Antipsychotics Prevent Psychosine Induced Toxicity in Glial Cells

This thesis submitted to University of Dublin, Trinity College

for the degree of

Doctor of Philosophy

Kapil Sharma

11th February 2023

Supervisor: Professor Kumlesh Dev

Discipline of Physiology, School of Medicine,

Trinity College Dublin, Ireland

Co-Supervisor: Professor Aidan Corvin

Discipline of Psychiatry, School of Medicine,

Trinity College Dublin, Ireland

Declaration and Statement of Plagiarism

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

I agree to deposit this thesis in the University's open access institutional repository or allow the library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

Signed Date

Kapil Sharma 11.02.2023

Acknowledgments

Sincerest thanks to my research supervisor and principal investigator Professor Kumlesh Dev. Your steadfast academic guidance and personal support was vital especially during challenging circumstances. Also, thanks to my co-supervisor Professor Aidan Corvin.

My gratitude to all staff, colleagues and friends in the School of Medicine, Department of Physiology and Trinity Biomedical Sciences Institute. Especially Dr Maria Velasco, Dr Sibylle Béchet, Dr Trisha Ang, Dr Johnny Fitzpatrick and Mr Luke Davison.

My appreciation to the Irish Clinical Academic Training (ICAT) Programme, supported by the Wellcome Trust and the Health Research Board (Grant Number 203930/B/16/Z), the Health Service Executive, National Doctors Training and Planning and the Health and Social Care, Research and Development Division Northern Ireland.

Finally, I would like to thank my friends and family for their tireless support and this thesis is dedicated to Ruth, Matthew and Lily Sharma.

Table of Contents

Declaration and Statement of Plagiarism ii
Acknowledgments..... iii
Table of Contents..... iv
List of Tables and Figures viii
List of Abbreviations xi
Scientific Abstract xiii
Lay Abstract..... xiv
Aims and Hypothesis..... xv
Value of Research xvi
Outputs xvii
Chapter 1 – Introduction..... 1
1. Schizophrenia. 2
1.1. Clinical presentation and diagnosis. 2
1.2. Aetiology of schizophrenia. 2
1.3. Clinical course and treatments for schizophrenia. 3
1.4. Pre-clinical research models of schizophrenia. 5
2. Neuropathology of schizophrenia - a focus on glial cell pathology. 6
2.1. Macroscopic and microscopic abnormalities. 6
2.2. Glial cells and their pathology in schizophrenia. 7
3. Effects of antipsychotics on glial cells. 16
3.1. Astrocytes. 16
3.2. Oligodendrocytes and oligodendrocyte progenitor cells. 17
3.3. Microglia. 19
4. Psychosine a toxic sphingolipid that accumulates abnormally in the leukodystrophy Krabbe disease. 20
Chapter 2 – Materials and Methods 31
1. Pharmacological compounds. 32
2. Antibodies. 32
3. Culture and treatment of human astrocytes and mouse organotypic cerebellar slices. 32
4. LDH assay. 34
5. MTT assay..... 34
6. Immunocytochemistry and fluorescent microscopy. 35

7. Animals. 36
8. Behavioural analysis. 37
9. Open Field Maze. 37
10. Statistical analysis. 38
11. Candidate's contribution statement. 39
Chapter 3 – Antipsychotics attenuate psychosine-induced cell toxicity and morphological damage in human astrocytes. 44
Chapter Aims 45
Abstract 46
1. Introduction. 47
2. Results. 49
2.1. Psychosine induces toxicity and reduces cell viability in human astrocytes. 49
2.2. Antipsychotics with primary D_2 receptor antagonism attenuate psychosine toxicity and improve astrocyte viability. 49
2.3. Antipsychotics with D_2 and $5HT_2$ receptor antagonism prevented psychosine toxicity and improved astrocyte viability. 50
2.4. Selective D_2 and $5HT_{2A}$ antagonists prevented psychosine induced toxicity. 51
2.5. Antipsychotics attenuate psychosine induced morphological changes in human astrocytes. 51
2.6. Psychosine induced morphological changes in human astrocytes are attenuated by D_2 and $5HT_{2A}$ selective antagonists. 52
3. Discussion. 60
3.1. Summary of Findings. 60
3.2. Current treatments and the role of glial cells in schizophrenia. 60
3.3. Psychosine toxicity as a model of glial cell dysfunction. 61
3.4. D_2 and $5HT_{2A}$ antagonism protects against psychosine induced glial cell toxicity. 62
3.5. Conclusion. 62
Chapter 4 – Antipsychotics attenuate psychosine-induced demyelination in organotypic cerebellar slice model. 63
Chapter Aims 64
Abstract 65
1. Introduction. 66
2. Results. 68
2.1. Haloperidol and clozapine attenuate psychosine induced demyelination in cultured cerebellar slices. 68

2.2. Haloperidol and clozapine reduce psychosine induced loss of GFAP and vimentin expression. 69

2.3. Haloperidol and clozapine attenuate psychosine-induced demyelination independent of changes to microglia. 69

2.4. Haloperidol and clozapine prevented psychosine induced axonal damage in white matter tracts of cultured cerebellar slices. 70

3. Discussion........ 77

3.1. Summary of findings. 77

3.2. Mechanisms of psychosine induced demyelination. 77

3.3. Psychosine deregulates astrocytes, which is attenuated by haloperidol and clozapine. 78

3.4. Microglia independent demyelination is regulated by haloperidol and clozapine. 79

3.5. Axonal damage associated with demyelination. 80

3.6. Conclusion. 80

Chapter 5 – The antipsychotic haloperidol improves survival, locomotion, and behaviour in twitcher mice – preclinical evidence for a potential novel treatment for Krabbe disease. 82

Chapter Aims........ 83

Abstract........ 84

1. Introduction. 85

2. Results. 87

2.1. Haloperidol ameliorated weight loss, improved twitching scores and immobility scores in twitcher mice. 87

2.2. Haloperidol improved behavioural metrics in twitcher mice. 87

2.3. Haloperidol improved survival in twitcher mice. 88

3. Discussion........ 96

3.1. Summary of findings Haloperidol improved survival, mobility and behaviour in twitcher mice. 96

3.2. Antipsychotics influence oligodendrocyte and oligodendrocyte progenitor cells. 96

3.3. Antipsychotics effects on astrocytes........ 98

3.4. Antipsychotics and microglia. 99

3.5. Conclusion. 100

Chapter 6 – Discussion. 102

1. Study background. 103

2. Summary of findings. 103

3. The role of astrocytes in schizophrenia and the effects of antipsychotics. 105

4. Oligodendrocyte pathology in schizophrenia and myelin protective effects of antipsychotics. 106
5. Antipsychotics as a potential novel therapy for Krabbe disease. 108
6. Dopamine antagonism as a possible mechanism for the protective effects of antipsychotics on psychosine induced glial cell toxicity. 109
7. Limitations and Future Directions. 109
8. Closing remarks. 111
References 114

List of Tables and Figures

Chapter 1 – Introduction.

Table 1.1 DSM-5 and ICD-11 diagnostic criteria for schizophrenia. 22

Figure 1.1 Clinical and aetiological overview of schizophrenia syndrome. 23

Table 1.2 Pharmacology Heat Map of receptor binding profiles of antipsychotics. 24

Table 1.3 Pharmacology of antipsychotics as per neuroscience based nomenclature (NbN) 25

Table 1.4 Genetic characteristics and main G-protein transduction of dopamine and serotonin receptors. 26

Figure 1.2 Intracellular signalling of D1 and D2 class G protein coupled dopamine receptors. 26

Table 1.5 Preclinical models of schizophrenia. 27

Figure 1.3 Homeostatic functions of astrocytes. 28

Figure 1.4 Astrocytes and the synapse. 29

Figure 1.5 The gliocentric model of schizophrenia. 30

Chapter 2 – Materials and Methods.

Table 2.1 List of compounds. 40

Table 2.2 List of antibodies. 40

Figure 2.1 Quantification of cellular processes to assess human astrocyte morphology. 41

Table 2.3 Behavioural scoring protocol. 42

Figure 2.2 In-vivo experimental design schematic. 43

Chapter 3 – Antipsychotics attenuate psychosine-induced cell toxicity and morphological damage in human astrocytes.

Figure 3.1 Psychosine reduces human astrocyte cell viability. 53

Figure 3.2 Antipsychotics with primary D_2 receptor antagonism prevented psychosine toxicity and improved astrocyte viability. 54

Figure 3.3 Antipsychotics with D_2 and $5HT_2$ antagonism prevented psychosine toxicity and improved astrocyte viability. 55

Figure 3.4 Selective D_2 and $5HT_{2A}$ antagonists prevented psychosine induced toxicity. 56

Figure 3.5 Psychosine induced morphological changes in GFAP and Vimentin were attenuated by antipsychotics with primary D_2 antagonism. 57

Figure 3.6. Psychosine induced morphological changes in GFAP and Vimentin were attenuated by antipsychotics with D2 and 5HT2 receptor antagonism. 58

Figure 3.7 Psychosine induced reduction in GFAP and Vimentin positive projections were attenuated by selective D_2 and $5HT_{2A}$ antagonists. 59

Chapter 4 – Antipsychotics attenuate psychosine-induced demyelination in organotypic cerebellar slice model.

Figure 4.1 Psychosine induces demyelination in organotypic cerebellar slices. 71

Figure 4.2 Haloperidol attenuated psychosine induced demyelination in cerebellar slices. 72

Figure 4.3 Clozapine attenuated psychosine induced demyelination in cerebellar slices. 73

Figure 4.4 Haloperidol and Clozapine reduce astrocyte marker loss caused by psychosine. 74

Figure 4.5 Psychosine, haloperidol and clozapine effects were independent of changes in microglia. 75

Figure 4.6 Psychosine induced increase in SMI-32 fluorescence is attenuated by haloperidol and clozapine. 76

Chapter 5 – The antipsychotic haloperidol improves survival, locomotion, and behaviour in twitcher mice – preclinical evidence for a potential novel treatment for Krabbe disease.

Figure 5.1 Haloperidol ameliorated weight loss in twitcher mice. 89

Figure 5.2 Haloperidol improved twitching and immobility scores in twitcher mice. 90

Figure 5.3 Haloperidol caused significant behavioural differences between treated and untreated twitcher mice. 91

Figure 5.4 Haloperidol improved survival in twitcher mice. 92

Table 5.1 Behavioural metrics for each treatment group for PND 25. 93

Table 5.2 Behavioural metrics for each treatment group for PND 28. 94

Table 5.3 Behavioural metrics for each treatment group for PND 30. 95

Chapter 6 – Discussion.

Figure 6.1 Hypothesised proposed mechanism for antipsychotic reversal of psychosine induced toxicity. 113

List of Abbreviations

5HT	Serotonin receptor
AC	Adenylate cyclase
AKT	Protein kinase B
Ami	Amisulpiride
ALDH	Aldehyde dehydrogenases
AMPK	Activated protein kinase
ANOVA	Analysis of variance
Ari	Aripiprazole
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
Chl	Chlorpromazine
Clo	Clozapine
CNS	Central nervous system
COMT	Catelchol-O-methyltransferase
Cx3cr1	Fractalkine receptor Cx3cr1
D_1	Dopamine 1 receptor
D_2	Dopamine 2 receptor
D_3	Dopamine 3 receptor
D_4	Dopamine 4 receptor
D_5	Dopamine 5 receptor
DISC-1	Disrupted in schizophrenia 1
DIV	Days in vitro
DMSO	Dimethyl sulfoxide
DSM-5	Diagnostics and Statistical Manual of Mental Disorders 5
EAAT	Excitatory amino-acid transporters
ECM	Extracellular matrix
ErbB4	Erb-B2 Receptor Tyrosine Kinase 4
ERK	Extracellular signal regulated kinase
GABA	γ-aminobutyric acid
GFAP	Glial fibrillary acid protein
GPCs	Glial progenitor cells

GWAS	Genome wide association studies
Hal	Haloperidol
Iba-1	Ionized calcium-binding adaptor
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10
KD	Krabbe disease also known as globoid cell leukodystrophy
LDH	Lactate Dehydrogenase
LPC	Lysophosphatidylcholine
LPS	Lipopolysaccharide
MAM	Methylazoxymethanol
MAG	Myelin associated glycoprotein
MBP	Myelin basic protein
MOG	Myelin oligodendrocyte glycoprotein
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NE	Norepinephrine
NRG1	Neuregulin 1
NFH	Neurofilament H
NMDA	N-methyl-D-aspartate
OFM	Open field maze
Ola	Olanzapine
OLIG	Oligodendrocyte lineage transcription factor
OPCs	Oligodendrocyte precursor cells
PBS	Phosphate buffered saline
PCP	Phencyclidine
PFA	Paraformaldehyde
PKA	Protein kinase A
PND	Post-natal day
PNS	Peripheral nervous system
Psy	Psychosine
Que	Quetiapine
Ris	Risperidone
S100β	S100 calcium-binding protein β
SEM	Standard error of mean
Sul	Sulpiride

Scientific Abstract

Glial cells are implicated in the neuropathophysiology of schizophrenia and other neuropsychiatric disorders. Also, the role of altered myelin in the onset and development of schizophrenia and changes in myelin due to antipsychotics remains unclear. There is growing interest in the effects of antipsychotics on glial cells, to explore the ways these drugs may act via non-neuronal mechanisms. To investigate if antipsychotic drugs modulate glial cell dysfunction, human astrocytes were treated with the toxin psychosine, with or without antipsychotics **(chapter 3)**. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and LDH (Lactate Dehydrogenase) assays showed that psychosine decreased cell viability and induced cell toxicity in human astrocytes. This was returned to almost control levels by antipsychotics. Immunocytochemical analysis showed that psychosine impaired astrocyte morphology which was reversed significantly by antipsychotics. Selective D_2 and $5HT_{2A}$ receptor antagonists were found to attenuate psychosine-induced decrease in cell viability, toxic effects, and reductions in astrocyte processes with advantage of dopamine over serotonin antagonism **(Results, chapter 3)**. The effects of the antipsychotics, haloperidol and clozapine, on levels of myelin using mouse organotypic cerebellar slices treated with the demyelinating agent psychosine was also investigated **(Chapter 4).** Psychosine induced a concentration-dependent loss of myelin. Importantly, both haloperidol and clozapine, reduced this psychosine-induced loss. Similarly, these drugs attenuated psychosine-induced loss of astrocyte markers. Psychosine also induced a decrease in nonphosphorylated neurofilament levels, which were restored by both haloperidol and clozapine, indicating neuroprotective and neurorestorative processes **(Results, chapter 4)**. Having shown that antipsychotics prevented psychosine toxicity to human astrocytes *in-vitro* and ameliorated psychosine induced demyelination in mouse organotypic cerebellar slice cultures *ex-vivo*. Another study aimed to examine if these findings would translate into improved survival, mobility, and behavioural metrics in an *in-vivo* model of psychosine toxicity i.e., the twitcher mouse model of Krabbe Disease **(Chapter 5).** The antipsychotic haloperidol increased survival, improved mobility, and positively influenced behaviours in twitcher mice **(Results, chapter 5)**. Overall, this work suggests that antipsychotics may regulate astrocyte cell damage and exert a protective effect on oligodendrocytes. Also, this suggests that antipsychotics or agents with similar pharmacology, namely $D_2/5HT_{2A}$ receptor antagonists, may be a potential novel treatment for KD, a leukodystrophy that leads to the accumulation of the toxin psychosine.

Lay Abstract

Schizophrenia is a mental illness that affects an individual's thoughts, feelings, and behaviour. It can be very disabling and about one in a hundred people worldwide develop this condition. It, as well as other mental health conditions can be treated with antipsychotic medication. The different types of these medications all seem to work by changing signalling chemicals in the brain and by interacting with different brain cells. These brain cells are nerve cells and cells that are important for nerve cell function called glia. How these drugs interact with nerve cells is well understood. However, how they interact with glia is an area of increased study. This work examined the effects of antipsychotic medication on glia after exposure to the toxin psychosine. Psychosine is a toxin that builds up in glia in a disease called Krabbe's disease (KD) and causes damage to these cells. This work showed that antipsychotics prevented psychosine toxicity in some glia cell types. Overall, this work suggests that antipsychotics may regulate damage as well as be protective towards glia and that antipsychotics or similar drugs may be a potential novel treatment for KD.

.

