

The molecular impact of physical exercise
on a rodent model of schizophrenia:
hippocampal gene expression and miRNA
levels.

Hemlata Bisnauthsing BSc, 4283081



**The University of
Nottingham**

UNITED KINGDOM • CHINA • MALAYSIA

Submitted to the University of Nottingham for the degree of
Master of Research

Words: 22858

The molecular impact of physical exercise on a rodent model of schizophrenia: hippocampal gene expression and miRNA levels.	1
Abstract	5
List of tables	8
Abbreviations	9
1.1 Introduction	10
1.1.1 Overview of Schizophrenia	10
1.1.2 The brain structure in SZ patients	12
1.1.3 The neurochemistry imbalance in the brain of SZ patients	14
1.1.4 Dopamine hypothesis of SZ	14
1.1.5 NMDA glutamate receptor hypothesis	18
1.2 Genes associated with Schizophrenia	21
1.2.1 NR1 (GRIN1)	21
1.2. 2 GRIN2A (GluN2A)	22
1.2. 3 GluN2A (GRIN2B)	23
1.2.4 COMT	24
1.2.5 DISC1	25
1.2.6 EGR-1	25
1.2.7 GAD1	26
1.2.8 PVALB (PV)	27
1.2.9 NTrK2	28
1.2.9 RELN	28
1.3 Epigenetics	30
1.3.1 Introduction to Epigenetics	30
1.3.2 Histone modification	30
1.3.3 DNA methylation	31
1.3.4 miRNA	31
1.3.5 miR137	31
1.3.6 miR132 and miR212	34
1.4 PCP treatment – An animal model of SZ	36
1.5 Exercise	37
1.6 Hypotheses	38
2. Methods	40
2.1 Experiments on animals	40
2.2 Extraction of DNA and RNA	42
2.3 PCR	44
2.3.1 Primer design	44
2.3.2 The annealing temperature	45
2.4 qPCR	48
Quantification of samples	49
2.5 Sequencing	50
2.5.1. PCR product purification	50
2.5.2 DNA Sequencing reaction	51

2.6 miRNA quantification	51
2.6.1 cDNA synthesis (for miRNA detection)	52
2.6.2 qPCR (for miRNA detection)	52
2.7 Normalisation and analysis of qPCR data	53
2.8 Statistical analysis	54
2.9 Sample size calculations	54
3. Results	56
3.1 The correct choice of primers	56
3.2 Results of gene expression in rat models	60
3.2.1 Expression levels of <i>Grin2A</i> , <i>Grin2B</i> , <i>COMT</i> , <i>EGR-1</i> , <i>DISC1</i> , <i>NTrK2</i> , <i>GAD-1</i> and <i>PV</i> in a rat model of schizophrenia with or without six weeks of voluntary exercise	60
3.2.1.1 Gene expression in a model of SZ (PCP treatment) in rats	60
3.2.1.2 Effect of exercise on gene expression	62
3.2.1.2 Effect of exercise on gene expression in a model of SZ (PCP treatment) in rats	64
3.2.2 The effect of exercise on <i>GRIN2A</i> , <i>GRIN2B</i> , <i>COMT</i> , <i>Egr-1</i> , <i>DISC1</i> , <i>NTrK2</i> , <i>GAD-1</i> and <i>PV</i> after six weeks of wheel running exercise followed by 2 weeks of no exercise.	66
3.2.2.1 Effect of PCP on gene expression, 6 weeks after the end of PCP treatment followed by 2 weeks of no exercise	66
3.2.2.2 Effect of exercise followed by 2 weeks of normal housing on hippocampal gene expression	68
3.2.2.3 Effect of exercise followed by 2 weeks of resting on gene expression in PCP treated rats	70
3.2.3 Results of miRNA expression in rat models	72
3.2.3.1 Hippocampal miRNA expression a model of SZ (PCP treatment)	72
3.2.3.2 Effect of exercise on miRNA expression	74
3.2.3.3 Effect of exercise on hippocampal miRNA expression in a rat model of SZ (PCP treatment)	76
3.3 Sample size analysis	81
4. Discussion	82
4.1 Effect of PCP and exercise alone and in combination on behaviour, gene and miRNA expression in the dorsal hippocampus in rats	83
4.1.2. Behavioural effects	83
4.1.3. Effects on gene expression of our genes of interest	84
4.1.3.1 Parvalbumin	85
4.1.4. MicroRNA expression	86
4.1.5. Effects maintained after wheel running stopped	87

4.2 Rodent models of schizophrenia and the relationship with schizophrenia in humans	88
4.3.1 Sample size and experimental design	90
4.3. Limitations of the study potentially leading to non-significant changes in expression	91
4.3.4 Methodological limitations or different approaches	91
4.3.5 Errors	93
4.4 Future studies	93
4.4.1 DNA methylation	93
4.4.2 miRNA-mRNA	95
4.5 Conclusion	96
5 References	97
Appendix	97
Normalising	123
The Sequence of the gene of interest	124

Abstract

Exercise is known to have positive attributions for humans, and many studies have examined the benefits of physical activity on the human physique and mentality as well as improvements in cognitive function and memory. Various studies on schizophrenia – a psychotic disorder – have shown downregulation in the expression of specific genes in the brain (e.g. *GRIN2A*, *GRIN2B*, *COMT*, *DICS1*, *Egr1*, *NTrK2*, *PV*, *RELN* and *GAD-1*). Studies have examined the manner in which physical activity can influence the expression of specific genes, but very little is known about the effect of physical exercise on the changes in gene expression in patients with schizophrenia.

This study hypothesised that there are effects of exercise on the hippocampal expression of selected genes and miRNAs (miR137, miR132 and miR212) in a rat model of schizophrenia. For this purpose, a phencycline (PCP) model of schizophrenia was used in adult female rats. In two studies, rats were divided into four groups; control rats (no exercise, n = 10), exercised rats (daily wheel running, saline injection, n = 10), schizophrenia model (PCP injection, no exercise, n = 10) and schizophrenia with exercise (PCP injection + wheel running, n = 10). Five rats in each group were humanely killed 6 weeks after PCP/saline injection, and the remaining rats were kept for a further two weeks without access to the running wheel in order to determine whether any changes in gene expression were maintained after the exercise was stopped. Hippocampal gene expression levels for *COMT*, *NTrK2*, *GRIN2A*, *GRIN2B*, *RELN*, *Erg1*, *PVALB* and *DISC1* and miRNAs miR-132, miR-212 and miR-137 were quantified through qPCR.

The results indicate that there were no statistically significant differences in the expression of any of these genes and miRNAs in

animals treated with PCP (compared to saline injected) or animals that exercised (compared to sedentary). Similarly, no significant differences were found after a period when the animals had no access to wheel running exercise.

Thus, based on the results, it can be concluded that neither PCP, the combination (exercise and PCP) nor exercise had a significant effect on specific gene or miRNA levels in the rat dorsal hippocampus. This could be due to the fact that the number of animals per group ($n = 5$) was low, and the experiment came with a lot of limitation.

Acknowledgements

I would like to thank Dr. Maria Toledo Rodriquez and Prof Lucy Donaldson for their support and guidance throughout the entire project. Further, I would also like to thank the University of Manchester for providing the tissues for the project

I am extremely grateful for the support and help I have received from both of my supervisors.

I am also very grateful to the B4 kitchen in Melton hall and E64 for their moral support throughout the project. I want to express my gratitude to Frankie North, Anne Finley, Philip Harrison, Dr. Evelina Kulcinskaja and Tony Chan for proofreading my thesis and Dr.Fan Wang and Corinna Bruce moral support.

List of tables

Table 1 The design of the primer

Table 2 PCR Reagents

Table 3 DNA dilution

Table 4 Reagent and volume

Table 5 miRNA reagent

Table 6 Reagent for miRNA cDNA qPCR

Table 7 Optimal parameter for genes

Table 8 Non-exercise versus exercise-PCP – Study 1

Table 9 Non-exercise versus exercise – Study 1

Table 10 Non-exercise-PCP versus exercise-PCP – Study 1

Table 11 Non-exercise versus non-exercise-PCP – Study 2

Table 12 Non-exercise versus exercise – Study 2

Table 13 Non-exercise-PCP versus exercise-PCP – Study 2

Table 14 miRNA non-exercise versus exercise-PCP – Study 1

Table 15 miRNA non-exercise versus exercise – Study 1

Table 16 miRNA non-exercise-PCP versus exercise-PCP – Study 1

Table 17 miRNA non-exercise versus exercise-PCP – Study 2

Table 18 miRNA non-exercise versus exercise – Study 2

Table 19 miRNA non-exercise-PCP versus exercise-PCP – Study 2

Table 20 Power analysis results

Abbreviations

Anterior Cingulate Cortex – ACC

cAMP response element binding protein – CREB

Catabolic enzyme catechol-O-methyl – COMT

Central nerve system – CNS

Disrupted in Schizophrenia – DISC1

Dopamine – Da

Dorsal hippocampus – DH

Dorsal hippocampus – DH

Early growth response transcription factor gene – EGR1

Glutamate – Glu

Glycine – Gly

Homovanilia acid – HVM

N-methyl-D-aspartate receptor – NMDAR

No template control – NTC

Parvalbumin – PV

Prefrontal cortex – PFC

Restive silence fact – REST

Schizophrenia – SZ

Single polymorphism nucleotide - SNP

Ventral hippocampus – VH

Zinc finger profile 268 – Zif268

1.1 Introduction

1.1.1 Overview of Schizophrenia

Schizophrenia (SZ) is a psychotic illness that onsets in early adulthood and persists throughout the patients' life. The symptoms exhibited are social withdrawal and an alteration in sleeping patterns. People often have an episode of SZ wherein the symptoms are severe, followed by a period with a few or no symptoms. Psychiatrists have divided the symptoms of SZ into three categories, namely the positive, negative and cognitive deficiency. These categories each describe a different aspect of SZ within the patients.

The positive symptoms are perhaps the most common symptoms, which include hallucinations wherein patients hear voices that cause harm, are abusive and can potentially instruct patients to undertake ill/self-harming behaviour. Patients may believe that external forces are controlling them and that they are victims of a particular form of threat or conspiracy. These symptoms respond to medication well (Vermeulen et al.,2017).

The negative symptoms include a lack of empathy, withdrawal from reality, self-neglect, loss of motivation and emotional blunting. Negative symptoms are believed to increase when patients have a reduced quality of life; such as poor life style (Penn et al., 2008). The cognitive deficit is a better indicator of the presence of both positive and negative symptoms (Bozikas et al., 2011). The cognitive deficits in working memory and the difficulty in concentrating and following instructions affect the patient's living skills (Van Snellenberg et al., 2016). Deficits in verbal memory are prominent in individuals with SZ. Psychological tests

demonstrate that when a list of words was given to healthy individuals, they tended to remember the positive words. Whereas, people with SZ tended to remember all the words equally, regardless of the connotations. Research suggests that they are unable to find pleasure in regular activity (Kurtz et al., 2013). SZ possesses a strong hereditary component. For example, the monozygotic twin of a patient with SZ will have a 40–50% chance of developing SZ (Barrès et al., 2016). Additional research has revealed that SZ is a complex multigenetic disorder (Tamminga, 2000) and more research is required to understand this complex disorder so as to find a better treatment for it. Medication, such as antipsychotic medication (e.g. Clozapine), is often used to control or minimise the symptoms by influencing the brain neurotransmitter dopamine. However, the medication of SZ causes severe side effects (e.g. weight gain, blurred vision, nausea and constipation) (Stroup et al., 2016).

Several studies have indicated exercise to be a complementary treatment that has a positive effect on patients with SZ. For example, certain studies have shown that physical exercise improved cognitive function in patients with SZ (Dauwan et al., 2016; Gorczynski and Faulkner, 2010). This study also reported changes in the brain structure that result in an increase of brain volume as well as in the brain-derived neurotrophic factor (BDNF) with exercise (Firth et al., 2017). Hence, exercise might be used as a possible adjuvant therapy alongside with medication to better treat SZ (see Section 1.2.9 and 1.5 for further information on BDNF).

1.1.2 The brain structure in SZ patients

SZ is a complex disorder that affects many parts of the brain. Brain volume in the frontal, parietal and temporal lobe are reduced in patients with SZ. Schizophrenic patients also have a smaller hippocampus volume as compared to healthy people (Gordon et al., 2008). Figure 1 illustrates that the overall volume, grey matter volume, white matter and whole brain volume are affected in patients with SZ. While the volumes of the dorsolateral prefrontal cortex (PFC) and Ventrolateral PFC increase, the volumes of the amygdala, thalamus, hippocampus, putamen, substantia nigra and globus pallidus decrease in patients with SZ. Conversely, the ventricular volume increases (Meyer-Lindenberg, 2010).

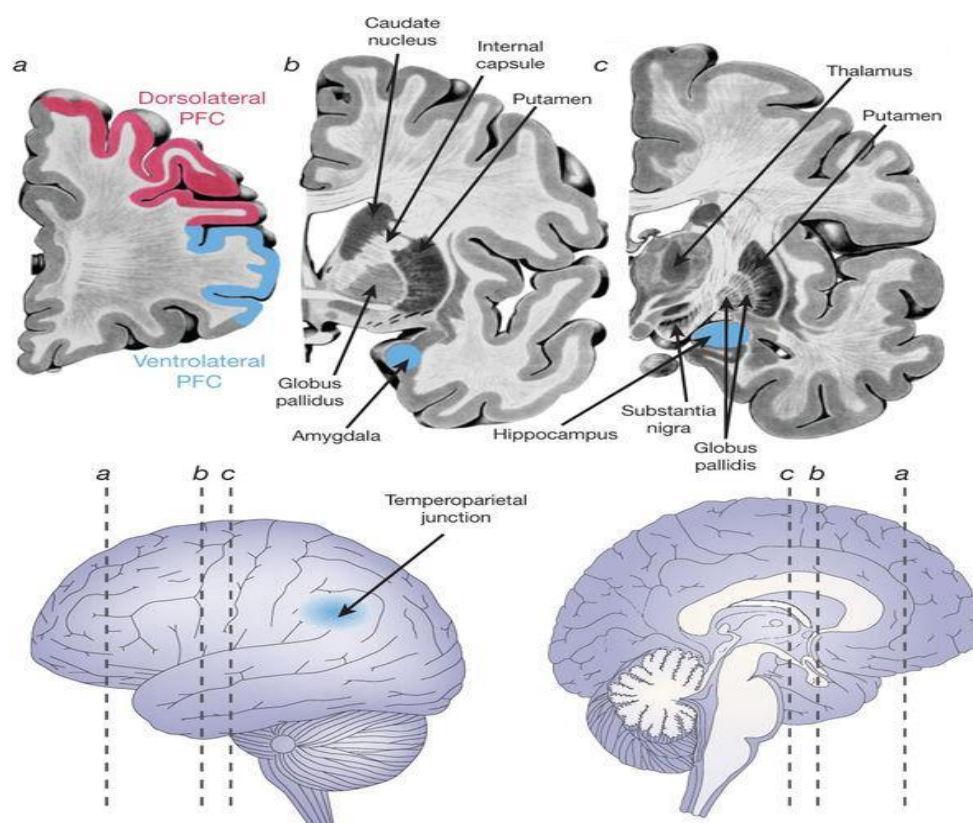


Figure 1: This figure illustrates the different brain sections that are affected in SZ patients. A–C are different coronal sections of the brain taken at the points indicated by the dotted lines in the diagrams (modified from Lindenberg, 2010).

In patients suffering from SZ for many years, additional reductions of the volume are observed in the cortex and the medial as well as left sections of the dorsal temporal gyrus (Meyer-Lindenberg, 2010).

Figure 2 shows the MRI scans illustrating the ventricular enlargement and a decrease in the overall hemispheric volume of a patient over a 15- year period since the first episode of SZ.

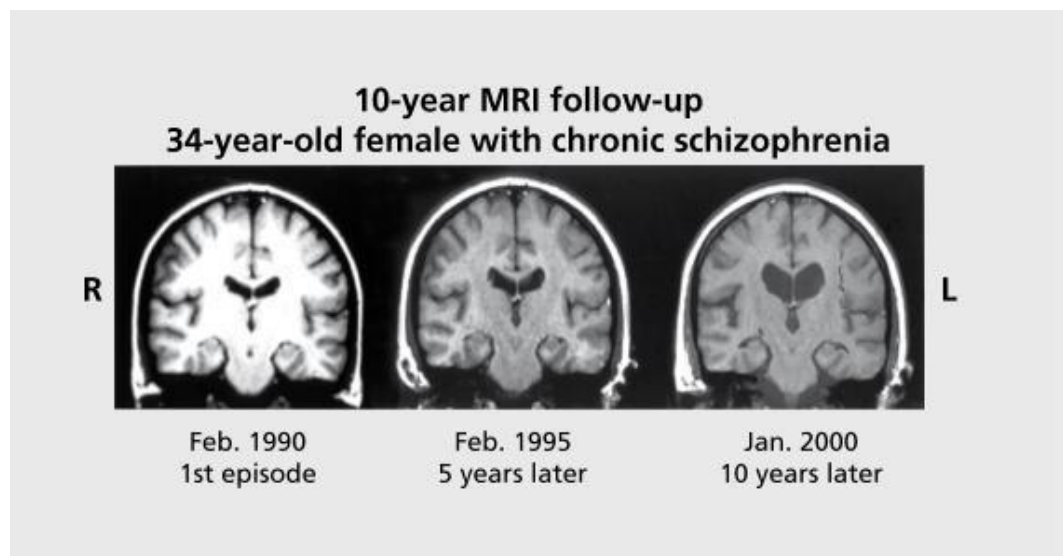


Figure 2: 34-year old female with chronic schizophrenia who has an enlargement of the ventricular region over a period of 10 years (modified from DeLisi et al., 2006).

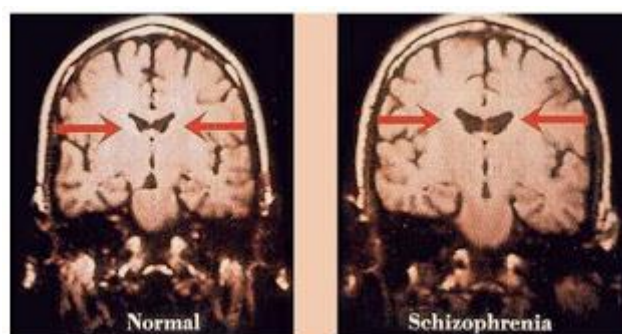


Figure 3: MRI scans of the brains from monozygotic twins. The twin whose brain scan is shown on the left does not have SZ, whereas the twin on the right does. Arrows indicate the enlargement in ventricle volume (modified from Fatemi, 2008).

The above MRI scan (Figure 2) are compared to Figure 3, which displays the hippocampus of a patient with SZ compared to their monozygotic twin who does not have the disorder. Figure 2 highlights the reduction in the volume of the hippocampus with the duration of the disorder (Pujol et al., 2014). These physical changes explain the impairment in working memory, attention and learning of novel tasks, in which the temporal lobe is particularly essential for memory formation (Kaalund et al., 2013).

1.1.3 The neurochemistry imbalance in the brain of SZ patients

Various existing theories have attempted to explain the difference in the neurochemistry of the brain between patients with SZ and individuals who do not have the disorder. There are two prevalent theories – an increase of dopamine levels and a malfunction of the NMDA glutamate receptor.

1.1.4 Dopamine hypothesis of SZ

Dopamine (DA) is a neurotransmitter released by neurons that activates the dopamine receptors, which are coupled to G-proteins and subsequently activate or inhibit adenylate cyclases (Girult and Greengard, 2004). There are five types of dopamine receptors, grouped into two families – D1-like family (D1 and D5) and D2-like family (D2, D3 and D4). The activation of the D1-like family receptors results in an increase of cAMP, while the activation of the D2-like family receptors results in a decrease in cAMP, (illustrated in Figure 4).

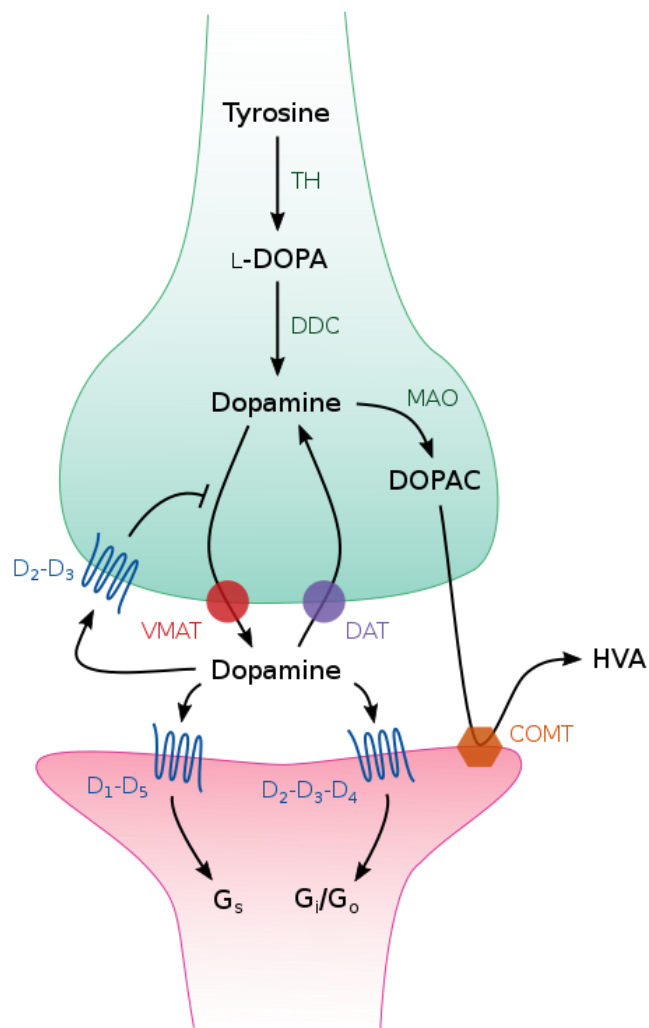


Figure 4: The diagram illustrates the synthesis, release and postsynaptic actions of dopamine. After synthesis, the dopamine is stored in synaptic vesicles. When presynaptic neurons are activated, Dopamine is released into the synaptic cleft through the vesicular monoamine transporter (VMAT2). Once in the synapse, dopamine binds to the dopamine receptors on the postsynaptic neuronal dendrites (D₁-D₅ and D₂, D₅, D₄). After the stimulation of the postsynaptic neurons, unbound dopamine molecules are taken up back into the presynaptic terminals. Dopamine can either be broken down by MAO to DOPAC and, ultimately, HVA or repackaged into vesicles (Blackstone, 2009). Abbreviations: COMT (Catechol-O-methyl), MAO (Monoamine oxidase), TH (tyrosine hydroxylase), DDC (DOPA decarboxylase), DOPAC (3, 4-dihydroxyphenyl-acetic acid), HVA (Homovanilic acid), VMAT (Vesicular monoamine transporter).

Many studies have suggested that the symptoms of SZ result from a malfunction in dopamine signalling (Belujon and Grace, 2017; Richtand et al., 2013).

Within the brain, there are several distinct dopaminergic pathways. Some of them are involved in reward-motivation, behavioural-emotions and pleasure movement (Blackstone, 2009). There are four major dopaminergic pathways in the brain: mesolimbic, mesocortical, nigrostriatal and tuberoinfundibular. These pathways consist of projection neurons that are located in specific areas of the brain whose axons project to release dopamine into remote brain areas that are located away from their soma (Figure 5) (Lievin-Le Moal, 2013).

Two of these pathways are related to SZ: a) the mesolimbic pathway, which projects from the ventral tegmental area (VTA) that is located in the midbrain to the ventral striatum, and b) the mesocortical pathway, which projects from the VTA to the prefrontal cortex (Figure 5) (Ikemoto et al., 2010).

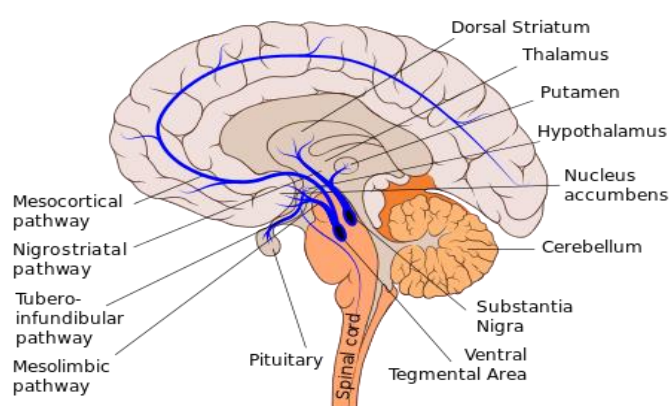


Figure 5: The dopaminergic pathways start from the VTA and project towards the mesocorticolimbic region or end in the prefrontal cortex. The dopaminergic pathways are marked in blue. Also shown are the nigrostriatal and tuberoinfundibular pathways, which have not been implicated in SZ (modified from Bearden et al., 2005).

The DA hypothesis of the SZ disorder states that overactive mesolimbic dopamine neurons cause the positive symptoms and that underactive mesocortical dopamine neurons cause the negative and cognitive symptoms associated with it (Vandenneuval & Pastekamp, 2008).

The basis of this hypothesis was formulated from studies with drugs, namely amphetamine and cocaine. The studies report an increase in DA and can also result in psychotic symptoms (Schramm-Sapota et al., 2009).

The antipsychotic drugs used for the treatment of the SZ disorder would decrease DA by antagonising the dopamine D2 receptors and reducing the positive symptoms and negative symptoms (Schwartz et al., 2008).

Figure 6 displays a schematic representation of the DA hypothesis through the over-activation of mesolimbic DA circuitry. Brain A shows the mesolimbic pathway in a healthy brain, whereas B shows the malfunction in the same in patients with SZ. It shows that an over-activation of the DA circuitry, which innervates the midbrain, allows the excessive release of DA and, in turn, over-activation, resulting in the positive symptoms of SZ (Stahl et al., 2013).

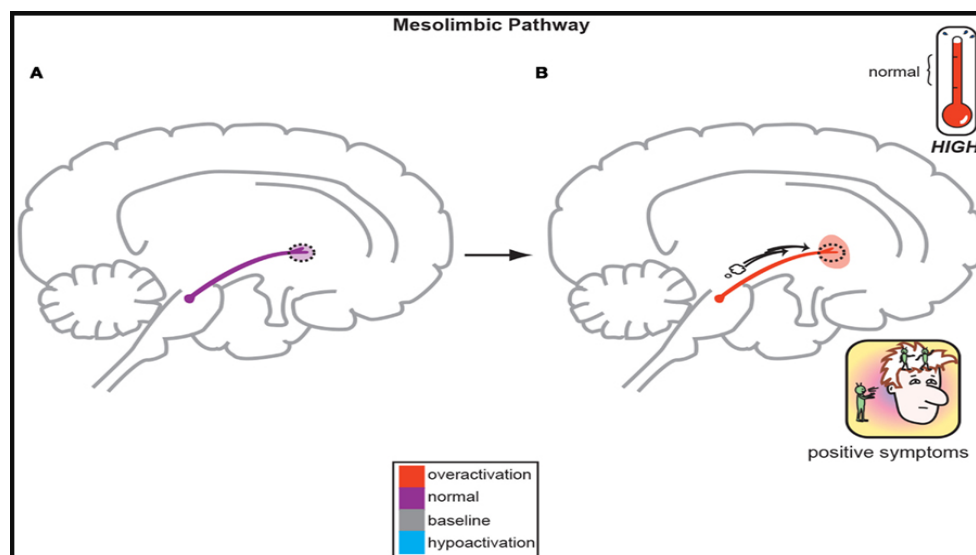


Figure 6: Illustration of the hypothesised malfunction of the DA pathway in the brain of SZ patients (B) versus normal brains (A). In B, there is an overproduction of DA compared to A, which may lead to the positive symptoms of SZ (modified from Stalh, 2013).

