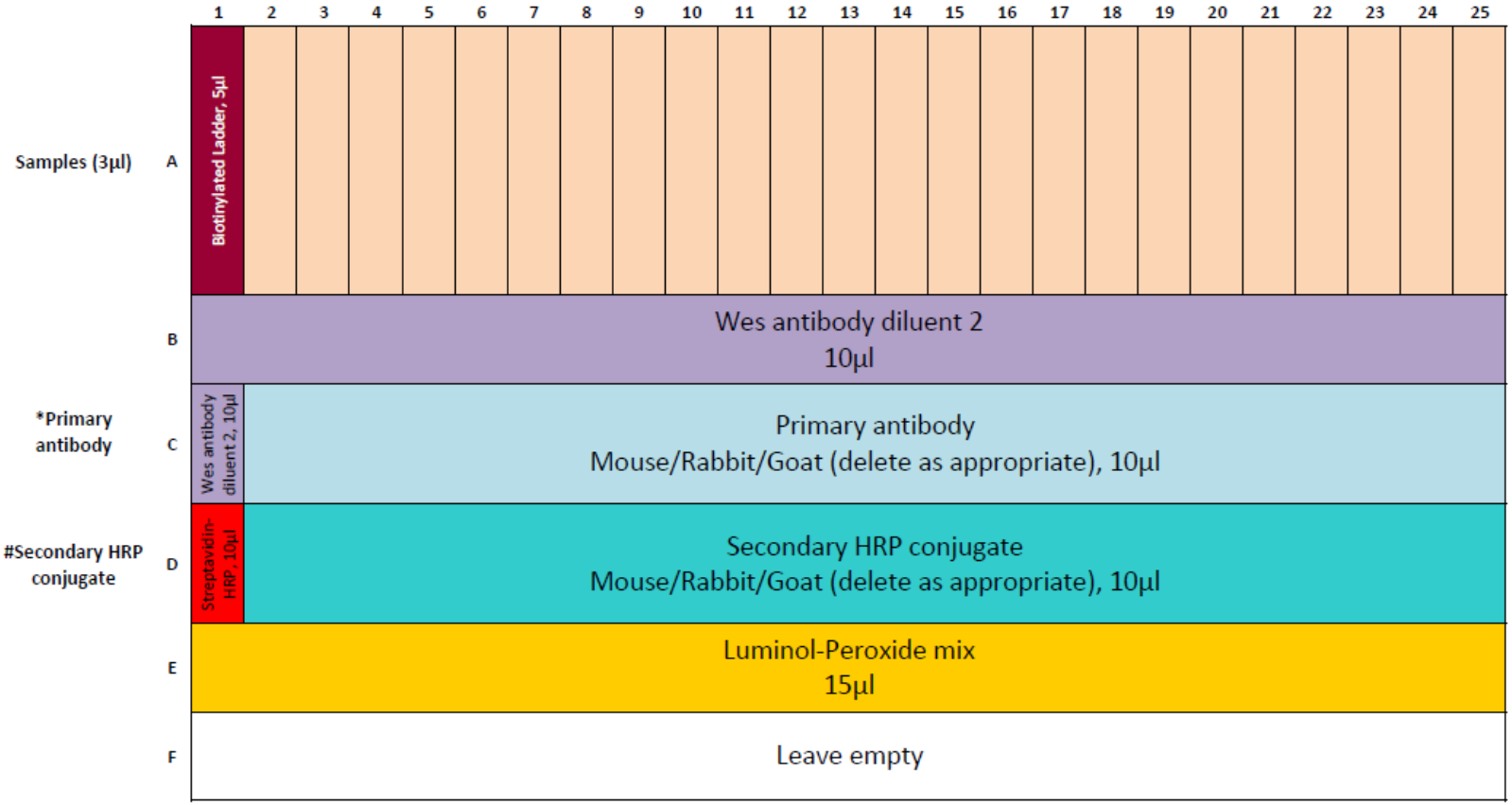
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Samples (3µl)	**A**	Biotinylated Ladder, 5µl																								
	B	Wes antibody diluent 2 10µl																								
***Primary antibody**	**C**	Wes antibody diluent 2, 10µl	Primary antibody Mouse/Rabbit/Goat (delete as appropriate), 10µl																							
#Secondary HRP conjugate	**D**	Streptavidin-HRP, 10µl	Secondary HRP conjugate Mouse/Rabbit/Goat (delete as appropriate), 10µl																							
	E	Luminol-Peroxide mix 15µl																								
	F	Leave empty																								

Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl
Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl
Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl

Leave empty	Leave empty	Leave empty	Leave empty	Leave empty
Leave empty	Leave empty	Leave empty	Leave empty	Leave empty

Wes Plate Template

Created by Lisa Heaney, 2017



Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl
Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl
Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl

Leave empty	Leave empty	Leave empty	Leave empty	Leave empty
Leave empty	Leave empty	Leave empty	Leave empty	Leave empty

Wes Plate Template

Created by Lisa Heaney, 2017

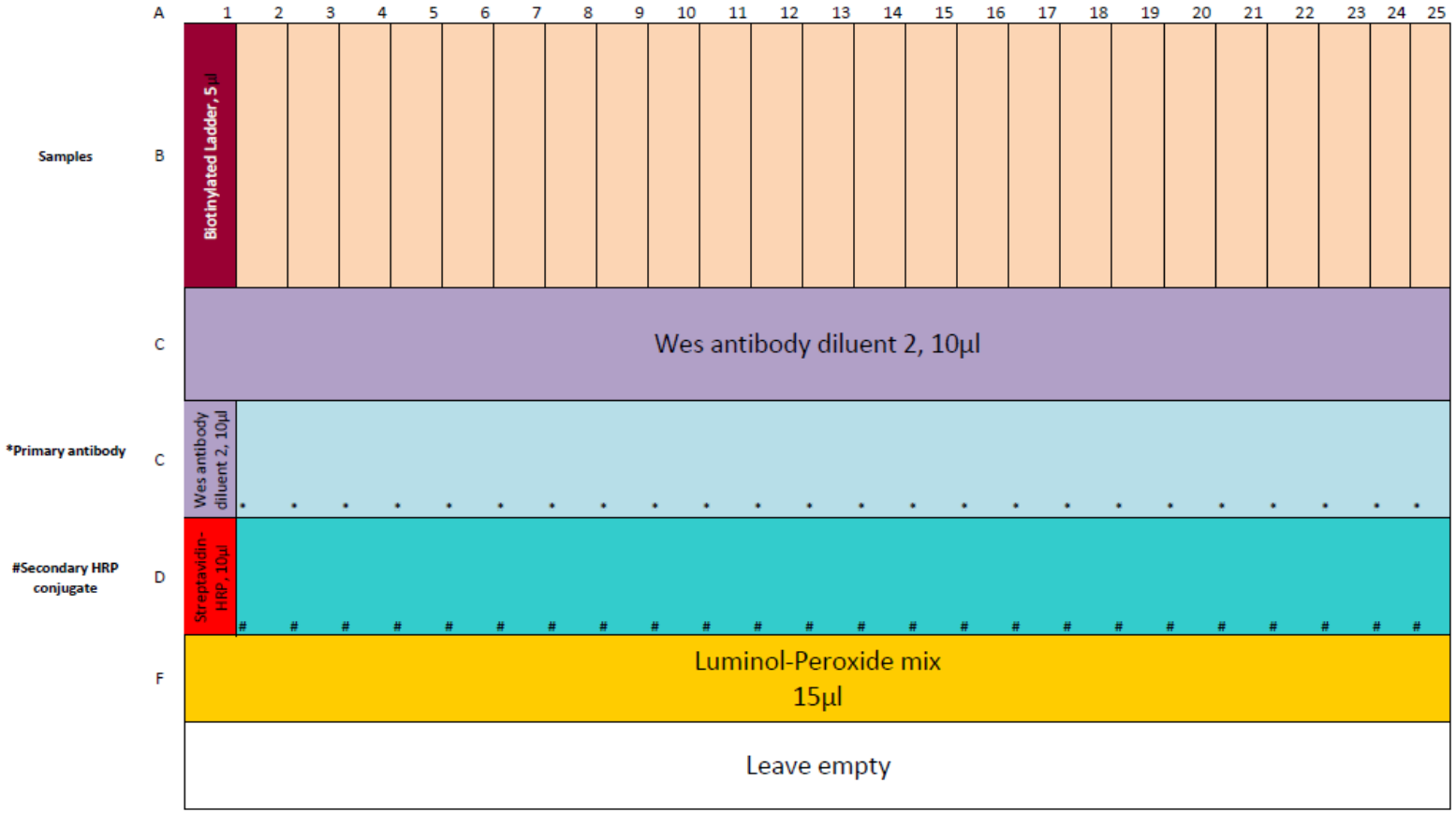
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Samples (3µl)	A	Biotinylated Ladder, 5µl																								
	B	Wes antibody diluent 2 10µl																								
*Primary antibody	C	Wes antibody diluent 2, 10µl	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
#Secondary HRP conjugate	D	Streptavidin-HRP, 10µl	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#	#
	E	Luminol-Peroxide mix 15µl																								
	F	Leave empty																								

Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl
Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl
Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl	Wash buffer 500µl

Leave empty	Leave empty	Leave empty	Leave empty	Leave empty
Leave empty	Leave empty	Leave empty	Leave empty	Leave empty

Wes Plate Template

Created by Liza Heaney, 2017



Wash buffer	Wash buffer	Wash buffer	Wash buffer	Wash buffer
Wash buffer	Wash buffer	Wash buffer	Wash buffer	Wash buffer
Wash buffer	Wash buffer	Wash buffer	Wash buffer	Wash buffer

Leave empty	Leave empty	Leave empty	Leave empty	Leave empty
Leave empty	Leave empty	Leave empty	Leave empty	Leave empty

[1] PBS has many uses because it is isotonic and non-toxic to cells. It can be used to dilute substances. It is used to rinse containers containing cells. Additives can be used to add function. For example, PBS with EDTA is also used to disengage attached and clumped cells. One of the most common preparations is described below.

A 10 litre stock of 10x PBS can be prepared by dissolving, in 8 litres of distilled water: 800 g NaCl; 20 g KCl; 144 g Na2HPO4·2H2O. When mixing is complete. Top up to 10 litres of liquid with distilled water.
After complete mixing, top up final solution to 10 L.

The pH of the 10X stock is will be approximately 6.8, but when diluted to 1x PBS it should change to 7.4.When making buffer solutions, it is good practice to always measure the pH directly using a pH meter. If necessary, pH can be adjusted using hydrochloric acid or sodium hydroxide.

On dilution, the resultant 1x PBS should have a final concentration of 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH of 7.4.
[2] We now weigh the tissue and add 10 times the weight to the sample, I believe. I have left homogenisation this way because this the way that I have processed my samples.

[3] Primary and secondary antibodies are two groups of antibodies that are classified based on whether they bind to antigens or proteins directly, or target another (primary) antibody that, in turn, is bound to an antigen or protein.

A **primary antibody** can be very useful for the detection of biomarkers (in our case, proteins) for diseases such as cancer and Alzheimer's disease. They can be used to quantify the amount of a protein that indicate other biological consequences. In our lab, we often look at synaptic markers, i.e. proteins that signal the state of health of synapses in our brain area of interest.

The secondary antibody is 'raised against' the host species used to generate the primary antibody, for instance, if you use a primary antibody raised in rabbit, you will need an anti-rabbit secondary antibody raised in a host species other than rabbit (e.g. donkey anti-rabbit secondary).

A **secondary antibody** is especially efficient in immuno-labelling. Secondary antibodies bind to primary antibodies, which are directly bound to the target antigen(s).