Aims and Hypothesis

The aims of the study are as follows:

- Confirm that psychosine is toxic and causes morphological changes to human astrocytes *in-vitro*. Also, confirm that psychosine causes de-myelination in mouse organotypic cerebellar slice cultures *ex-vivo*.
- Determine if antipsychotics prevent psychosine induced toxicity and morphological changes in human astrocytes.
- Investigate whether antagonism at dopamine-2 (D_2) or serotonin ($5HT_{2A}$) receptors prevent psychosine induced toxicity and morphological changes in human astrocytes.
- Determine if antipsychotics prevent psychosine induced demyelination in organotypic slices.
- Observe whether these drugs effect other glia cell markers in the mixed glial organotypic mouse cerebellar slice culture. Specifically, astrocytes, microglia, and markers for axonal damage.
- Investigate if antipsychotic medication improve survival, twitching scores, immobility scores and subtle behavioural metrics on open field testing in a murine model of psychosine toxicity i.e., twitcher mice.

We hypothesise that in line with previous studies (Clementino, Velasco-Estevez, Buttini, Sonvico, & Dev, 2021; Misslin, Velasco-Estevez, Albert, O’Sullivan, & Dev, 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan, Schubart, Mir, & Dev, 2016) psychosine would reduce cell viability, be toxic to and impair the morphology of human astrocytes *in-vitro*. Also, that psychosine would cause demyelination in mouse organotypic cerebellar slice culture *ex-vivo*. We hypothesise that antipsychotics would prevent such reductions in cell viability, toxicity, and morphological impairments in human astrocytes. Also, we hypothesised that the drugs would prevent demyelination as well as correcting other deleterious effects of psychosine on other glial markers in slices. Finally, we examined if antipsychotic medication would improve survival, twitching scores, immobility scores and subtle behavioural metrics on open field testing in a murine model of psychosine toxicity i.e., twitcher mice.

Value of Research

Schizophrenia is a mental illness that affects an individual's thoughts, feelings and behaviour. It can be very disabling and about one percent of people worldwide have the condition. It, as well as other mental health conditions can be treated with antipsychotic medication. However, although beneficial, these medications are far from optimal as they have significant limitations. They are not as successful in treating cognitive and negative symptoms compared to positive symptoms. They have a range of side effects that may reduce adherence and a proportion of people remain treatment resistant. The different types of these medications all seem to work by changing the levels of dopamine and other signalling chemicals in the brain. However, how they exactly work is not fully understood. These medications may effect non-nerve cell types called glial cells. These cells do many things in the brain including supporting nerve cells, forming myelin and activate the immune system. The aim of this work is to examine the effects of antipsychotic medication on glial cells with the goal of understanding the ways these drugs may act via non-nerve cells. This work examined the effects of antipsychotic medication on these cells after exposure to the toxin psychosine. Psychosine builds up in individuals with Krabbe's disease (KD) and causes damage to these cells. This work showed that antipsychotics prevented psychosine toxicity in astrocytes and oligodendrocytes but did not effect microglia. This work suggests that antipsychotics may regulate astrocyte cell damage, be protective towards oligodendrocytes and that antipsychotics or similar drugs may be a potential novel treatment for KD. Although the exact mechanism for this requires further investigation, we propose that antipsychotics may interact with glial cells and exert a myelin protective effect. This study prompts further research in this area examining the potential for antipsychotics or agents with similar pharmacology in the treatment for KD. It also suggests that antipsychotics function in part by positively effecting glial cells and prompts continued research of glial cell targets for novel antipsychotic medications in the treatment of schizophrenia.

Outputs

Manuscripts

- Sharma, K., & Dev, K. K. (2022). Antipsychotics attenuate psychosine-induced cell toxicity and morphological damage in human astrocytes. Manuscript submitted for publication.
- Sharma, K., & Dev, K. K. (2022). Antipsychotics attenuate psychosine-induced demyelination in organotypic cerebellar slice model. Manuscript submitted for publication.

Presented posters/talks

- Antipsychotics ameliorate glial cell dysfunction in human astrocytes and improve myelination in mouse organotypic cerebellar slices. Federation of European Neuroscience Societies conference November 2020.
- The effects of antipsychotics on glial cell dysfunction College of Psychiatrist of Ireland and Royal College of Psychiatrists joint winter conference November 14th and 15th 2019.
- The effects of antipsychotics on glial cells and neuroinflammation. British Neuroscience Association Festival of Neuroscience 2019. 14-17th April 2019. Poster number PS158.
- Do typical and atypical antipsychotics alter the expression of IL-6 in human astrocytes under pro-inflammatory conditions? European Meeting on Glial Cells in Health and Disease. July 10th -13th 2019. Poster Abstract number 442.

Scholarships and Awards

- Neuroscience Ireland award for Federation of European Neuroscience Societies conference 2020. Effected by COVID-19 held virtually.
- Best presentation at ICAT annual conference November 2020.

Chapter 1 – Introduction

1. Schizophrenia.

1.1. Clinical presentation and diagnosis.

Schizophrenia is a severe and chronic mental illness that manifests with positive (delusions and hallucinations), negative (apathy and avolition), and cognitive symptoms (working memory deficits) as well as difficulties in social and occupational functioning. Its lifelong prevalence is approximately one percent, estimated incidence rate is 1.5 per 10,000 per year and it is associated with significant disability among affected individuals. Furthermore, those suffering from schizophrenia have increased morbidity and mortality, dying on average 10-15 years sooner than those without schizophrenia as well as having up to a ten percent chance of dying by suicide (McCutcheon, Reis Marques, & Howes, 2020). The condition is diagnosed using standard diagnostic criteria set out in the Diagnostic and Statistical Manual of Mental Disorders (Association, 2013) as well as the International Classification of Diseases (Organization, 2019). Although there are some differences between these diagnostic criteria (table 1.1) both require abnormalities in at least two of the following areas: thought content (delusions); perceptual abnormalities (hallucinations); emotional expression (negative symptoms); behaviour and speech (Gaebel & Zielasek, 2015). Contemporary thinking is that subtle features of social and cognitive dysfunction may present in early adolescence before the onset of firm diagnostic symptoms which usually present in early adulthood. This “prodromal” phase may present as early as a decade before the onset of diagnostic psychotic symptoms (Kahn & Keefe, 2013) (figure 1.1).

1.2. Aetiology of schizophrenia.

Environmental and genetic risk factors play a role in the development of schizophrenia. Environmental factors for developing schizophrenia include childhood trauma, urban living, later paternal age, illicit substance use, migration status, prenatal events, perinatal issues, or being born in winter/spring. Genetic risk factors have been investigated by numerous twin, family, and genome wide association studies (GWAS) which have all highlighted the polygenetic risk factors for schizophrenia. The condition is highly heritable with point estimates of heritability being about 81% (Sullivan, Kendler, & Neale, 2003) and GWAS have identified hundreds of potential gene loci associated with the disorder (Dennison, Legge, Pardinas, & Walters, 2020). Genetics alone do not completely explain the aetiology of schizophrenia, for example a monozygotic twin is only 40-50% likely to develop schizophrenia if their

corresponding twin has the disorder, despite sharing 98% genetic structure (Cunningham & Peters, 2014). Therefore, it is thought that schizophrenia is a disorder that develops due to the complex interactions between environment and genetic risk factors over the course of an individual's development which likely leads to abnormalities in neurodevelopment that manifest as the syndrome of schizophrenia, see figure 1.1 adapted from (Stilo & Murray, 2019).

Of particular interest to this work are the abnormalities in neurochemistry that are linked with developing schizophrenia. Increased dopamine in the subcortex, including the basal ganglia lead to the manifestation of positive symptoms e.g., amphetamines that increase dopamine levels have generated psychotic symptoms in those without schizophrenia (Connell, 1957). This "dopamine hypothesis" has led to the mainstay of pharmacological treatment being dopamine receptor (D) antagonism which is traditionally a feature of so called "typical" antipsychotics (Toda & Abi-Dargham, 2007). While "atypical" antipsychotics antagonise dopamine and serotonin receptors e.g., $5HT_{2A}$ implicating serotonin receptor pathways either in the development and/or treatment of schizophrenia (Tsoi, Hunter, & Woodruff, 2008). The issues with classifying antipsychotics as "typical" and "atypical" are addressed in section 1.3. There is growing evidence that cortical neurochemical dysfunction also plays a role in schizophrenia especially in relation to cognitive symptoms with the hypothesis being that schizophrenia is in part, caused by abnormalities in synaptic development and pruning (Kambeitz et al., 2016). Electrophysiology data suggests abnormalities in GABA (γ-aminobutyric acid) interneurons lead to dysfunction of neural oscillations in those with schizophrenia (Uhlhaas & Singer, 2010). Also strong glutamate N-methyl-D-aspartate (NMDA) antagonists like ketamine and PCP (phencyclidine) have led to psychotic symptoms leading to the theory that Glutamate NMDA receptor dysfunction is associated with schizophrenia perhaps leading to cortical glutamate increase and excitotoxicity (Stone, Morrison, & Pilowsky, 2007).

1.3. Clinical course and treatments for schizophrenia.

Longitudinal studies have found that the clinical course of people with schizophrenia is variable with up to half of those diagnosed making good recovery in symptomatology and functioning (Hopper, Harrison, Janca, & Sartorius, 2007). However, a reasonably consistent finding is that social, occupational, and cognitive functioning are difficult to recover early in the course of the illness (Robinson, Woerner, McMeniman, Mendelowitz, & Bilder, 2004). This leads to significant disease burden and disability. Access to early intervention by a multidisciplinary team,

avoidance of stressors, less prominent negative symptoms and adherence to antipsychotic medications have been shown to be positive prognostic factors that improve recovery. Treating schizophrenia involves biological, psychological, and social interventions. Psychological interventions may include cognitive behavioural therapy for psychosis or a range of multimodal interventions that suit the needs of the individual. Social interventions include psychoeducation, occupational support, housing assistance and addiction interventions. Generally, a combination of interventions is required for optimal chance of recovery and has been shown to be more effective that singular treatments alone (Norman, Lecomte, Addington, & Anderson, 2017).

The main biological treatment for schizophrenia is pharmacotherapy with antipsychotic medications. The first antipsychotic, chlorpromazine, was synthesised in the 1950s and since then the field of psychopharmacology has developed as its own scientific discipline with dozens of approved antipsychotics for the treatment of schizophrenia as well as agents to treat other mental illnesses (Ban, 2007). Antipsychotics may be classified into “typical” (first generation) and “atypical” (second generation) compounds, but this method is limited and largely based upon chronology i.e., the time they were marketed. One group has not been shown to be consistently more efficacious than the other, except for the “atypical” clozapine, which is reserved for treatment resistant cases. “Atypicals” cause fewer extrapyramidal side effects but increased metabolic side effects (Rang, Dale, Ritter, Flower, & Henderson, 2011) (Meltzer & Gadaleta, 2021). A more scientifically informed method of classifying antipsychotics is by using neuroscience-based nomenclature (NbN) which incorporates up to date neuroscientific knowledge and classifies antipsychotics as A) D_2 antagonists B) D_2 and $5HT_2$ antagonists C) D_2 and $5HT_{1A}$ partial agonists D) D_2, $5HT_2$ and $NE_{\alpha 2}$ antagonists and E) D_2, $5HT_2$ antagonists and NE reuptake inhibitors (Zohar & Kasper, 2016).

Table 1.2 shows a pharmacological heat map summarising commonly used antipsychotics and their receptor profiles. The issues with differentiating such drugs as “typical” and “atypical” is highlighted here as these medications have a broad receptor profile which often overlap. Primary relief from positive symptoms is thought to derive from D_2 receptor antagonism in the mesolimbic areas of the brain. Motor side effects occur from D_2 antagonism in the nigrostriatal zones, increased prolactin/endocrinological side effects by D_2 antagonism in the tuberoinfundibular pathway and D_2 antagonism in the mesocortical areas leads to ineffective treatment of negative symptoms. “Atypical” antipsychotics attempt to combat some of these side effects and try to treat negative and cognitive symptoms by antagonising serotonin

(e.g.,$5HT_{2A}$) receptors in addition to dopamine receptors. $5HT_{2A}$ antagonism in the nigrostriatal area releases dopamine decreasing motor side effects while in the mesocortical pathway $5HT_{2A}$ antagonism is thought to improve negative and cognitive symptoms. However, negative and cognitive symptoms are generally treatment resistant and there is not much evidence that antipsychotics are very effective in treating these deficits (Stahl, 2008).

Dopamine is a monoamine neurotransmitter and table 1.3 summarises details of the five dopamine receptors (D_1-D_5) as adapted from (J.-M. Beaulieu & Gainetdinov, 2011). Dopamine receptors are G protein coupled receptors which are expressed throughout the peripheral (PNS) and central nervous system (CNS). Their functions are numerous and beyond the scope of this work but briefly they regulate movement, sleep, cognitive function, feeding, executive function and reward mechanisms, more details can be found in reviews including by (J. M. Beaulieu, Espinoza, & Gainetdinov, 2015). They are categorised into D_1 type (D_1 and D_5) and D_2 type (D_2, D_3 and D_4) receptors mainly based on shared genetic, structural, and functional characteristics in each type. Notably, D_1 type receptors stimulate adenylate cyclase (AC) while D_2 type receptors inhibit AC. This leads to upregulation and downregulation of Cyclic adenosine monophosphate (cAMP) respectively. Figure 1.2 illustrates this signalling pathway of D_1 and D_2 type receptors as adapted from (J.-M. Beaulieu & Gainetdinov, 2011). Serotonin, like dopamine is a monoamine neurotransmitter and table 1.3 summarises the details of some subtypes of serotonin G protein coupled receptors. There are seven broad serotonin receptor “super families” ($5HT_1$ – $5HT_7$) each further subdivided e.g., $5HT_{2A}$ depending on genetic, structural, and functional characteristics. They are broadly expressed in the PNS and CNS and their functions are also multiple including regulating mood, perception, reward, appetite, and attention, a detailed review can be found here (Berger, Gray, & Roth, 2009).

1.4. Pre-clinical research models of schizophrenia.

Preclinical models of schizophrenia are important for the development of treatments that may be more efficacious or have fewer side effects and to increase knowledge of the neurobiological basis of the disorder. However, valid cellular and animal models are difficult to develop due to its multifactorial aetiology and the fact that the condition only fully manifests in humans, limiting animal model analysis to observing abnormal behaviours which may not fully reflect complex symptoms (Jones, Watson, & Fone, 2011). Table 1.4 summarises some cellular and animal models of schizophrenia as adapted from (Jones et al., 2011; Koszła, Targowska-Duda,

Kędzierska, & Kaczor, 2020). *In-vitro* models include nerve cell cultures derived from mice (HT22) and rats as well as human cells derived from tumours that have neuronal features (SH-SY5Y). Ethical considerations can constrain use of human tissue for research, but the use of stem cells is more acceptable for example multipotent and pluripotent stem cells can develop into varied cell types for research. The use of 3D cell culture systems over single layer 2D cultures can also be useful for *in-vitro* modelling of schizophrenia (Koszła et al., 2020). *In-vivo* animal models are broadly categorised into pharmacological, genetic, and neurodevelopmental models. Pharmacological models include the PCP and amphetamine models where animals are exposed to these drugs which induce psychosis. Experimental treatments are then administered followed by assessment of memory, attention and behavioural metrics. Genetic models include the disrupted in schizophrenia 1 (DISC-1), neuregulin 1 and ErbB4 knock out models. Finally, neurodevelopmental models aim to mimic foetal factors that may lead to schizophrenia in humans. This is done for example by prenatal administration of methylazoxymethanol (MAM) or lipopolysaccharide (LPS) or by neonatal ventral hippocampal lesion models (Sarnyai et al., 2011).

2. Neuropathology of schizophrenia - a focus on glial cell pathology.

2.1. Macroscopic and microscopic abnormalities.

Many studies have shown gross and microscopic changes in those with schizophrenia. However, the condition does not have a defined “diagnostic” neuropathological set of characteristics and study findings can be variable. Macroscopically: ventricular enlargement; reductions in brain volume; reduced brain weight; cortical thickening; reduced cortical gyrification; cerebellar asymmetry as well as abnormalities in the hippocampus, association neocortex and thalamus have all been found to be associated with the condition. Histologically, once again studies differ but: clustered neurons in cortical white matter tracts; small cell bodies in pyramidal neurons of the hippocampus and neocortex; reduced density of some interneurons and synaptic projections as well as fewer thalamic neurons have been observed. Abnormalities in glial cells have also been implicated and this is further discussed in detail in the next section. Overall, the various implicated neuropathologies have led to the idea that schizophrenia is a disorder of neuronal connectivity with pathology possible all along the neuronal path from dendrite, cell body, axon to synapse and accompanying glial cells (Harrison & Weinberger, 2005).