COMT and *DISC1* are genes whose malfunction is linked to the DA hypothesis of SZ. These will be introduced and discussed later in this thesis (see chapters 1.2.4–1.2.5).

1.1.5 NMDA glutamate receptor hypothesis

Glutamate is particularly essential for learning and memory, as it is a major neurotransmitter in the central nervous system (CNS) and activates the ionotropic and metabotropic glutamate receptors. There are three pharmacological subtypes of glutamate receptors, which are named according to their preferred agonists that bind to their respective receptor: AMPA, Kainite and N-methyl-D-aspartate receptor (NMDAr) (Coyle, 2012). The NMDAr is a transmembrane protein and is a specific ionotropic glutamate receptor. As an agonist molecule NMDA binds selectively to the NMDAr (Paoletti and Neyton, 2007). The ionotropic NMDA receptors consist of multiple sub-units: NR1 (*GRIN1*), NR2

(*GRIN2A*, *GRIN2B*, *GRIN2C* and *GRIN2D*) and NR3 (*GRIN3A* and *GRIN3B*). These are channel proteins and in order to open their pores, NMDARs require glutamate and one of the two agonists (D-serine or glycine) to bind to their corresponding binding sites. Thereafter, the channels open, and positively charged ions can flow through the cell membrane (see figure 7) (Teng et al., 2010). The NMDAR dysfunction is associated with SZ and the hypothesis explores the possibility that the disease results from the abnormality in the NMDA glutamate receptors. It was first proposed when a significant decrease was found in the glutamatergic (GLU) signalling of SZ patients (Kim et al., 2010).

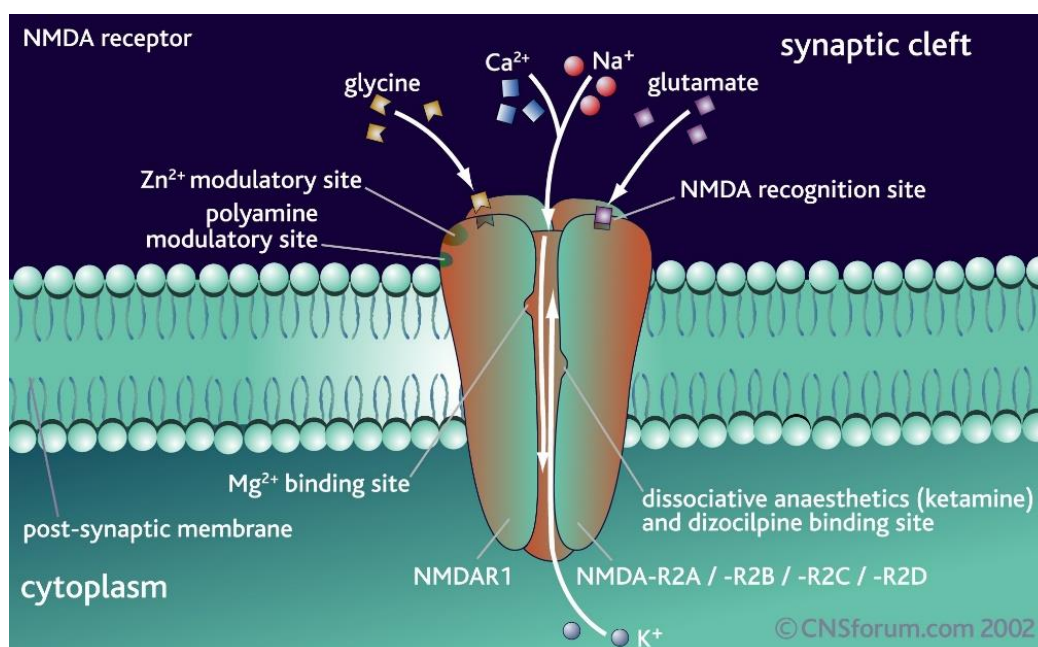


Figure 7: Illustration of the NMDAR wherein glycine and glutamate bind to the receptor and allow the positive ions (Ca^{2+} and Na^{+}) to flow into cells and depolarise the neuron (Leiff, 2011).

Post-mortem examinations of SZ patients, human studies and animal model studies found that the NMDAR expression was reduced in the superior frontal cortex as well as the temporal cortex (Bygrave et al., 2016). Additionally, it was revealed that abnormalities in *GRIN2A* could lead to changes in the trafficking

of glutamate receptor molecules and could be associated with SZ symptoms (Funk, 2009; Howes et al., 2015).

In order to regulate GLU signalling, GABA is the primary inhibitory neurotransmitter. It allows the flow of negatively charged Cl^- into the postsynaptic neuron and thus, the hyperpolarised neuron becomes less likely to respond to new stimulation (see Figure 8) (Kelsom and Lu, 2013).

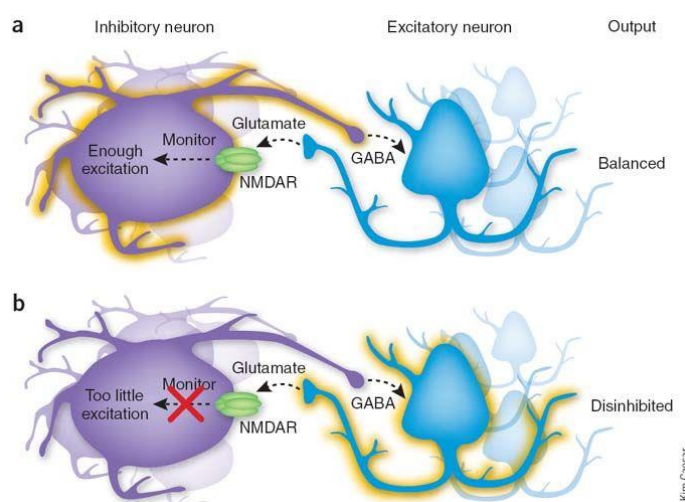


Figure 8: (a) Inhibitory neurons monitor the levels of excitatory activity via the NMDA receptor. Under normal circumstances, the inhibitory neuron maintains adequate GABA levels in order to balance the inhibition with excitation. The glutamate released from the mitral cell excites dendritic spines of granule cells that release GABA back onto the mitral cells. The feedback circuit is predominantly mediated by NMDA receptors. However, when voltage-gated Ca^{2+} channels are blocked, it results in the triggering of the presynaptic GABA to be released for local dendritic feedback inhabitation. (b) Individuals with SZ have their metabolic process changed. There is a mutation of the NMDAR on the subunit NR1 and a decreased expression of the GABAergic tone as well as a reduced expression of 67 KDa isoform of the GABA synthesizing enzyme glutamic acid decarboxylase (GAD67). This is demonstrated in the PFC and hippocampus of SZ (Emamian et al., 2004; Chen et al., 2005).

It was found that disruption of the GABAergic signalling can cause the hypo-reactivity of NMDARs (Ripke et al., 2013)

A study in humans that measured GABA in vivo using proton MRS (¹H-MRS) reported a reduction of GABA in SZ patients as compared to healthy controls (de Jonge et al., 2017). Post-mortem studies in SZ patients found a reduction in the GABA-synthesizing enzyme – GAD67 mRNA and protein (Hashimoto et al., 2003). The change in the receptor would lead to a GABAergic dysfunction that in turn leads to the inhibition of the NMDAR and disinhibition of glutamatergic pyramidal neurons (Lisman et al., 2008; Woo et al., 2004) (see chapter 1.2.7).

Interestingly, a recent study has suggested that GABAergic abnormalities may be linked to the positive symptoms of SZ, but this has not been confirmed and more research is required for the same (Tso et al., 2015). There are multiple genes involved in the NMDAR hypothesis that will be investigated in this study – *GRIN2A*, *GRIN2B*, *GAD-1*, *ErG1* and *PV* (explained further in chapters 1.2.1–1.2.10).

1.2 Genes associated with Schizophrenia

There are many genes potentially involved in SZ. In this study, we chose to focus only on the ones which show extensive evidence for supporting an association with SZ. These were selected based on the evidence from both the human and the experimental models.

1.2.1 NR1 (GRIN1)

NR1 is encoded by the *GRIN1* gene, which is located in the chromosome 9q34.3 (Rice et al., 2001). The protein is a subunit

of NMDA receptor and is a member of the glutamate receptor channel. The subunits play an important role in the plasticity of synapses with regards for memory and learning (Chen et al., 2017). The knockout of *GRIN1* in mice produces elevated locomotor activity. In humans, a *GRIN1* malfunction is linked to deficiencies in social interaction in schizophrenic patients (Martucci et al., 2003).

Studies in Chinese SZ patients have discovered variation in both *GRIN1* and *GRIN2B*. Only one polymorphism seems to have functional significance, G100C, and is located in the promoter region of *GRIN1*. It was found that the interaction between *GRIN1* and *GRIN2B* association and the interaction of G100C, T4197 and T5988C polymorphism in *GRIN2B* was the aetiology of SZ and linked to the positive and negative symptoms (Qin et al., 2005). Additionally, the rs11146020 in *GRIN1* polymorphism is linked to experiencing episodes of major depression, which is one of the negative symptoms of SZ (Georgi et al., 2007). Additionally, *GRIN1* mutations are associated with a reduction in the volume of the prefrontal cortex of patients suffering from SZ (Weickert et al., 2012).

1.2. 2 GRIN2A (GluN2A)

GRIN2A encodes for the protein *Grin2A*, which is mostly found in the hippocampus and the spinal cord (Weickert et al., 2012). It is another subunit of the NMDA receptor, which plays an important role in learning and memory (Chen et al., 2017). Studies have found that, in humans, a deficiency of *GRIN2A* can influence cognitive processes and result in dysregulated glutamatergic transmission in interneurons, which affects the inhibitory control of excitatory cortical circuits (Weickert et al., 2012). Interestingly,

it seems that there is a relationship between the number of GT repeats in the *GRIN2A* promoter and SZ. While usually there would be 19 GT repeats, the offspring of SZ parents had 21 to 23 GT repeats (Tang et al., 2006).

Researchers found that the longer length of the repeats were, the more prominent the SZ-like symptoms were. Moreover, the increase in the range of the GT repeat caused by a reduction in the *GRIN2A* protein impaired the NMDA receptor's response to GLUT stimulation (Itokawa et al., 2003).

Additionally, the rs11146020 in *GRIN1* polymorphism is linked to the experiencing of episodes of major depression, which is one of the negative symptoms of SZ (Georgi et al., 2007). Additionally, *GRIN1* mutations are associated with a reduction in the prefrontal cortex volume of patients having SZ (Weickert et al., 2012).

1.2.3 GluN2A (GRIN2B)

GRIN2B encodes for the protein GluN2A, and some studies reveal its association with the SZ disorder (Li & He, 2007). It is part of the NMDA receptor subunit and is important for learning and memory (Chen et al., 2017). *GRIN2B* is located in the chromosome 12p12 and consists of 13 exons. It is expressed in the hippocampus, basal ganglia, and cerebral cortex (Li & He, 2007). It is associated with neuronal plasticity and spatial learning, and it was found that mice lacking the *GRIN2B* have impaired memory (Martucci et al., 2006).

The linking of multiple single polymorphism nucleotide (SNPs) in the *GRIN2B* gene to cognitive dysfunction/impairment as well as positive and negative symptoms (rs2098469, rs12820037 and rs7298664 (Yang et al., 2015) reduces *GRIN2B* mRNA and protein levels in the Dorsolateral prefrontal cortex (DLPFC), along with

verbal memory, in SZ patients (rs1805502) (rs220599) (Weickert et al., 2012).

1.2.4 COMT

The catabolic enzyme Catechol-O-Methyl Transferase (*COMT*) is an enzyme that is involved in the degradation of catecholamines as well as the neurotransmitters dopamine, epinephrine and norepinephrine (see figure 9).

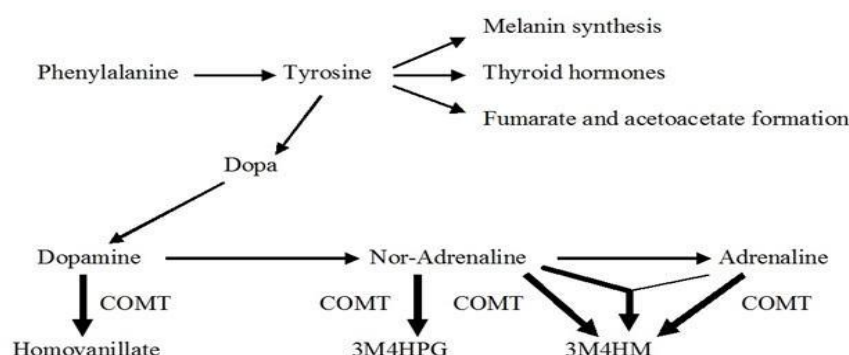


Figure 9: DA pathway. Notice that *COMT* degrades the neurotransmitter (Modified from McGregor, 2014).

The *COMT* gene is located on chromosome 22q11. Its low activity has been associated with the obsessive-compulsive disorder and SZ (Green et al., 2014).

There are two *COMT* protein isoforms (Val108/158 Met) (Egan et al., 2014; Green et al., 2014). Egan et al. (2014) examined the *COMT* alleles of 175 patients with SZ and found that the *COMT* Val allele causes an increase in the DA catabolism in the prefrontal cortex. This mutation causes the impairment of the cognitive function of the prefrontal cortex and increases the risk of SZ (Egan et al., 2001). Usually, individuals with homozygous Val allele have a higher *COMT* activity and lower dopaminergic signalling in the

prefrontal cortex, compared to those with the heterozygous Met allele. This abnormality results in cognitive dysfunction (Bowie & Harvey, 2006) and affects the daily behaviours such as the deficiency of the working memory.

1.2.5 DISC1

The Disrupted in Schizophrenia 1 (*DISC1*) is a protein that exerts its effect on many other proteins it interacts with, modulating their functional state and biological activities (Dahoun et al., 2017).

Several studies have shown that the unregulated expression or an alteration in the protein structure of *DISC1* can increase the chances of developing SZ, clinical depression and bipolar disorder (Kim et al., 2010).

DISC1 is also involved in dopamine signalling (Kang et al., 2011).

A few studies that use the mouse model of SZ show an increased level of dopamine and hyperlocomotive activity when the mice lacked *DISC1* (Sakae et al., 2008). It was also found that the D2 receptor forms a complex with *DISC1* that contributes to the signalling of GSK-3. Post-mortem studies of the striatum of SZ patients and *DISC1*-L100P mutant exhibit a decrease in the GSK-3 (Ermamian, 2012; Lipina et al., 2010).

Additional studies found that, in SZ, a significant translocation (1:11q42;q14:3) was linked to both the mutation of the *DISC1* protein and disruption in the working memory (Cui et al., 2016; Blackwood et al., 2001)

1.2.6 EGR-1

The Early Growth Response transcription factor gene (*EGR-1*) is also known as the zinc finger protein 225 (Zif268). It is suggested

that *EGR-1* is associated with neuronal activity, and many studies have emphasised its link to neuronal plasticity (Bramham et al., 2008).

Dysfunction of *EGR-1* contributes SZ (Duclot and Kabbaj, 2017). A study on mice that underwent social isolation showed a down-regulation in the expression of the *EGR-1* protein as well as the *EGR-1* gene transcript in the frontal cortex (Hattori et al., 2014).

Ramaker and his colleagues compared post-mortem human brain samples between patients with SZ and unaffected patients. They noticed that the transcription factor *EGR-1* was significantly down-regulated in patients with SZ (Kirby et al., 2017). The various *EGR-1* phenotypes altered in SZ patients include neural differentiation, emotional memory formation (Nakazawa et al., 2012) and the loss of *EGR-1* regulation of the NMDA receptor pathways that influence synaptic plasticity and memory formation (Zhao et al., 2015).

1.2.7 GAD1

GAD1 encodes for the Glutamate Acid Decarboxylase enzyme and synthesises gamma-aminobutyric acid (GABA) from L-glutamate (Hettema et al., 2006). GABA is the primary inhibitory neurotransmitter in the mammalian central nervous system. The synthesis of GABA is primarily performed by the 67 kDa and 65 kDa isoforms of the glutamic acid decarboxylase enzymes (*GAD67* and *GAD65*) that are encoded by *GAD1* and *GAD2* genes (Brown et al., 2015).

Studies of the variation in the *GAD* gene reveal that it contributes to an increase in the risk of developing SZ and thus, *GAD1* has been implicated in the neuropathology of the SZ disorder (Tao et al., 2017). Additionally, dysfunction of the prefrontal cortex in SZ

can include decreased *GAD1* mRNA expression (Staube et al., 2007), where the malfunction is thought to be due to the changes in DNA methylation. The DNA methylation levels of two CpG loci within the *GAD1* promoter link to the SZ SNP rs3749034, which is expressed in the DLPFC (Akbarain and Huang, 2006). DNA methylation changes a subset of the *GAD1* regulatory network genes in the hippocampus of SZ patients. This can lead to dysfunction of the GABAergic neurons within the hippocampus (Duclot et al., 2017).

1.2.8 PVALB (PV)

Parvalbumin (*PV*) is a calcium-binding protein that is expressed in GABAergic interneurons and plays a role in cell-cycle regulation and second messenger production (Cates et al., 2002).

A decrease in the neuronal *PV* allows for a reduction in the density of neurons containing detectable levels of *GAD1* mRNA. The study looked at the expression level of *PV*, *GAD1* and the calcium-binding protein calretinin (CR) in the post-mortem brain of 15 patients with SZ (Hashimoto et al., 2003).

Additionally, mice studies saw that knock out of *GAD1* led to a reduction in the GABAergic transmission from *PV+* interneurons, which had an impact on behavioural domains, such as fear, which is consistent with the SZ phenotype (Brown et al., 2015). A study conducted on transgenic mice with NMDAR deletion from cortical and hippocampal GABAergic interneurons (the majority of which were *PV* positive) revealed that the mice developed SZ-like characteristics (Nakazawa et al., 2012)

1.2.9 NTrK2

The Neurotrophic receptor Tyrosine Kinase2 (*NTrk2*) is a receptor for the Brain Derived Neurotrophic Factor (*BDNF*) (Squinto et al., 1991). It is a membrane-bound receptor, which phosphorylates itself and activates the MAPK pathway, leading to cell differentiation and synaptic plasticity. It is modulated in different brain regions, including the amygdala – a region of the brain that processes emotions (Musumeci et al., 2009). *NTrk2* also modulates glutamate receptor function in the amygdala (Bickart et al., 2014).

Numerous studies have indicated the existence of a link between *NTrk2* and several psychiatric disorders such as SZ and depression (Andres-Leon et al., 2008). It was found that deletion in the human *NTrk2* promoter is linked to traits of anxiety (Spalek et al., 2016) as well as a decreased level of *NTrk2* and *BDNF* in patients with SZ (Hashimoto et al., 2005).

Multiple SNPs in the *NTrk2* gene have been linked to SZ and have been associated with emotional arousal and other psychotic conditions (rs1387923 rs2579372, rs1565445 and rs2579372) (Spalek et al., 2016). Additionally, SNP was found in *BDNF* and *NTrk2* genes (*BDNF*-rs1387923, *NTrk2*-rs2579372 and *NTrk2*-rs1565445) of patients with SZ, and these mutations influence mood disorders (Zhang et al., 2015).

1.2.9 RELN

The *RELN* gene encodes for the protein Reelin, an extracellular matrix-associated glycoprotein expressed in the cortex and hippocampus (Negrón-Oyarzo et al., 2016). *RELN* controls glutamatergic neurotransmission (Chen et al., 2005). It is also

involved in neuronal migration, synaptic plasticity, learning and memory (Weeber et al., 2002).

It was found that the downregulation of *RELN* expression and methylation of its promoter in the prefrontal cortex resulted in the impairment of behavioural tasks in a rodent model of SZ (Negrón-Oyarzo 2016).

Additionally, a study examined the allelic expression of *RELN* in post-mortem brain samples from patients with SZ. It was found that, while there was no significant difference in the total appearance of *RELN*. There was a significant reduction in the proportion of the short *RELN* isoform, examined in the post mortem brain sample from patient with SZ and bipolar, except for in the C-terminal region. (Ovadia and Shifman, 2011), whereas the mutation of *RELN*, leading to overexpression, enhanced glutamatergic transmission and simultaneously reduced the *GRIN2B* subunit in NMDAr (Li et al., 2010). Additionally, it showed that mice with an overexpression of *RELN* spend less time floating in the forced swim test than normal mice with a normal expression of *RELN* – a model representing the development of helplessness behaviour (Teixeira et al., 2011), which is one of the cognitive symptoms of SZ.

Thus, changes in the expression of the genes mentioned above can affect glutamatergic, dopaminergic and GABAergic systems, all of which are of significance in SZ.

1.3 Epigenetics

1.3.1 Introduction to Epigenetics

Epigenetics is the study of alterations in the gene expression that are not due to changes in the DNA sequence but instead due to modifications in the transcriptional or translational processes (Weinhold, 2006). There are three main types of epigenetic modifications: DNA methylation, histone modifications and non-coding RNAs (miRNAs) (Mazzio & Soliman, 2012). Several studies have shown that epigenetic changes might be involved in the SZ pathology (Dempster et al., 2013).

1.3.2 Histone modification

Histone modifications are post-translational modifications to the histone proteins, which include methylation, phosphorylation, acetylation, ubiquitylation and sumoylation (Bannister and Kouzarides, 2011).

The modification can neutralise the histones positively charged N-terminal tails, thus changing the strength of their interaction with the DNA molecules and affecting transcription. Histone acetylation is performed by acetyltransferases (HAT) that catalyse acetylation of lysine amino acid in the N-terminal tails of histone 3 and 4. Further, histone deacetylation is performed by histone deacetylases (HDACs), which removes the acetyl groups in the chromatin, resulting in decreased gene expression (Peterson and Laniel, 2004)

Histone methylation consists of the transfer of methyl groups from S-adenosyl-L-methionine to lysine or arginine residues of histones (Greer et al., 2012). Histone methylation is best characterised in terms of histones H3 and H4. H3 undergoes methylation on lysines

4, 9, 27 and 36 and arginine 2, 17 and 26. H4 undergoes methylation on lysine 20 and arginine 3 (Jenuwein, 2001).

Overall, histone methylation can impact gene expression by altering the chromatin structure, affecting diverse biological processes (Garvin and Sharma et al., 2009).

Studies have shown changes in histone methylation in the *GAD1*, *GAD2*, *NPY* and *SST* genes in the prefrontal cortex of patients with SZ, wherein there was evidence that H3K4 methylation is upregulated in the human prefrontal cortex in the promoter region of GABAergic genes (*GAD1/ GAD2/ NPY/ SST*) (Lee et al., 2015).

1.3.3 DNA methylation

DNA methylation is the most studied epigenetic modification and consists of the addition of a methyl group to cytosine residues of the DNA. DNA methylation occurs in CpG dinucleotides, which are located in the regulatory or promoter regions, where they tend to be grouped in CpG islands (Bird, 1986). The methylation of CpG islands in promoter regions usually results in the transcriptional silencing of the associated genes (Teroganova et al., 2016).

Post-mortem studies found that patients with SZ have hypermethylation in *RELN* and *GAD67* promoters in the prefrontal cortex (Nishioka et al., 2012). Additionally, other rodent studies revealed significantly higher methylation of one of the two *PV* CpG sites in the hippocampus and prefrontal cortex after the administration of PCP to mice (Fachim et al., 2016).

1.3.4 miRNA

miRNAs are known to play an essential regulatory role by targeting the messenger RNAs of protein-coding genes, resulting in translational repression (Yekta et al., 2004). miRNAs are small

non-coding RNA molecules, with a length of around 21–25 nucleotides (Conaco et al., 2006), that are synthesised in the nucleus (as shown in Figure 10). Each primary transcript (pri-miRNA) is a short stem-loop structure known as a pre-miRNA, comprising a 5' cap and poly-A-tail. Pre-miRNAs are processed in the nucleus by the microprocessor complex Drosha/DgCR8 (Lewis et al., 2003). Pri-miRNA results in a fragment that is 70 bp in length and folded into a stem-loop structure, which processes the pri-miRNA in the nucleus. The pri-miRNA gets exported into the cytoplasm and cut by the type III ribonuclease Dicer to produce a 22 bp product (Hammond et al., 2000). The mature miRNA assembles into an RNA-induced silencing complex (RISC) with additional proteins, which is responsible for gene silencing (Freidman et al., 2009). RISC delivers mature miRNA to their mRNA targets, binding them to a complementary 6–8 bp 'seed region' that is located in the 3' UTR (Siergert et al., 2015). See figure 10 below for a graphic summary of the process. Several reports link miR-137 and miR-132/212 to SZ (Miller et al., 2012).

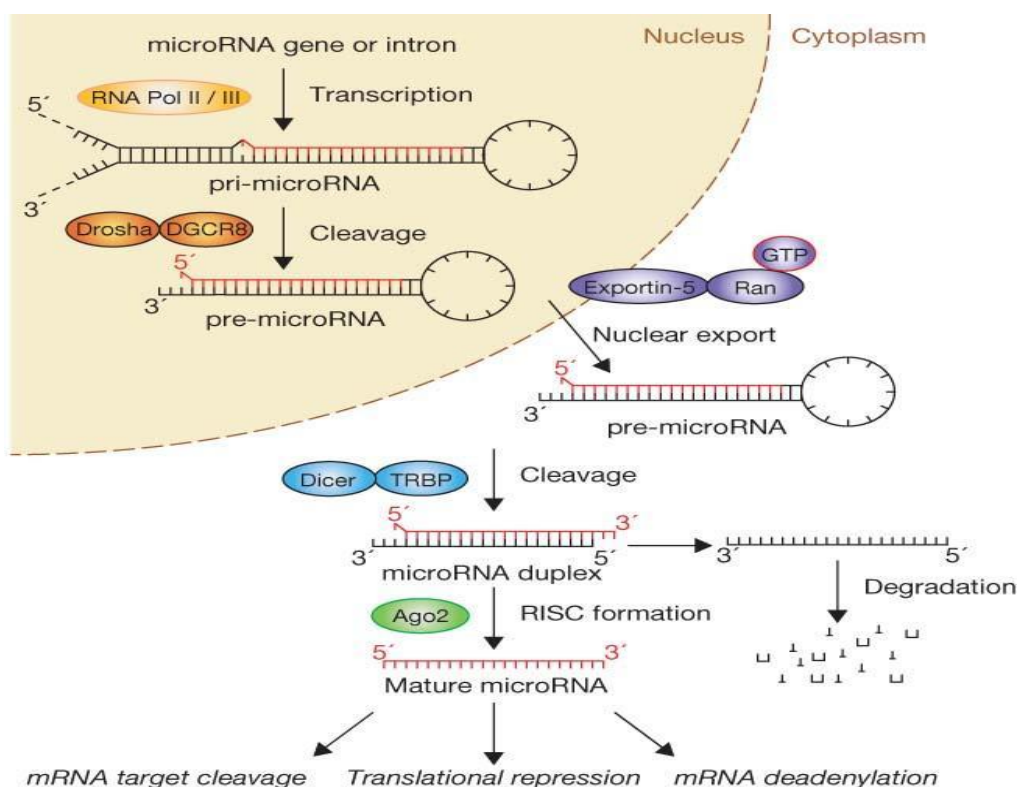


Figure 10: Overview of the processing of the miRNA. It is transcribed by RNA polymerase II. The nuclease consists of a complex (Drosha/DGCR8) that processes the pri-miRNA transcription to 70 bp RNA before exporting it out to the cytoplasm, where DICER cuts the dsmiRNA into 22 bp. Further, each strand in it works as a ssmiRNA, although only one can join the RISC and act on the target mRNA (modified from Winter et al., 2009).