The domain that the secondary antibody binds to (the Fc domain) is constant within the same animal class e.g. anti-rabbit or anti-goat. Therefore, only one type of secondary antibody is required to bind to many types of primary antibodies. This reduces the cost by labelling only one type of secondary antibody, rather than labelling various types of primary antibodies. Secondary antibodies help increase sensitivity and signal amplification due to multiple secondary antibodies binding to a primary antibody.
As mentioned above, the secondary antibody has to be directed against the isotype (immunoglobin phenotype) of the primary antibody. Polyclonal primary antibodies are generally raised in rabbit, goat, sheep or donkey and are generally IgG isotypes. The secondary antibody therefore, will typically be an anti-IgG H&L (Heavy & Light chains) antibody. Monoclonal primary antibodies are commonly raised in mouse, rabbit and rat. For example, if the primary monoclonal antibody is a mouse IgG1, you will need an anti-mouse IgG or a less specific F(ab) fragment anti-mouse IgG. Note: F(ab) is a domain like Fc.

Secondary antibodies are generated by immunizing a host animal with a pooled population of immunoglobulins (Ig) from the target species. In this case, the Ig would come from rabbits and be introduced into one of the available host species: goat, donkey, mouse, chicken, or sheep. After the host animal's immune system has responded to the rabbit Ig, the resulting antibodies are collected and purified. Secondary antibodies can be conjugated to enzymes such as horseradish peroxidase (HRP) or alkaline

phosphatase (AP); or fluorescent dyes such as fluorescein isothiocyanate (FITC), rhodamine derivatives, Alexa Fluor dyes; or other molecules to be used in various applications.

Secondary antibodies are used in many biochemical assays, including: ELISA, Western blot, immunostaining, immunohistochemistry, immunocytochemistry.

Adapted from https://en.wikipedia.org/wiki/Primary_and_secondary_antibodies, https://www.thermofisher.com/uk/en/home/life-science/antibodies/secondary-antibodies/secondary-antibodies-target-species/anti-rabbit-secondary-antibodies.html and https://en.wikipedia.org/wiki/Isotype_(immunology) . All accessed 23/12/2017.

[4]Do not use filtered pipette tips (they are more expensive).

[5] The Wes product insert says to make 150µl but we usually make just 100µl.

[6] If you are not confident that the pipettes that you are using are accurate enough to reliably create the correct concentration in 4µl of liquid, you can double all of the liquids and make the sample it up to 8µl. If you do this, you will need to add 2µl of fluorescence to make your final sample, buffer and fluorescence solution to 10µl.

[7] DTT (DL-Dithiothreitol; Cleland's reagent) is used to stabilize enzymes and other proteins, which possess free sulfhydryl groups. It has been shown to restore activity lost by oxidation of these groups *in vitro*. DTT quantitatively reduces disulphide bonds and maintains monothiols in a reduced state (source). 1,4-dithiothreitol is used in: Isolation, purification and characterization of proteins and enzymes; measurement of enzyme activities (reactivation of enzymes); determination of disulphide groups in proteins and enzymes; DNA extraction prior to amplification (source).

[8] Proteins that, in theory, should remain constant in our sample as they are not affected by treatment. The levels of/reading from the protein of interest is compared to the levels of the housekeeping protein to control for different amounts/densities of proteins between samples.

[9] In the unlikely event that your samples are saturated, you need to dilute the samples more. If it is only the last time point/ last two time points, you don't need to dilute it very much, if it is all time points, you need to dilute your samples a lot. If only one time point is saturated, you could pick an exposure time that isn't saturated and analyse that one across all your results.

[10] Previously, it would be important to check 'all exposures' to see if the samples have saturated at any exposure time (this will appear as a trough in the peak, in the shape of an 'm', as you should have nice bell-shaped peaks that reduce in size as the exposure time increases). Since a 2018 software upgrade that affected the Compass program, oversaturation should no longer be an issue.