2.2. Glial cells and their pathology in schizophrenia.

Glial cells are essential in the growth and structure of neural systems and influence information processing as well as organisational and functional plasticity of these systems (de Hoz & Simons, 2014; Hill & Nishiyama, 2014; Schafer et al., 2012; Verkhratsky & Nedergaard, 2018). There is growing data about the influence of glial cells on the neuropathophysiology of schizophrenia with all glial cell types as well as their progenitor cells showing functional and structural abnormalities, for comprehensive reviews see (H. G. Bernstein, J. Steiner, P. C. Guest, H. Dobrowolny, & B. Bogerts, 2015; A. G. Dietz, Goldman, & Nedergaard, 2020; Liu, Du, Chen, & Cheng, 2022). This section outlines the structure and function of macroglia: astrocytes, oligodendrocytes, and their progenitor cells, as well as microglia. It also summarises current evidence from genetic, post-mortem and animal studies for pathology in these cells being associated with schizophrenia.

2.2.1. Astrocyte structure and function.

Astrocytes take their name from the ancient Greek for star, this reflects their structure of numerous cellular processes that reach out and interact with nerve cells, synapses, and other elements in the CNS. They are the most numerous of all the glial cells, are distributed widely in the CNS and they characteristically express glial fibrillary acid protein (GFAP). Several morphologically different astrocyte types have been characterised depending on which layer of the CNS is examined. Interlaminar astrocytes, containing long vertical processes and oblique extensions that spread radially predominate in layers 1 and 2. Protoplasmic astrocytes are the most numerous of all astrocytes in the CNS and can be found mainly in layers 3 and 4. They have many projections that can interact with vasculature and synaptic structures, it is estimated that one protoplasmic astrocyte cell could interface with as many as 2,000,000 synapses in human brains. Astrocytes with varicose projections can be seen in layers 5 and 6, they project fewer, less branched but longer extensions than protoplasmic astrocytes as they interface with less structures. Finally, fibrous astrocytes are mostly located in white matter and are relatively larger cells with fewer, shorter, and less branched projections (Vasile, Dossi, & Rouach, 2017).

The physiological functions of astrocytes are numerous and have been excellently outlined by (Verkhratsky & Nedergaard, 2018). Astrocytes are integral in the maintenance of ion, water, reactive oxygen species and neurotransmitter homeostasis. Figure 1.3 summarises the systemic,

organ, metabolic, cellular, and molecular homeostatic functions of astrocytes demonstrating their importance in multiple areas of the CNS. Another key role of astrocytes involves the control of synaptic networks including the forming and eradication of synapses. Figure 1.4 outlines astrocytes' function in the "tripartite synapse" (showing their importance in controlling neurotransmitter release and uptake) and the "multipartite synapse" (showing their role in forming and eradicating synapses). In the former, excess neurotransmitter released from the presynaptic terminal communicates with neurotransmitter receptors in nearby astrocytes and this causes increased intracellular Ca^{2+} and the release of glutamate, which communicates with the presynaptic nerve to regulate neurotransmission (Araque, Parpura, Sanzgiri, & Haydon, 1999). In the latter an astroglia "cradle" constitutes presynaptic terminals, postsynaptic cells, adjacent astroglia processes with sheath, microglial processes, and an extracellular matrix. The astroglial sheath regulates synaptic genesis, maturation, maintenance, and extinction (Verkhratsky & Nedergaard, 2014). In addition to influencing neurotransmission and synaptic regulation astrocytes can also directly influence transmission by the release of their own neurotransmitters or "gliotransmitters" which unlike those released from nerve cells act more gradually (Verkhratsky & Nedergaard, 2018). Astrocytes provide metabolic support to neuronal tissue by being the brain's only deposit of glycogen as well as by making and moving lactate from the blood to neurons via the astrocyte-neuron lactate shuttle system (Figley, 2011). They communicate with vasculature in the central nervous system likely forming "neuro-glia-vascular" units which manage local blood circulation (Muoio, Persson, & Sendeski, 2014). Astrocytes are key in the separation of peripheral and central blood and lymph circulations, they form essential parts which regulate the blood brain barrier as well as the "glymphatic" system (Abbott, Rönnbäck, & Hansson, 2006; Hablitz et al., 2020). Finally, there is evidence that astrocytes are involved in higher order cognitive operations with evidence that they can modify neuron excitability and synaptic transmission (De Pittà, Brunel, & Volterra, 2016). Moreover, chimeric mice imbedded with human astrocyte precursor cells outdid chimeric mice implanted with mouse astrocyte precursor cells in cognitive assessment suggesting that evolutionary changes to the cells are associated with higher order brain functions such as learning, memory and possibly emotion (Han et al., 2013).

2.2.2. Astrocyte abnormalities and schizophrenia.

Many studies have implicated astrocyte dysfunction in the pathology of neuropsychiatric disorders including schizophrenia (H.-G. Bernstein, J. Steiner, P. C. Guest, H. Dobrowolny, & B.

Bogerts, 2015; A. G. Dietz et al., 2020; Verkhratsky & Nedergaard, 2014) although there remains uncertainty surrounding the specific pathological mechanisms. Evidence from genomic and GWAS have confirmed that astrocyte related genes are implicated in schizophrenia. Pertinent findings from large scale genetic studies are excellently and succinctly summarised in a review by (Notter, 2021). The strongest evidence comes from a functional gene set analysis using GWAS data carried out by (Goudriaan et al., 2014) which showed that astrocyte related gene sets for synaptic signalling, glial development and epigenetic factors are connected to a higher risk of schizophrenia. These findings have been confirmed by other genetic studies including in transcriptome wide association studies (RNA sequencing studies) (Gandal et al., 2018) which show that expression of astrocyte associated genes were increased in schizophrenia for example in the cingulate cortex and appear to occur irrespective of medication dosage (González-Peñas, Costas, Villamayor, & Xu, 2019; Ramaker et al., 2017; Toker, Mancarci, Tripathy, & Pavlidis, 2018). There is increasing data that suggest that increased expression of astrocyte related genes are key to the pathology of schizophrenia which expands the need for research beyond neuronal only mechanism for the disease.

Post-mortem studies of patients with schizophrenia have demonstrated alterations in astrocyte density, structure, and release of characteristic astrocyte markers (Liu et al., 2022; X. Zhang, Alnafisah, Hamoud, Shukla, McCullumsmith, et al., 2021). Initially, it was thought that schizophrenia was associated with astrogliosis however this finding was not confirmed in repeat experiments and in fact a decreased density of astrocytes in certain CNS areas like the motor cortex, nucleus accumbens and substantia nigra are now thought to be associated with the disease (Tarasov et al., 2019; M. Williams et al., 2013; M. R. Williams et al., 2013). There is variation in studies looking at the release of GFAP in autopsy brain samples of patients with schizophrenia depending on the region examined. In a review by (X. Zhang, Alnafisah, Hamoud, Shukla, McCullumsmith, et al., 2021) only three studies showed increased GFAP levels (two in the dorsolateral prefrontal cortex and one in the olfactory tubercle of the mesolimbic system). The other 14 studies reviewed showed decreased or no significant changes in GFAP expression in multiple CNS areas. This heterogeneity between brain regions appears to be consistent when other astrocyte markers such as S100 calcium-binding protein β (S100β), excitatory amino-acid transporters (EAAT) and aldehyde dehydrogenases (ALDH) are investigated in post-mortem samples. It is thought that confounding influences of smoking, medication, disease state and other person related issues may explain the inconsistencies in post-mortem analyses. However

overall, there is ample evidence from these types of studies to implicate astrocytes as being involved in the pathology of schizophrenia.

Animal experiments also provide evidence for abnormalities in astrocytes being connected to schizophrenia with impairments in astrocyte structure and number leading to schizophrenia like phenotypes in animals. Rats injected with the astrocyte specific toxin L-α-aminoadipate or mice bred with disorders that reduce astrocyte numbers e.g., by causing tetanus toxin release in astrocytes, show cognitive deficits associated with schizophrenia (H. S. Lee et al., 2014; Lima et al., 2014). Both overexpression and elimination of S100β in mice has shown to produce electrophysiological and behavioural changes hypothesised to be linked to schizophrenia pathology. Including deficits in short term memory in the former and increased long term potentiation in the hippocampus as well as better fear linked memory in the latter (Nishiyama, Knöpfel, Endo, & Itohara, 2002; Roder, Roder, & Gerlai, 1996). The DISC1 gene was one of the first outlined to be associated with an increased risk of schizophrenia and it is also linked to overall CNS development. Mice bred with altered DISC1 gene in astrocytes have been shown to have schizophrenia like phenotypes (Terrillion et al., 2017). Finally, in one study induced pluripotent stem cells from patients with schizophrenia and those from age matched controls were used to make glial precursor cells which were subsequently grafted onto mice. Schizophrenia derived chimeric mice demonstrated slowed astrocytic differentiation and abnormal astrocyte morphology compared to those from controls, they also had behavioural and gene expression abnormalities associated with schizophrenia (Windrem et al., 2017). Taken together there is evidence to suggest that astrocytes play a role in the pathology of schizophrenia.

2.2.3. Oligodendrocyte structure and function.

Oligodendrocytes are glial cells that form myelin in the central nervous system, this process is dynamic, and each cell can myelinate several axons at multiple layers. Their development takes place in four phases: firstly; oligodendrocyte precursor cells (OPCs) are formed and propagate; secondly, structural change occurs that allows the cells to form multiple extensions; thirdly, these extensions interact with axons forming myelin and finally; these cells provide support for the now fully formed myelinated axon segment (Michalski & Kothary, 2015). OPC's key function is to produce new oligodendrocytes and they develop in "waves", initially in the forebrain around six weeks of gestation, there is then a second wave in the dorsal ventricular zone and

then a third in the cortex during the postnatal period. In adulthood oligodendrocytes make up about 10% of total glial cells but they are the most propagating of all cells in the CNS and, they are uniformly organised in white and grey matter. They readily move along vasculature elements in the CNS so that they can reach white matter tracts for axonal myelination. Their key function is to form myelin which facilitates speedy transmission of action potentials as well as offering metabolic supports to the segment of axon it surrounds. Oligodendrocytes characteristically express myelin related proteins such as myelin basic protein (MBP), myelin associated glycoprotein (MAG) and myelin-oligodendrocyte glycoprotein (MOG) (Kuhn, Gritti, Crooks, & Dombrowski, 2019). Oligodendrocytes may also serve functions beyond myelination, there is evidence that they may modulate an immune response with OPCs able to move to sites of insult (Hughes, Kang, Fukaya, & Bergles, 2013). Also, they may play a role in sensory perception as outlined in an *in-vivo* study which suggests that oligodendrocytes may take part in cortical information processing (Moore et al., 2020).

2.2.4. Oligodendrocyte dysfunction in schizophrenia.

Genetic studies looking at oligodendrocyte associated genes have shown that many are differently expressed in patients with schizophrenia (H. G. Bernstein et al., 2015; Andrea G Dietz, Goldman, & Nedergaard, 2019). The most compelling evidence comes from a GWAS by (Goudriaan et al., 2014) which implicated 29 oligodendrocyte associated gene sets with schizophrenia. Interestingly, gene sets relating to oligodendrocyte lipid metabolism, oxidation-reduction, and gene transcription as well as those found to be associated directly with myelin production were shown to be associated with schizophrenia. Studies have shown decrease in MOG, oligodendrocyte lineage (OLIG) and proteolipid protein (PLP) genes in the prefrontal cortex of patients with schizophrenia. Also, suppression of MOG in the hippocampus and MBP in the dorsolateral prefrontal cortex has been shown to be associated with schizophrenia (Martins-de-Souza et al., 2009; Marui et al., 2018). A tabular summary of studies implicating oligodendrocyte gene related abnormalities being associated with schizophrenia can be found in (H. G. Bernstein et al., 2015; Liu et al., 2022). Taken together, although exact abnormalities may depend on study methodology and area of the CNS examined the evidence from such genetic studies to support oligodendrocyte dysfunction in schizophrenia appears robust.

Post-mortem studies of patients with schizophrenia have demonstrated alternations in oligodendrocyte density, structure, and release of characteristic markers in different brain areas. In a recent systematic review by (Liu et al., 2022), 20 out of 30 studies showed decreases

in oligodendrocyte associated markers in patients with schizophrenia and most of the studies reviewed noted alterations in oligodendrocyte density. Decreases were noted in the anterior thalamic nucleus, thalamus, hippocampus, white matter areas and areas of the prefrontal cortex as well as others. Other areas such as the anterior cingulum bundle, substantia nigra and others did not show reductions in oligodendrocyte concentrations. Morphological changes in oligodendrocytes are also observed in electron microscopy studies with significant changes in ultra-structure noted in oligodendrocytes in the prefrontal cortex white matter compared to control (Uranova, Vikhreva, Rakhmanova, & Orlovskaya, 2018; Vikhreva, Rakhmanova, Orlovskaya, & Uranova, 2016). These studies as well as others further support the role of oligodendrocyte dysfunction in schizophrenia.

Animal models also provide evidence for an association between oligodendrocyte dysfunction and the pathology of schizophrenia. A tabular summary of animal models of schizophrenia based on oligodendrocyte and myelin dysfunction can be found in (Takahashi, Sakurai, Davis, & Buxbaum, 2011) and a general summary of animal models of schizophrenia is discussed in section 1.4. Importantly, evidence has accumulated from animal models where specific toxins are used to induce demyelination and these have been shown to provide insights into the pathophysiology of schizophrenia and highlight mechanisms by which treatments may function (Winship et al., 2019). Mice given the toxin cuprizone to induce demyelination had cognitive shortfalls which can be characteristic in schizophrenia. The toxin lysophosphatidylcholine (LPC) has also been used to induce demyelination in rats who have subsequently demonstrated schizophrenia like behaviours. Schizophrenia like characteristics were seen in NRG1 and ERBB4 knock out mice (a myelin linked animal model for schizophrenia) including prepulse inhibition, increased activity as well as showing movement and working memory deficits. Taken together animal studies provide additional support for the link between oligodendrocyte pathology and schizophrenia.

Finally, some of the most compelling evidence for oligodendrocyte dysfunction and schizophrenia comes from imaging studies that highlight white matter pathology also being associated with the condition. Diffusion tensor imaging can quantify changes in white matter, such changes have been noted to develop prior to symptoms and develop along with the clinical course of the illness (Podwalski et al., 2021; Samartzis, Dima, Fusar-Poli, & Kyriakopoulos, 2013). Subcortical white matter deficits are seen in patients with schizophrenia as are white matter deficits in the occipital areas, parietal areas, and the prefrontal cortex. It is thought that these

deficits particularly in the frontoparietal connections may lead to the working memory issues experienced in people with schizophrenia (Katherine H Karlsgodt et al., 2008; Shergill et al., 2007). Finally, an interesting observation is that symptoms of schizophrenia manifest in other diseases with white matter pathologies such as metachromatic leukodystrophy, Niemann Pick's disease and multiple sclerosis (Walterfang, Wood, Velakoulis, Copolov, & Pantelis, 2005). This further adds to the multitude of evidence for oligodendrocyte abnormalities as well as issues with myelin being implicated in the pathology of schizophrenia.

2.2.5. Microglia structure and function.

Microglia are the immune cell (macrophages) of the CNS, they begin development early in embryo formation and before other "macroglial" cells or their precursors. They form from erythroid myeloid precursors which go on to develop into pre-macrophages that migrate to the brain, and it is thought that adult microglia in the CNS self-renew from these embryological sources. However, it is also possible for peripheral blood monocytes to enter that CNS in specific situations, and these then appear and act like microglia. The structure of microglia is varied and can change readily for example amoeboid microglia can move and phagocytose, while ramified microglia remain relatively still but have processes that continuously examine the environment (Prinz, Jung, & Priller, 2019). They characteristically express certain markers including fractalkine receptor (Cx3cr1) and ionized calcium-binding adaptor (Iba-1). Microglia serve many functions in the CNS as reviewed by (Nayak, Roth, & McGavern, 2014), two broad themes are homeostatic and immune related. In the former microglia play key roles in regulating neuronal survival and death as well as generating or pruning synapses. In the latter microglia protect the CNS from various threats by implementing an immune response to viral, bacterial, or parasitic pathogens. Dysfunction in microglia is known to be associated with neuropsychiatric disorders like obsessive compulsive disorder and Rett syndrome, as well as neurodegenerative disorders like Alzheimer's and Parkinson's disease (Nayak et al., 2014). There is also evidence of microglia dysfunction being linked to the pathology of schizophrenia.