1.3.5 miR-137

miR-137 plays a role in brain function and is involved in disorders such as schizophrenia and cancer (Mahmoudi and Cairns, 2016).

A recent study demonstrated that miR-137 could target mRNA modulates and regulate protein expression at the post-transcriptional level, thereby altering the development of the neurological signalling of the brain (He et al., 2018; Caputo et al., 2015).

Moreover, the upregulation of miR-137 leads to the reduction of mouse neural stem cell proliferation and accelerated neural differentiation in embryonic neural stem cells as well as the brain (Sun et al., 2011). Additionally, a mouse model showed that the upregulation of miR-137 is linked to SZ because it downregulates the presynaptic target genes complexin-1 (*Cplx1*), *Nsf* and synaptotagmin-1 (*Syt1*), which leads to impaired vesicle release and can reduce synaptic plasticity in the hippocampus (Siegert et al., 2015; He et al., 2018). The inhibition of miR-137 disrupts the mRNA translation of the GLUA1 receptor and dendritic outgrowth that links with the NMDA hypothesis of SZ (Thomas et al., 2017). Additionally, miR-137 suppresses *GRIN2A*, which causes abnormalities in the NMDA receptor (Mahmoudi and Cairin, 2016).

1.3.6 miR-132 and miR-212

miR-132 is another miRNA that is associated with SZ and is shown to influence NMDA receptors (Mirnics et al., 2001).

A study found that the upregulation of miR-132 inhibits the signalling of NMDA. Additionally, *DNMT3A*, *GATA2*, and *DPYSL3* are regulated by miR-132, which can alter their expression during normal neurodevelopment in SZ subjects (Miller et al., 2012).

Moreover, it was shown that miR-132 is required to control NMDA receptor depolarisation (Cheng et al., 2007). *GRIN2B* is a part of the heterotetramer in the NMDAr. The role of these heterotetramers is to mediate a slow Ca^{2+} that is permeable to the excitatory synaptic transmission in the central nervous system. *GRIN2B* expresses prenatally, and this expression begins to decline after birth. It is suggested to play a role in brain development and circuit formation. The failure of proper brain development can cause disorders such as SZ and autism spectrum disorder, etc. (Hu et al., 2014). Research reports that miR-132 can influence *GRIN2B* by creating a disturbance in glutamatergic neurotransmission. It is the downregulation by miR-132 in the *DNMT3A*, *GATA2*, and *DPYSL3* that causes the dysfunction of *GRIN2B* (Miller et al., 2012).

Additionally, Phencyclidine (PCP) SZ-like symptoms in rats. Phencyclidine (PCP) treatment in rats led to an increase in the phencyclidine-responsive transcript prt6 levels in the thalamus. Prt6 is a non-coding RNA that includes two miRNAs, miR-132 and miR-212, that are highly expressed in the brain. This study suggested that prt6 is involved in the onset of drug-induced SZ-like symptoms in rats (Takebayashi et al., 2014).

1.4 PCP treatment – An animal model of SZ

Animal models have often been used in research to mimic SZ symptoms in humans. PCP is an NMDA receptor antagonist that can cause psychosis in humans with SZ. It is assumed to do this by increasing the mesocortical pathway DA release in the PFC (Howes et al., 2015). This project focuses on the phencyclidine (PCP) rodent model of SZ disorder, where PCP treatment produces SZ-like symptoms in mice and rats. This model does not show the full spectrum of the SZ symptoms, as many reports state that PCP cannot provide the same aspects of the SZ disorder as seen in humans (Jones et al., 2011). However, many of the symptoms produced by PCP are similar to SZ. Various studies indicated that PCP does mimic SZ-like symptoms in animals; in monkeys treated with PCP, for example, there was a reduced DA turnover and a decrease in the BDNF mRNA as well as loss of the dendritic spine synapse in the dorsolateral PFC (Matsuzaki and Dowling, 1984). Further, another study showed a decreased expression of GABAergic markers after PCP treatment (Amitai et al., 2012), which is associated with cognitive impairments linked to SZ. Treatment with PCP can also induce an enduring deficit of *PV* neurons and a reduction in *PV* gene expression, resulting in cognitive deficits similar to the ones seen in SZ patients (Karlund et al., 2012).

Behaviourally, PCP affects the sensitisation of the locomotor responses in rodents and causes deficits in novel object recognition. The deficiency caused by PCP is linked to a reduction in the basal and stress-induced DA and glutamate release as well as a decrease in synaptic spines and *PV* neurons (Jentsch and Taylor, 2014). Additionally, it was found that the PCP animal model of SZ results in hypermethylation in the *PV* promoter, which contributes to *PV* deficits (Jones et al., 2011).

Subchronic dosing with PCP (2 mg/kg, twice daily for a week) is a model devised by Dr Neill and colleagues, that not only induces behavioural changes similar to those seen in humans with SZ but also shows effects on the parvalbumin neurons in the hippocampus (Fachim et al., 2016)

Overall, PCP treatment in rodents produces a model that mimics SZ reasonably well in terms of behaviour and gene expression.

1.5 Exercise

Environmental factors often trigger epigenetic changes. Smoking and stress, for example, have adverse effects on human health.

In contrast, physical exercise has shown to provide many positive health benefits for humans (Barrès et al., 2016).

Many studies have observed exercise benefit cognitive domains of SZ (Erickson, 2015) as well as result in an increase in hippocampal volume and improve short-term memory function that is correlated with improved aerobic fitness (Firth et al., 2014).

Many papers indicate an active link between memory and exercise. A recent meta-analysis on schizophrenia and exercise analysed all the controlled trials that investigated the cognitive outcomes of exercise interventions in SZ (Malchow et al., 2013). This study showed that individuals with SZ had experienced degradation in their physical health, specifically in cardiorespiratory fitness and metabolic health. Additionally, the study showed that better physical fitness was linked to better cognitive performance, which results in higher grey and white matter volumes as well as higher levels of a neurotrophic factor (BDNF) and supports the growth, survival, and differentiation of

both developing and mature neurons, promotes plasticity and results in improved social cognition and working memory (Firth et al., 2016).

Dietich and colleagues showed an exercise-induced increase in the expression of hippocampal NMDAr and *BDNF* in a rodent model of SZ (Dietich et al., 2005). After the mice were treated with MK-801 (a drug that can antagonise the NMDA receptor), they exhibited a reduction in NMDAr expression and an enhanced expression of the tyrosine hydrolysate, which resulted in an increased rate of DA synthesis and reduction of BDNF receptor expression (Dietich et al., 2005; Park et al., 2014).

Gavish and colleagues conducted an experiment on female mice that showed that exercise improved the cognitive deficits of dominant-negative DISC1 transgenic mice (Segal-Gavish et al., 2017).

Additionally, our collaborators noticed a positive effect of its use on cognitive tasks in rats treated with PCP (Heaney et al., 2018). Overall, exercise appears to improve cognitive performance. In addition, the specific genes affected by exercise are also associated with an improvement in DA and NMDAr.

1.6 Hypotheses

The work presented in this thesis focuses on the “possible” changes in expression of specific genes and miRNAs in the context of a rodent model of SZ after exercising. The work aimed to determine whether exercise exerts positive benefits on “epigenetic” changes in this model of SZ and extends the report from Dr Neill from the University of Manchester based on a behavioural study of an SZ model using PCP-treated rodents. That study observed an alleviation of schizophrenia symptoms after rodents exercised (Hearney et al., 2018). As exercise exerts profound effects on the

hippocampus and memory, we decided to investigate the expression of specific genes of interest in the hippocampus of the PCP-treated rats with or without access to the running wheel.

These hypotheses were tested by quantifying the expression of *GRIN2A*, *GRIN2B*, *COMT*, *Egr-1*, *DISC1*, *NTrK2*, *GAD-1* and *PV* as well as miRNAs miR-132, miR-137 and miR-212 in a rat PCP model of schizophrenia. Two studies were conducted – impact after six weeks of voluntary exercise (Study 1) and impact after six weeks of exercise followed by two weeks of no exercise (Study 2).

Thus, the hypotheses were as follows:

1. In a model of SZ (PCP treatment), specific genes of interest (*GRIN2A*, *GRIN2B*, *COMT*, *Egr-1*, *DISC1*, *NTrK2*, *GAD-1*, *RELN* and *PV*) will be downregulated and particular miRNAs will be upregulated (miR-132, miR-137, miR-212).
2. Exercise (wheel running) will reverse the effects of PCP treatment on the expression of those genes and miRNAs.
3. The effect of exercise will continue during the two weeks of rest when the animals do not have access to a running wheel. As to see whether any changes have occurred during the rest period.

2. Methods

2.1 Experiments on animals

Dr Neil and her team conducted the animal (behavioural) experiments and dissection of the dorsal hippocampus at the University of Manchester. All procedures and animal care were in accordance with the UK Animal (Scientific Procedures) Act 1986 and EU Directive 2013/63- and were approved by the University of Manchester Animal Welfare and Ethical Review Board (AWERB). 40 female Lister-hooded rats (Charles River, UK), (265 +/-50 g at the start of the experiment) were housed in four groups. All the rats were housed in a temperature- and humidity-controlled environment with food and water ad libitum. The four groups were – non-exercise (non-ex) (n = 10) (control), non-exercise-PCP (sed-PCP) (n = 10), exercise (ex) (n = 10) and exercise-PCP (ex-PCP) (n = 10) and housed in groups of five per cage. The University of Nottingham was assigned 40 rats to perform the genetic analysis. Therefore, the design of this project adhered to the following pattern: Study 1 had a total of 20 rats, with each group containing five rats, and Study 2 followed the same pattern, as shown in Figure 11.

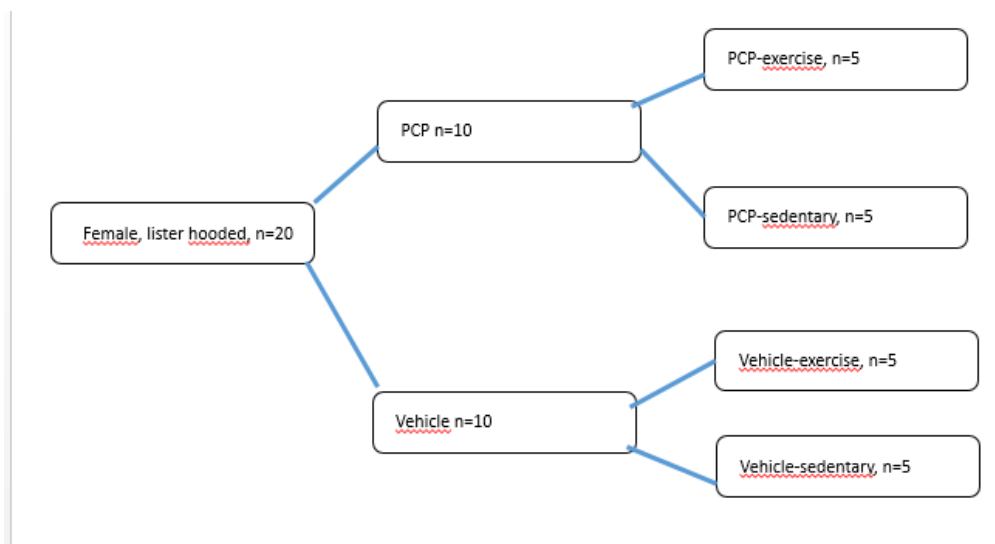


Figure 11: Study 1 (20 rats) and Study 2 (20 rats) both follow the same procedure, with the only exception being that, in Study 1, the rats were killed after six weeks exercise while, in study 2, the rats had a rest period of 2 weeks after the 6 weeks of exercise intervention.

Twenty animals were given PCP, administered at a dose of 2 mg/kg intraperitoneal twice on a daily basis, between 09:30–16:30, for seven days (Heaney et al., 2018). The other twenty animals received vehicle (0.9% saline) intraperitoneal injections (PCP and saline were at 1 ml/kg dose). After a seven-day washout period to ensure the drugs did not influence the behaviour of the rats, the rats were assigned to either voluntary the exercise (wheel running, 20 rats) or control (non-exercise, 20 rats) group. Rats in the exercise group were permitted to run on the wheel for one hour a day, five days a week during the intervention phase, whereas the control group was placed in individual cages with an upturned plastic tunnel, for one hour a day for five days a week, during the intervention phase. The exercise intervention lasted for six weeks.

Six weeks after starting the exercise intervention, half of the rats (Study 1) were humanely killed by cervical dislocation; their

brains were extracted, the dorsal hippocampus dissected and immediately frozen (at -80 °C) until further analysis. The remaining 20 rats (Study 2) remained undisturbed in their cages for two more weeks (without exercising) before being humanely killed, their brains being extracted and their dorsal hippocampus being dissected and immediately frozen (at -80 °C) until further analysis.

2.2 Extraction of DNA and RNA

Frozen tissues were transferred from Manchester University to the University of Nottingham, where they were processed. RNA was extracted from the dorsal hippocampus using Trizol (Sigma) and following the manufacturer's instructions. Thereafter, the genomic DNA was isolated from the intermediate phase (Trizol separation) following the Back Extraction protocol (Chang lab, Stanford). Below is a detailed protocol.

1) Tissue homogenisation.

Frozen samples were weighed; if the tissues weighed between 50–100 mg, 1 ml of TRI reagent was added. If the tissues weighed between 25–50 mg, 0.8 ml TRI reagent was added. Right after, the tissues were homogenised manually. Homogenates were frozen at -80 °C until further processing.

2) Phase separation.

Depending on the weight of the starting material, 200 µl of chloroform per ml of TRI reagent was added (i.e. 200 µl for tissues weighing more than 50 mg and 160 µl for tissues weighing 25–50 mg). Samples were mixed and then left for 2–15 min at room

temperature, followed by centrifugation at 12,000 g for 15 min at 2–8 °C. After centrifugation, there were three phases: a lower, red phase or organic phase (containing proteins), interphase (containing the DNA) and, finally, a colourless upper aqueous phase (containing the RNA).

3) RNA precipitation.

In order to precipitate the RNA, the aqueous phase was transferred to another tube, and 500/400 µl isopropanol (500 µl isopropanol per ml of TRI reagent) was added to it. Samples were mixed gently and then incubated for 10 minutes at room temperature. Thereafter, the samples were centrifuged at 12,000 g for 10 minutes at 4 °C, and the supernatant was removed. This left behind a white pellet (RNA). The RNA pellet was washed by adding 1 ml/0.75 ml of 70% ethanol (1 ml per ml of TRI reagent) and mixed by inverting the sample and centrifuging for 5 minutes at 4 °C. The supernatant was carefully removed, and the RNA pellet was air-dried under the hood. Further, 100 µl of Millipore H₂O was added to the pellet and mixed using pipetting in order to resuspend the RNA. It was left for 10–15 minutes at 55–60 °C to allow it to dissolve thoroughly.

4) DNA isolation.

After removing the aqueous phase (containing the RNA) 500/400 µl ml back extraction buffer (BEB) (500 µl BEB per 1 ml of Trizol) was added to the remaining interphase as well as organic phase and mixed on a shaker for 10 minutes. The samples were centrifuged at 12,000 g for 30 minutes at room temperature. Afterwards, the upper phase (containing DNA) was transferred to another tube where 500/400 µl chloroform (500 µl per 1 ml of Trizol) was added and mixed before being centrifuged for 2

minutes at 16,000 g. The upper phase was transferred again to a new tube, and 400/320 µl of 100% ethanol was added (400 µl per 1 ml of Trizol) before being incubated for 5 minutes at room temperature. The samples were then centrifuged at 12,000 g for 15 minutes at 4 °C, followed by the removal of the supernatant. The pellet (DNA) was washed with 500/400 µl of 70% ethanol (500 µl per 1ml of Trizol). The sample was then spun down at 12,000 g for 5 minutes at 4 °C, the supernatant was removed, and the pellet (DNA) was allowed to air dry for 10 minutes before being dissolved in 50 µl of elution buffer (Qiagen).

The RNA and DNA quality and concentration were determined using the spectrophotometer (ND-1000, Nanodrop, UK).

2.3 PCR

2.3.1 Primer design

PCR primers were designed and tested in silico in order to ensure that their amplification was specific and there were no dimers formed during amplification as follows.

The sequence of the genes of interest was found using the gene database at NCBI (<https://www.ncbi.nlm.nih.gov/>). Primers were designed with the aim of including an intron in the PCR product (i.e. each primer sits in a different exon), thus minimising the amplification of genomic DNA. The program serial cloner (http://serialbasics.free.fr/Serial_Cloner.html) was used to store and edit the intron/exon gene sequences, while Primer3 was used to design the primers (<http://primer3.ut.ee/>). The criteria for primer design was – 18–20/23 nucleotides as primer size and the product size range was 75–200 bp. The in silico testing of

the primers in order to determine whether they would produce dimers during amplification was performed using Amplify (<http://amplifx.software.informer.com/1.7/>).

Additionally, the primer sequence was blasted against the entire rat genome (using https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=OGP__10116__10621&LINK_LOC=blasthome) to confirm whether they were specific to the gene of interest.

2.3.2 The annealing temperature

To determine the optimum annealing temperature for each primer, different PCR reactions were performed (each with a different annealing temperature). The PCR reactions contained a buffer (1.5 mM), MgCl₂ (25 mM), dNTP (10 µM), primer (10µM) and TAQ (1X standard). The PCR amplification cycle consisted of the following steps: (1) 2 min at 98 °C (enzyme activation) followed by 35 cycles of 96 °C (2 minutes, denaturalization), (2) 54–64 °C (20 seconds, gradient annealing) and (3) 55 °C (30 seconds, elongation) followed by 10 min at 72 °C (final elongation). Thereafter, amplicons were stored at 4 °C and then run on an agarose gel (2%) to determine whether the amplified product was of the correct size. Table 2 lists the reagents used to perform the PCR (Sigma, UK; Thermofisher, UK).

Table 1 summarises the designed primers and their characteristics. The housekeeping genes used were *GAPDH*, *PGK1* and *HrPT1* (Gaskin et al., 2016). We included *PV* as a positive control because it has been shown to get downregulated by a similar PCP treatment as in our study (i.e. 2 mg/kg PCP, twice a day for seven days) (Fachim et al., 2016).

Gene	Primer ID	Primer sequence	Amplicon Size Bp	Annealing temp/°C
GAD-1*	PGF-21, PGR-22	F:CAAAGGACCAATAGCCTG GA R:CGTCTTGCGGACATAGTT GA	240	61
PV*	PGF-26, PGR-27	F:GCGGATGATGTGAAGAAG GT R:AGTCAGCGCCACTTAGCT TT	225	63
GRIN2 B	PGF-139, PGR-138	F:CAGCGCTGAATGGCTCTA AG R: CTGGAATGGCATGATCGGT G	174	58
GRIN2 A	PGF-158, PGR-159	F: CCTGGCTACCGAGACTTCAT R: CACGTTCTGCATATCCCAGC	137	58
NTrK2	PGF-142, PGR-143	F:CTAACACATTGACCCAGA GAAC R: AAGCCGGAATCCACAATTGT AAG	130	83

ERG1	PGF-144, PGR-145	F:GCTCACTCCACTATCCAC TATCA R: ACTGGTAGGTGTTAAGAGC C	169	83
COMT	PGF-146, PGR-147	F: AATCTGACCCTTTCTATGCT CCT R: CCTCAATTCAGCCTTTCATC CAT	169	58
RELN	PGF-156, PGR-15	F: GTGGCCAGAAGTACACTCC T R: CGGTCACTCTTCTCCAATGT	180	65
DISC1 **	PGF-162, PGR-163	F:CAGCTCTAAGTCTCTG GCTTCTA R:GCTGAAGTTGGAAGTA AAGGTGT	157	-
DISC1 **	PGF-164, PGR-165	F:GAGATTTGGGCCGTGT CATC R:CCCAGTCTTCTCGTGTT CCT	91	-

*Table 1: Primers designed and/or used in this study (sequence and direction of each primer). Annealing temperature shows the temperature where the best amplification occurred (highest yield and specificity). Primers for GAD-1 and PV were designed by Phil Gaskin (Gaskin et al., 2016). DISC1 primers were discarded as they did not produce multiple amplicons. ** Neither of the tested DISC1 primers provided specific amplification and were therefore not included in this study.*

2.4 mRNA Assays (qPCR)

Afterwards the optimal primer concentrations for qPCR were determined for each primer pair. For this purpose, qPCR reactions were performed in triplicate with the SensiMIX Plus SYBR Green PCR kit (Bioline, UK) in a RotorGene 6000 cycler (Qiagen, Germany). The concentration of the reagent used in the Master mix was as follows: SensiMix (1X), primers (50–800 nM), cDNA (1:4 dilution of a pool of all samples). The qPCR amplification cycle consisted of the following steps: 2 min at 95 °C (enzyme activation) followed by a total of 40 cycles of (a) 10 seconds at 95 °C (denaturalization), (b) 1 min (for annealing temperature for each primer see Table 2) and (c) 1 min at 72 °C (elongation) followed with a melt curve analysis (to confirm single product amplification). Samples were run in duplicates.

Finally, a standard curve for each primer pair was performed in order to calculate the primer efficiency and determine the dilution to use when testing the samples. For this purpose, we created a pool with 3 µl of each sample cDNA and subsequently performed a serial dilution of 1 to 4 from the pool (see Table 3). Thereafter, 1.5 µl from each dilution was used for the standard curve qPCR (additional reagents, SensiMix 1X and primers at the concentration level determined above and water to reach a volume of 15 µl). The cycling was performed (steps, times and temperatures) as described above.

Ratio	Code
1:4	B
1:16	C
1:64	D
1:256	E
1:1024	F
1:4096	G

Table 2: The above table displays the values for the 1:4 serial dilution used in this study.

2.4.1 mRNA Quantification

In order to quantify the expression of each gene, 3 µl of each sample's cDNA was added per tube, followed by 12 µl of Master mix (containing SensiMix (X1), H₂O, Primer (concentration as determined before) and Betaine (1 M). Samples from all groups were evenly distributed through the rotor of the qPCR machine to ensure that there were no artefacts caused by virtue of location. All samples were run in triplicate. Due to a large number of samples, we performed two runs for each gene. In order to obtain precise results, two control samples were included in each run. The CT of these controls was set at the same values in both runs to allow the comparison of the CT of the samples. Amplification was performed in the RotorGene 6000 cyclers (Qiagen, Germany) and the qPCR amplification cycle was identical to the one that was used for the standard curve (see above).

The value obtained from the qPCR would be used in statistical calculation, in order to understand the significance of the results (see 2.7 and 2.8).

2.5 Sequencing

For quality control purposes (confirm sequence), we sequenced the amplification product for each primer pair in order to verify the identity of the product amplified by the primers. For this, we first purified the amplified PCR product and then sequenced it.

2.5.1. PCR product purification

The amplified PCR samples for each primer pair were purified using the QIAquick PCR purification kit (Qiagen, Germany) and following the manufacturer's instructions. Briefly, the PCR product was mixed with five times the volume of buffer PBI. The mixture was placed in a QIAquick column and centrifuged for 45 seconds at 13,000 rpm. The flow-through was discarded, and the QIAquick column was placed back in the same tube. 750 µl of PE buffer was used to wash the sample and was then again centrifuged for 1 min at 13,000 rpm. The flow-through was discarded, and the column spun for another minute. Finally, the QIAquick column was removed and placed in a clean 1.5 ml microcentrifuge tube before 30 µl of EB buffer (10 mM Tris-Cl, pH 8.5) was added into each column. It was allowed to stand for 1 min with a closed lid and centrifuged for 1 min at 13,000 rpm to elute the PCR product. DNA quality and quantity were measured on a Nanodrop (ND-1000, Nanodrop, UK).

2.5.2 DNA Sequencing reaction

The sequencing reaction was performed using the BIGDYE kit (Life Technologies, USA) by following the manufacturer's instructions. In brief, reagents were added to a tube as described in Table 3. The sample was amplified in the PCR machine (25 cycles for – (1) 96 °C (30 seconds), (2) 50 °C (15 seconds) and (3) 60 °C (4 min)). Thereafter, the samples were given to the Sequencing Facility SoLS to be run on a 3130xl ABI PRISM Genetic Analyzer (Life Technologies, USA).

Reaction	µl of sample
Big Dye	1
Seq Buffer	2
Template	1
Primer	1
PCR grade water	5

Table 3: The reagents and volumes for the DNA sequencing reaction

2.6 miRNA quantification

The detection of miRNA was performed with 203301 Universal cDNA synthesis (reverse transcription) and PCR with ExiLENT

SYBR Green Master 203420 kits EXiqon (qPCR) both from EXiqon (Qiagen, Denmark) by following the manufacturer's instructions.

2.6.1 cDNA synthesis (for miRNA detection)

The total template RNA was diluted to a concentration of 5 ng/ μ L using nuclease-free water. Primers were re-suspended by adding 80 μ l of nuclease-free water to the tube and mixing thoroughly. Before the RT reaction, 1 μ l of the synthetic spike was added per 20 ng of total sample RNA to the control sample. For the sample, the reagents were combined as described in Table 4, with the enzyme mix added last. The reaction was incubated for 60 min at 42 °C, and then the reverse transcriptase was heat inactivated for 5 min at 95 °C before being immediately cooled to 4 °C and stored in a -20 °C freezer until the qPCR analysis (which took place within two weeks).