2.2.6. Microglia pathology in schizophrenia.

The potential role of microglia in schizophrenia has been outlined in many studies as reviewed by (H. G. Bernstein et al., 2015; A. G. Dietz et al., 2020; C. Wang, Aleksic, & Ozaki, 2015). The GWAS carried out by (Goudriaan et al., 2014) showed that microglia related gene sets were not linked to a greater risk of schizophrenia. But genes that are associated with human leukocyte

antigen (HLA) complex, which has been shown to interact with activated microglia, specifically the HLA-DRβ1 gene has been shown to be linked to the development of schizophrenia (Wright, Nimgaonkar, Donaldson, & Murray, 2001). So too were genes related to the major histocompatibility complex in a GWAS by (Stefansson et al., 2009). Decreased microRNA levels of cluster of differentiation - an indicator for microglia (CD68) have been observed in several studies of patients with schizophrenia including in the dorsolateral prefrontal cortex and the corpus callosum. These data have been used to suggest that there is a significant immune and inflammatory component to the pathology of schizophrenia and studies have shown a number of pro-inflammatory cytokines in biological samples of patients with the condition (Howes & McCutcheon, 2017; K. E. O'Connell, Thakore, & Dev, 2014).

In a systematic review of post-mortem studies half of the publications analysed showed that microglia specific markers were increased in the brains of patients with schizophrenia and a meta-analysis observed that there was a rise in the amount of microglia in the temporal cortex of those with schizophrenia (Trépanier, Hopperton, Mizrahi, Mechawar, & Bazinet, 2016; Van Kesteren et al., 2017). IBA1 levels were reported to be increased in multiple brain regions of patients with schizophrenia including the prefrontal cortex, hippocampus and amygdala (Howes & McCutcheon, 2017), while other studies have shown no difference in IBA1 levels in areas such as the superior temporal gyrus, medial frontal gyrus and anterior cingulate cortex (Sneeboer et al., 2020; Snijders et al., 2021). Findings also suggest that there may be more microglia density in those who are suicidal compared to those who are not. Also some studies have shown more microglia activation on electron microscopy in schizophrenic patients (Petrasch-Parwez et al., 2020; Uranova, Vikhreva, Rakhmanova, & Orlovskaya, 2020). Although data from post-mortem studies can vary depending on methodology and CNS area studied there is evidence for microglial involvement in the pathology of schizophrenia.

In-utero, perinatal and postnatal stresses like infections or obstetric complications have been associated with the development of schizophrenia (Howes & McCutcheon, 2017) and animal models have tried to experimentally examine the potential mechanisms of this association. Polyinosinic–polycytidilic acid (poly(I:C)) injections administered to pregnant mice are used to experimentally simulate maternal immune activation. In studies where poly(I:C) has been administered to pregnant mice more microglia density and activation has been observed in the offspring postnatally but not in-utero compared to controls (Juckel et al., 2011; Manitz et al., 2013; Smolders et al., 2015). These changes appear to develop as the offspring age and this is

thought to occur due to the interaction between microglia and dopamine receptors which lead to altered dopamine concentrations and may explain some of the pathology in developing schizophrenia (Vidal & Pacheco, 2020). Cumulatively, these suggest evidence that microglia which mediate immune systems and inflammation in the CNS play a role in the pathology of schizophrenia.

2.2.7.Glial progenitor cells and the gliocentric model of schizophrenia.

Glial progenitor cells (GPCs) are macroglia precursor cells that can form oligodendrocytes and astrocytes depending on certain conditions, for example exposure to oligodendrocyte lineage transcription factor (OLIG2) causes development of oligodendrocytes but reduces astrocyte development. Neuron activity, presence of specific neurotransmitters, oxidative state as well as the presence of certain growth factors can all also environmentally influence their differentiation (Gibson et al., 2014; Hill & Nishiyama, 2014; Wegener et al., 2015). GPCs are promoted to differentiate by cyclins D and E, but are hindered by kip cell cycle inhibitors (Lim & Kaldis, 2013) and like mature macroglial cells abnormalities in GPCs have been implicated in the pathology of schizophrenia. Genetic studies have shown increased levels of genes that code for cyclins and decreases in those that code for cell cycle inhibitors in patients with schizophrenia suggesting sustained proliferation of GPCs in those with the condition (Katsel et al., 2008). Also, evidence suggests that OLIG2 differences individually as well as by interacting with genes linked to oligodendrocytes such as 2',3'-Cyclic-Nucleotide 3'-Phosphodiesterase (CNP), Erb-B2 Receptor Tyrosine Kinase 4 (ERBB4) but not Neuregulin 1 (NRG1) may increase the chance of developing schizophrenia (Georgieva et al., 2006). Also, deficits in white matter were observed by diffusion tensor imaging between those with a single nucleotide polymorphism in OLIG2 compared to controls (Prata et al., 2013). These data together support the idea of GPCs as well as mature macroglia being linked to the pathology of schizophrenia.

Given the abundance of evidence for glial cell abnormalities being associated with schizophrenia (A. G. Dietz et al., 2020) have proposed a gliocentric model of schizophrenia where microglial activation during development leads to dysfunction in GPCs. This subsequently effects the development of oligodendrocytes and astrocytes, the former causing deficits with myelination and the latter issues with homeostasis and synaptic function. This model states that early or prodromal schizophrenia may be influenced by glial cell dysfunction (impaired differentiation)

as opposed to other disorders that result from abnormalities in maturely differentiated glial cells see figure 1.5 as adapted from (A. G. Dietz et al., 2020).

3. Effects of antipsychotics on glial cells.

Given the ample evidence for glial cell dysfunction being associated with schizophrenia there is also data regarding the effects of antipsychotic medications on glial cells. Such studies attempt to mechanistically understand the effects of these medications as well as potentially identify glial targets that may improve the clinical course of schizophrenia or improve the side effects of current treatments.

3.1. Astrocytes.

D-serine is an amino acid gliotransmitter secreted by astrocytes, that is involved in interactions between neurons and astrocytes by being a co-agonist at N-methyl-D-aspartate (NMDA) receptors. Clozapine but not haloperidol was shown to promote d-serine release in rat cortical astrocytes suggesting an interaction between “atypical” agents and astrocyte-neuron signalling specifically via NMDA receptor stimulation (Tanahashi, Yamamura, Nakagawa, Motomura, & Okada, 2012). D-serine as an augmentation to antipsychotic treatments was shown to be beneficial in a recent systematic review and meta-analysis by (Goh, Wu, Chen, & Lu, 2021) as well as at high doses in a clinical trial by (Kantrowitz et al., 2010). The suggestion is that d-serine works along with antipsychotic treatment e.g., clozapine to correct low levels of glutamate seen in schizophrenia, by influencing factors such as glutamate uptake in astrocytes via decreasing glutamate transporter (GLT-1) expression. The ability of clozapine to reduce GLT-1 expression has been confirmed *in-vitro* using rat cortical astrocytes (Vallejo-Illarramendi, Torres-Ramos, Melone, Conti, & Matute, 2005) and *in-vivo* using adult wistar rats (da Rocha et al., 2021).

In concordance with such evidence other antipsychotics like zotepine (Kouji Fukuyama & Motohiro Okada, 2021) as well as clozapine and quetiapine (K. Fukuyama & M. Okada, 2021) have been shown to increase release of another astrocyte linked transmitter L-glutamate by activating astrocytic hemichannels. Also, a collection of agents including clozapine, olanzapine, aripiprazole, risperidone, haloperidol, and chlorpromazine have also been shown to stimulate the release of macrophage migration inhibitory factor (MIF), a cytokine expressed in astrocytes, neurons, and immune cells in primary cultured mouse astrocytes (Okazaki et al., 2021). This

suggests that mechanistically antipsychotics may function in part by influencing astrocyte expression on MIF. A study also suggests that haloperidol (D2 antagonist) and risperidone (D2,5HT2 antagonist) may act differentially, the former promoting the release of inflammatory cytokines while the latter suppressing them compared to controls in primary astrocyte culture (Bobermin, da Silva, Souza, & Quincozes-Santos, 2018). Further evidence for immune and anti-inflammatory mechanisms of antipsychotics is discussed in section 4.3 when outlining their effects on microglia. Animal studies using macaque monkeys treated long-term with haloperidol and olanzapine demonstrated reduced brain volume (Dorph-Petersen et al., 2005). Interestingly, it was observed that this treatment led to reduced astrocyte amounts in the parietal cortex as well as reduced volume in that area (Konopaske et al., 2008).

There are few human CNS studies that examine effects of antipsychotics on astrocytes. Human imaging studies have shown grey and white matter reductions in those prescribed antipsychotic treatment. In a longitudinal imaging study of patients presenting with first-episode schizophrenia and treated “as usual” with antipsychotics, longer use of these medications was associated with deficits in grey and white matter (Ho, Andreasen, Ziebell, Pierson, & Magnotta, 2011). Another study observed that haloperidol but not olanzapine was associated with reduced grey matter in those with first episode psychosis (Lieberman et al., 2005). It has been suggested that such deficits may be due to the effects of antipsychotics on astrocytes as well as other glial cells (X. Zhang, Alnafisah, Hamoud, Shukla, Wen, et al., 2021) but further research especially using human samples is required to confirm this. Collectively, these data suggest that not only do astrocytes play a role in the development of schizophrenia but also that antipsychotic treatment effects may be explained mechanistically via astrocyte interaction. It also highlights the importance of further research in this area for potential drug development.

3.2. Oligodendrocytes and oligodendrocyte progenitor cells.

Many *in-vitro* studies have shown the influence of antipsychotics on oligodendrocytes and have proposed that fixing damaged myelin by revitalising these cells may be one of the mechanisms by which the antipsychotics exert a neuroprotective effect (A. T. Chen & Nasrallah, 2019). Quetiapine and clozapine but not olanzapine, were shown to reverse the negative effects of cuprizone on oligodendrocyte progenitor cells development to mature oligodendrocytes *in-vitro* (Xu, Yang, & Li, 2014). A comprehensive proteomic analysis of developing oligodendrocytes (human MO3.13 cells) treated with chlorpromazine, haloperidol, quetiapine and risperidone

implicated numerous cellular biological systems that the drugs may influence including but not limited to, cell communication, protein metabolism and cell growth or maintenance (Brandão-Teles, De Almeida, Cassoli, & Martins-de-Souza, 2019). These pathway links were also confirmed in mature oligodendrocytes (human MO3.13 cells) in a proteomic analysis by (Seabra et al., 2020) using haloperidol and clozapine. Haloperidol and clozapine prevented apoptotic cell death in oligodendrocytes that were cultured under glucose deprived conditions, highlighting their potential protective effects (Johann Steiner et al., 2011). Studies have shown that haloperidol (Niu et al., 2010) and olanzapine (Kimoto et al., 2011) are able to induce propagation of oligodendrocytes progenitor cells while both did not aid in the development of these cells into mature oligodendrocytes in primary culture of rat cells.

In-vivo studies have shown the potential benefits of antipsychotics on oligodendrocytes and myelin. Quetiapine halted cuprizone induced demyelination in mice and positively influenced their behaviour on Y-maze tasks, by increasing propagation of precursor cells (Xiao et al., 2008). This finding has been replicated and it has been suggested that quetiapine exerts such benefits via the notch signalling pathway (involved in oligodendrocyte development and myelin formation) highlighting this pathway as a potential target for novel drugs for schizophrenia (H.-n. Wang et al., 2016). Another method by which quetiapine might improve oligodendrocyte function is by influencing epigenetic impairments associated with schizophrenia by influencing histone methylation, as seen in a study involving socially isolated mice (X. Chen et al., 2020). Further, quetiapine was shown to directly improve the developmental morphology of oligodendrocytes towards myelination, by increasing the activity of OLIG1 (oligodendrocyte transcription factor 1 – promotes maturation) and decreasing the activity of GPR17 (OLIG1 regulated G protein coupled receptor 17 -inhibits maturation) in mouse oligodendrocytes (X. Wang et al., 2021). Quetiapine also prevented demyelination in an immune mouse model for multiple sclerosis via downregulation of T-cell propagation implicating immune interactions of antipsychotics may also lead oligodendrocyte improvements (Mei et al., 2012). Section 4.3 further discusses immune effects of antipsychotics outlining their effects on microglia. Olanzapine increased the amount of precursor cells in the hypothalamus of mice and clozapine aided the recovery of mice exposed to the demyelinating toxin cuprizone (Templeton, Kivell, McCaughey-Chapman, Connor, & La Flamme, 2019; Yamauchi et al., 2010). Haloperidol was noted to increase the amount of oligodendrocyte precursor cells in the corpus callosum, hippocampus and cerebral cortex of mouse brains (H. Wang et al., 2010).

Olanzapine was associated with more retained brain volume in those with an initial episode of psychosis compared to the haloperidol (Lieberman et al., 2005). Those prescribed clozapine, were shown to have more peripheral BDNF (brain derived neurotrophic factor – involved in many CNS pathways) compared to those prescribed "typical" agents (Pedrini et al., 2011). While antipsychotic treatment especially with "typical" agents or a combination of "typical" and "atypical" drugs appears to promote grey matter loss in imaging studies (Vita, De Peri, Deste, Barlati, & Sacchetti, 2015), the medications seem to correct white matter changes including those that occur during initial presentations of schizophrenia in drug naïve individuals (Sagarwala & Nasrallah, 2021). This may indicate a preferential effect of these medications on oligodendrocytes and myelin relative to other glial cells. Interestingly, clozapine and haloperidol were shown to prevent autophagy and mitophagy in multiple sclerosis in a study by (Patergnani et al., 2021) using human cerebrospinal fluid samples as well as animal *in-vitro*, *ex-vivo* and *in-vivo* experiments. This highlights the possible benefits of such medication in this condition as well as illuminating how they may function to improve other leukodystrophies as well as schizophrenia. Cumulatively, this evidence suggests that antipsychotics act, in part, by influencing oligodendrocytes and their precursor cells.

3.3.Microglia.

It has been proposed that antipsychotics may act in part, by having an anti-inflammatory effect with agents decreasing the expression of inflammatory cytokines and influencing microglia response *in-vitro* (A Kato et al., 2011). Cellular studies for example in cultured rat microglia, that were immune activated by exposure to lipopolysaccharide (LPS), flupentixol (Kowalski, Labuzek, & Herman, 2003) and chlorpromazine (Labuzek, Kowalski, Gabryel, & Herman, 2005) reduced tumour necrosis factor α (TNF-α), nitric oxide (NO) and interleukin (IL-1) and IL-2 release respectively. Similar effects in reducing expression of NO and inflammatory cytokines in immune induced microglia was seen with drugs like olanzapine (Hou et al., 2006), risperidone (Takahiro Kato, Monji, Hashioka, & Kanba, 2007), quetiapine (Bian et al., 2008) and aripiprazole (T. Kato et al., 2008). It is hypothesised that this may occur by the influence of antipsychotics on calcium signalling as well as mitogen-activated protein kinase (MAPK), protein kinase C (PKC) and NF-κB pathways within microglia (A Kato et al., 2011; Mizoguchi, Kato, Horikawa, & Monji, 2014). Animal studies have shown that rats treated with haloperidol and olanzapine show increased IBA1 expression in certain areas like the striatum, somatosensory cortex, and hippocampus (Cotel et al., 2015) while another study showed that clozapine reversed microglial activation in

the striatum and hippocampus (Ribeiro et al., 2013). Finally, the tetracycline antibiotic minocycline is known to be an inhibitor of microglia and exert neuroprotective effects (Domercq & Matute, 2004). Its use has been proposed as a novel antipsychotic as well as an augmentation to antipsychotic medications. So too has the use of anti-inflammatory agents like cyclooxygenase-2 (COX-2) inhibitors and these agents have been shown to be effective as an augmentation strategy for schizophrenia in a recent systematic review and meta-analysis (Jeppesen et al., 2020). The COX-2 inhibitor celecoxib was shown to be effective in a clinical trial of inpatients in an active phase of the illness (Akhondzadeh et al., 2007) while not effective in those symptomatic but in an outpatient setting (Rapaport et al., 2005) suggesting the clinical state and trait of individuals may influence response. Taken together the above evidence highlights the effects that antipsychotic medications may have via microglia and suggests further study in this area for novel mechanistic insights or drug development.

4. Psychosine a toxic sphingolipid that accumulates abnormally in the leukodystrophy Krabbe disease.

Krabbe disease (KD) also known as globoid cell leukodystrophy is a rare autosomal recessive disease caused by mutations in the *GALC* gene which codes for the enzyme galactosylceramidase. It has an incidence of 1 per 100,000 births and therefore is designated an orphan disease for drug development research. The genetic abnormalities associated with the disorder lead to the accumulation of lipids, including psychosine, which is toxic and causes demyelination in the central and peripheral nervous system (Béchet, O'Sullivan, Yssel, Fagan, & Dev, 2020). This pathology leads to those afflicted with infantile forms of the disease manifesting with seizures (including febrile seizures), muscle frailty, irritability, vomiting, feeding difficulties with eventual mortality usually prior to two years of age. A minority have a slower disease progression or later-onset of the disease and can present with delayed developmental milestones. There is no cure for the disorder, clinical interventions focus on screening for the disease in those deemed to be at risk, providing general symptomatic support and treatment with haematopoietic stem cell transplantation (HSCT). HSCT has shown to increase longevity in those with the disease but those with KD still have serious mobility and speech deficits as well as a much-reduced life expectancy (D. A. Wenger, Luzi, & Rafi, 2021). Therefore, there is a need for continued research for novel therapies for KD and murine models provide a useful tool for investigating potential therapies.

Twitcher mice (C57BL/6J) are a naturally occurring mouse mutant that contain a mutation in the *GALC* gene, which leads to the accumulation of psychosine in homozygous mice and associated toxicity centrally and peripherally. Homozygous mice manifest symptoms from post-natal day (PND) 20 and rapidly progress usually not surviving past PND 40 (Duchen, Eicher, Jacobs, Scaravilli, & Teixeira, 1980). The genetic pathology in twitcher mice approximates to human KD with psychosine induced toxicity especially towards oligodendrocytes leading to progressive demyelination. They are an established and often used murine model for the study of KD pathology and potential treatments (D. A. Wenger, Rafi, & Luzi, 2016). Psychosine (galactosylsphingosine), is a toxic lipid that accumulates abnormally in those with KD. It has been shown to be toxic to oligodendrocytes and cause demyelination by various proposed mechanisms. These include activating apoptotic processes, inhibiting protein kinase c activity, forming lysophosphatidylcholine resulting in cytotoxicity and causing phosphorylation leading to cell death (Cantuti-Castelvetri et al., 2012; Davenport, Williamson, & Taylor, 2011; S. Giri, Khan, Rattan, Singh, & Singh, 2006; Haq, Giri, Singh, & Singh, 2003). Previously, our research group has shown that psychosine causes demyelination in mouse organotypic cerebellar slice cultures and that psychosine causes toxicity in mouse and human astrocytes (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K. Dev, 2015; C. O'Sullivan et al., 2016).

Interestingly, schizophrenia type symptoms have been observed in those with different leukodystrophies including metachromatic leukodystrophy and Nieman-pick disease type C but not in KD directly (Walterfang et al., 2005). This may be because the majority of those with KD have a rapid progressive illness with mortality usually prior to two years of age. Despite this the study of leukodystrophies is useful in understanding the potential neuropathological aetiology of schizophrenia. This work employed an *in-vitro* psychosine toxicity model to exam whether antipsychotics prevented astrocyte toxicity (chapter 3). An *ex-vivo* organotypic cerebellar slice model was used to examine if haloperidol and clozapine prevented psychosine glia cell toxicity (chapter 4). Finally, the twitcher mouse model was used to examine if the *in-vitro* and *ex-vivo* findings translated into increased survival in an *in-vivo* model of psychosine toxicity (chapter 5).

ICD-11 Schizophrenia	DSM-5 Schizophrenia
At least 2 of (1 must be A-D) for 1 month: A. Delusions B. Hallucinations C. Disorganised thinking D. Passivity or control phenomena E. Negative symptoms F. Disorganised behaviour G. Psychomotor abnormalities	A. 2 or more (1 must be 1, 2 or 3) present for 1 month: 1. Delusions 2. Hallucinations 3. Disorganised speech 4. Disorganised behaviour 5. Negative symptoms B. Social/occupational dysfunction C. Some residual symptoms persist for 6 months

Table 1.1. DSM-5 and ICD-11 diagnostic criteria for schizophrenia.
Abridged and adapted from (Gaebel & Zielasek, 2015). Although there are some differences between diagnostic criteria both require abnormalities in at least two of the following areas: thought content (delusions); perceptual abnormalities (hallucinations); emotional expression (negative symptoms); behaviour and speech.

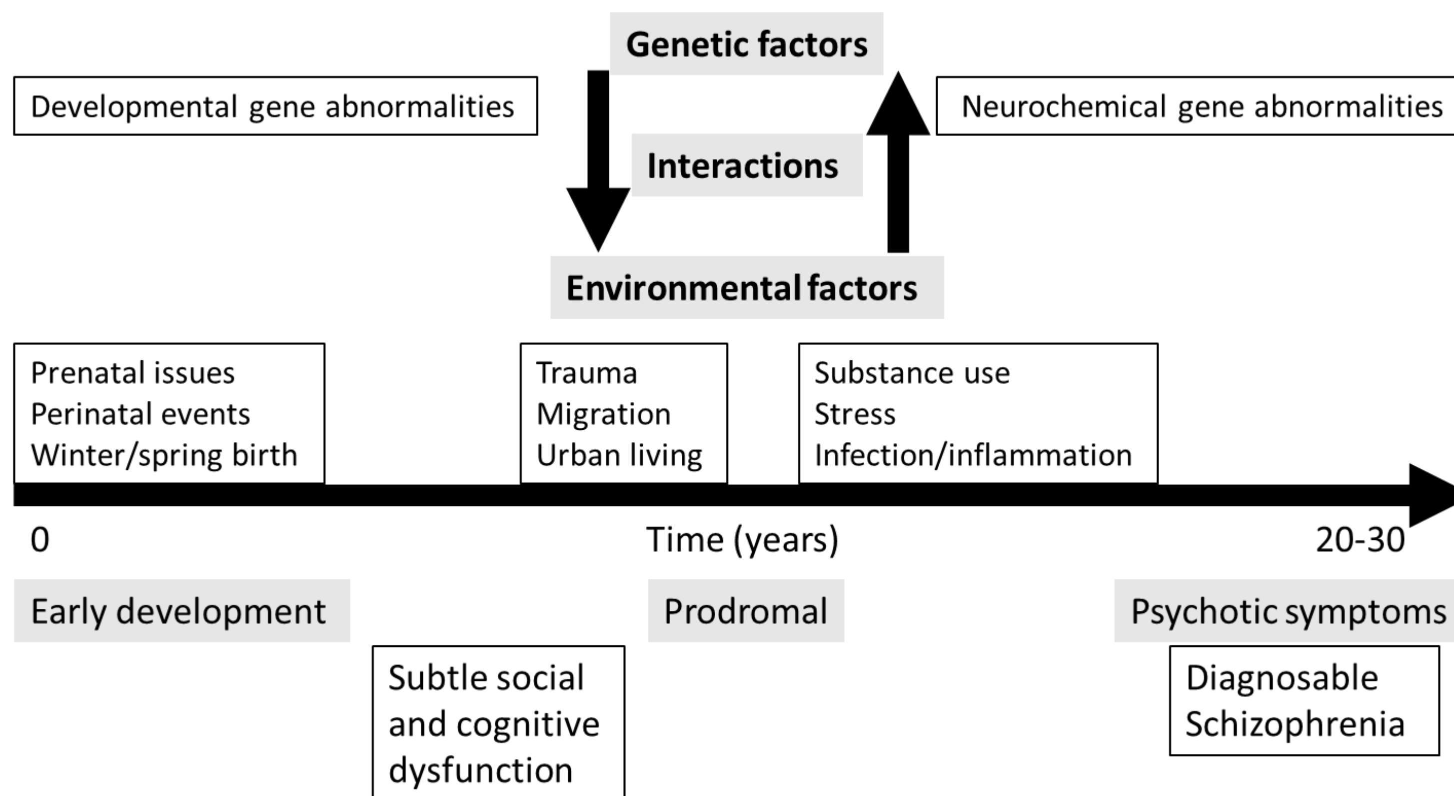


Figure 1.1 Clinical and aetiological overview of schizophrenia syndrome.
Subtle features of social and cognitive dysfunction may present in early adolescence possibly a decade before the onset of diagnostic psychotic symptoms which usually present in early adulthood. Schizophrenia is a disorder that develops due to the complex interactions between environment and genetic risk factors over the course of an individual's development which likely leads to abnormalities in neurodevelopment that manifest as the syndrome of schizophrenia. Edited and adapted from (Stilo & Murray, 2019).

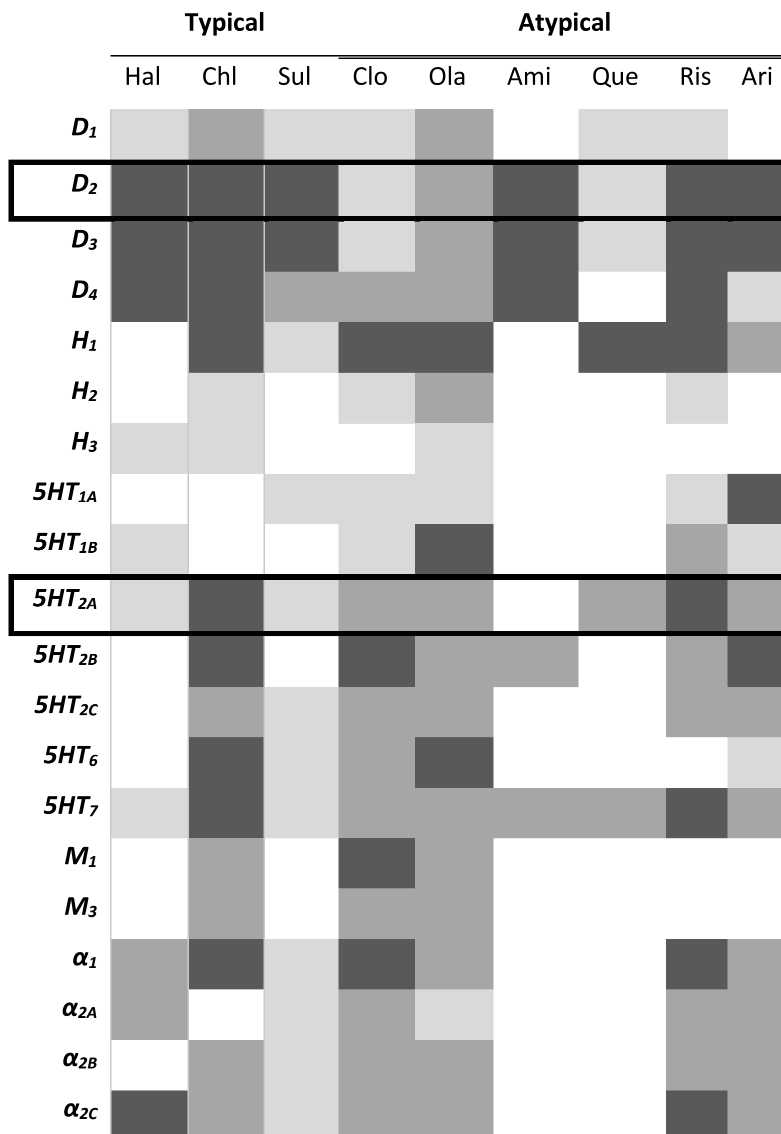


Table 1.2 Pharmacology heat map of receptor binding profiles of antipsychotics.
Inhibitory constant (Ki): 100nM < Ki < 10000nM (weak, light grey), 10nM < Ki < 100 (moderate, mid grey), 1 > Ki < 10 (strong, dark grey). All drugs are antagonists or inverse agonists at D_2 and/or $5HT_{2A}$ receptors apart from aripiprazole which is a partial agonist at these receptors. Those traditionally termed "typical" are Haloperidol (Hal), chlorpromazine (Chl), sulpiride (Sul). Those traditionally termed "atypical" are clozapine (Clo), olanzapine (Ola), amisulpride (Ami), quetiapine (Que), risperidone (Ris) and aripiprazole (Ari). The issues with differentiating such drugs as "typical" and "atypical" is highlighted here as these medications have a broad receptor profile which often overlap therefore differences in receptor profile are not captured by this classification. Adapted from (Siafis, Tzachanis, Samara, & Papazisis, 2018)

Antipsychotic	Neuroscience based nomenclature (NbN) pharmacology
Haloperidol	Dopamine antagonist
Chlorpromazine	Dopamine, serotonin antagonist
Sulpiride	Dopamine antagonist
Clozapine	Dopamine, serotonin, norepinephrine antagonist
Olanzapine	Dopamine, serotonin antagonist
Amisulpride	Dopamine antagonist
Quetiapine	Dopamine, serotonin antagonist and norepinephrine reuptake inhibitor
Risperidone	Dopamine, serotonin, norepinephrine antagonist
Aripiprazole	Dopamine, serotonin partial agonist and antagonist

Table 1.3 Pharmacology of antipsychotics as per neuroscience based nomenclature (NbN)
Those traditionally termed "typical" are Haloperidol, chlorpromazine, sulpiride. Those traditionally termed "atypical" are clozapine, olanzapine, amisulpride, quetiapine, risperidone and aripiprazole. The issues with differentiating such drugs as "typical" and "atypical" is further highlighted here as it does not factor receptor profile differences within "typical" and "atypical" agents. The NbN classification provides a more scientific method to categorise antipsychotics. (Zohar & Kasper, 2016)

Dopamine receptors	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**		
Class	D_1 class	D_2 class	D_2 class	D_2 class	D_1 class		
Gene symbol	DRD1	DRD2	DRD3	DRD4	DRD5		
Chromosome locus	5q35.1	11q23.1	3q13.3	11p15.5	4p16.1		
G-protein	$G_{\alpha s}$	$G_{\alpha i}$	$G_{\alpha i}$	$G_{\alpha i}$	$G_{\alpha s}$		
Serotonin receptors	**$5HT_{1A}$**	**$5HT_{1B}$**	**$5HT_{2A}$**	**$5HT_{2B}$**	**$5HT_{2C}$**	**$5HT_6$**	**$5HT_7$**
Gene symbol	HTR1A	HTR1B	HTR2A	HTR2B	HTR2C	HTR6	HTR7
Chromosome locus	5q12.3	6q14.1	13q14.2	2q37.1	Xq23	1p36.13	10q23
G-protein	$G_{\alpha i}$	$G_{\alpha i}$	$G_{\alpha q}$	$G_{\alpha q}$	$G_{\alpha q}$	$G_{\alpha s}$	$G_{\alpha s}$

Table 1.4 Genetic characteristics and main G-protein transduction of dopamine and serotonin receptors.

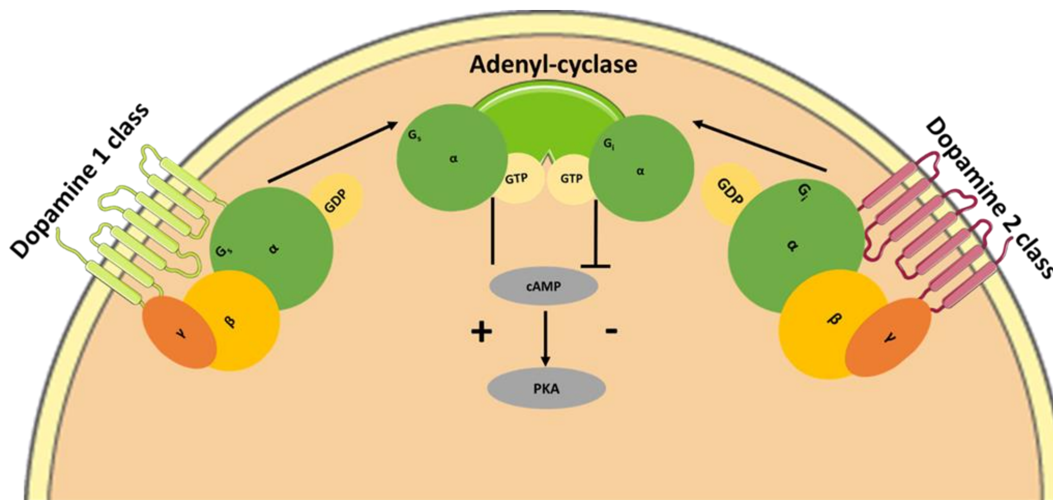


Figure 1.2 Intracellular signalling of D_1 and D_2 class G protein coupled dopamine receptors. D_1 type receptors stimulate adenylate cyclase (AC) while D_2 type receptors inhibit AC. This leads to upregulation and downregulation of cAMP respectively which in turn effects on protein kinase A (PKA) downstream effects, as well as other processes. Adapted from (J.-M. Beaulieu & Gainetdinov, 2011) Art adapted from Servier medical art under creative commons license (Servier).