Reagent	Volume μ l, RT reaction
5X reaction buffer	2
Nuclease-free water	4.5
Enzyme mix	1
Synthetic RNA spike	0.5
Template total RNA (5ng/ μ l)	2
Total	10

Table 4: The reagents for miRNA reverse transcription

2.6.2 qPCR (for miRNA detection)

The cDNA was diluted 80X in nuclease-free water in a low-nucleic acid binding tube. The primers were re-suspended by adding nuclease-free water to the tube and mixing thoroughly. The cDNA, nuclease-free water and PCR master mix were placed on ice and allowed to thaw for 15–20 minutes. Thereafter, the reagents were mixed as described in Table 5 and amplified in the qPCR machine using 40 cycles of 95 °C (10 minutes) followed by 60 °C (1 minute). UniSp6 RNA was used as the house-keeping gene control. miR137 was used as the positive control for exercise because previous literature links it with SZ and our unpublished results suggests that exercise upregulates it (He et al., 2018; Seigert et al., 2015). Additionally, a non-template control (NTC) was also used for each miRNA tested.

Reagent	µl Volume for miRNA master mix	µl Volume for RNA spike master mix
PCR master mix	5	5
PCR primer mix	1	1
Diluted cDNA template	4	4
Total	10	10

Table 5: The reagent mix for miRNA qPCR reactions

2.7 Normalisation and analysis of qPCR data

In order to normalise the qPCR data (gene expression and miRNA), we used a modified version of the Pfaffl method (mathematical model for relative quantification in real-time RT-PCR, seen below) (Pfaffl, 2001).

$$ratio = \frac{(E_{ref})^{\Delta CP_{ref}(control-sample)}}{(E_{ref})^{\Delta CP_{ref}(control-sample)}}$$

For gene expression, the relative expression of the genes was normalised to the geometric mean of the control genes following the GeNorm normalisation (Vandesompele et al., 2002).

2.8 Statistical analysis

Due to the sample size, the data was analysed using non-parametric Mann-Whitney tests, run in Prism 7 (<https://www.graphpad.com/scientific-software/prism/>). This was chosen because the data did not show a normal distribution. The significance was set at $p < 0.05$, and the Mann-Whitney was calculated as $U_{crit} = 2$, which is the U value for whether the null hypothesis is rejected. The data was represented as the mean $\pm$ the standard deviation (mean $\pm$ SD).

2.9 Sample size calculations

In order to understand the correct sample size to obtain statically significant. It was ideal to calculate the power of the sample size and should ideally be calculated before the experiment is proceeded. (Ideally, during the behaviour study). However, as this experiment takes place after the behaviour study analysis, this power analysis refers to chapter 2.4, 2.7 and 2.8.

Using the data produced by our experiment, we calculated the optimal sample sizes for an adequately powered future experiment using the software G*power (<http://www.gpower.hhu.de/>).

The settings used were – acceptable power 0.8 (beta) and alpha 0.05 in the G*power software. Additionally, effect sizes were calculated in G*power from the data derived from these studies(Scheewe et al., 2013; Thoma et al., 2009).

3. Results

The results show that, while there were slight changes in gene expression with exercise and PCP treatment, very few of these differences are statistically significant.

Sections 3.1 to 3.2 describe the results from primer design. The results of the gene/miRNA expression assays in the rats are described in section 3.2.–3.5

3.1 The correct choice of primers

The correct design of primers was essential for the project. Agarose gels confirmed that the specific PCR products for the genes (*RELN*, *GRIN2A*, *GRIN2B*, *EGR-1*, *DISC1*, *NTrK2*, *COMT*, *Gad-1*, *PV*) were amplified (see Figure 12). However, despite multiple attempts, it was not possible to obtain a single PCR product of the correct size for *DISC1*, and this gene was not further studied (see Figure 13).

Sequencing confirmed the identity of the PCR products (see appendix).

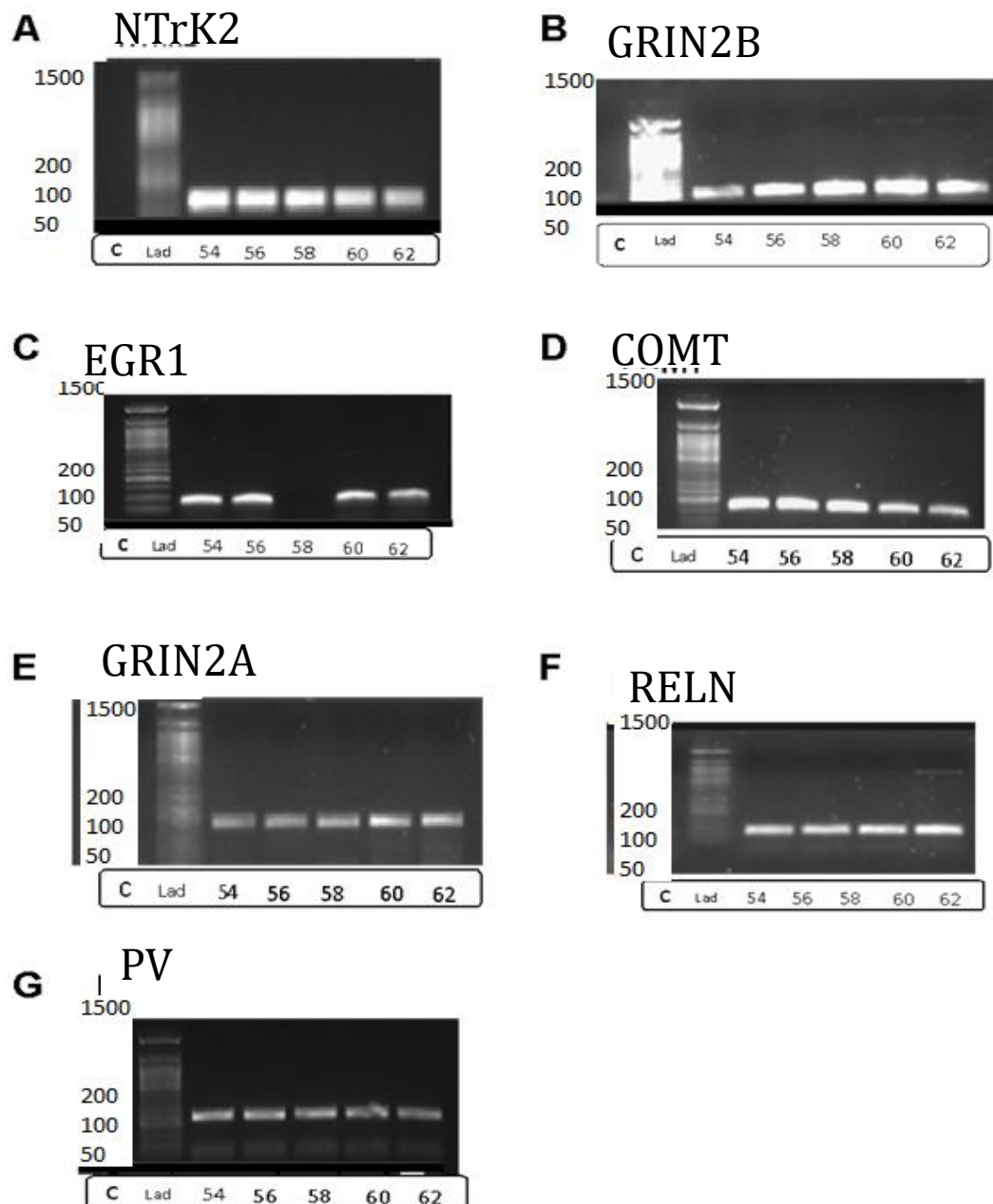


Figure 12. Images of PCR products (RELN, GRIN2A, GRIN2B, EGR-1, DISC1, NTrK2, COMT, Gad-1, PV) run on a 2% agarose gel with 50 bp ladder. The annealing temperature were from 54°C–62°C.

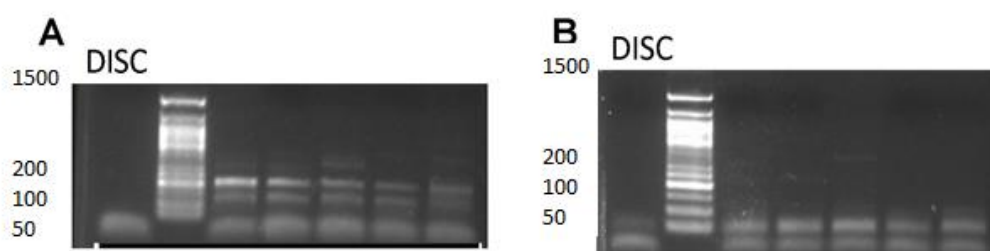


Figure 13: Images of PCR products of DISC that failed to provide single bands of the correct molecular weight and thus could not be used for further analysis. The annealing temperatures were from 54°C–62°C. The PCR products were run on a 2% agarose gel with a 50 bp ladder.

Further, the optimal primer concentration for qPCR was determined (see Table 7). The range of the optimal primer concentrations was between 500–800 nM except for COMT, which was lower. The optimal primer concentrations for the housekeeping genes (GAPDH-5', HrPT1 and PGRK1) was known thanks to the previous calibrations performed in the lab (300–400 nM). Finally, the efficiency of the primers and the best dilution of the sample cDNA was determined (see Table 6).

Gene	Primer	$T_m(^{\circ}\text{C})$	stdcv effect	stdcv dilution	CT	log	conc	gain
Gad-1	PGP21 PGP22	61/62	0.77	c(1:16)	22.81	4	500	9
PV	PGP26 PGP27	63	0.87	c(1:16)	27.60	4	800	9
Grin2B	PGP38 PGP39	58	0.84	c(1:16)	27.88	2	500	9
Grin2A	PGP58 PGP59	58	1.01	c(1:16)	22.01	4	500	9
NTrK2	PGP41 PGP43	54	0.82	c(1:16)	23.00	3	400	9
COMT	PGP46 PGP47	58	0.91	c(1:16)	23.58	3	200	9
Egr1	PGP144 PGP145	54	0.97	c(1:16)	21.93	3	400	9
Reln	PGP58 PGP59	65	0.89	c(1:16)	29.70	3	500	9

Table 6: Optimal parameters for amplifying each gene of interest in qPCR. The temperature is T_m , standard efficiency is StdCv effic and standard dilution is STD CV Dilu ideal. Further, cycle threshold (CT) and CT log was used to determine the expected range of CT for the samples. The optimal primer concentration (Conc) and gain were used as settings for the qPCR machine for each run.

3.2 Results of gene expression in rat models

In this chapter, I will present the results of the expression of genes of interest (*Grin2A*, *Grin2B*, *COMT*, *EGR-1*, *DISC1*, *NTrK2*, *GAD-1*, *RELN* and *PV*) in Study 1 and Study 2

3.2.1 Expression levels of *GRIN2A*, *GRIN2B*, *COMT*, *EGR-1*, *DISC1*, *NTrK2*, *GAD-1*, *RELN* and *PV* in a rat model of schizophrenia with or without six weeks of voluntary exercise

3.2.1.1 Gene expression in a model of SZ (PCP treatment) in rats

It was hypothesised that the expression of our genes of interest (*GRIN2A*, *GRIN2B*, *COMT*, *EGR-1*, *DISC1*, *NTrK2*, *GAD-1* and *PV*) would downregulate after the PCP treatment. Six weeks after PCP treatment, there was a tendency of reduction in the *RELN*, *GAD-1*, *NTrK2*, *GRIN2A* and *COMT* gene expression, but this was not statistically significant (Figure 14, Table 7). *PV* mRNA (positive control based on the previous finding) showed no downregulation on expression. There was no significant change in any of the genes of interest (Figure 14, Table 7).

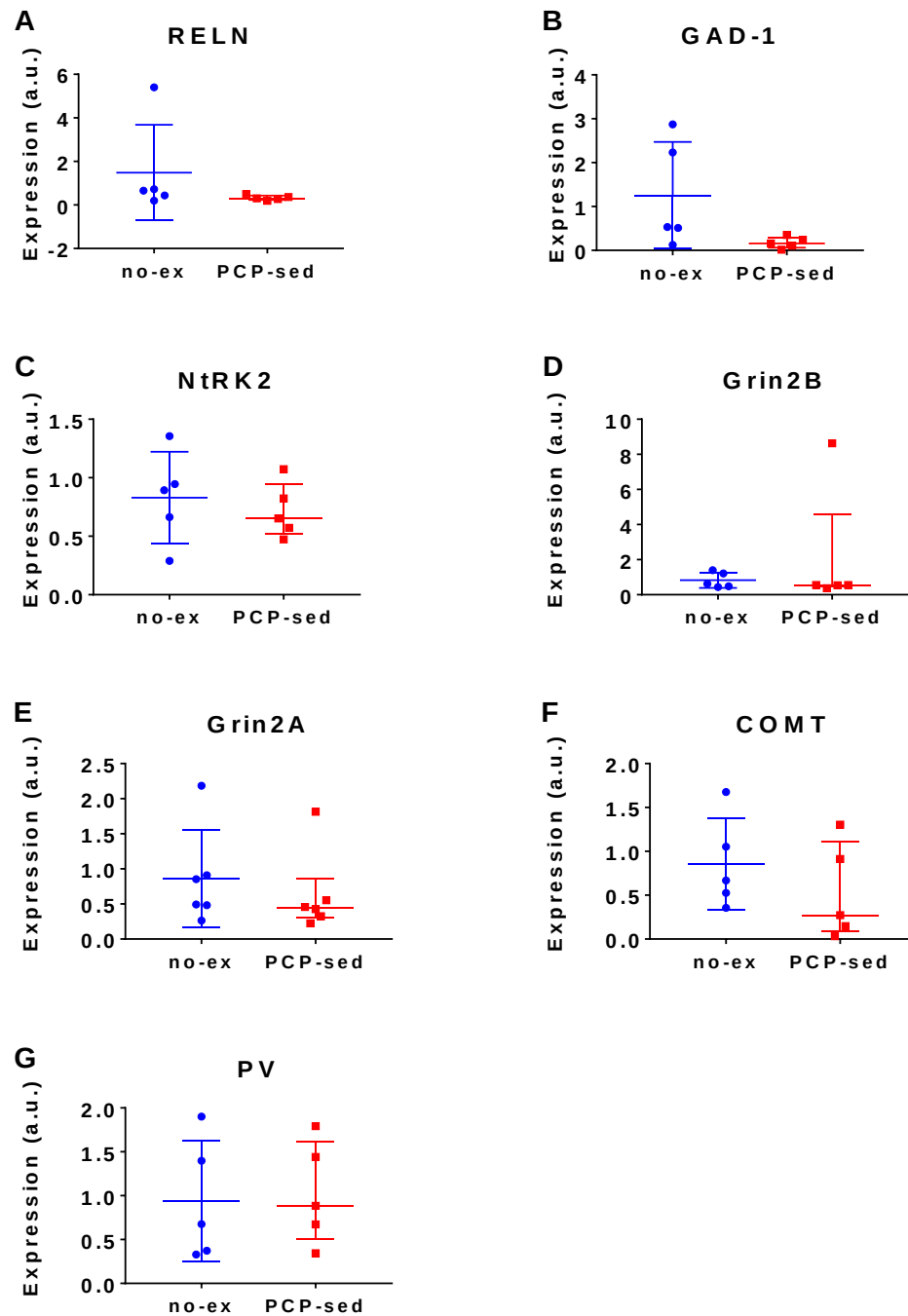


Figure 14: Effect of PCP on the hippocampal expression of our genes of interest. Animals were treated with PCP or vehicle for 1 week, followed by a washout period and 6 weeks of normal housing conditions with no opportunity to exercise. Panels: RELN (A), GAD-1 (B), NtrK2 (C), COMT (D), GRIN2A (E), GRIN2B (F) and PV (G). Mean ($\pm$ SD).

Gene	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
RELN	no-ex	0.3048	Sed-PCP	0.5847	5	0.1508
GAD-1	no-ex	0.158	Sed-PCP	0.562	3	0.0556
NTrK2	no-ex	0.652	Sed-PCP	0.874	9	0.5476
Grin2A	no-ex	0.458	Sed-PCP	0.819	11	0.3095
Grin2B	no-ex	0.553	Sed-PCP	0.806	11	0.7973
PV	no-ex	0.884	Sed-PCP	0.572	11	0.8413
COMT	no-ex	0.273	Sed-PCP	0.654	7	0.3095

Table 7: Mann-Whitney results from comparing non-exercise (no ex) with non-exercise-PCP (PCP-sed) when the rats underwent PCP treatment

3.2.1.2 Effect of exercise on gene expression

It was hypothesised that exercise would increase the expression levels of the genes of interest in the animals that are not receiving PCP treatment. Exercise alone had no statistically significant effects on the expression of the genes of interest in animals that had free access to a running wheel (Figure 15, Table 8).

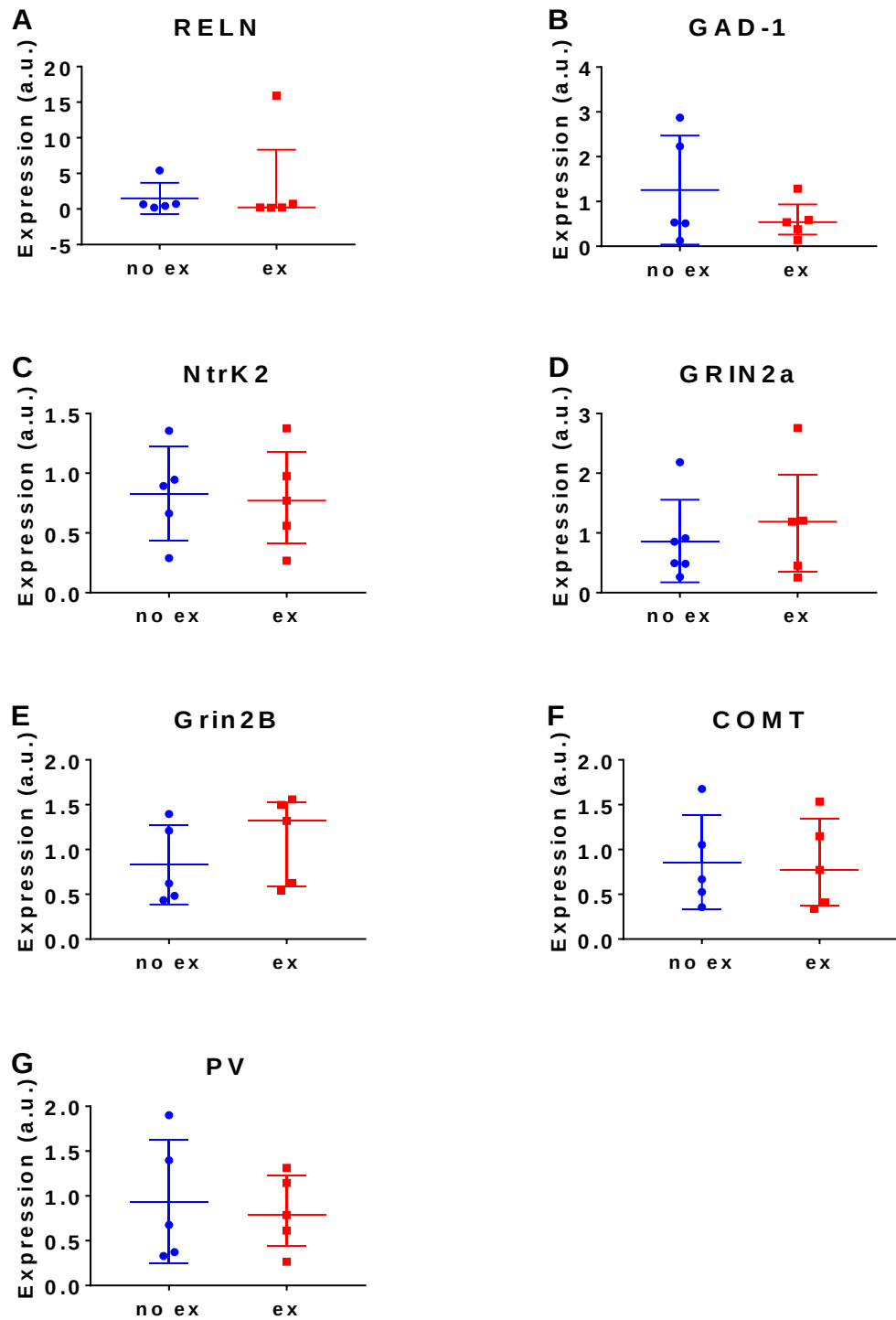


Figure 15: The effect of 6 weeks of wheel running exercise on the hippocampal expression of our genes of interest. Panels: RELN (A), GAD-1 (B), NTrK2 (C), COMT (D), GRIN2a (E), GRIN2B (F) and PV (G). Mean ($\pm$ SD). These are all none treated animals.

Gene	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
RELN	no ex	0.547	ex	0.214	6	0.685
GAD-1	no ex	0.530	ex	0.538	11	0.842
NtrK2	no ex	0.893	ex	0.770	12	0.999
Grin2A	no ex	0.493	ex	0.822	9	0.902
Grin2B	no ex	0.620	ex	1.319	6	0.222
PV	no ex	0.676	ex	0.786	11	0.841
COMT	no ex	0.667	ex	0.773	12	0.999

Table 8: Mann-Whitney values for the comparison between non exercise (no ex) with exercise (ex) rats.

3.2.1.2 Effect of exercise on gene expression in a model of SZ (PCP treatment) in rats

The exercise was hypothesised to reverse the effects of the PCP treatment and was expected to produce an increase in the gene expression in the exercised animals.

Figure 16 shows that there were no significant changes in gene expression when comparing PCP-exercised rats that exercised with PCP-treated animals who did not have access to a running wheel(non-exercise-PCP).

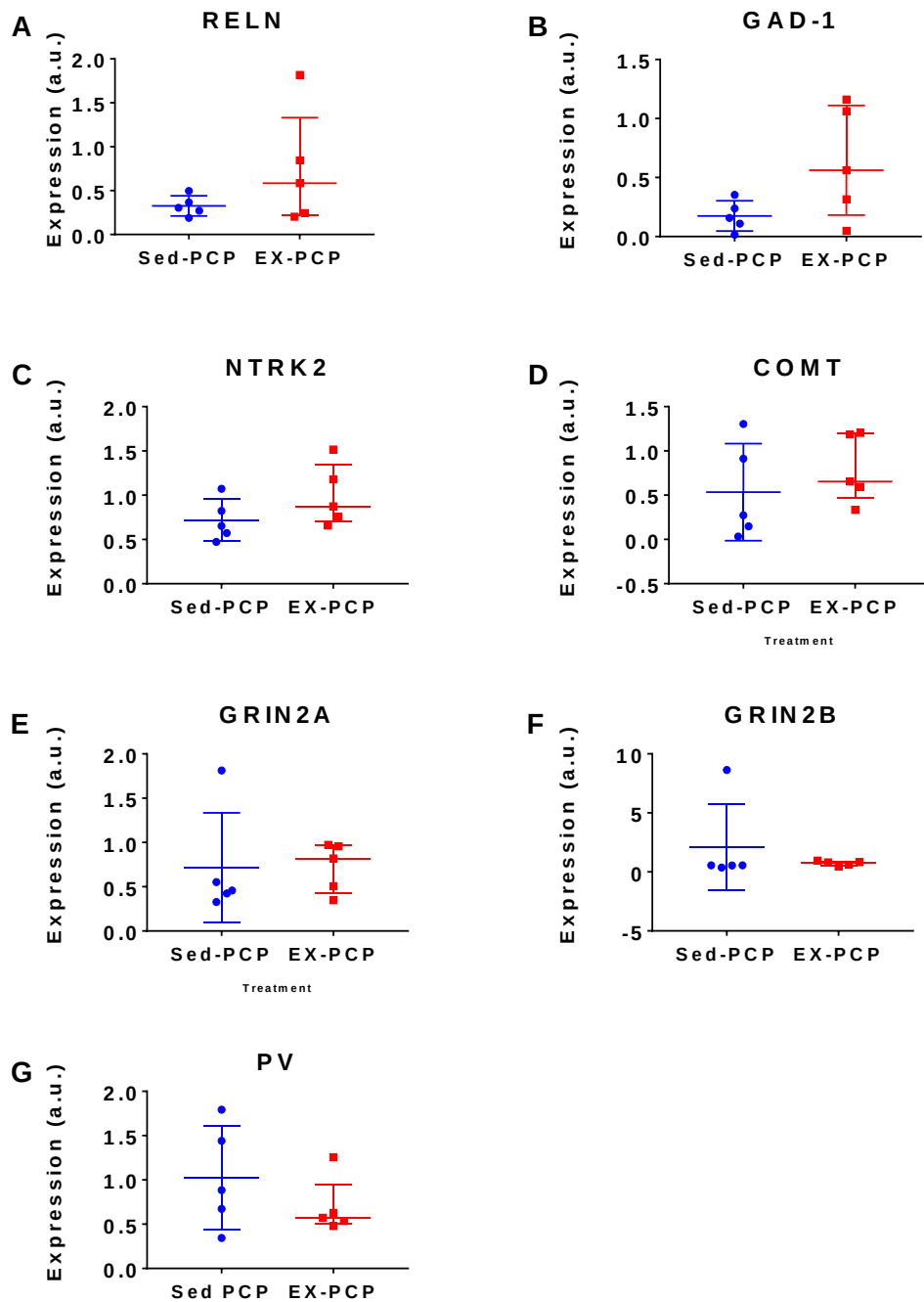


Figure 16: Effect of exercise on hippocampal expression in PCP-treated animals. Animals were treated with PCP, followed by a washout period of one week, then 6 weeks of wheel running. Panels: RELN (A), GAD-1 (B), NTrk2 (C), COMT (D), GRIN2a (E), GRIN2B (F) and PV (G). Mean ($\pm$ SD).

Gene	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
RELN	sed-PCP	0.6589	ex-PCP	0.3048	8	0.1508
GAD-1	sed-PCP	0.53	ex-PCP	0.158	5	0.0556
NTrK2	sed-PCP	0.893	ex-PCP	0.652	5	0.5476
GRIN2A	sed-PCP	0.6735	ex-PCP	0.4415	9	0.3095
GRIN2B	sed-PCP	0.62	ex-PCP	0.553	5	0.7937
PV	sed-PCP	0.676	ex-PCP	0.884	7	0.842
COMT	sed-PCP	0.667	ex-PCP	0.273	8	0.309

Table 9 lists the P-value and the Mann-Whitney-value for each gene for non-exercise-PCP (sed-PCP) and exercise-PCP (ex-PCP).

3.2.2 The effect of exercise on *GRIN2A*, *GRIN2B*, *COMT*, *EGR-1*, *DISC1*, *NTrK2*, *GAD-1*, *RELN* and *PV* after six weeks of wheel running exercise followed by 2 weeks of no exercise.

3.2.2.1 Effect of PCP on gene expression, 6 weeks after the end of PCP treatment followed by 2 weeks of no exercise

It was hypothesised that gene expression would be downregulated six weeks after PCP treatment (Fig. 14) and that any change in gene expression would be maintained further during a two-week rest. The effect of the PCP treatment, followed by eight weeks of no exercise (Study 2) showed subtle but again non-significant differences in the PV (control) gene expression as compared to the shorter time of the study (6 weeks).

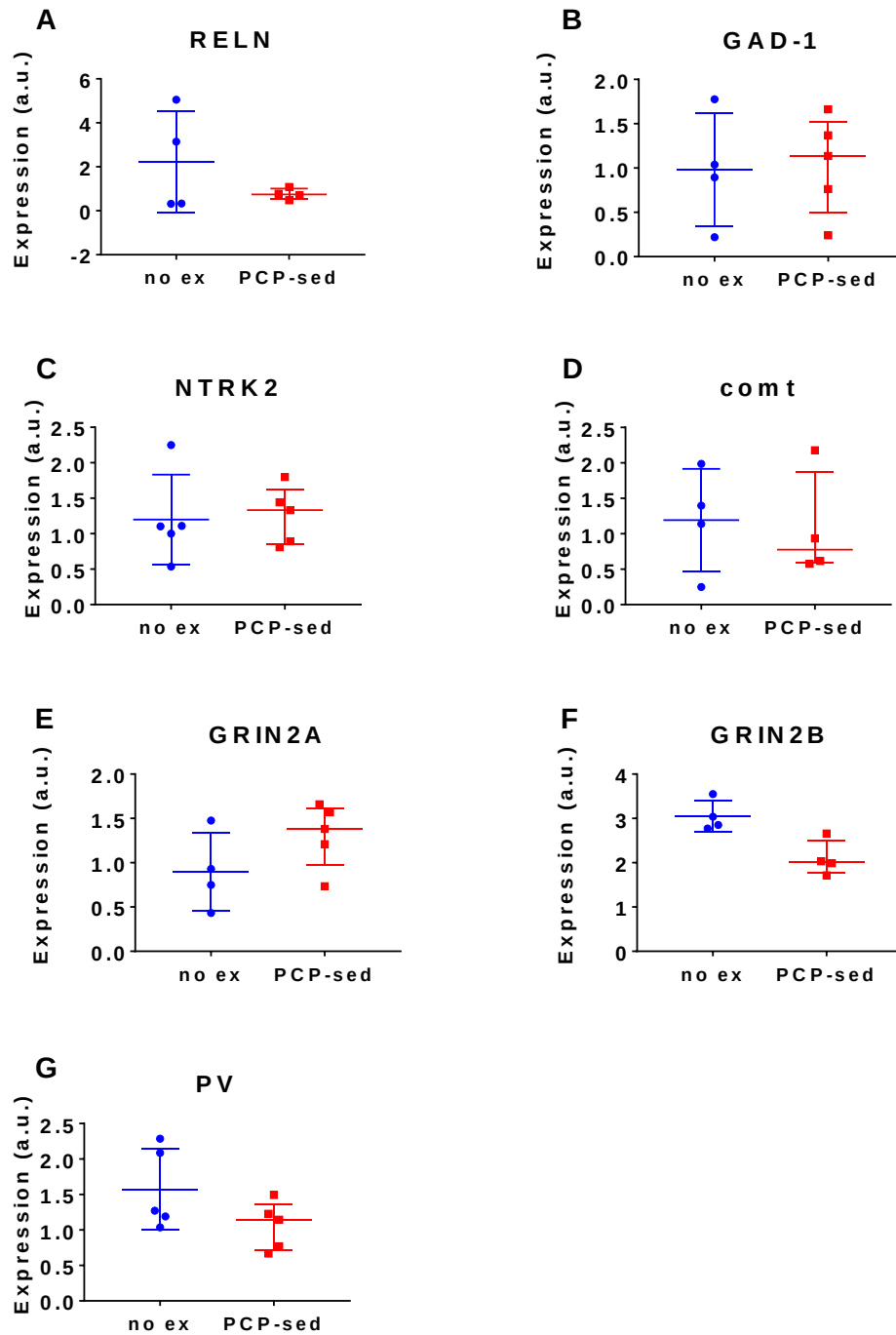


Figure 17: Effect of PCP on hippocampal expression of our genes of interest on animals treated with PCP, followed by no opportunity for exercise for 8 weeks. Panels: RELN (A), GAD-1 (B), NTRK2 (C), COMT (D), GRIN2a (E), GRIN2B (F) and PV (G). Mean ($\pm$ SD).

Gene	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
RELN	no ex	3.146	PCP-sed	0.778	8	0.999
GAD-1	no ex	1.038	PCP-sed	1.136	9	0.841
NTrK2	no ex	1.104	PCP-sed	1.332	11	0.841
Grin2A	no ex	0.929	PCP-sed	1.381	5	0.690
Grin2B	no ex	2.85	PCP-sed	1.987	0	0.095
PV	no ex	1.272	PCP-sed	1.144	6	0.222
COMT	no ex	1.397	PCP-sed	0.935	7	0.999

Table 10: Mann-Whitney values for the comparison between non-exercise (no ex) with non-exercise-PCP (PCP-sed) after 2 weeks of rest.

3.2.2.2 Effect of exercise followed by 2 weeks of normal housing on hippocampal gene expression

As was the case with six weeks of exercise, exercised animals left for a further two weeks of housing with no opportunity for exercise had no significant changes in gene expression as compared to animals that did not exercise at all (compare Figure 15, Table 8 and Figure 18, Table 11).

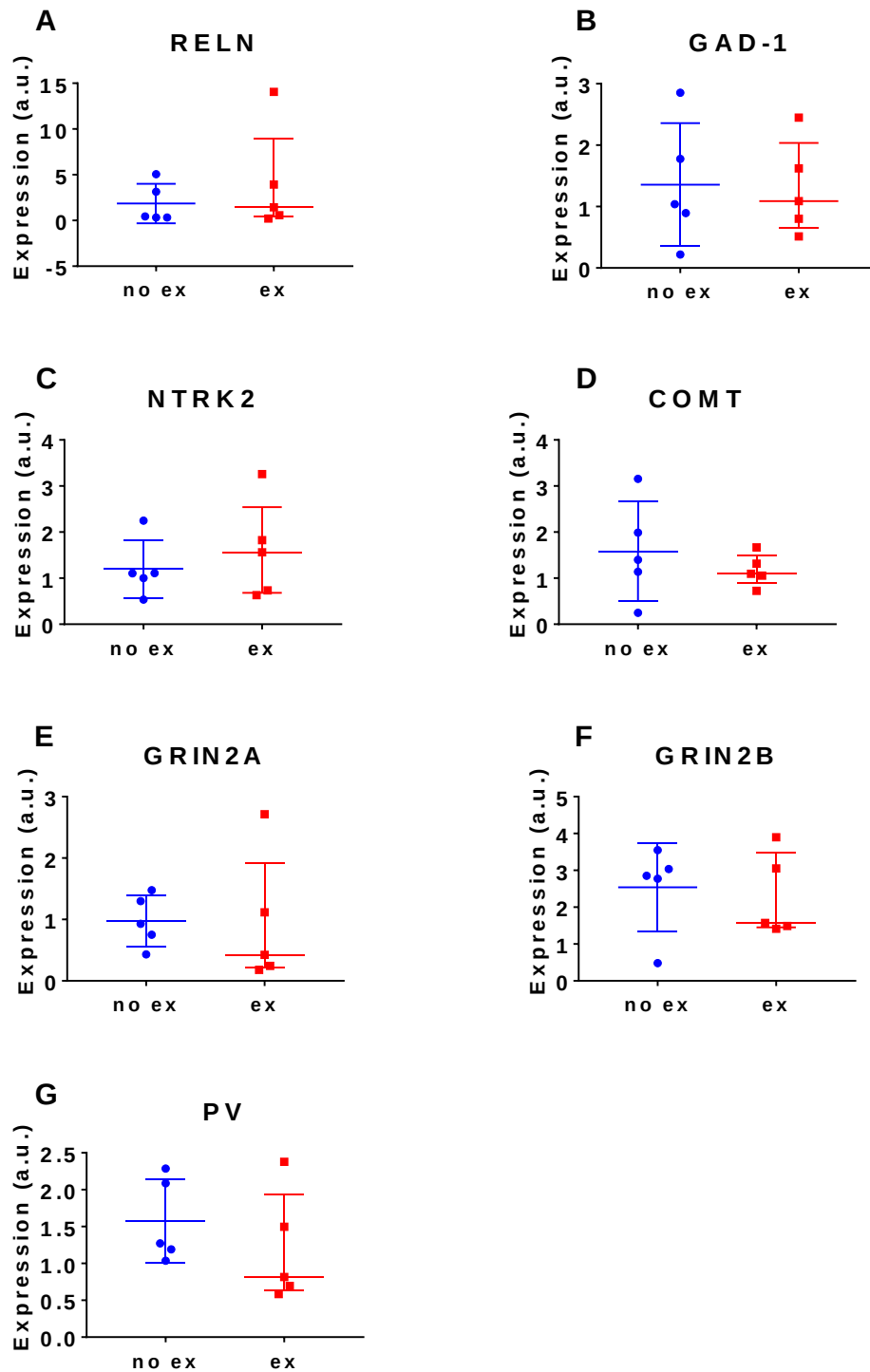


Figure 18: Effect of 6 weeks of exercise followed by 2 weeks rest on hippocampal expression of our genes of interest. Panels: RELN (A), GAD-1 (B), NTrK2 (C), COMT (D), GRIN2a (E), GRIN2B (F) and PV (G). Mean ($\pm$ SD).

Gene	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
RELN	no ex	1.736	ex	1.009	7	0.886
GAD-1	no ex	0.966	ex	1.355	5	0.486
NTrK2	no ex	1.104	ex	1.692	6	0.413
Grin2A	no ex	0.839	ex	0.423	3	0.400
Grin2B	no ex	2.945	ex	2.312	7	0.886
PV	no ex	1.272	ex	1.156	8	0.730
COMT	no ex	1.397	ex	1.185	7	0.556

Table 11: Mann-Whitney values for the comparison between non-exercise (no ex) with exercise (ex) after 2 weeks of rest

3.2.2.3 Effect of exercise followed by 2 weeks of resting on gene expression in PCP treated rats

It was hypothesised that exercise on PCP treated rats would reverse the effects of PCP on our genes of interest and that this effect would be maintained for two weeks after exercise stopped (running wheel removed). However, we found that there were no statistically significant changes in our genes of interest (Table 12, Fig. 19).

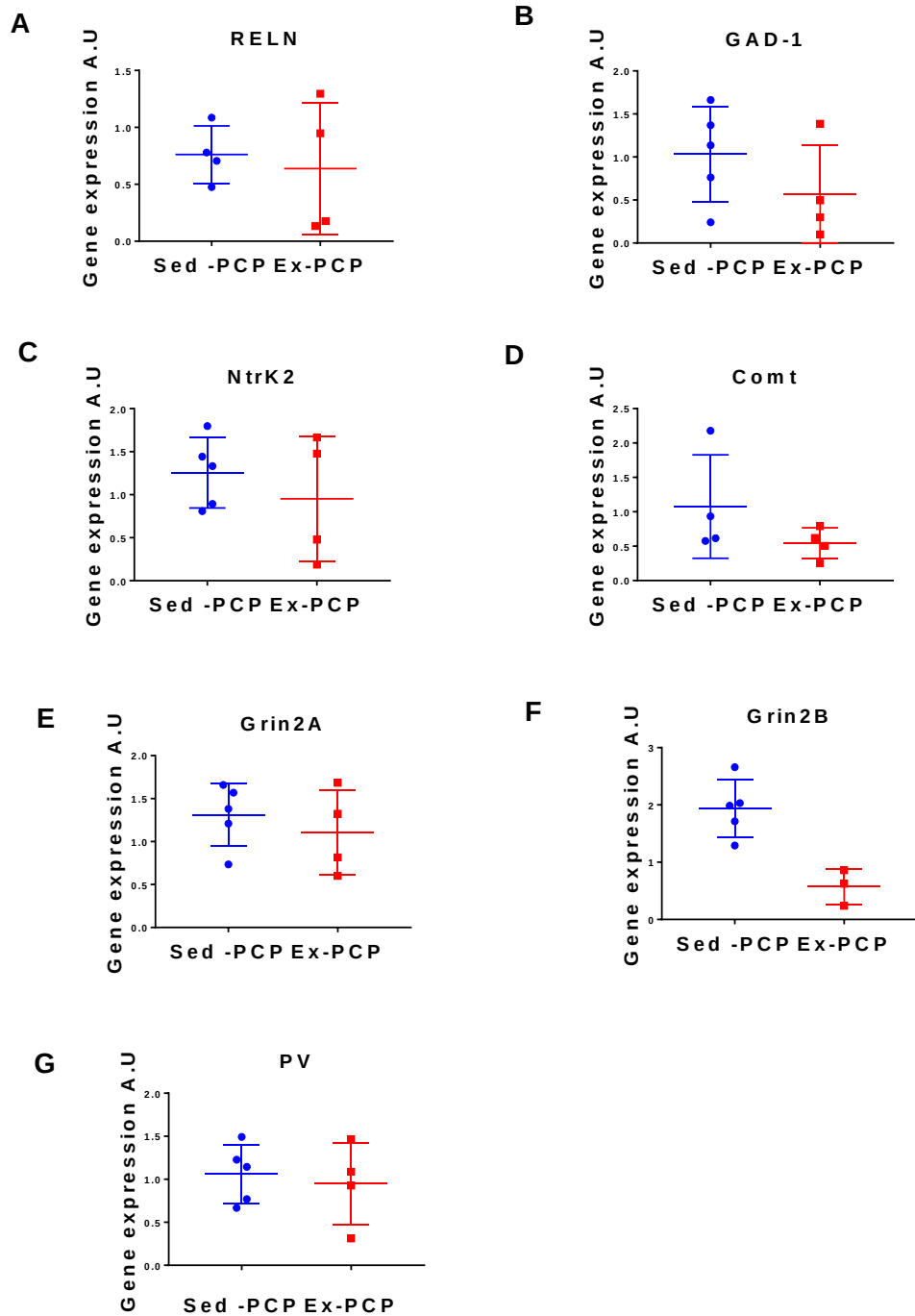


Figure 19: Effect of 6 weeks of exercise followed by 2 weeks of rest on the hippocampal expression of genes of interest of PCP-treated rats. Panels: RELN (A), GAD-1 (B), NTrK2 (C), COMT (D), GRIN2a (E), GRIN2B (F) and PV (G). Mean ($\pm$ SD).

Gene	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
<i>RELN</i>	sed-PCP	0.778	ex-PCP	0.947	12	0.999
<i>GAD-1</i>	sed-PCP	1.136	ex-PCP	0.495	11	0.841
<i>NTrk2</i>	sed-PCP	1.322	ex-PCP	1.478	12	0.999
<i>GRIN2A</i>	sed-PCP	1.381	ex-PCP	1.325	12	0.999
<i>GRIN2B</i>	sed-PCP	1.997	ex-PCP	0.858	10	0.691
<i>PV</i>	sed-PCP	1.144	ex-PCP	1.088	12	0.999
<i>COMT</i>	sed-PCP	0.935	ex-PCP	0.620	8	0.421

Table 12: Mann-Whitney values for the comparison between non-exercise-PCP (sed-PCP) and exercise-PCP (ex-PCP).

3.2.3 Results of miRNA expression in rat models

3.2.3.1 Hippocampal miRNA expression a model of SZ (PCP treatment)

Based on previous literature and unpublished results, it was hypothesised that the hippocampal expression of miR137, miR-132 and miR-212 would be upregulated after PCP treatment in animals that did not exercise. However, six weeks after PCP treatment, there were no significant differences in the hippocampal expression of these miRNAs (Figure 20, Table 13).

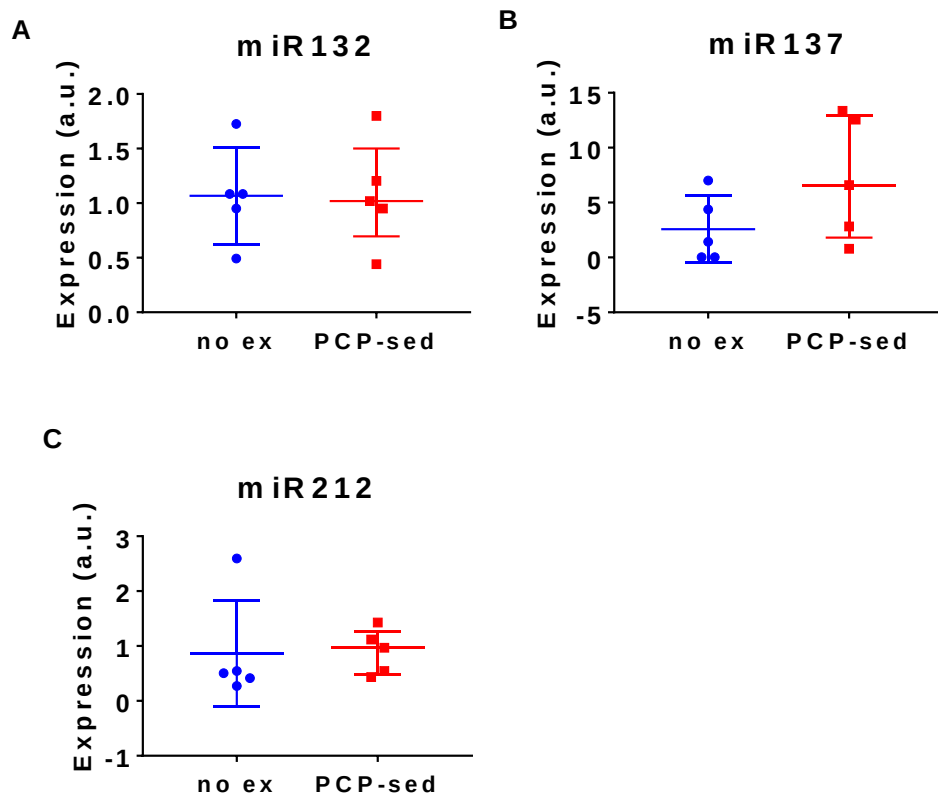


Figure 20: Hippocampal miRNA expression of miR-132 (A), miR137 (B), miR212 (C) in PCP treated animals (without exercise). Results are shown as mean $\pm$ SD.

miRNA	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
132	no ex	2.067	sed-PCP	1.026	7	0.309
137	no ex	3.435	sed-PCP	2.928	4	0.628
212	no ex	1.104	sed-PCP	0.564	6	0.222

Table 13: Mann-Whitney values for the comparison between non-exercise (no ex) with non-exercise-PCP (sed-PCP).

3.2.3.2 Effect of exercise on miRNA expression

It was hypothesised that exercise would downregulate the expression of the miRNAs that were of interest to us in the hippocampus of saline-treated animals. Again, there were no significant differences in the expression of these miRNAs (Figure 21, Table 14).

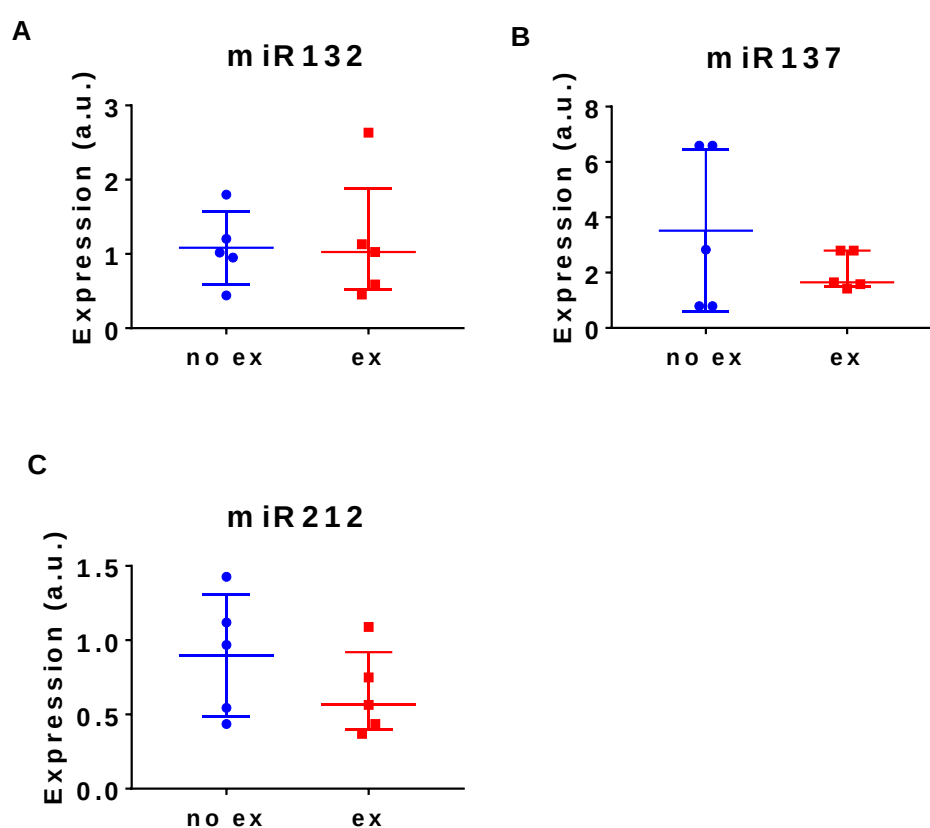


Figure 21: Level of hippocampal miRNA expression for miR-132 (A), miR-137 (B) and miR-212 (C) in animals that had free access to a running wheel and those that did not. Results are shown as mean $\pm$ SD.

miRNA	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
132	no ex	1.019	ex	1.026	12	0.999
137	no ex	2.828	ex	1.616	4	0.628
212	no ex	1.104	ex	0.564	6	0.222

Table 14: Mann-Whitney values for the comparison between non-exercise (no ex) and exercise (ex)

3.2.3.3 Effect of exercise on hippocampal miRNA expression in a rat model of SZ (PCP treatment)

The exercise was initially hypothesised to downregulate the effects of PCP treatment in our SZ model. However, as there were no effects caused by PCP or exercise, it is not surprising that there was no significant effect of the combination of these two interventions (Figure 22, Table 15).

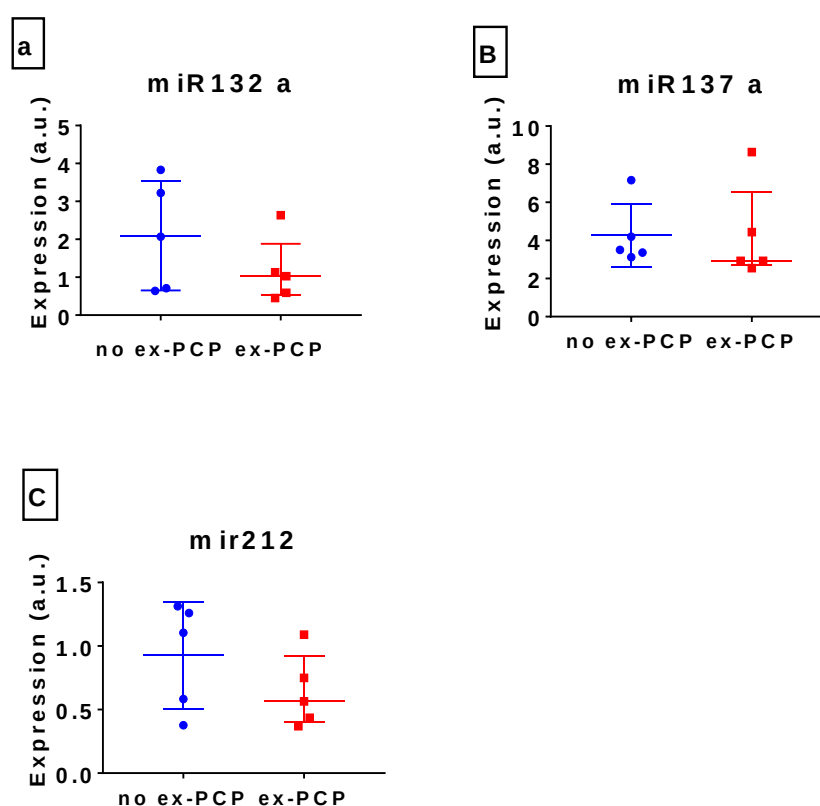


Figure 22: Level of hippocampal miRNA expression (*miR-132* (A), *miR137* (B) and *miR212* (c)) in PCP-treated animals (with and without exercise). Results are shown as mean $\pm$ SD.

miRNA	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
132	no ex-PCP	2.067	ex-PCP	1.026	7	0.309
137	no ex-PCP	3.435	ex-PCP	2.928	4	0.628
212	no ex - PCP	1.104	ex-PCP	0.564	6	0.222

Table 15: Mann-Whitney values for the comparison between non-exercise-PCP (no ex-PCP) with exercise-PCP (ex-PCP)

Similarly, there was no effect on hippocampal miRNA in animals treated with PCP (Figure 23, Table 16), in animals with or without exercise (Figure 24, Table 17) or PCP treatment with and without exercise for six weeks followed by two weeks rest (Figure 25, Table 18).

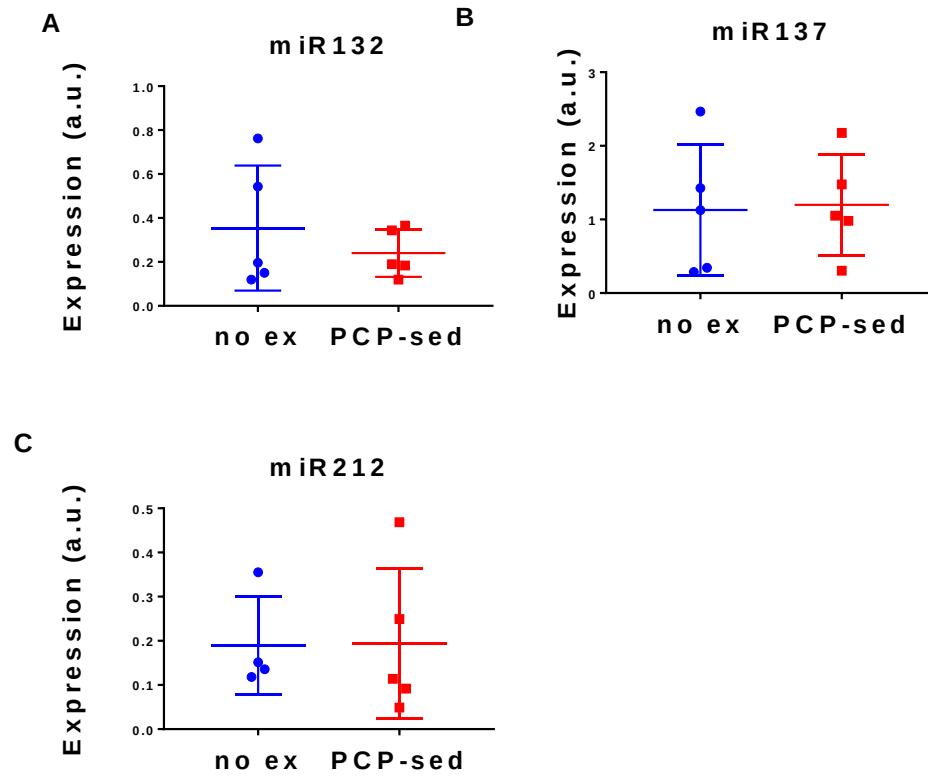


Figure 23: Level of hippocampal miRNA expression for miR-132 (A), miR-137 (B) and miR-212 (C) in PCP-treated versus saline-treated animals (without exercise). Results are shown as mean $\pm$ SD.

miRNA	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
132	no ex	0.369	(sed-PCP)-	0.189	6	0.412
137	no ex	1.127	(sed-PCP)-	1.051	12	0.999
212	no ex	0.143	(sed-PCP)-	0.113	7	0.556

Table 16: Mann-Whitney values for the comparison between non-exercise (no ex) with non-exercise-PCP (sed-PCP) after a duration of 2 weeks of rest.