Cellular models	**Description**	**Advantages**	**Disadvantages**
SH-SY5Y	Human cells derived from tumours that have neuronal features	Neuron characteristics	Abnormal Genetics
HT-22	Nerve cells derived from mice and rats	Routine experimental procedure	Human applicability
Stem cells	Multipotent and pluripotent cells for developmental and drug discovery studies	Differentiation potential	Protocol validity under development
3D culture	Cellular models for toxicity studies and drug discovery	Cell to cell interactions	Need for even nutrition
Animal models	**Description**	**Advantages**	**Disadvantages**
Pharmacological	Amphetamine or Phencyclidine administration	Valuable preclinical as mimic human symptomology	Predictive validity to full human condition
Neuro-developmental	Prenatal MAM or LPS administration or neonatal ventral hippocampal lesion		
Genetic	DISC-1, Neuregulin-1, ErB4 knock out		

Table 1.5 Preclinical models of schizophrenia.

Edited and adapted from (Jones et al., 2011; Koszła et al., 2020)

-

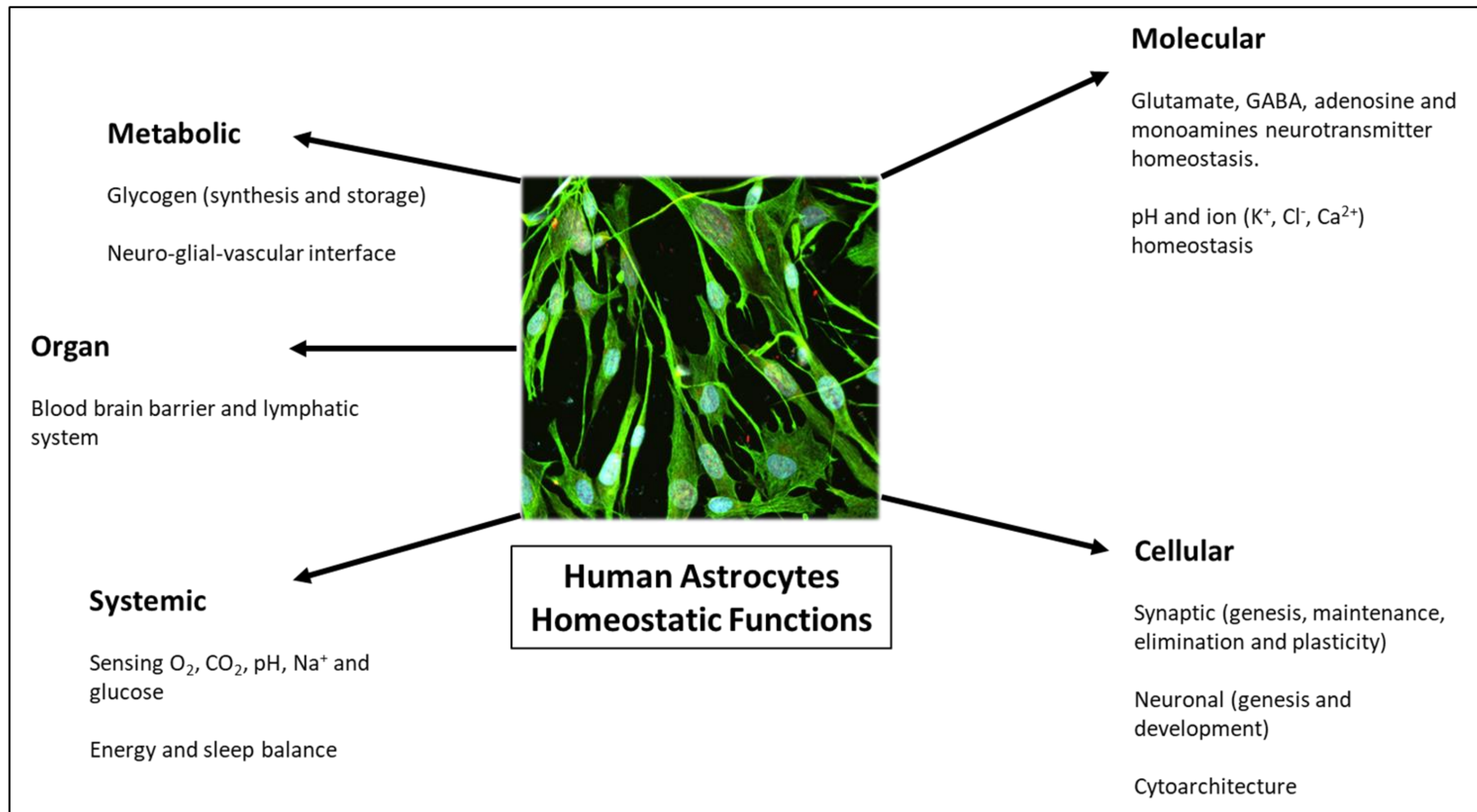


Figure 1.3 Homeostatic functions of astrocytes.
The systemic, organ, metabolic, cellular and molecular homeostatic functions of astrocytes demonstrating their importance in multiple areas of the CNS adapted from (Verkhratsky & Nedergaard, 2018). Image from immunocytochemistry experiments showing astrocytes expressing GFAP and Hoescht.

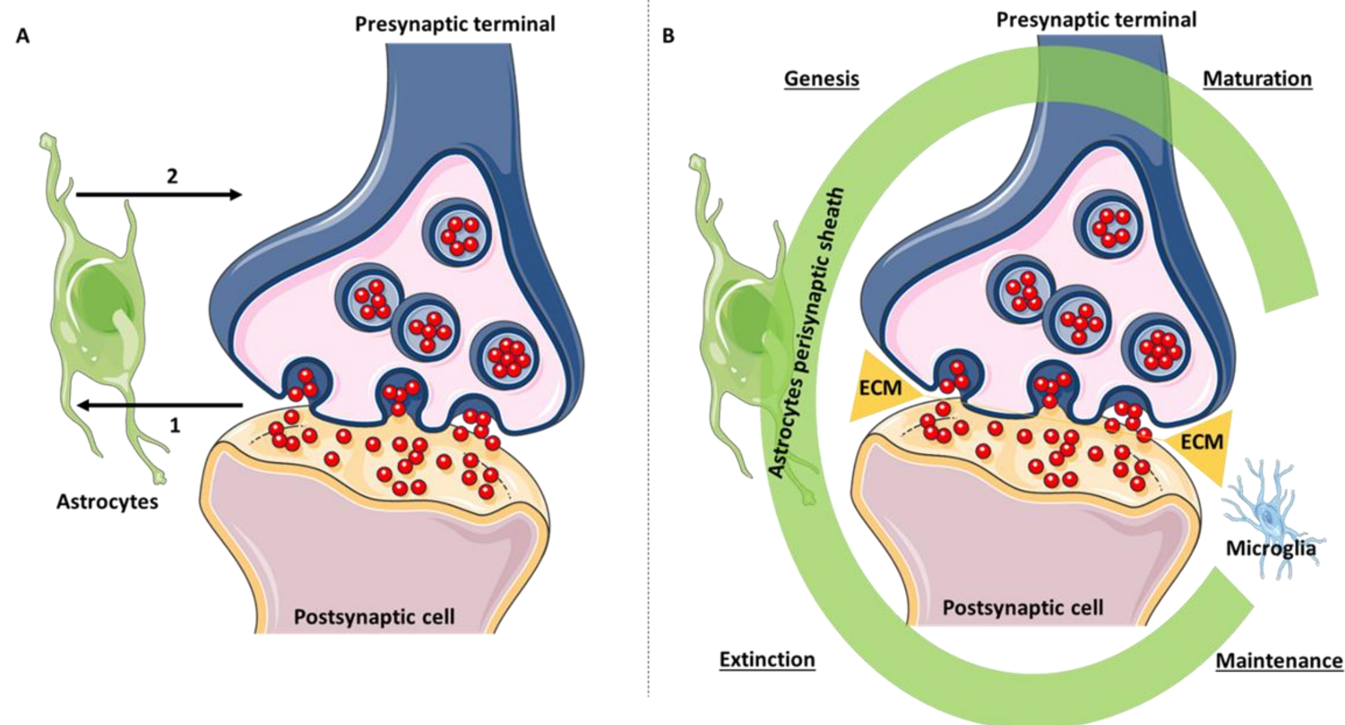


Figure 1.4 Astrocytes and the synapse.

A) Demonstrates the traditional tripartite synapse model where (1) excess neurotransmitter released from the presynaptic terminal communicates with neurotransmitter receptors in nearby astrocytes. This causes increased intracellular Ca2+ and (2) the release of glutamate, which communicates with the presynaptic nerve to regulate neurotransmission. B) Illustrates an astroglia "cradle" linked to the multipartite synapse model, where synapses constitute presynaptic terminals, postsynaptic cells, adjacent astroglia processes (sheath), microglial processes and an extracellular matrix (ECM). The astroglial sheath regulates synaptic genesis, maturation, maintenance, and extinction. Adapted from (Verkhratsky & Nedergaard, 2018) and art adapted from (Servier)

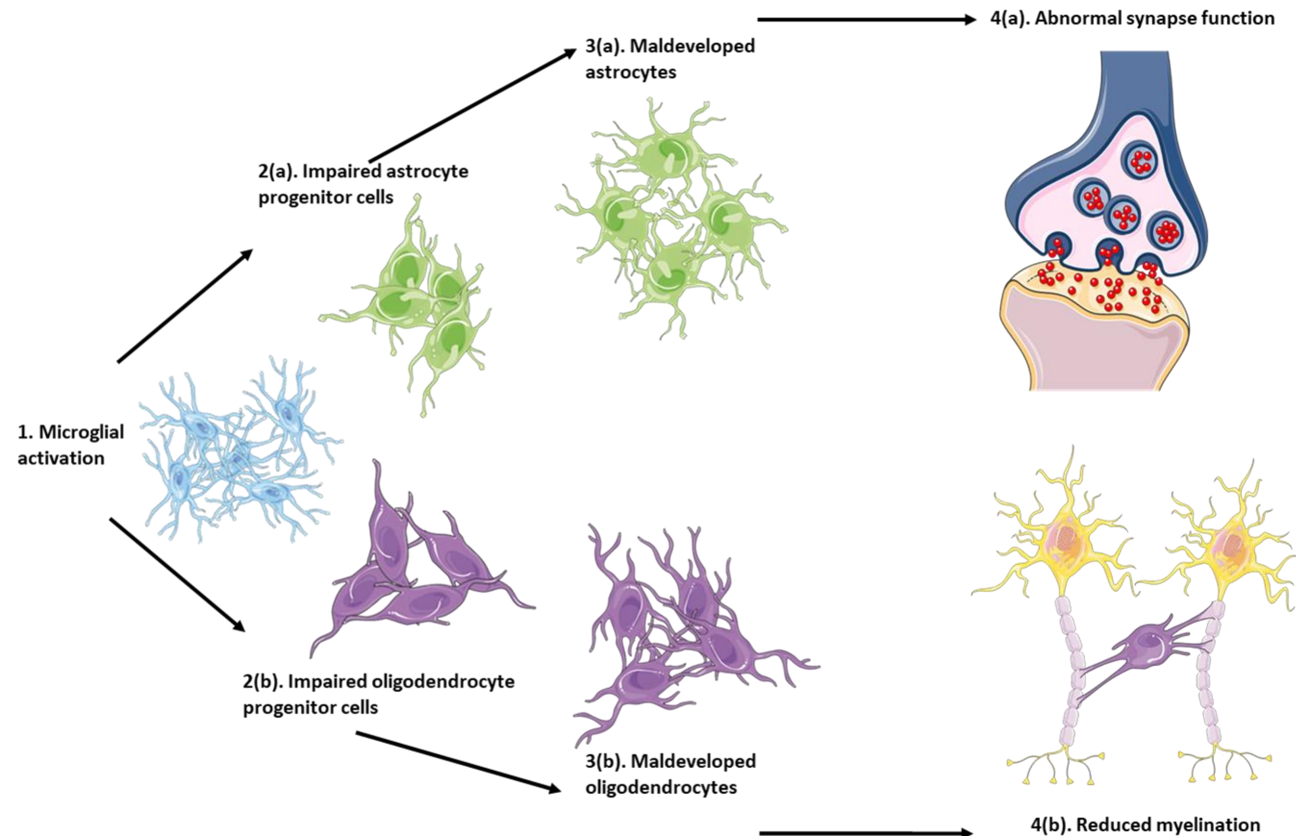


Figure 1.5 The gliocentric model of schizophrenia.

A gliocentric model of schizophrenia proposed by and adapted from (A. G. Dietz et al., 2020). (1) Microglia are activated in early development by prenatal issues, perinatal events or other predisposing factors that lead to the development of schizophrenia. (2) This activation leads to impaired maturation of (2a) astrocyte and (2b) oligodendrocyte progenitor cells which in turn leads to Immature (3a) astrocytes and (3b) oligodendrocytes. (4) These immature cells cause impaired synapse functions like altered glutamate metabolism or response to various neuromodulators in the case of (4a) astrocytes and impaired myelination with associated dysfunction in neuro-connectivity in the case of (4b) oligodendrocytes.

Chapter 2 – Materials and Methods

1. Pharmacological compounds.

Compounds and their working concentrations are summarised in table 2.1. Clozapine (Cayman; 12059), Olanzapine (Cayman; 11937), Amisulpride (Cayman; 14619), Quetiapine (Acros organics; 462400050), Risperidone (TCI; R0087), Aripiprazole (TargetMol; T1566), Haloperidol (TCI; H0912), Chlorpromazine (TargetMol; T1384), Sulpiride (TargetMol; T1201), Eticlopride (Merck; E101) and Volinanserin (Merck; M3324) were reconstituted in dimethyl sulfoxide (DMSO, Sigma; D8418) at stock concentrations of 5mM and 20mM depending on solubility. For animal studies veterinary Haloperidol (Ratiopharm; V27029A) was prescribed and used in accordance with regulations 44 and 45 of the animal remedies regulations 2005. Psychosine (ChemCruz; sc-202781A) was prepared as 10mM stock concertation in DMSO. Working concentrations from stock compounds were made using serum-free media prior to treatments.

2. Antibodies.

Primary and secondary antibodies for immunocytochemistry and confocal microscopy are summarised in table 2.2. Primary antibodies were mouse anti-vimentin (Vimentin, Santa-Cruz; Sc-373717), chicken anti-glial fibrillary acidic protein (GFAP, Abcam; ab4674), mouse anti-myelin oligodendrocyte glycoprotein (MOG, Millipore; MAB5680), rabbit anti-myelin basic protein (MBP, Abcam; ab40390), chicken anti-neurofilament H (NFH, Millipore; ab5539), rabbit anti-ionized calcium binding adaptor molecule 1 (Iba1, Wako; 019-19741) and mouse anti-neurofilament H non-phosphorylated (anti-SMI32, Millipore; NE1023). Secondary antibodies were, goat alexa-549 anti-mouse (Jackson immunoresearch; 115-5060-068), goat alexa-633 anti-chicken (Invitrogen; A21103) and goat alexa-488 anti-rabbit (Invitrogen; A11008).

3. Culture and treatment of human astrocytes and mouse organotypic cerebellar slices.

Cell culture treatments were utilised to investigate whether antipsychotics or receptor specific antagonists prevented psychosine toxicity in human astrocytes and were prepared as described previously (Elain, Jeanneau, Rutkowska, Mir, & Dev, 2014). Briefly, human astrocytes isolated from the cerebral cortex were purchased (ScienCell research laboratories; 1800), cryopreserved in liquid nitrogen and thawed as per guidelines. Cells were cultured in media (GE healthcare hyclone; 10770245) supplemented with 1% penicillin/streptomycin (Sigma; p4333), 10% foetal

bovine serum (biosera; fb1090) and 1% astrocyte growth supplement (ScienCell research laboratories; 1852). Cells were maintained in T-75 culture flasks (5%CO2 at 37°C) and media changed every two to three days. When confluent cells were sub-cultured by incubating with 4mls of trypsin-EDTA solution (Sigma; 59418c) for 5 minutes at 37°C. Complete medium was used to deactivate trypsin and cells were centrifuged and resuspended in 1ml of medium. Cells were then counted using a haemocytometer and seeded at densities of $0.01x10^6$ and $0.1x10^6$ in 96 and 12 well culture plates respectively. After three days or when greater than 80% confluent cells were cultured in serum free media for four hours and then treated as specified in the figure legends. 96 well plates were used for MTT assays of cell viability, LDH cytotoxicity assays of cell toxicity and 12 well plates were used for immunocytochemistry and confocal microscopy. All cell culture work was done under sterile conditions.

Organotypic cerebellar slice culture treatments were utilised to investigate whether Clozapine and/or haloperidol prevented psychosine induced de-myelination and were prepared using published protocols (Clementino et al., 2021; Sinead A. O'Sullivan & Dev, 2017; Sheridan & Dev, 2014; Velasco-Estevez et al., 2020). All animal work was done in agreement with EU guidelines permitted by Trinity College Dublin ethics committee. 10-day old (P10) C57BL/6J mice were humanely euthanised by decapitation and the cerebellum was surgically removed from other hindbrain structures under sterile conditions. The cerebellum was then cut into 400µm parasagittal sections using a McIlwain tissue chopper. Five slices were placed per cell culture insert (Millicell; PICMORG50). Serum based medium consisting of 50% Opti-MEM (Invitrogen; 31985047), 25% Hanks' balanced salt solution (HBSS, Invitrogen; 14025050), 25% heat-inactivated horse serum (Molecular probes; 26050088) supplemented with 2nM GlutaMAX (Invitrogen; 35050038), 28mM D-glucose (Sigma; g8769), 100U/l penicillin/streptomycin (Sigma; p4333) and 25mM HEPES buffer solution (HEPES, Molecular probes; 15630056) was used for first three days in vitro (DIV). Slices were cultured using serum-free medium consisting of 98% Neurobasal-A medium (Invitrogen; 10888022), 2% B27 (Molecular probes; 17504044) supplemented with 2nM GlutaMAX, 28mM D-glucose, 100U/l penicillin/streptomycin and 25mM HEPES after 3 DIV. One millilitre of medium per 35mm well was used to culture slices using an interface method and media was changed every three days. At 12 DIV slices were treated as per figure legends and slices were prepared for immunohistochemistry at 14 DIV.

4. LDH assay.

LDH (Lactate Dehydrogenase) assays provide a means to assess cell toxicity. LDH is released into the extracellular space upon damage to the cell membrane, which is a marker for cell damage, apoptosis, and necrosis. This extracellular LDH can be quantified by measuring the amount of formazan formed by enzymatic reaction and serves as a reliable marker for the number of dead cells. Invitrogen CyQUANT™ LDH cytotoxicity assay kits were utilised to quantify cellular toxicity as per manufacturer's protocol. The optimum number of human astrocytes for LDH cytotoxicity assay was investigated. Cells were serially diluted from 0 to $0.01x10^6$ cells per 100µl of media and seeded in 96 well plates in triplicates. One set of cells were lysed to determine the maximum LDH response, and another set were used to determine spontaneous LDH release. Cells were incubated overnight at 37°C, 10µl of sterile water was added to spontaneous group and 10µl of lysis buffer was added to maximum group. Cells were incubated for 45 minutes. 50µl of media from each sample was transferred to new 96 well plate along with positive control. 50µl of reaction mixture was added to each sample and gently mixed. Plates were protected from light and incubated at room temperature for 30 minutes after which 50µl of stop solution was added to each well. Absorbance at 680nM was subtracted from 490nM to find LDH activity and maximum LDH minus spontaneous LDH activity was plotted vs cell number to determine optimal number of cells for assays.

For experimental assays cells were seeded at $0.01x10^6$ per 100µl of media in 96 well plates in triplicate and treated as per figure legends after overnight incubation at 37°C. Cytotoxicity was measured using 50µl aliquots of the cellular supernatant and LDH activity for each sample was determined as outlined above (with averages of each triplicate per treatment group taken). Experimental group or "n" were cells cultured at different passage and at different times on individual 96 well plates. Percentage cytotoxicity was then calculated by the formula:

$$\%\ cytotoxicity = [\frac{[Treated\ LDH - Spontaneous\ LDH]}{[Maximum\ LDH - Spontaneous\ LDH]}] * 100$$

5. MTT assay.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays provide a measure of assessing cell viability. MTT enters the mitochondria of living cells and is reduced to formazan

by these metabolically active cells. The level of formazan can be quantified to give a marker of cell viability. MTT assays of cell viability were conducted as outlined previously (Misslin et al., 2017). Human astrocytes were seeded in 96 well plates at a density of $0.01x10^6$ in triplicate per well and cultured for 24 hours until greater than 80% confluent. The cells were serum starved for four hours and then treated as per figure legends. After, treatment media was removed and replaced with 100µl of fresh serum free media supplemented with 10µl of 12mM MTT Formazan (Sigma; m2003) and plates were incubated for two and a half hours at 37°C. Subsequently, 75µl of media was removed and 50µl of DMSO was added per well. Cells were incubated for ten minutes, and the absorbance was read at 540nm. Average absorbance levels were taken per each triplicate treatment group and one experimental group or "n" were cells cultured at different passage and at different times on individual 96 well plates.

6. Immunocytochemistry and fluorescent microscopy.

Fixing, blocking, incubating, mounting and imaging of cells and slices was carried out as outlined previously (Misslin et al., 2017; C. O'Sullivan et al., 2016; Sinead A. O'Sullivan & Dev, 2017; Rutkowska, Preuss, Gessier, Sailer, & Dev, 2014). These experiments were utilised to investigate glial cell morphological differences in each treatment group and glial cell specific fluorescent marker differences between treatment groups.

Human astrocytes were cultured at a density of $0.1x10^6$ in 12 well culture plates with wells lined with sterile 18mm coverslips (VWR; 6310153). After treatments (as per figure legends) cells were rinsed with PBS and fixed with 4% PFA for five minutes. Nonspecific binding was reduced by incubating with blocking buffer (1% BSA and 0.01% triton-x in PBS) for one hour. Human astrocytes were incubated with appropriate primary antibodies overnight. Cells were then rinsed with PBS and incubated with secondary antibody for 60 minutes. After further washes in PBS cells were incubated with Hoescht nuclear stain for thirty minutes. The coverslips were placed onto glass microscope slides with SlowFade® reagent (Invitrogen; s36936) and edges were sealed using varnish. Slides were stored in the dark until imaged using confocal microscopy. Images of cells were acquired using scanning confocal microscopy (Leica; SP8) at 10x and 20x magnification. For cells the number of astrocyte projections of 20-30 cells per treatment group (figure 2.1) was analysed. Image analysis was carried out using ImageJ software (https://imagej.nih.gov/ij/) (Rueden & Eliceiri, 2019; Schindelin et al., 2012).

Cerebellar slices were washed with PBS and then fixed with 4% PFA for 10 minutes. Slices were then blocked and permeabilised with 10% BSA and 0.05% triton-x in PBS overnight. Slices were then incubated for 48 hours in appropriate primary antibody diluted in 2% BSA + 0.1% triton-x and then washed and incubated for 24 hours in appropriate secondary antibody. The slices were rinsed and placed on glass microscope slides with SlowFade® (Invitrogen; s36936) and edges were closed with varnish. Slides were stored in the dark until imaged. Images of slices were acquired using scanning confocal microscopy (Leica; SP8) at 10x and 20x magnification the mean fluorescence for each treatment group was analysed by examining regions of interest (ROI). Image analysis was carried out using ImageJ (https://imagej.nih.gov/ij/) and Imaris® software (Rueden & Eliceiri, 2019; Schindelin et al., 2012).

7. Animals.

All animal work was carried out in compliance with EU legislation approved by Trinity College Dublin ethics committee and in accordance with guidelines from the Health Products Regulatory Authority (HPRA) under project authorisation number AE19136/P123. Twitcher mice were utilised to investigate whether haloperidol improved survival, twitching scores, immobility scores and subtle behavioural metrics on open field testing in this murine model (a naturally occurring mouse mutant that contain a mutation in the *GALC* gene which codes for galactosylceramidase). Psychosine (galactosylsphingosine) accumulates in homozygous twitcher mice and is associated with toxicity in the CNS and PNS. The genetic pathology in twitcher mice equates to human globoid cell leukodystrophy also known as Krabbe's disease (KD). A colony of heterozygous twitcher mice originally obtained from Jackson Laboratory (B6.CE-Galctwi/J Stock no:000845) were maintained for breeding by qualified staff in the comparative medicine unit at Trinity College Dublin under pathogen free conditions. Ear punch tissue samples were acquired from mice and sent for commercial automated genotyping by TransnetYX (www.transnetyx.com) using real time PCR. Homozygous mice and their wild-type litter mates were treated with haloperidol at a dose of 1mg/kg/day in drinking water or just water control via suckling pipette (figure 2.2). During experimental conditions mice were kept in grouped cages, had constant access to food and water and were under a 12-hour light/dark timetable. Humane endpoints were specified prior to experimentation as per table 2.3.

8. Behavioural analysis.

From PND 21 onwards mice were subject to behavioural observations one to three times daily. Modified established protocols (Wicks et al., 2011) previously published by our research group (Béchet et al., 2020) were utilised to record twitching severity scores and immobility severity scores over time (table 2.3). A score of 0 meant a complete absence of twitching or mobility issues. For twitching scores: very mild fine twitching was scored as 1; mild intermittent twitching as 2; constant fine twitching as 3; constant moderate twitching as 4 (animals were humanely euthanised if this was observed on 4 independent occasions within 24 hours) and constant severe uncontrollable twitching was scored as 5 (animals were humanely euthanised immediately). For locomotor scores: complete limp tail was scored as 1; mild hind limb paralysis as 2; moderate hind limb paralysis as 3 (animals were humanely euthanised if this was observed on 4 independent occasions within 24 hours); complete hind limb paralysis as 4 and complete hind limb paralysis with front limb paralysis was scored as 5 (animals were humanely euthanised immediately). Animal's body weight, as a marker of thriving, was also recorded daily during behavioural observations and animals were humanely euthanised if they lost more than 20% of their body weight during the experiment. Although blinding was not fully possible as phenotypic changes between twitcher and wild-type mice became observable over time, therefore mice genotypes became identifiable. The experimenter also prepared the treatments, so no blinding was possible at the drug treatment phase. However, data were pseudo-anonymised by using animal numbers and data analysis was carried out blinded to genotype and treatment.

9. Open Field Maze.

Open field maze (OFM) testing was conducted on animals to obtain more behavioural, locomotion and anxiety related data. Animals were habituated to the OFM apparatus on PND 21 and 22 by placing them in pairs in the OFM apparatus for 5 minutes. On PND 25, 28 and 30 animals were tested in the OFM apparatus, this was before the emergence of any severe twitching or immobility issues. Animals were placed in the centre of the OFM apparatus, a 44cm X 44cm cage with high darkened walls, for 5 minutes. A video was recorded by a camera hanging above and out of sight. All tests were done using the same protocol, under the same environmental conditions at approximately the same time of day and the OFM apparatus was cleansed with ethanol between different animals. Recordings were analysed using ANYmaze tracking software (Stoelting) examining: distance (m); mean speed (m/s); max speed (m/s); time

mobile (%); centre entries and corner time (%). An experimental unit or "n" was one individual animal per treatment group and 8 animals were utilised per group based on sample size calculations.

10. Statistical analysis.

All data was analysed and graphed using GraphPad Prism 9 (GraphPad Software, Inc.), values shown are means +/- standard error of the mean and details of analyses are located within figure legends. Normality was assessed by generating normal QQ plots for data and assessing for any obvious skewness. Clear outliers where identified were removed. Formal normality tests were carried out using D'Agostino-Pearson or Anderson-Darling tests for experiments with sufficient n numbers. In experiments with smaller sample size, but still suitably powered, normality was assessed using Shapiro-Wilk or Kolmogorov-Smirnov tests. Experiments involved the comparison of means of more than two groups and one or two factors. Therefore, parametric ANOVA tests or non-parametric Kruskal-Wallis tests followed by Tukey's or Dunn's multiple comparisons tests were carried out for experiments comparing means from selected groups. For cell studies an experimental group or "n" were cells cultured at different passage in triplicate and at different times on individual 96 or 12 well plates. For slice studies one experimental unit or "n" was 4/5 cerebellar slices cultured per treatment group in 6-well plates. For animal studies an experimental unit or "n" was one individual animal per treatment group and 8 animals were utilised per group based on sample size calculations. Previous work in our laboratory examined other potential treatments for psychosine toxicity in-vitro, ex-vivo and in-vivo (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015). With reference to these as well as literature review sample size calculation utilised a range of estimated mean change and standard deviation was set as 20-25% and 5-8% respectively. A power of 0.8 and a stricter than conventional alpha of 0.01 was utilised to ensure experiments were not underpowered (Button et al., 2013). Survival analysis was carried out using Kaplan-Meier curve with a log rank Mantel-Cox test. In all cases, the significance levels (alpha) were fixed at $p<0.05$*, $p<0.01$**, $p<0.001$*** and $p<0.0001$****. Further information on statistical methods is given in results sections and in figure legends.

11. Candidate's contribution statement.

The supervisor conceived the idea. The supervisor and the candidate designed the methodology of this work. The candidate performed the experiments, analysed the data, and wrote the manuscript.

Compound	Details	Working concentration
Clozapine	Cayman; 12059	1µM-10µM
Olanzapine	Cayman; 11937	1µM-10µM
Amisulpride	Cayman; 14619	1µM-10µM
Quetiapine	Acros organics; 462400050	1µM-10µM
Risperidone	TCI; R0087	1µM-10µM
Aripiprazole	TargetMol; T1566	1µM-10µM
Haloperidol	TCI; H0912 / Ratiopharm; V27029A	1µM-10µM
Chlorpromazine	TargetMol; T1384	1µM-10µM
Sulpiride	TargetMol; T1201	1µM-10µM
Eticlopride	Merck; E101	1µM-10µM
Volinanserin	Merck; M3324	1µM
Psychosine	ChemCruz; sc-202781A	0.1µM-100µM
Hoescht	Thermo Scientific; 62249	4µM

Table 2.1. List of compounds.

Primary Antibodies						
Target	Type	Host	Supplier	Cat No.	RRID	Dilution
Vimentin	Polyclonal	Mouse	Santa Cruz	Sc-373717	AB_10917747	1/1000
GFAP	Polyclonal	Chicken	Abcam	Ab4674	AB_304558	1/1000
MOG	Monoclonal	Mouse	Millipore	MAB5680	AB_1587278	1/1000
MBP	Polyclonal	Rabbit	Abcam	ab40390	AB_1141521	1/1000
NFH	Polyclonal	Chicken	Millipore	ab5539	AB_177520	1/1000
Iba1	Polyclonal	Rabbit	Wako	019-19741	AB_839504	1/1000
SMI32	Monoclonal	Mouse	Millipore	NE1023	AB_2715852	1/1000

Secondary Antibodies				
Target	Host	Supplier	Cat No.	Dilution
Alexa 549 anti-mouse	Goat	Jackson	115-5060-068	1/1000
Alexa 633 anti-chicken	Goat	Invitrogen	A21103	1/1000
Alexa-488 anti-rabbit	Goat	Invitrogen	A11008	1/1000

Table 2.2. List of antibodies.