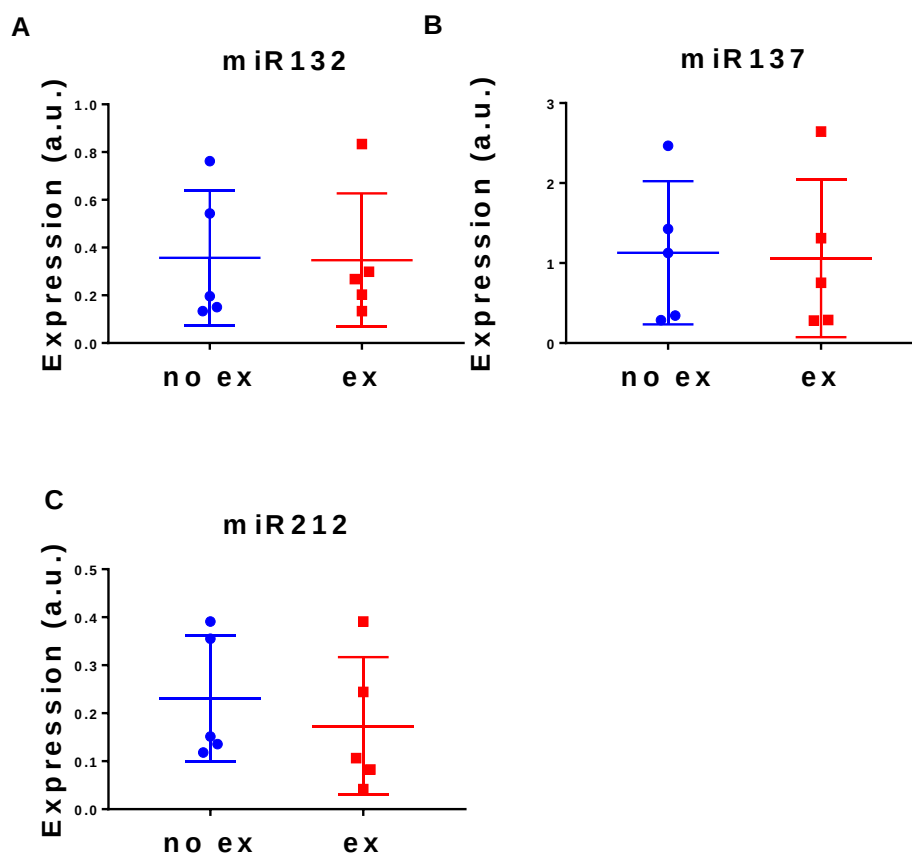


Figure 24: Level of hippocampal miRNA expression for miR-132 (A), miR-137 (B) and miR-212 (C) in saline-treated animals that either exercised for 6 weeks and then remained sedentary or animals that did not exercise for 8 weeks). Results are shown as mean $\pm$ SD.

miRNA	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
132	no ex	0.369	ex	0.267	10	0.999
137	no ex	1.127	ex	0.754	11	0.841
212	no ex	0.143	ex	0.106	7	0.556

Table 17: Mann-Whitney values for the comparison between non-exercise (no ex) with exercise (ex) after 2 weeks of rest without PCP treatment.

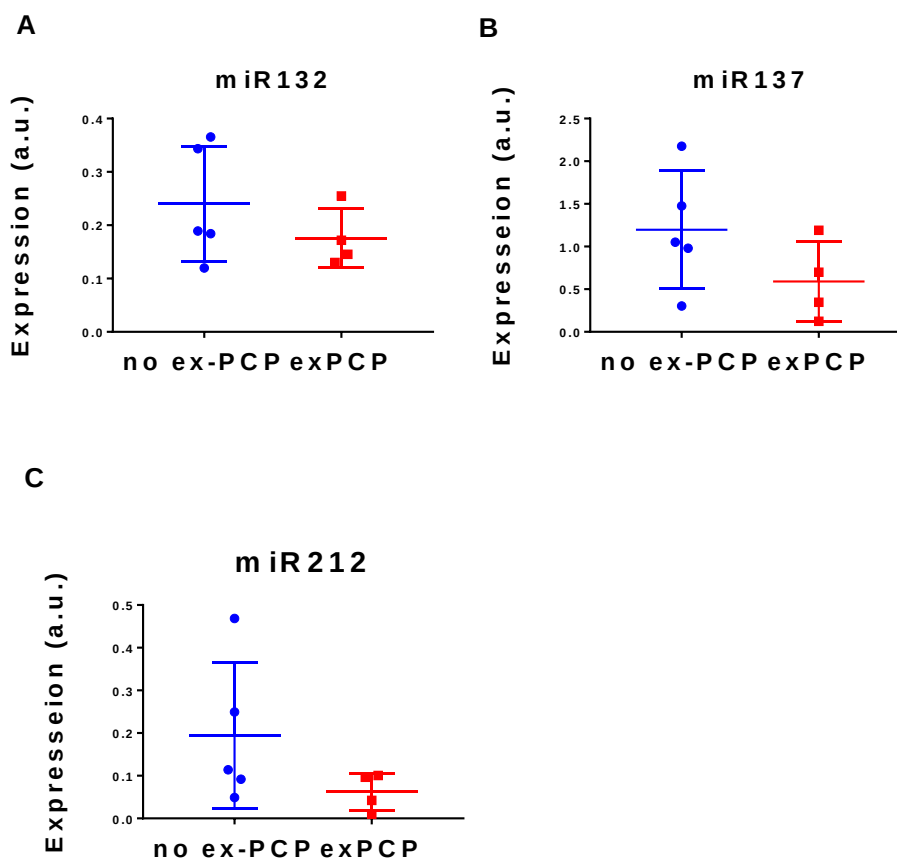


Figure 25: Level of hippocampal miRNA expression for miR-132 (A), miR-137 (B) and miR-212 (C) in PCP-treated animals (without and with exercise for 6 weeks followed by 2 weeks rest). Results are shown as mean $\pm$ SD.

miRNA	Treatment A	Median A	Treatment B	Median B	Mann-WhitneyU	P-value
132	(sed-PCP)	0.189	(ex-PCP)	0.158	6	0.413
137	(sed-PCP)	1.051	(ex-PCP)	0.523	5	0.285
212	(sed-PCP)	0.114	(ex-PCP)	0.069	4	0.191

Table 18: Mann-Whitney values for the comparison between non-exercise-PCP (sed-PCP) with exercise-PCP (ex-PCP) after a duration of 2 weeks of rest.

3.3 Sample size analysis

We used our data to perform sample size analyses. We found that, for many genes, the required sample sizes in order to obtain sufficient power in a repeat experiment would have to be between 11 and 500 (See table 19). In some cases (see asterisks in the table), the calculated required sample size was genuinely enormous (> 100000). The large volume of the required sample size can be interpreted to indicate that the treatments would not affect the variable to the extent that would be physiologically relevant, given the low-effect sizes and similar variability in groups.

	Hypothesis A: PCP treatment of our genes of interest will be upregulated			Hypothesis B: Exercise will reverse the effect on PCP			Hypothesis C: The effect of exercise will continue after a period of two weeks		
Gene	effect size	sample size	total sample size	effect size	sample size	total sample size	effect size	sample size	total sample size
RELN	0.742	30	60	0.875	11	22	0.321	154	308
GAD-1	1.248	12	24	1.302	20	40	0.275	209	418
NTrK2	0.342	*	*	0.929	54	108	0.412	94	188
COMT	0.502	64	128	0.549	*	*	0.546	54	108
Grin2A	0.361	123	246	0.011	*	*	0.479	70	140
Grin2B	0.597	45	90	0.541	55	110	0.699	34	68
PV	0.143	*	*	0.706	33	66	0.622	42	84

Table 19: Power analysis calculation for each hypothesis. Asterisks indicate extremely large sample sizes (> 100000), which were therefore discarded. Sample size refers to a function of power level $1 - \beta$, significance level α , and the wanted detected population effect size. The total sample size means the value of the baseline correlation expected in the null hypothesis.

4. Discussion

The overall aims of this project are listed as follows: a) To study whether exercise results in changes of gene expression and miRNA levels in the dorsal hippocampus of a rat model of SZ and b) to determine whether the changes mentioned above would persist after two weeks of rest. The background of this project was to extend the behavioural study conducted by Dr Neill's group at the University of Manchester. It demonstrated a positive effect of exercise on the memory recognition test – Novel Object Location – wherein PCP-treated rodents that underwent exercise spent more time exploring the novel object than the familiar object as compared to the non-exercise-PCP control (Hearney et al., 2018).

This project explored the potential molecular mechanisms that could explain the results of the behavioural study. The initial aim was to study the expression of 9 genes (*COMT*, *NTrK2*, *GRIN2A*, *GRIN2B*, *EGR1*, *DISC1*, *RELN*, *GAD-1* and *PV*), as they related to the two hypotheses concerning SZ, i.e. Dopaminergic and Glutamatergic theories. The chosen genes affect dopamine as well as glutamate, and the changes in the expression of these genes could result in the less active signalling of the neurotransmitters (Carlson, 1988; Richtand et al., 2013; Blackstone, 2009). Additionally, the three miRNAs (miR-137, miR-132 and miR-212) were chosen based on their association with the downregulation or upregulation of genes that have been found to be linked to the SZ disorder in numerous studies and reports related to the disorder (Mahmoudi and Cairns, 2016; Zhang et al., 2015).

It was not possible to design reliable primers to detect *DISC1* and *EGR1*. Thus, these could not be studied further. For the remaining seven genes and three miRNAs, extremely few

significant differences were found in gene expression compared to the control data. *GRIN2B* was the only gene of interest that displayed any changes of significance. Further, surprisingly, our positive control PV showed no significant changes at all. While the number of animals was sufficient to exhibit the significant effects of exercise on cognitive behaviour in the PCP-treated animals, the gene expression results they produced were underpowered. There were trends in the expression of some genes that could be used as the basis for follow-up studies with a higher power in order to further investigate the effects of exercise on the hippocampal gene expression in rodent models of SZ.

4.1 Effect of PCP and exercise alone and in combination on behaviour, gene and miRNA expression in the dorsal hippocampus in rats

4.1.2. Behavioural effects

Our collaborators report noticing an improvement in the PCP-treated rats after exercise. They found that rats performing the exercise with the PCP-treatment had more positive responses to the novel object test as compared to PCP-treated rats that did not (Heaney et al., 2018).

Human studies have shown that exercise had a significant improvement on cognition, such as on the working memory and processing speed (Vakhrusheva et al., 2016), with the same positive benefits reported along with an increase in the hippocampus volume in adults (Firth et al., 2018). Animal

studies show that exercised mice treated with MK-801 (which also produces SZ-like symptoms in animal models) performing exercise showed an improvement in memory and novel recognition (Park et al., 2014).

However, the lack of any significant differences in gene and miRNA expression from our results leads to the inability to provide a specific explanation for the molecular mechanisms underlying the behavioural effects in the rats reported by our collaborators (i.e. recovery in PCP-induced memory impairment in animals that exercised). Our results indicated a tendency for exercise to affect a small number of our genes of interest, which could influence DA and NMDAr. These tendencies could potentially explain the positive results that were noticed in the work of our collaborators and others (Gomes da Silva et al., 2010; Arida et al., 2007; Park et al., 2014).

4.1.3. Effects on gene expression of our genes of interest

PCP treatment has been shown to have long-lasting effects on gene expression in the brain. PCP treatment or drugs equivalent to PCP have been previously reported to downregulate our genes of interest. Studies have shown that the administration of PCP decreases *GAD-1* and *PV*, similar to that seen in SZ patients (Bullock et al., 2009; Wang et al., 2007). Our data shows no statistically significant decrease in *PV* (Figure 14), with a borderline significant decrease in *GAD-1* after PCP treatment ($p = 0.055$).

Bullock et al. (2009) demonstrated that PCP caused a decrease in the expression of *GAD67* and *GAD65*, which is similar to that seen in SZ patients as well as in mRNA levels for the NMDAr

subunits *GRIN2B* and *GRIN2A*. However, we saw no change in *GRIN2B* and *GRIN2A* (Figure 14).

Moreover, Kobil et al. (2011) reported an increase in hippocampal *GRIN2A* and *GRIN2B* mRNAs in mice that had free access to a running wheel. Additionally, exercise led to an increase in *GRIN2A* mRNA levels in the hippocampus both during low and moderate intensity treadmill running in comparison to the control mice that had no exercise (Kobil et al., 2011). However, our data indicated no significant changes in the expression of any of our genes of interest as a result of voluntary exercise.

4.1.3.1 Parvalbumin

We used *PV* as a positive control for PCP-induced changes, as studies on the rat PCP model have resulted in the decrease of *PV* mRNA in the hippocampus, prefrontal cortex and thalamus (Cochrain et al., 2003; Fachim et al., 2016). Nevertheless, we found no significant change in *PV* expression in rats who did not exercise and were treated with PCP or saline, which disagrees with the results published by Fachim et al. (2016), which Dr Neill was a part of and had noticed a decrease of *PV* in the hippocampus after administering PCP (2 mg/kg) to the rats. One explanation for the lack of change in the *PV* expression in our study could be found in the dose of PCP (which is a 'subchronic' dose) or the length of time between PCP treatment and gene expression analyses. However, Neill and colleagues have frequently reported decreased hippocampal *PV* expression using this model of low-dose PCP administration and have shown continued effects of PCP on hippocampal *PV* neurons over prolonged time periods (Neill et al., 2016).

It is also difficult to draw any conclusions about the expression of other genes and miRNAs as this positive control did not exhibit the expected changes. Although some of the genes (*RELN*, *GAD-1*, *COMT* and *NTrK2*) might show a slight tendency towards upregulation in animals that exercised (non-exercise-PCP vs exercise-PCP), there was no statistically significant difference, and we can therefore draw no conclusions regarding the involvement of these genes in the behavioural outcomes of these animals.

4.1.4. MicroRNA expression

Our data showed no upregulation of miR-132, miR-212 or miR-137 (miR-137 is a positive control). It has been reported that miR-132, miR-212 and miR-137 are upregulated in the human post-mortem SZ hippocampus (Wright et al., 2013). miR-137 is associated with SZ and is involved in neurogenesis, along with being highly expressed in the hippocampus, prefrontal cortex and amygdala (Yin et al., 2014). Other studies report that the upregulation of human miR137 causes a decrease of *GRIN2A* (Siegert et al., 2015; Beveridge and Cairns, 2011). It is surprising that there was no significant change in the miR-137 expression given the previous literature findings.

The same could be said with Hsa-miR-132 and Hsa-miR-212 (human), where the literature has also reported the upregulation of hsa-miRNA-132 (Kim et al., 2010) as well as the upregulation of miR-212 in animals (Perkins et al., 2007) in models of SZ. Interestingly, these studies did not use the same model as this thesis. Perkins et al. injected the animals daily with haloperidol 1 mg/kg/d ($n = 6$) or saline 0.9% ($n = 6$) for 4 weeks before sacrifices (Perkins et al., 2007). Siegert et al. used post mortem to investigate miRNA changes (Siegert et al., 2015).

Understanding miRNA expression changes with the model presented in our studies was not possible.

The lack of significance in our results is likely due to the small sample sizes of the study (see section 4.3.1).

4.1.5. Effects maintained after wheel running stopped

As exercise did not result in significant changes on gene expression right after the six weeks intervention, it is logical to assume that the 2 weeks sedentary period would not make any difference (as exercise did not produce an effect, lack of exercise will not have any impact).

We did not find any literature in the SZ models indicating whether the positive effect of exercise in our genes of interest would remain after a period of rest. Whether the positive impacts of exercise remain is something that needs to be addressed in the future, as most studies have focused solely on the immediate benefits of exercise. However, some literature does point out that physical activity appears to reduce sympathetic system activity and increase the parasympathetic activity even during rest in young and middle-aged subjects (Gregoire et al., 1996). Other human studies have found that school children engaging in physical activity 2 or 3 times per week with a rest period of a day exhibited an improvement in muscular strength and endurance during childhood and adolescence (Strong et al., 2005). However, this study only focused on the physical performance of the human body and not the neurological changes in the brain, which are more critical for the studies of SZ. Nevertheless, based on the literature, it can be speculated that any positive effect on the expression of our genes/miRNA of interest would remain during a rest period.

4.2 Rodent models of schizophrenia and the relationship with schizophrenia in humans

In this project, a rodent model of SZ was chosen, as it is easier to test brain development and behaviour for observation and experimental processes in rodents (Logde et al., 2013). The drug that was chosen to mimic SZ was PCP, as it possesses the ability to mimic SZ-like symptoms and targets genes that are specifically of interest to us (Fachim et al., 2016; Heanley et al., 2018). Previous reports show that PCP-induced decrease of PV in rats' PFC is similar to that seen in patients with SZ (Nomura et al., 2017). *COMT* and *NTrk2* were also reported to get decreased in expression using PCP as a rodent model of SZ (Parker et al., 2018).

At a physiological level, SZ has previously been reported as a dysfunction of glutamatergic pathways, and PCP can mimic a similar pathway dysfunction, i.e. NMDR dysfunction (Frohlich and Van Horn, 2014). PCP has also been reported to produce pathological disturbances, such as cognitive deficits and negative symptoms, which are similar to some of the symptoms in SZ (Howes et al., 2015). With the above reasons, PCP was considered the best substance for this experiment to trigger SZ-like symptoms within the rat model.

Another drug that has been considered and also used to model SZ is ketamine. Ketamine has been reported to result in biochemical brain changes similar to those seen in patients with SZ. Ketamine treatment results in the upregulation of the D1 receptor in the frontal cortex, which is associated with cognitive impairment and dopaminergic hypofunction (Narendran et al., 2005). Ketamine also binds to NMDA receptors (Stone et al., 2012) and leads to increased D2 receptor binding in the hippocampus along with a decrease in glutamate receptor

binding in the frontal cortex, which is similar to the effects of PCP (Becker et al., 2003). Further, similar to PCP, ketamine has been found to exert structural changes associated with SZ, such as reduction in thalamic and prefrontal grey matter volume (Liao et al., 2011). There are also reports of reductions in the GABAergic interneuron function (Homayoun and Moghaddam, 2007).

Comparing both ketamine and PCP as models of SZ, ketamine treatment is as effective as PCP in inducing SZ-like symptoms. However, ketamine has fewer toxicity effects as compared to PCP. PCP was developed as a veterinary anaesthetic but is no longer used and is primarily known as a drug of abuse. PCP is described as a dissociative anaesthetic (Marcotte et al., 2001), as it causes hallucinations, confusion and violent behaviour (Bush, 2013). It also causes neurotoxicity (FDA, 2014, Rowland, 2005; Frohlich and von Hann, 2014) with a significant downregulation of BDNF mRNA for an administration of 2 mg/kg; i.p. twice daily for seven days. Ketamine is an NMDA receptor antagonist, which causes neurotoxicity that results in neurodegeneration in the hippocampus (Aydin and Deveci, 2016). The dosing also induces robust behavioural and hippocampal PV deficits in female rats (Abdul-Monim et al., 2007; Xia et al., 2008).

Additionally, it was found that ketamine effects can support the glutamate hypothesis, as ketamine acts at the NMDAr and results of ketamine use have effects that are similar to NMDAr hypofunction in SZ. Therefore, the ketamine model is also useful to study the positive, negative and cognitive symptoms as well as the dopaminergic and GABAergic dysfunction in SZ (Morgan et al., 2003).

PCP may have more severe neurotoxicity and provide more robust deficits that mimic phenotypic aspects of SZ. Both models

may allow the investigation of the dopaminergic and glutamatergic hypothesis of SZ, including understanding the underlying effects on the mesolimbic and mesocortical pathways. Some studies using the PCP model showed impairments in social interaction, learning and memory, which links to the negative and cognitive symptoms (Murray et al., 2002). Therefore, in a rodent model, PCP provides a well-rounded understanding of the SZ disorder.

4.3.1 Sample size and experimental design

The design of the project resulted in limitations, as we were constrained by the study design chosen by our collaborators from the University of Manchester. It would have been ideal to know the effect size and power before starting the project, and the power analysis should have been conducted before starting the experiment. Based on our results, it can be strongly argued the sample size for each group should be increased to provide more reliable results. Most studies using rodent models of SZ use larger sample sizes than 5 per group (Scheewe et al., 2013; Thoma et al., 2009).

The lack of statistical significance is possibly due to the small sample size ($n = 5$ per group). However, this sample size was powered for the behavioural experiment. Sample size calculations indicate that for some gene expression targets, a much higher number of animals would be required, such as for studying the effect of PCP on *RELN* (requires 30 animals per group) and our positive control (*PV*) requires 84 animals per group.

There may be a question as to whether the PCP administration had the expected effect on the rats. The same experimental design showed an effect of exercise on the cognitive behaviours in PCP-

treated rats, but we do not know if that also holds true for the rats used for this study. This PCP dosing regime usually has reproducible effects on behaviour and parvalbumin neurons (Abdul-Monim et al., 2007; Xia et al., 2008; Neill et al., 2010).

4.3. Limitations of the study potentially leading to non-significant changes in expression

4.3.4 Methodological limitations or different approaches

This project used qPCR in order to study the changes in gene expression. qPCR has its advantages and disadvantages. The advantage of qPCR is that it uses fluorescence, whose detection is sensitive and allows the discrimination between the levels of expression across a range of concentrations (Smith et al., 2009). However, qPCR is only semi-quantitative due to the kinetics of PCR product accumulation (Fuijii et al., 2004). One of the limitations of qPCR, among others, is that it requires expensive equipment and reagents and, due to its high sensitivity, experimental design and understanding of normalisation techniques are crucial to obtain accurate conclusions from the collected data (Wong and Medrano, 2005).

Most studies on SZ have used qPCR (Cattane et al., 2015; Hattori et al., 2014). We had the benefit of the equipment being provided in the laboratory. Hence, qPCR and PCR was chosen for the study of the gene expression.

However, there is room for improvement, and other techniques could have been used to improve the study in order to increase

its quality. Northern blot provides information regarding the transcript size within an RNA mixture and can determine the levels of RNA expression of individual genes but requires higher amounts of sample (Sharkey et al., 2004).

SAGE sequencing is a transcriptomic technique that allows a snapshot view of the RNA levels (Saha et al., 2002). It allows the analysis of the expression of thousands of genes in parallel, which makes it suitable to measure the global gene expression profile of a specific tissue, including specific genes and miRNAs (Gowda et al., 2004). SAGE could have been used to examine the genes in parallel. It is also as accurate as qPCR (Shendure et al., 2008)

DNA microarrays are another technology that can measure the expression of thousands of genes at the same time. Microarrays can be classified according to the length of the probe, which can either be cDNA of hundreds or thousands of base pairs or oligonucleotide arrays that are short (25–50 bp). Additionally, microarrays can be classified according to the number of samples that can be simultaneously profiled. Single-channel arrays can analyse one sample at a time, whereas multiple-channel arrays can analyse two or more samples (Tarca et al., 2009).

While we performed the study using PCR, other studies have used Western blot (Park et al., 2014; Scheewe et al., 2013; Thoma et al., 2009). Using the Western blot method could provide an understanding of the protein expression (as the mRNA might not be translated) and its location in the cell/tissue of the protein (Shi et al., 2015). However, the disadvantage of using Western would be that we would not be able to study miRNA levels.

Hence, with the above considerations, we chose to use qPCR to perform the study.

4.3.5 Errors

In many cases, there was a loss of sample during RNA extraction, while contamination occurred for some samples. Outliers were not removed, due to small sample sizes, but may have affected the findings. Non-parametric tests were used to minimise the effects of outliers, but these are less powerful than parametric tests, particularly with small groups. They are also more susceptible to Type 2 errors.

4.4 Future studies

4.4.1 DNA methylation

Due to time constraints and insufficient resources, we were not able to investigate the total epigenetic impact of exercise on PCP treated animals. For example, we did not have the time to study the impact on DNA methylation due to the time it would take to design and calibrate the primers.

The DNA methylation could have been studied and the impact of exercise and PCP could have been determined. If our positive control (*PV*) had shown a significant change in expression, we could have investigated the changes in DNA methylation at the CpGs on the promoter site. Moreover, previous studies have shown hypermethylation in one or two *PV* CpG sites in both the PFC and hippocampus in animal models of SZ and have undergone the same treatment as performed in this experiment (Fachim et al., 2016). Studies have shown a decrease in the methylation of *PV* (Teroganova et al., 2016; Ripke et al., 2013)

DNA methylation can allow us to understand the changes in the gene expression level, as a change in DNA methylation will be translated into a changed gene expression only if and when the gene is transcribed (i.e. the transcription factor comes in contact with the promoter).

It could explain how our collaborators found positive changes in behaviour, which have been reported in other papers as well. They report that parvalbumin gene promoter is hypermethylated in this model (Fachim et al., 2016). Despite this, we could not find any significant changes in gene expression during exercise and non-exercise and only observed a tendency towards changes.

The changes in DNA methylation were present, but there was no increase in expression. Therefore, we could not conclude whether methylation prevented the increase in expression. Apart from considering the post hoc analysis to redo the experiment, several techniques can be used to study changes in DNA methylation.

Bisulfite pyrosequencing is sequencing using the synthesis method. It quantitatively determines the methylation of individual cytosines. DNA samples are treated with sodium bisulfite, which changes any unmethylated cytosine into uracil (Bassil et al., 2013). The cytosine that is methylated is resistant to the changes and would remain as cytosine after bisulfite treatment (Delaney, 2016).

Thereafter, the DNA methylation status can be determined by PCR sequencing (Li and Tollesfbol, 2011). The PCR process amplifies the DNA regions and will change to thymines for any uracils. Whereas, 5-mc or 5-hmc are amplified as cytosines (Huang et al., 2010), thus determining the methylation status in the loci (Li and Tollesfbol, 2011).

Another technique to study DNA methylation is liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). It requires smaller quantities of the hydrolysed DNA

sample and can detect all methylated cytosine residues, with a detection level between 0.05–10% (Song et al., 2013).

Methylated DNA Immunoprecipitation (MeDIP) also detects methylated DNA. It precipitates genomic DNA that is shared by sonication and immunoprecipitated with antibodies that recognise 5-methylcytidine. Subsequently, qPCR quantifies the amount of DNA precipitated by the antibodies (Mohn et al., 2009).

These methods could be used to investigate whether exercise results in changes in DNA methylation in PCP-treated animals.

Therefore, if we expand on the project to investigate DNA methylation, these techniques can allow us to understand DNA methylation and its impact during PCP treatment with and without exercise.

4.4.2 miRNA-mRNA

This thesis studied the impact of exercise and PCP treatment on miRNA levels (miR-132/-212/-137). The project was never allowed to examine whether those miRNA interacted with our genes of interest. It would be beneficial to combine both aspects to see if the changes in the miRNA levels could explain the tendencies of expression changes for our genes of interest.

There are several techniques that can be employed to study miRNA-mRNA interactions. For example, Choi et al. (2007) described a method to detect miRNA-mRNA interactions by using zebrafish miR430. The method detects the dampened expression of the TGF-beta Nodal agonist *squint* and the TGF-beta nodal antagonist *left*. They developed a target protector for the miRNA binding sites of the target mRNA and disrupted the interaction of specific miRNA-mRNA pairs. They observed that the protection from the miR430 resulted in the enhancement of nodal

signalling, while the lack of miRNA protection caused a reduction in the nodal signalling (Choi et al., 2007).

Another method for studying miRNA-mRNA interactions is by using a computer model, which was suggested and developed by Agarwal et al. (2015). This method is based on the theory that the miRNA region, known as 'seed', binds to a complementary sequence in the target mRNA, known as 'Canonical sites'. From this knowledge and the results collected by Agarwal et al. (2015), a static model was developed that predicts the effect of miRNA binding to the canonical sites. The model is a part of the resource called TargetScan (Agarwal et al., 2015). However, these methods could be used in our experiments to determine whether the miRNA of interest and our gene of interest had a miRNA-mRNA interaction.

Moreover, expanding the study and looking at other brain areas might provide more information about the triggers and the influence that exercise has on the model of SZ.

4.5 Conclusion

This project aimed to study whether exercise causes any significant changes in the expression of genes (*GRIN2A*, *GRIN2B*, *COMT*, *EGR-1*, *DISC1*, *NTrK2*, *GAD-1*, *RELN* and *PV*) and miRNAs (miR-132/-137/-212) in the dorsal hippocampus of a rat model of schizophrenia (PCP treatment). Despite some trends for altered miRNA and mRNA expression when the PCP-treated rodents performed the exercise, our results exhibited no statistically significant differences in all datasets. It is concluded that exercise does not upregulate genes expression after PCP treatment.

5 References

- Abdul-Monim, Z., Neill, J., & Reynolds, G. (2007). Sub-chronic psychotomimetic phencyclidine induces deficits in reversal learning and alterations in parvalbumin-immunoreactive expression in the rat. *Journal of Psychopharmacology*, 21(2), 198–205. doi: 10.1177/0269881107067097
- Agarwal, V., Bell, G., Nam, J., & Bartel, D. (2015). Predicting effective microRNA target sites in mammalian mRNAs. *Elife*, 4. doi: 10.7554/elife.05005
- Akbarian, S., & Huang, H. (2006). Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. *Brain Research Reviews*, 52(2), 293–304. doi: 10.1016/j.brainresrev.2006.04.001
- Amitai, N., Kuczenski, R., Behrens, M., & Markou, A. (2012). Repeated phencyclidine administration alters glutamate release and decreases GABA markers in the prefrontal cortex of rats. *Neuropharmacology*, 62(3), 1422–1431. doi: 10.1016/j.neuropharm.2011.01.008
- Andrés-León, E., Cases, I., Alonso, S., & Rojas, A. (2017). Novel miRNA-mRNA interactions conserved in essential cancer pathways. *Scientific Reports*, 7(1). doi: 10.1038/srep46101
- Arida, R., Scorza, C., Scorza, F., Gomes da Silva, S., da Graça Naffah-Mazzacoratti, M., & Cavalheiro, E. (2007). Effects of different types of physical exercise on the staining of parvalbumin-positive neurons in the hippocampal formation of rats with epilepsy. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 31(4), 814–822. doi: 10.1016/j.pnpbp.2007.01.021

- Aydin, M., & Deveci, U. (2016). Pathophysiology of Ketamine Neurotoxicity. *Neuropathology of Drug Addictions and Substance Misuse*, 563–572. doi: 10.1016/b978-0-12-800212-4.00052-2
- Bannister, A., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Research*, 21(3), 381–395. doi: 10.1038/cr.2011.22
- Barrès, R., & Zierath, J. (2016). The role of diet and exercise in the transgenerational epigenetic landscape of T2DM. *Nature Reviews Endocrinology*, 12(8), 441–451. doi: 10.1038/nrendo.2016.87
- Bassil, C., Huang, Z., & Murphy, S. (2013). Bisulfite Pyrosequencing. In Malek, A., and Tchernitsa, O. (Eds.), *Ovarian Cancer: Methods and Protocols* (pp. 95–107). Totowa, NJ: Humana Press.
- Bearden, C., Jawad, A., Lynch, D., Monterosso, J., Sokol, S., & McDonald-McGinn, D. et al. (2005). Effects of Comt Genotype on Behavioral Symptomatology in the 22q11.2 Deletion Syndrome. *Child Neuropsychology*, 11(1), 109–117. doi: 10.1080/09297040590911239
- Becker, A., Peters, B., Schroeder, H., Mann, T., Huether, G., & Grecksch, G. (2003). Ketamine-induced changes in rat behaviour: A possible animal model of schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 27(4), 687–700. doi: 10.1016/s0278-5846(03)00080-0
- Belujon, P., & Grace, A. (2017). Dopamine system dysregulation in major depressive disorders. *International Journal of Neuropsychopharmacology*, 20(12), 1036–1046. doi: 10.1093/ijnp/pyx056

- Beveridge, N., & Cairns, M. (2012). MicroRNA dysregulation in schizophrenia. *Neurobiology Of Disease*, 46(2), 263-271. doi: 10.1016/j.nbd.2011.12.029
- Bickart, K., Dickerson, B., & Feldman Barrett, L. (2014). The amygdala as a hub in brain networks that support social life. *Neuropsychologia*, 63, 235–248. doi: 10.1016/j.neuropsychologia.2014.08.013
- Bird, A. (1986). CpG-rich islands and the function of DNA methylation. *Nature Volume*, 321, 209–213.
- Blackstone, C. (2009). Infantile parkinsonism-dystonia: a dopamine “transportopathy”. *Journal of Clinical Investigation*. doi: 10.1172/jci39632
- Blackwood, D., Fordyce, A., Walker, M., St. Clair, Porteous, & Muir, W. (2001). Schizophrenia and affective disorders— cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: Clinical and P300 findings in a family. *American Journal of Human Genetics*, 69(2), 428–433.
- Bowie, C., & Harvey, P. (2006). Cognitive deficits and functional outcome in schizophrenia. *Journal Listneuropsychiatr Dis Treat*, 2(4), 531–536.
- Bozikas, V., & Andreou, C. (2011). Longitudinal studies of cognition in first episode psychosis: A systematic review of the literature. *Australian & New Zealand Journal of Psychiatry*, 45(2), 93–108. doi: 10.3109/00048674.2010.541418
- Bramham, C., Worley, P., Moore, M., & Guzowski, J. (2008). The immediate early gene arc/arg3.1: Regulation, mechanisms, and function. *Journal of Neuroscience*, 28(46), 11760–11767. doi: 10.1523/jneurosci.3864-08.2008

- Brown, J., Ramikie, T., Schmidt, M., Baldi, R., Garbett, K., & Everheart, M. (2015). Inhibition of parvalbumin-expressing interneurons results in complex behavioral changes. *Mol Psychiatry*, (20), 1499–1507.
- Bullock, W., Bolognani, F., Botta, P., Valenzuela, C., & Perrone-Bizzozero, N. (2009). Schizophrenia-like GABAergic gene expression deficits in cerebellar Golgi cells from rats chronically exposed to low-dose phencyclidine. *Neurochemistry International*, 55(8), 775–782. doi: 10.1016/j.neuint.2009.07.010
- Bush, M. (2013). *Emergency Department Visits Involving Phencyclidine (PCP)*. The CBHSQ Report.
- Bygrave, A.M., Masiulis, S., Nicholson, E., Berkemann, M., Barkus, C., Sprengel, R., et al. (2016) Knockout of NMDA-receptors from parvalbumin interneurons sensitizes to schizophrenia-related deficits induced by MK-801. *Transl Psychiatry*, 6, e778–e778.
- Caputo, V., Ciolfi, A., Macri, S., & Pizzuti, A. (2015). The emerging role of microRNA in Schizophrenia. *CNS & Neurological Disorders - Drug Targets*, 14(2), 208–221. doi: 10.2174/1871527314666150116124253
- Carlson, R. (1988). Exemplary lives: the uses of psychobiography for theory development. *Journal of Personality*, 56(1), 105–138. doi: 10.1111/j.1467-6494.1988.tb00464.x
- Cates, M., Teodoro, M., & Phillips, G. (2002). Molecular mechanisms of calcium and magnesium binding to parvalbumin. *Biophysical Journal*, 82(3), 1133–1146. doi: 10.1016/s0006-3495(02)75472-6
- Cattane, N., Minelli, A., Milanesi, E., Maj, C., Bignotti, S., & Bortolomasi, M. et al. (2015). Altered gene expression in schizophrenia: Findings from transcriptional signatures in

fibroblasts and blood. *PLOS ONE*, 10(2), e0116686. doi: 10.1371/journal.pone.0116686

Chen, W., Shieh, C., Swanger, S., Tankovic, A., Au, M., & McGuire, M. et al. (2017). GRIN1 mutation associated with intellectual disability alters NMDA receptor trafficking and function. *Journal Of Human Genetics*, 62(6), 589-597. doi: 10.1038/jhg.2017.19

Chen, Y. (2005). Reelin modulates NMDA receptor activity in cortical neurons. *Journal of Neuroscience*, 25(36), 8209–8216. doi: 10.1523/jneurosci.1951-05.2005

Cheng, H., Papp, J., Varlamova, O., Dziema, H., Russell, B., & Curfman, J. et al. (2007). MicroRNA modulation of circadian-cock period and entrainment. *Neuron*, 54(5), 813–829. doi: 10.1016/j.neuron.2007.05.017

Choi, W., Giraldez, A., & Schier, A. (2007). Target protectors reveal dampening and balancing of nodal agonist and antagonist by miR-430. *Science*, 318(5848), 271–274. doi: 10.1126/science.1147535

Conaco, C., Otto, S., Han, J., & Mandel, G. (2006). Reciprocal actions of REST and a microRNA promote neuronal identity. *Proceedings of the National Academy of Sciences*, 103(7), 2422–2427. doi: 10.1073/pnas.0511041103

Coyle, J. (2012). NMDA receptor and schizophrenia: A brief history. *Schizophrenia Bulletin*, 38(5), 920–926. doi: 10.1093/schbul/sbs076

Cui, L., Sun, W., Yu, M., Li, N., Guo, L., Gu, H., & Zhou, Y. (2016). Disrupted-in-schizophrenia1 (DISC1) L100P mutation alters synaptic transmission and plasticity in the hippocampus and causes recognition memory deficits. *Molecular Brain*, 9(1). doi: 10.1186/s13041-016-0270-y

Dahoun, T., Trossbach, S., Brandon, N., Korth, C., & Howes, O. (2017). The impact of Disrupted-in-Schizophrenia 1

(DISC1) on the dopaminergic system: A systematic review. *Translational Psychiatry*, 7(1), e1015–e1015. doi: 10.1038/tp.2016.282

Dauwan, M., Begemann, M., Heringa, S., & Sommer, I. (2015). Exercise improves clinical symptoms, quality of life, global functioning, and depression in schizophrenia: A systematic review and meta-analysis. *Schizophrenia Bulletin*, 42(3), 588–599. doi: 10.1093/schbul/sbv164

de Jonge, J., Vinkers, C., Hulshoff Pol, H., & Marsman, A. (2017). GABAergic mechanisms in schizophrenia: Linking postmortem and in vivo studies. *Frontiers in Psychiatry*, 8. doi: 10.3389/fpsy.2017.00118

Delaney, C., Garg, S., & Yung, R. (2015). Analysis of DNA methylation by pyrosequencing. *Methods in Molecular Biology*, 249–264. doi: 10.1007/978-1-4939-2963-4_19

DeLisi, L., Szulc, K., Bertisch, H., Majcher, M., & Brown, K. (2006). Understanding structural brain changes in schizophrenia. *Dialogues in Clinical Neuroscience*, 8(1), 71–78.

Dempster, E., Viana, J., Pidsley, R., & Mill, J. (2012). Epigenetic studies of schizophrenia: Progress, predicaments, and promises for the future. *Schizophrenia Bulletin*, 39(1), 11–16. doi: 10.1093/schbul/sbs139

Duclot, F., & Kabbaj, M. (2017). The role of early growth response 1 (EGR1) in brain plasticity and neuropsychiatric disorders. *Frontiers in Behavioral Neuroscience*, 11. doi: 10.3389/fnbeh.2017.00035

Egan, M., Goldberg, T., Kolachana, B., Callicott, J., Mazzanti, C., & Straub, R. et al. (2001). Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proceedings of the National Academy of*

- Sciences*, 98(12), 6917–6922. doi: 10.1073/pnas.111134598
- Emamian, E. (2012). AKT/GSK3 signaling pathway and schizophrenia. *Frontiers in Molecular Neuroscience*, 5. doi: 10.3389/fnmol.2012.00033
- Erickson, K., Hillman, C., & Kramer, A. (2015). Physical activity, brain, and cognition. *Current Opinion In Behavioral Sciences*, 4, 27-32. doi: 10.1016/j.cobeha.2015.01.005
- Fachim, H., Srisawat, U., Dalton, C., Harte, M., Marsh, S., Neill, J., & Reynolds, G. (2016). Subchronic administration of phencyclidine produces hypermethylation in the parvalbumin gene promoter in rat brain. *Epigenomics*, 8(9), 1179–1183. doi: 10.2217/epi-2016-0050
- Fatemi, S., King, D., Reutiman, T., Folsom, T., Laurence, J., & Lee, S. et al. (2008). PDE4B polymorphisms and decreased PDE4B expression are associated with schizophrenia. *Schizophrenia Research*, 101(1–3), 36–49. doi: 10.1016/j.schres.2008.01.029
- Firth, J., Stubbs, B., Vancampfort, D., Schuch, F., Lagopoulos, J., Rosenbaum, S., & Ward, P. (2018). Effect of aerobic exercise on hippocampal volume in humans: A systematic review and meta-analysis. *Neuroimage*, 166, 230–238. doi: 10.1016/j.neuroimage.2017.11.007
- Friedman, R., Farh, K., Burge, C., & Bartel, D. (2008). Most mammalian mRNAs are conserved targets of microRNAs. *Genome Research*, 19(1), 92-105. doi: 10.1101/gr.082701.108
- Frohlich, J., & Van Horn, J. (2013). Reviewing the ketamine model for schizophrenia. *Journal of Psychopharmacology*, 28(4), 287–302. doi: 10.1177/0269881113512909
- Fujii, T., Nakashima, K., & Hayashi, N. (2005). Random amplified polymorphic DNA-PCR based cloning of markers to identify

the beer-spoilage strains of *Lactobacillus brevis*, *Pediococcus damnosus*, *Lactobacillus collinoides* and *Lactobacillus coryniformis*. *Journal of Applied Microbiology*, 98(5), 1209–1220. doi: 10.1111/j.1365-2672.2005.02558.x

Funk, A., Rumbaugh, G., Harotunian, V., McCullumsmith, R., & Meador-Woodruff, J. (2009). Decreased expression of NMDA receptor-associated proteins in frontal cortex of elderly patients with schizophrenia. *Neuroreport*, 20(11), 1019–1022. doi: 10.1097/wnr.0b013e32832d30d9

Gaskin, P., Toledo-Rodriguez, M., Alexander, S., & Fone, K. (2016). Down-regulation of hippocampal genes regulating dopaminergic, GABAergic, and glutamatergic function following combined neonatal phencyclidine and post-weaning social isolation of rats as a neurodevelopmental model for schizophrenia. *International Journal of Neuropsychopharmacology*, 19(11), pyw062. doi: 10.1093/ijnp/pyw062

Georgi, A., Jamra, R., Schumacher, J., Becker, T., Schmael, C., & Deschner, M. et al. (2006). No association between genetic variants at the GRIN1 gene and bipolar disorder in a German sample. *Psychiatric Genetics*, 16(5), 183–184. doi: 10.1097/01.ypg.0000242194.36150.2b

Gomes da Silva, S., Doná, F., da Silva Fernandes, M., Scorza, F., Cavaleiro, E., & Arida, R. (2010). Physical exercise during the adolescent period of life increases hippocampal parvalbumin expression. *Brain and Development*, 32(2), 137–142. doi: 10.1016/j.braindev.2008.12.012

Gordon, G., Choi, H., Rungta, R., Ellis-Davies, G., & MacVicar, B. (2008). Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature*, 456(7223), 745–749. doi: 10.1038/nature07525

- Gowda, M. (2004). Robust-LongSAGE (RL-SAGE): A substantially improved LongSAGE method for gene discovery and transcriptome analysis. *Plant Physiology*, 134(3), 890–897. doi: 10.1104/pp.103.034496
- Green, M., Chia, T., Cairns, M., Wu, J., Tooney, P., Scott, R., & Carr, V. (2014). Catechol-O-methyltransferase (COMT) genotype moderates the effects of childhood trauma on cognition and symptoms in schizophrenia. *Journal of Psychiatric Research*, 49, 43–50. doi: 10.1016/j.jpsychires.2013.10.018
- Greer, E., & Shi, Y. (2012). Histone methylation: a dynamic mark in health, disease and inheritance. *Nature Reviews Genetics*, 13(5), 343–357. doi: 10.1038/nrg3173
- Gregoire, A., Kumar, R., Everitt, B., Henderson, A., & Studd, J. (2018). Transdermal oestrogen for treatment of severe postnatal depression. *Lancet* 1996, Apr 6;347(9006), 930–933.
- Hammond, S., Bernstein, E., Beach, D., & Hannon, G. (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature*, 404(6775), 293–296. doi: 10.1038/35005107
- Hashimoto, T., Volk, D., Eggan, S., Mirnics, K., Pierri, J., & Sun, Z. et al. (2003). Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *The Journal of Neuroscience*, 23(15), 6315–6326. doi: 10.1523/jneurosci.23-15-06315.2003
- Hattori, T., Shimizu, S., Koyama, Y., Emoto, H., Matsumoto, Y., & Kumamoto, N. et al. (2014). DISC1 (Disrupted-in-Schizophrenia-1) regulates differentiation of oligodendrocytes. *Plos ONE*, 9(2), e88506. doi: 10.1371/journal.pone.0088506

- He, E., Lozano, M., Stringer, S., Watanabe, K., Sakamoto, K., & den Oudsten, F. et al. (2018). MIR137 schizophrenia-associated locus controls synaptic function by regulating synaptogenesis, synapse maturation and synaptic transmission. *Human Molecular Genetics*, 27(11), 1879–1891. doi: 10.1093/hmg/ddy089
- Heaney, L., Neill, J., Gonzales, A., Yung, A., Allan, S., & Harte, M. (2018). F231 Gym rats: Exercise reverses cognitive impairment in the phencyclidine rat model of schizophrenia. *Schizophrenia Bulletin*, 44(suppl_1), S312–S312. doi: 10.1093/schbul/sby017.762
- Hettema, J., Neale, M., Myers, J., Prescott, C., & Kendler, K. (2006). A population-based twin study of the relationship between neuroticism and internalizing disorders. *American Journal of Psychiatry*, 163(5), 857–864. doi: 10.1176/ajp.2006.163.5.857
- Homayoun, H., & Moghaddam, B. (2007). NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *Journal of Neuroscience*, 27(43), 11496–11500. doi: 10.1523/jneurosci.2213-07.2007
- Howes, O., McCutcheon, R., & Stone, J. (2015). Glutamate and dopamine in schizophrenia: An update for the 21st century. *Journal of Psychopharmacology*, 29(2), 97–115. doi: 10.1177/0269881114563634
- Hu, W., MacDonald, M., Elswick, D., & Sweet, R. (2014). The glutamate hypothesis of schizophrenia: Evidence from human brain tissue studies. *Annals of the New York Academy of Sciences*, 1338(1), 38–57. doi: 10.1111/nyas.12547
- Huang, Y., Pastor, W., Shen, Y., Tahiliani, M., Liu, D., & Rao, A. (2010). The behaviour of 5-Hydroxymethylcytosine in

bisulfite sequencing. *Plos ONE*, 5(1), e8888. doi: 10.1371/journal.pone.0008888

Ikemoto, S. (2010). Brain reward circuitry beyond the mesolimbic dopamine system: A neurobiological theory. *Neuroscience & Biobehavioral Reviews*, 35(2), 129–150. doi: 10.1016/j.neubiorev.2010.02.001

Itokawa, M., Yamada, K., Iwayama-Shigeno, Y., Ishitsuka, Y., Detera-Wadleigh, S., & Yoshikawa, T. (2003). Genetic analysis of a functional GRIN2A promoter (GT)_n repeat in bipolar disorder pedigrees in humans. *Neuroscience Letters*, 345(1), 53–56. doi: 10.1016/s0304-3940(03)00501-9

Jentsch, J., & Pennington, Z. (2014). Reward, interrupted: Inhibitory control and its relevance to addictions. *Neuropharmacology*, 76, 479–486. doi: 10.1016/j.neuropharm.2013.05.022

Jenuwein, T. (2001). Translating the Histone Code. *Science*, 293(5532), 1074–1080. doi: 10.1126/science.1063127

Jones, C., Watson, D., & Fone, K. (2011). Animal models of schizophrenia. *British Journal of Pharmacology*, 164(4), 1162–1194. doi: 10.1111/j.1476-5381.2011.01386.x

Kaalund, S., Riise, J., Broberg, B., Fabricius, K., Karlsen, A., & Secher, T. et al. (2012). Differential expression of parvalbumin in neonatal phencyclidine-treated rats and socially isolated rats. *Journal of Neurochemistry*, 124(4), 548–557. doi: 10.1111/jnc.12061

Kang, E., Burdick, K., Kim, J., Duan, X., Guo, J., & Sailor, K. et al. (2011). Interaction between FEZ1 and DISC1 in regulation of neuronal development and risk for schizophrenia. *Neuron*, 72(4), 559–571. doi: 10.1016/j.neuron.2011.09.032

- Kelsom, C., & Lu, W. (2013). Development and specification of GABAergic cortical interneurons. *Cell & Bioscience*, 3(1), 19. doi: 10.1186/2045-3701-3-19
- Kim, A., Reimers, M., Maher, B., Williamson, V., McMichael, O., & McClay, J. et al. (2010). MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders. *Schizophrenia Research*, 124(1–3), 183–191. doi: 10.1016/j.schres.2010.07.002
- Kirby, M., Ramaker, R., Roberts, B., Lasseigne, B., Gunther, D., & Burwell, T. et al. (2017). Genome-wide DNA methylation measurements in prostate tissues uncovers novel prostate cancer diagnostic biomarkers and transcription factor binding patterns. *BMC Cancer*, 17(1). doi: 10.1186/s12885-017-3252-2
- Kobilo, T., Yuan, C., & van Praag, H. (2011). Endurance factors improve hippocampal neurogenesis and spatial memory in mice. *Learning & Memory*, 18(2), 103–107. doi: 10.1101/lm.2001611
- Kurtz, M., Olfson, R., & Rose, J. (2013). Self-efficacy and functional status in schizophrenia: Relationship to insight, cognition and negative symptoms. *Schizophrenia Research*, 145(1–3), 69–74. doi: 10.1016/j.schres.2012.12.030
- Lee, D., Shin, J., Tonge, P., Puri, M., Lee, S., & Park, H. et al. (2014). An epigenomic roadmap to induced pluripotency reveals DNA methylation as a reprogramming modulator. *Nature Communications*, 5(1). doi: 10.1038/ncomms6619
- Lewis, B., Shih, I., Jones-Rhoades, M., Bartel, D., & Burge, C. (2003). Prediction of mammalian microRNA targets. *Cell*, 115(7), 787–798. doi: 10.1016/s0092-8674(03)01018-3

- Li, L., & He, D. (2007). Association study between the NMDA receptor 2B subunit gene (GRIN2B) and schizophrenia: A HuGE review and meta-analysis. *Genet Med., Jan, 9*(1), 4–8.
- Li, N., Lee, B., Liu, R. J., Banasr, M., Dwyer, J. M., Iwata, M., Li, X. Y., Aghajanian, G., ... Duman, R. S. (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science (New York, N.Y.)*, 329(5994), 959-64.
- Li, Y., & Tollefsbol, T. (2011). DNA methylation detection: Bisulfite genomic sequencing analysis. *Methods in Molecular Biology*, 11–21. doi: 10.1007/978-1-61779-316-5_2
- Liao, Y., Tang, J., Corlett, P., Wang, X., Yang, M., & Chen, H. et al. (2011). Reduced dorsal prefrontal gray matter after chronic ketamine use. *Biological Psychiatry*, 69(1), 42–48. doi: 10.1016/j.biopsych.2010.08.030
- Lievin-Le Moal, V., & Servin, A. (2013). Pathogenesis of human enterovirulent bacteria: Lessons from cultured, fully differentiated human colon cancer cell lines. *Microbiology and Molecular Biology Reviews*, 77(3), 380–439. doi: 10.1128/mmb.00064-12
- Lipina, T., Niwa, M., Jaaro-Peled, H., Fletcher, P., Seeman, P., Sawa, A., & Roder, J. (2010). Enhanced dopamine function in DISC1-L100P mutant mice: Implications for schizophrenia. *Genes, Brain and Behavior*, 9(7), 777–789. doi: 10.1111/j.1601-183x.2010.00615.x
- Lisman, J., Coyle, J., Green, R., Javitt, D., Benes, F., Heckers, S., & Grace, A. (2008). Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends in Neurosciences*, 31(5), 234–242. doi: 10.1016/j.tins.2008.02.005

- Lodge, D. (2013). The MAM rodent model of schizophrenia. *Current Protocols in Neuroscience*. doi: 10.1002/0471142301.ns0943s63
- Mahmoudi, E., & Cairns, M. (2017). MiR-137: An important player in neural development and neoplastic transformation. *Molecular Psychiatry*, 22, 44–55.
- Malchow, B., Reich-Erkelenz, D., Oertel-Knöchel, V., Keller, K., Hasan, A., & Schmitt, A. et al. (2013). The effects of physical exercise in schizophrenia and affective disorders. *European Archives Of Psychiatry And Clinical Neuroscience*, 263(6), 451-467. doi: 10.1007/s00406-013-0423-2
- Marcotte, E., Pearson, D., & Srivastava, . (2001). Animal models of schizophrenia: A critical review. *Journal of Psychiatry and Neuroscience*, 26(5), 395–410.
- Martucci, L., Wong, A., De Luca, V., Likhodi, O., Wong, G., King, N., & Kennedy, J. (2006). N-methyl-d-aspartate receptor NR2B subunit gene GRIN2B in schizophrenia and bipolar disorder: Polymorphisms and mRNA levels. *Schizophrenia Research*, 84(2–3), 214–221. doi: 10.1016/j.schres.2006.02.001
- Martucci, L., Wong, A., Trakalo, J., Cate-Carter, T., Wong, G., Macciardi, F., & Kennedy, J. (2003). N-methyl-d-aspartate receptor NR1 subunit gene (GRIN1) in schizophrenia: TDT and case-control analyses. *American Journal of Medical Genetics*, 119B(1), 24–27. doi: 10.1002/ajmg.b.20014
- Matsuzaki, M., & Dowling, K. (1984). Phencyclidine (PCP): Effects on limbic afterdischarges in the rhesus monkey. *Brain Research Bulletin*, 13(5), 635–641. doi: 10.1016/0361-9230(84)90195-3
- Mazzio, E., & Soliman, K. (2012). Basic concepts of epigenetics. *Epigenetics*, 7(2), 119–130. doi: 10.4161/epi.7.2.18764

- McGregor, N. (2014). Catechol O-Methyltransferase: A review of the gene and enzyme. *OPEN Access Journal of Dentistry and Clinical Research*, 1(007).
- Meyer-Lindenberg, A. (2010). From maps to mechanisms through neuroimaging of schizophrenia. *Nature*, 468(7321), 194–202. doi: 10.1038/nature09569
- Miller, B., Zeier, Z., Xi, L., Lanz, T., Deng, S., & Strathmann, J. et al. (2012). MicroRNA-132 dysregulation in schizophrenia has implications for both neurodevelopment and adult brain function. *Proceedings of the National Academy of Sciences*, 109(8), 3125–3130. doi: 10.1073/pnas.1113793109
- Mirnics, K., Middleton, F., Lewis, D., & Levitt, P. (2001). Analysis of complex brain disorders with gene expression microarrays: Schizophrenia as a disease of the synapse. *Trends in Neurosciences*, 24(8), 479–486. doi: 10.1016/s0166-2236(00)01862-2
- Mirnics, K., Middleton, F., Lewis, D., & Levitt, P. (2001). Analysis of complex brain disorders with gene expression microarrays: Schizophrenia as a disease of the synapse. *Trends in Neurosciences*, 24(8), 479–486. doi: 10.1016/s0166-2236(00)01862-2
- Mohn, F., Weber, M., Schübeler, D., & Roloff, T. (2009). Methylated DNA immunoprecipitation (MeDIP). *Methods in Molecular Biology*, 55–64. doi: 10.1007/978-1-59745-522-0_5
- Morgan, C., Mofeez, A., Brandner, B., Bromley, L., & Curran, H. (2003). Acute effects of ketamine on memory systems and psychotic symptoms in healthy volunteers. *Neuropsychopharmacology*, 29(1), 208–218. doi: 10.1038/sj.npp.1300342

- Murray, J. (2002). Phencyclidine (PCP): A dangerous drug, but useful in schizophrenia research. *The Journal of Psychology*, 136(3), 319–327. doi: 10.1080/00223980209604159
- Musumeci, G., Grasselli, G., Rossi, S., De Chiara, V., Musella, A., & Motta, C. et al. (2011). Transient receptor potential vanilloid 1 channels modulate the synaptic effects of TNF- α and of IL-1 β in experimental autoimmune encephalomyelitis. *Neurobiology of Disease*, 43(3), 669–677. doi: 10.1016/j.nbd.2011.05.018
- Nakazawa, K., Jeevakumar, V., & Nakao, K. (2017). Spatial and temporal boundaries of NMDA receptor hypofunction leading to schizophrenia. *npj Schizophrenia*, 3(1). doi: 10.1038/s41537-016-0003-3
- Narendran, R., Frankle, W., Keefe, R., Gil, R., Martinez, D., & Slifstein, M. et al. (2005). Altered prefrontal dopaminergic function in chronic recreational ketamine users. *American Journal of Psychiatry*, 162(12), 2352–2359. doi: 10.1176/appi.ajp.162.12.2352
- Negrón-Oyarzo, I., Lara-Vásquez, A., Palacios-García, I., Fuentealba, P., & Aboitiz, F. (2016). Schizophrenia and reelin: A model based on prenatal stress to study epigenetics, brain development and behavior. *Biological Research*, 49(1). doi: 10.1186/s40659-016-0076-5
- Neill, J., Barnes, S., Cook, S., Grayson, B., Idris, N., & McLean, S. et al. (2010). Animal models of cognitive dysfunction and negative symptoms of schizophrenia: Focus on NMDA receptor antagonism. *Pharmacology & Therapeutics*, 128(3), 419–432. doi: 10.1016/j.pharmthera.2010.07.004
- Nishioka, M., Bundo, M., Kasai, K., & Iwamoto, K. (2012). DNA methylation in schizophrenia: progress and challenges of

- epigenetic studies. *Genome Medicine*, 4(12), 96. doi: 10.1186/gm397
- Nomura, J., Kannan, G., & Takumi, T. (2017). Rodent models of genetic and chromosomal variations in psychiatric disorders. *Psychiatry and Clinical Neurosciences*, 71(8), 508–517. doi: 10.1111/pcn.12524
- Ovadia, G., & Shifman, S. (2011). The genetic variation of RELN expression in schizophrenia and bipolar disorder. *Plos ONE*, 6(5), e19955. doi: 10.1371/journal.pone.0019955
- Paoletti, P., & Neyton, J. (2007). NMDA receptor subunits: function and pharmacology. *Current Opinion In Pharmacology*, 7(1), 39-47. doi: 10.1016/j.coph.2006.08.011
- Park, J., Lee, S., & Kim, T. (2014). Treadmill exercise enhances NMDA receptor expression in schizophrenia mice. *Journal of Exercise Rehabilitation*, 10(1), 15–21. doi: 10.12965/jer.140088
- Park, S., Lee, Y., Oh, H., Lee, H., Lee, Y., & Ko, S. et al. (2014). Oleanolic acid attenuates MK-801-induced schizophrenia-like behaviors in mice. *Neuropharmacology*, 86, 49–56. doi: 10.1016/j.neuropharm.2014.06.025
- Parker, C., Chen, H., Flagel, S., Geurts, A., Richards, J., & Robinson, T. et al. (2018). Rats are the smart choice: Rationale for a renewed focus on rats in behavioral genetics. *Neuropharmacology*, (76), 250–258.
- Penn, D., Sanna, L., & Roberts, D. (2007). Social cognition in schizophrenia: An overview. *Schizophrenia Bulletin*, 34(3), 408–411. doi: 10.1093/schbul/sbn014
- Perkins, D., Jeffries, C., Jarskog, L., Thomson, J., Woods, K., & Newman, M. et al. (2007). MicroRNA expression in the prefrontal cortex of individuals with schizophrenia and

- schizoaffective disorder. *Genome Biology*, 8(2), R27. doi: 10.1186/gb-2007-8-2-r27
- Peterson, C., & Laniel, M. (2004). Histones and histone modifications. *Current Biology*, 14(14), R546–R551. doi: 10.1016/j.cub.2004.07.007
- Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Research*, 29(9), e45.
- Pujol, N., Penadés, R., Junqué, C., Dinov, I., Fu, C., & Catalán, R. et al. (2014). Hippocampal abnormalities and age in chronic schizophrenia: Morphometric study across the adult lifespan. *British Journal of Psychiatry*, 205(05), 369–375. doi: 10.1192/bjp.bp.113.140384
- Qin, S., Zhao, X., Pan, Y., Liu, J., Feng, G., & Fu, J. et al. (2005). An association study of the N-methyl-D-aspartate receptor NR1 subunit gene (GRIN1) and NR2B subunit gene (GRIN2B) in schizophrenia with universal DNA microarray.
- Rice, S., Niu, N., Berman, D., Heston, L., & Sobell, J. (2001). Identification of single nucleotide polymorphisms (SNPs) and other sequence changes and estimation of nucleotide diversity in coding and flanking regions of the NMDAR1 receptor gene in schizophrenic patients. *Molecular Psychiatry*, 6(3), 274–284. doi: 10.1038/sj.mp.4000838
- Richtand, N., Ahlbrand, R., Horn, P., Stanford, K., Bronson, S., & McNamara, R. (2011). Effects of risperidone and paliperidone pre-treatment on locomotor response following prenatal immune activation. *Journal of Psychiatric Research*, 45(9), 1194–1201. doi: 10.1016/j.jpsychires.2011.02.007
- Ripke, S., O'Dushlaine, C., Chambert, K., Moran, J., Kähler, A., &

- Rönn, T., & Ling, C. (2013). Effect of exercise on DNA methylation and metabolism in human adipose tissue and skeletal muscle. *Epigenomics*, 5(6), 603–605. doi: 10.2217/epi.13.61
- Rowland. (2015). Retrieved from <https://www.fda.gov/downloads/medicaldevices/safety/alertsandnotices/ucm393589.pdf>
- Saha, S., Sparks, A., Rago, C., Akmaev, V., Wang, C., & Vogelstein, B. et al. (2002). Using the transcriptome to annotate the genome. *Nature Biotechnology*, 20(5), 508–512. doi: 10.1038/nbt0502-508
- Sakae, N., Yamasaki, N., Kitaichi, K., Fukuda, T., Yamada, M., & Yoshikawa, H. et al. (2008). Mice lacking the schizophrenia-associated protein FEZ1 manifest hyperactivity and enhanced responsiveness to psychostimulants. *Human Molecular Genetics*, 17(20), 3191–3203. doi: 10.1093/hmg/ddn215
- Scheewe, T., van Haren, N., Sarkisyan, G., Schnack, H., Brouwer, R., & de Glin, M. et al. (2013). 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), 675–685. doi: 10.1016/j.euroneuro.2012.08.008
- Schramm-Sapota NL, Walker QD, Caster JM, Levin ED, Kuhn CM. Are adolescents more vulnerable to drug addiction than adults? Evidence from animal models. *Psychopharmacology (Berl)* 2009;206:1–21
- Segal-Gavish, H., Barzilay, R., Rimoni, O., & Offen, D. (2017). Voluntary exercise improves cognitive deficits in female dominant-negative DISC1 transgenic mouse model of neuropsychiatric disorders. *The World Journal of Biological Psychiatry*, 1–10. doi: 10.1080/15622975.2017.1323118

- Sharkey, F., Banat, I., & Marchant, R. (2004). Detection and quantification of gene expression in environmental bacteriology. *Applied and Environmental Microbiology*, 70(7), 3795–3806. doi: 10.1128/aem.70.7.3795-3806.2004
- Shendure, J. (2008). The beginning of the end for microarrays? *Nature Methods*, 5(7), 585–587. doi: 10.1038/nmeth0708-585
- Shi, H., Yang, F., Li, W., Zhao, W., Nie, K., Dong, B., & Liu, Z. (2015). A review: Fabrications, detections and applications of peptide nucleic acids (PNAs) microarray. *Biosensors and Bioelectronics*, 66, 481–489. doi: 10.1016/j.bios.2014.12.010
- Siegert, S., Seo, J., Kwon, E., Rudenko, A., Cho, S., & Wang, W. et al. (2015). The schizophrenia risk gene product miR-137 alters presynaptic plasticity. *Nature Neuroscience*, 18(7), 1008–1016. doi: 10.1038/nn.4023
- Smith, C., & Osborn, A. (2009). Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. *FEMS Microbiology Ecology*, 67(1), 6–20. doi: 10.1111/j.1574-6941.2008.00629.x
- Song, S., Ito, K., Ala, U., Kats, L., Webster, K., & Sun, S. et al. (2013). The oncogenic microRNA miR-22 targets the TET2 tumor suppressor to promote hematopoietic stem cell self-renewal and transformation. *Cell Stem Cell*, 13(1), 87–101. doi: 10.1016/j.stem.2013.06.003
- Spalek, K., Coynel, D., Freytag, V., Hartmann, F., Heck, A., & Milnik, A. et al. (2016). A common NTRK2 variant is associated with emotional arousal and brain white-matter integrity in healthy young subjects. *Translational Psychiatry*, 6, e758.

- Squinto, S., Stitt, T., Aldrich, T., Davis, S., Blanco, S., & Radziejewski, C. et al. (1991). trkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. *Cell*, 65(5), 885–893. doi: 10.1016/0092-8674(91)90395-f
- Stahl, S. (2013). *Stahl's essential psychopharmacology print and online bundle*. Cambridge: Cambridge University Press.
- Stone, J., Dietrich, C., Edden, R., Mehta, M., De Simoni, S., & Reed, L. et al. (2012). Ketamine effects on brain GABA and glutamate levels with 1H-MRS: Relationship to ketamine-induced psychopathology. *Molecular Psychiatry*, 17(7), 664–665. doi: 10.1038/mp.2011.171
- Strong, W., Malina, R., Blimkie, C., Daniels, S., Dishman, R., & Gutin, B. et al. (2005). Evidence based physical activity for school-age youth. *The Journal of Pediatrics*, 146(6), 732–737. doi: 10.1016/j.jpeds.2005.01.055
- Stroup, T., Gerhard, T., Crystal, S., Huang, C., & Olfson, M. (2016). Comparative effectiveness of Clozapine and standard antipsychotic treatment in adults with schizophrenia. *American Journal of Psychiatry*, 173(2), 166–173. doi: 10.1176/appi.ajp.2015.15030332
- Sun, G., Ye, P., Murai, K., Lang, M., Li, S., & Zhang, H. et al. (2011). miR-137 forms a regulatory loop with nuclear receptor TLX and LSD1 in neural stem cells. *Nature Communications*, 2(1). doi: 10.1038/ncomms1532
- Takebayashi, H., Yamamoto, N., Umino, A., & Nishikawa, T. (2009). Developmentally regulated and thalamus-selective induction of leiomodin2 gene by a schizophrenomimetic, phencyclidine, in the rat. *The International Journal of Neuropsychopharmacology*, 12(08), 1111. doi: 10.1017/s1461145709009997

- Tamminga, C. (2000). The biology of schizophrenia. *Dialogues Clin Neurosci*, Dec, 2(4). 339–348.
- Tang, J., Chen, X., Xu, X., Wu, R., Zhao, J., Hu, Z., & Xia, K. (2006). Significant linkage and association between a functional (GT)_n polymorphism in promoter of the N-methyl-d-aspartate receptor subunit gene (GRIN2A) and schizophrenia. *Neuroscience Letters*, 409(1), 80–82. doi: 10.1016/j.neulet.2006.09.022
- Tao, R., Davis, K., Li, C., Shin, J., Gao, Y., & Jaffe, A. et al. (2017). GAD1 alternative transcripts and DNA methylation in human prefrontal cortex and hippocampus in brain development, schizophrenia. *Molecular Psychiatry*, 23(6), 1496–1505. doi: 10.1038/mp.2017.105
- Tarca, A., Draghici, S., Khatri, P., Hassan, S., Mittal, P., & Kim, J. et al. (2008). A novel signaling pathway impact analysis. *Bioinformatics*, 25(1), 75–82. doi: 10.1093/bioinformatics/btn577
- Teixeira, C., Martín, E., Sahún, I., Masachs, N., Pujadas, L., & Corvelo, A. et al. (2011). Overexpression of Reelin prevents the manifestation of behavioral phenotypes related to schizophrenia and bipolar disorder. *Neuropsychopharmacology*, 36(12), 2395–2405. doi: 10.1038/npp.2011.153
- Teng, H., Cai, W., Zhou, L., Zhang, J., Liu, Q., & Wang, Y. et al. (2010). Evolutionary mode and functional divergence of vertebrate NMDA receptor subunit 2 genes. *Plos ONE*, 5(10), e13342. doi: 10.1371/journal.pone.0013342
- Teroganova, N., Girshkin, L., Suter, C., & Green, M. (2016). DNA methylation in peripheral tissue of schizophrenia and bipolar disorder: A systematic review. *BMC Genetics*, 17(1). doi: 10.1186/s12863-016-0332-2

- Thoma, R., Monnig, M., Hanlon, F., Miller, G., Petropoulos, H., & Mayer, A. et al. (2009). Hippocampus volume and episodic memory in schizophrenia. *Journal of the International Neuropsychological Society*, 15(02), 182. doi: 10.1017/s1355617709090225
- Thomas, K., Anderson, B., Shah, N., Zimmer, S., Hawkins, D., & Valdez, A. et al. (2017). Inhibition of the schizophrenia-associated microRNA miR-137 disrupts Nrg1a neurodevelopmental signal transduction. *Cell Reports*, 20(1), 1–12. doi: 10.1016/j.celrep.2017.06.038
- Tso, I., Fang, Y., Phan, K., Welsh, R., & Taylor, S. (2015). Abnormal GABAergic function and face processing in schizophrenia: A pharmacologic-fMRI study. *Schizophrenia Research*, 168(1–2), 338–344. doi: 10.1016/j.schres.2015.08.022
- Vakhrusheva, J., Marino, B., Stroup, T., & Kimhy, D. (2016). Aerobic exercise in people with schizophrenia: Neural and neurocognitive benefits. *Current Behavioral Neuroscience Reports*, 3(2), 165–175. doi: 10.1007/s40473-016-0077-2
- Van Snellenberg, J., Girgis, R., Horga, G., van de Giessen, E., Slifstein, M., & Ojeil, N. et al. (2016). Mechanisms of working memory impairment in schizophrenia. *Biological Psychiatry*, 80(8), 617–626. doi: 10.1016/j.biopsych.2016.02.017
- Vandenheuvel, D., & Pasterkamp, R. (2008). Getting connected in the dopamine system. *Progress in Neurobiology*, 85(1), 75–93. doi: 10.1016/j.pneurobio.2008.01.003
- Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). *Genome Biology*, 3(7), research0034.1. doi: 10.1186/gb-2002-3-7-research0034

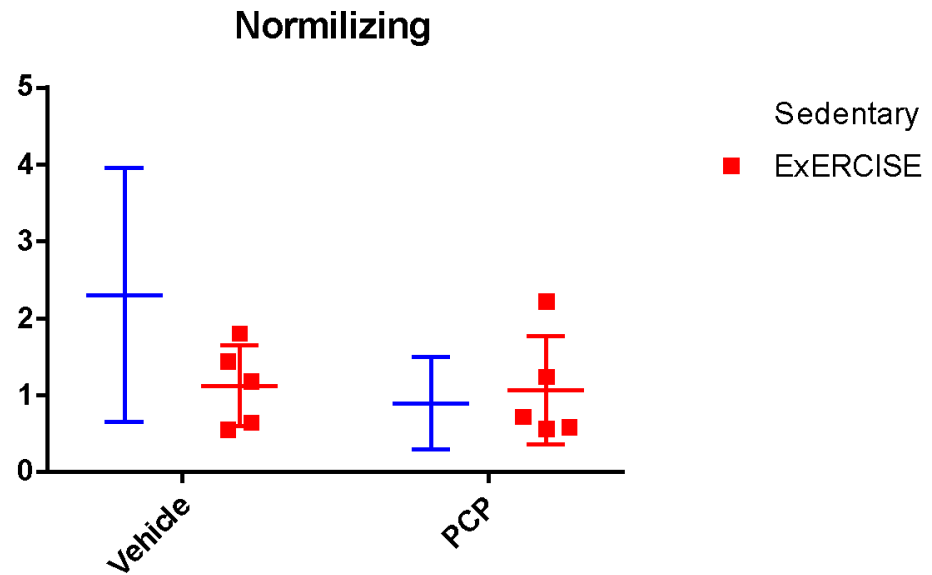
- Vermeulen, J., van Rooijen, G., Doedens, P., Numminen, E., van Tricht, M., & de Haan, L. (2017). Antipsychotic medication and long-term mortality risk in patients with schizophrenia; a systematic review and meta-analysis. *Psychological Medicine*, 47(13), 2217–2228. doi: 10.1017/s0033291717000873
- Wang, C., Yang, S., Xia, Y., & Johnson, K. (2007). Postnatal phencyclidine administration selectively reduces adult cortical parvalbumin-containing interneurons. *Neuropsychopharmacology*, 33(10), 2442–2455. doi: 10.1038/sj.npp.1301647
- Weeber, E., Beffert, U., Jones, C., Christian, J., Förster, E., Sweatt, J., & Herz, J. (2002). Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *Journal of Biological Chemistry*, 277(42), 39944–39952. doi: 10.1074/jbc.m205147200
- Weickert, C., Fung, S., Catts, V., Schofield, P., Allen, K., & Moore, L. et al. (2012). Molecular evidence of N-methyl-D-aspartate receptor hypofunction in schizophrenia. *Molecular Psychiatry*, 18(11), 1185–1192. doi: 10.1038/mp.2012.137
- Weinhold, B. (2006). Epigenetics: The science of change. *Environ Health Perspect*, 114(3), A160–A167.
- Winter, J., Jung, S., Keller, S., Gregory, R., & Diederichs, S. (2009). Many roads to maturity: MicroRNA biogenesis pathways and their regulation. *Nature Cell Biology*, 11(3), 228–234. doi: 10.1038/ncb0309-228
- Wong, M., & Medrano, J. (2005). Real-time PCR for mRNA quantitation. *Biotechniques*, 39(1), 75–85. doi: 10.2144/05391rv01

- Woo, K. (2004). Effects of diet and exercise on obesity-related vascular dysfunction in children. *Circulation*, 109(16), 1981–1986. doi: 10.1161/01.cir.0000126599.47470.be
- Wright, S., Thompson, R., & Galloway, T. (2013). The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution*, 178, 483–492. doi: 10.1016/j.envpol.2013.02.031
- Xia, S., Zhou, X., Wang, T., Zhang, Q., Li, Q., & Liu, Y. et al. (2015). Experimental validation of candidate schizophrenia gene CALN1 as a target for microRNA-137. *Neuroscience Letters*, 602, 110–114. doi: 10.1016/j.neulet.2015.07.001
- Yang, Y., Li, W., Zhang, H., Yang, G., Wang, X., & Ding, M. et al. (2015). Association study of N-Methyl-D-Aspartate receptor subunit 2B (GRIN2B) polymorphisms and schizophrenia symptoms in the Han Chinese population. *PLOS ONE*, 10(5), e0125925. doi: 10.1371/journal.pone.0125925
- Yekta, S. (2004). MicroRNA-directed cleavage of HOXB8 mRNA. *Science*, 304(5670), 594–596. doi: 10.1126/science.1097434
- Yin, J., Lin, J., Luo, X., Chen, Y., Li, Z., Ma, G., & Li, K. (2014). miR-137: A new player in schizophrenia. *International Journal of Molecular Sciences*, 15(2), 3262–3271. doi: 10.3390/ijms15023262
- Zhang, Y., Fan, M., Wang, Q., He, G., Fu, Y., Li, H., & Yu, S. (2015). Polymorphisms in microRNA genes and genes involving in NMDAR signaling and schizophrenia: A case-control study in Chinese Han population. *Scientific Reports*, 5(1). doi: 10.1038/srep12984
- Zhao, D., Lin, M., Chen, J., Pedrosa, E., Hrabovsky, A., & Fourcade, H. et al. (2015). MicroRNA profiling of neurons generated using induced pluripotent stem cells derived

from patients with schizophrenia and schizoaffective disorder and 22q11.2 Del. PLOS ONE, 10(7), e0132387.
doi: 10.1371/journal.pone.0132387

Appendix

Normalising



The normalising of the data allow us to understand whether the data can be trusted. The data must be read reversed compared to the existing data that is found in the results chapter.

The Sequence of the gene of interest

The DNA sequence of the genes of interest. The red text marks the primer sequence. It was only possible to perform the reverse sequence in most of the genes of interest. Further, some were discarded because they were impossible to sequence. The reason behind performing the sequencing was to ensure that the right sequence was amplified in the qPCR.

Gene	Sequences
NTrk	F: 5'- CGAATGCCAAAGCTAGCTTCGACATCATCTTC ATTGATGATTTCTAACCTTTTCTGGTTTGCAAT GAGAATTCGGTGAT gttctctgggtcaatgctgtagAAAGGTCTATGTAG ACGTTGTCGCAGCTTATGTGGGGCTGAAAAA CCTTACAATTGT GGATTCCGCCTTAAATACA-3' R: CAATATTTCTATTTGCAACCAGAAAAGGTTA GAAATCATCAATGAAGATGATGTCTGAAGCTT ACGTGGGGCTGAAAAACcttacaattgtggattccg gcttA

PVALB	R: 5'- CATTTGTAAAATTCTTTCACCCCAAATCTTGC CGTCCCCGTCCTTGTCTCCAGCAGCCATCAG CGTCTTTGTTTCCTTAG CAGACAAGTCTCTGGCATCTGAGGAGAAGCC CTTCAGAATGGACCCCAGCTCATCCTCCTCAA TGAAGCCACTTTTGTCT TTGTCCAGAATGTGGAAcAccttcttcacatcatccg cA -3'
	GGACTTACCCTCAGTAGACTGCACAGTTTGC TCCTCCCCGTTCTTAGCTGGAAGCAGATCTTG AGCAAACAGGTTGGAGA AGTCCGTCTCTGTGCGCCGGAAGCGGGCACC AGGGTCACTGTTTTACAGGAAAGCAGGTTC TTGGAGGCCTGCCTCTCC CTGAAGGCGCTCACAAGACGACTCTTCTCTtc caggctattggcctttgA
GAD-1	GGACTTACCCTCAGTAGACTGCACAGTTTGC TCCTCCCCGTTCTTAGCTGGAAGCAGATCTTG AGCAAACAGGTTGGAGA AGTCCGTCTCTGTGCGCCGGAAGCGGGCACC AGGGTCACTGTTTTACAGGAAAGCAGGTTC TTGGAGGCCTGCCTCTCC CTGAAGGCGCTCACAAGACGACTCTTCTCTtc caggctattggcctttgA

EGR1	None
DISC1	None
RELN	R: 5'AAAGGTTAACATGGAATATTCCACCAACCA CGGGCTCACGTGGCACCTTGTACAAGACGAG TGCCTTCCCAGTATGCCAA GTTGCCAGGAATTTACATCGGCCAGCATTTAC CACGCCAGTGAGTTCACACAGTggagaagagtg accgATGTAAATTCC TGGCAACTTGGCATACTGGGAAGGCACTCGT CTTGTACAAGGTGCCACGTGAGCCCGTGGTT GGTGGAATACTCCAGCTT CACCTGGTTGTCCATGTGAGGAGTGTA-3'
COMT	R: 5'- TCCCGGGGGCTGTGGTTCAGACTGTCATACA CTGGCACATTTTAAAGGTAGTGAGCCCACTAT GCAAAATCACTTCAATA ACCCTGAAAAGCACCTatggatgaaaggctgaatt gaggACAAGCGCGAGGAGCATAGAAAGGGTC AGATTGGG-3'
GRIN2B	R: 5'- CTGCTCGCTGGACACAGCTTTGTGGgctgggatat gcagaacgtgAGCGGGTGGTTAGGCTTGTCTTGAT AAGCTGATGA AGTCTCGGTAGCCAGGAC-3'

GRIN2A	R: 5'- GAAACTGGCATCAGTGTCATGGTATCTCGCA GCAATGGGACTGTGTCACCTTCTGCCTTcttag agcc-3'
--------	---