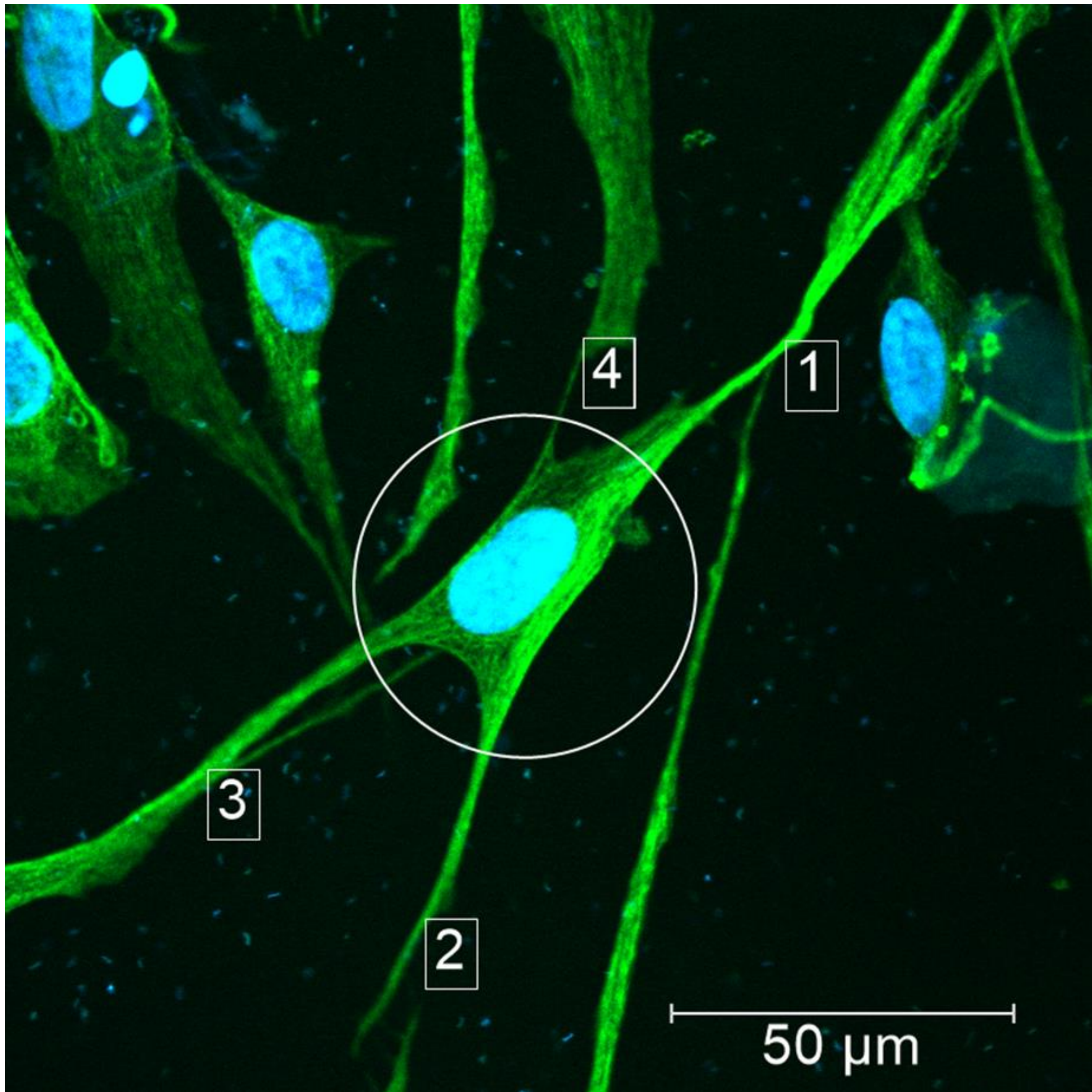


Figure 2.1 Quantification of cellular processes to assess human astrocyte morphology. The number of astrocyte projections of 20-30 cells per treatment group was analysed e.g., four for the example above. Images were analysed using ImageJ software. Above image of astrocyte expressing GFAP and Hoescht from immunocytochemistry experiments.

ANNEX 1 Observational Score Sheet for Mobility, Weight and Twitching

ANIMAL SCORE SHEET AND MANAGEMENT OF OUTCOMES

Project – Investigating new therapies for the fatal degenerative disorder Krabbe disease using the twitcher mouse module (Animal Model: Twitcher)

ANIMAL NUMBER/CODE: ___________________________ **DATE OF BIRTH:** ___________________________ **GENDER:** ___________________________

EVALUATION AND RECORDING WEIGHT, TWITCHING AND MOBILITY SCORES OF ANIMALS (PLEASE USE TABLE BELOW)

	With Mother/WEANING PERIOD																				EARLY POST WEANING (MONITOR 2 x day)									
AGE (DAY)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
TWITCHING SCORE*																														
MOBILITY SCORE*																														
WEIGHT OF ANIMAL																														
OPEN FIELD MAZE TEST																					5 min	5 min			5 min			5 min		5 min

	EXPECTED INCREASE IN SCORE & FATALITIES (MONITOR 3 x day)																POSSIBLE INCREASED LIFESPAN: DRUG TREATED ONLY (MONITOR 3 x day)													
AGE (DAY)	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
TWITCHING SCORE*																														
MOBILITY SCORE*																														
WEIGHT OF ANIMAL																														
OPEN FIELD MAZE TEST										5 min																				

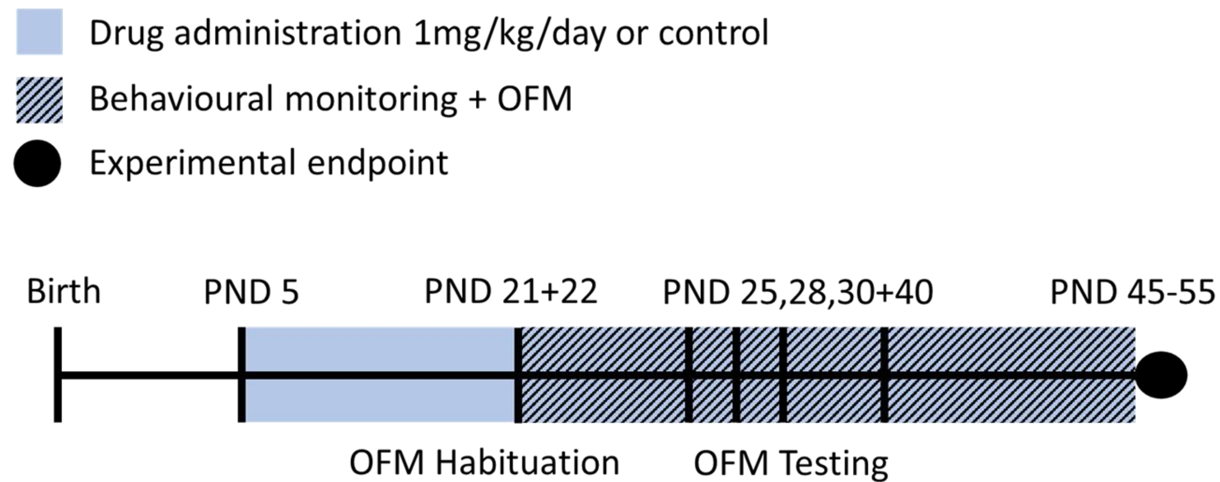
	UNEXPECTED BUT POSSIBLE INCREASED LIFESPAN: DRUG TREATED ONLY (MONITOR 3 x day)																				
AGE (DAY)	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	
TWITCHING SCORE*																					
MOBILITY SCORE*																					END OF STUDY
WEIGHT OF ANIMAL																					
OPEN FIELD MAZE TEST																					

BEHAVIOURAL SCORE SYSTEM AND MANAGEMENT OF ADDITIONAL OUTCOMES (SCORE 1/day & MONITOR 1-3/day)

Score	GENERAL Description	Expected Time of Score Progression; Monitoring Level	ACTION TO BE TAKEN AND HUMANE ENDPOINTS
0	None: complete absence of twitching/mobility score (pre-weaning)	Days 01-18; Monitor 1/day	n/a
X	Fatality	Days 34-46; Monitor 3/day	n/a
X	20% loss body weight over course of experiment	Days 34-46; Monitor 3/day	Euthanize immediately
X	Age 80 days (end of study)	Day 80	Euthanize immediately
Score	*TWITCHING BEHAVIOURAL Description	Expected Time of Score Progression; Monitoring Level	ACTION TO BE TAKEN AND HUMANE ENDPOINTS
1	Very mild rare, fine twitching (post-weaning)	Days 18-22; Monitor 2/day	Allow to continue
2	Mild: intermittent fine twitching	Days 22-26; Monitor 2/day	Allow to continue
3	Constant fine twitching	Days 26-30; Monitor 2/day	Allow to continue
4	Constant moderate twitching	Days 30-42; Monitor 3/day	Euthanize upon 4th independent observation, no more than 24h
5	Constant severe trembling and uncontrollable twitching	Days 32-46; Monitor 3/day	Euthanize immediately
Score	*MOBILITY BEHAVIOURAL Description	Expected Time of Score Progression; Monitoring Level	ACTION TO BE TAKEN AND HUMANE ENDPOINTS
1	Complete limp tail	Days 30-46; Monitor 3/day	Allow to continue
2	Mild hind limb paralysis	Days 30-46; Monitor 3/day	Allow to continue
3	Moderate hind limb paralysis	Days 30-46; Monitor 3/day	Euthanize upon 4th independent observation, no more than 24h
4	Complete hind limb paralysis	Days 30-46; Monitor 3/day	Euthanize immediately
5	Hind limb and front limb paralysis and incontinence	Days 40-46; Monitor 3/day	Euthanize immediately
Score	MANAGEMENT OF ADDITIONAL OUTCOMES	Monitoring Time	ACTION TO BE TAKEN AND HUMANE ENDPOINTS
X	Abnormal development (twitching/mobility) during weaning period	Days 01-20 (during weaning period)	Euthanize immediately
X	Moribund state	Days 01-80 (anytime throughout experiement)	Euthanize immediately
Score	OPEN FIELD MAZE TEST	Monitoring Time	
See Annex 2	Habituation Days	Days 21, 22; Monitor 5 min/day	Please see Annex 2
See Annex 2	Testing Days	Days 25, 28, 30, 40; Monitor 5 min/day	Please see Annex 2

Table 2.3 Behavioural scoring protocol.

Animals were monitored one to three times a day and records of twitching and mobility severity were kept. Animal weights were also recorded. OFM habituation and testing was carried out on PND 21/22 and PND 25,28,30 and 40 respectively. Humane endpoints were established prior to experimentation and animals meeting such criteria were euthanised via CO_2 inhalation.



PND = Post Natal Day
OFM = Open Field Maze

Figure 2.2 *In-vivo* experimental design schematic.
PND 5 twitcher mice and wild-type littermates were treated with haloperidol (1mg/kg/day) or control (water) by suckling pipette. Daily behavioural monitoring of mobility scores, twitching scores and weight were carried out from PND 21 onwards. Open field maze habituation and testing occurred on PND 21,22 and PND 25, 28, 30, 40 respectively.

Chapter 3 – Antipsychotics attenuate psychosine-induced cell toxicity and morphological damage in human astrocytes.

Chapter Aims

- To confirm that psychosine reduces cell viability and is toxic to human astrocytes *in-vitro*.
- To confirm that psychosine impairs astrocyte morphology.
- To investigate if antipsychotics with primarily D_2 receptor antagonism haloperidol, chlorpromazine and sulpiride can prevent psychosine induced toxicity and morphological impairments in human astrocytes.
- To investigate if antipsychotics with D_2 and $5HT_2$ receptor antagonism clozapine, olanzapine, amisulpride, quetiapine, risperidone and aripiprazole can prevent psychosine induced toxicity and morphological impairments in human astrocytes.
- To determine if the selective D_2 antagonist eticlopride can prevent psychosine induced toxicity and morphological impairments in human astrocytes.
- To investigate if the selective $5HT_{2A}$ antagonist volinanserin can prevent psychosine induced toxicity and morphological impairments in human astrocytes.

Abstract

Glial cells are implicated in the neuropathophysiology of schizophrenia and other neuropsychiatric disorders. There is interest in the effects of antipsychotics on these cells, to explore the ways these drugs may act via non-neuronal mechanisms. To investigate if antipsychotic drugs modulate glial cell dysfunction, in this study, human astrocytes were treated with the toxin psychosine, with or without antipsychotics. An MTT assay showed that psychosine decreased cell viability compared to control and that antipsychotics significantly attenuated this cell damage. Similarly, an LDH assay showed that psychosine induced cell toxicity in human astrocytes, which was returned to almost control levels by antipsychotics. Immunocytochemical analysis also showed that psychosine impaired astrocyte morphology, as determined by the reduction in GFAP and vimentin positive astrocyte extensions, was reversed significantly by antipsychotics. Pharmacological analysis indicates that antipsychotics inhibit activity of multiple receptors, with dopamine D_2 and/or serotonin $5HT_{2A}$ receptors being most common. Here, selective D_2 and $5HT_{2A}$ receptor antagonists were found to attenuate psychosine-induced decrease in cell viability, toxic effects, and reductions in astrocyte processes. Overall, this work points toward the role of D_2 and/or serotonin $5HT_{2A}$ receptors in regulating astrocyte cell damage and highlights the direct effects of a broad selection of commonly prescribed antipsychotics on human astrocytes.

Keywords: astrocytes, antipsychotics, psychosine, D_2 receptors, $5HT_{2A}$ receptors

1. Introduction.

Schizophrenia is traditionally characterised as a chronic condition, but it is thought to be a neurodevelopmental disorder that progresses from a prodromal (prepsychotic) phase to a first episode (acute) psychosis phase, before manifesting as a chronic illness (Goff et al., 2005), involving genetic, environmental, immune and metabolic factors (K. E. O'Connell et al., 2014). Individuals with schizophrenia present with positive, negative as well as cognitive symptoms (Patel, Cherian, Gohil, & Atkinson, 2014). Prevalence and incidence rates vary between studies, but it is thought the global prevalence approaches 1%, with an incidence of 1.5 per 10,000 per year (McGrath, Saha, Chant, & Welham, 2008). Twin, family and genome wide association studies (GWAS) have underlined the hereditability of schizophrenia as well as it being a polygenic disorder (Schwab & Wildenauer, 2013). Imaging studies have highlighted several anatomical abnormalities associated with schizophrenia (Katherine H. Karlsgodt, Sun, & Cannon, 2010) including abnormalities in grey and white matter, cerebral volume loss, ventricular enlargement as well as temporal lobe asymmetry (Ross, Margolis, Reading, Pletnikov, & Coyle, 2006).

Abnormalities in neurochemistry are linked with schizophrenia, especially hyper-dopaminergia in the basal ganglia leading to the manifestation of positive symptoms. Although antipsychotics have a broad receptor profile it is thought that primary relief from positive symptoms derives from dopamine D_2 receptor subtype antagonism in the mesolimbic areas. Consequently, motor side effects derive from D_2 receptor antagonism in the nigrostriatal zones, increased prolactin via D_2 antagonism in the tuberoinfundibular and ineffective negative symptom relief from D_2 antagonism in the mesocortical areas. Antipsychotics with D_2 and $5HT_2$ receptor antagonism aim to combat some of these side effects as well as attempting to treat negative and cognitive symptoms. The antagonism of $5HT_{2A}$ receptors in the nigrostriatal area releases dopamine, potentially decreasing motor side effects, while in the mesocortical pathway potentially improving negative and cognitive symptoms (Stahl, 2008). Strong glutamate N-methyl-D-aspartate (NMDA) antagonists like ketamine and phencyclidine (PCP) have led to psychotic symptoms leading to the theory that glutamate NMDA receptor dysfunction is associated with schizophrenia perhaps leading to cortical glutamate increase and excitotoxicity (Stone et al., 2007).

There is growing data about the influence of glial cells on the neuropathophysiology of schizophrenia (H. G. Bernstein et al., 2015; Andrea G Dietz et al., 2019). Various studies have shown astrogliapathology being associated with schizophrenia. Astrocytes were found in lower numbers in white matter of cases and had altered morphology (Merenlender-Wagner et al., 2015; M. Williams et al., 2013; M. R. Williams et al., 2013). The expression of several astrocyte related genes was altered in cases (Goudriaan et al., 2014). Lower glial fibrillary acid protein (GFAP) was noted in white matter of people with the disorder, with corresponding reduction in GFAP in that brain region on western blot (Bruneau, McCullumsmith, Haroutunian, Davis, & Meador-Woodruff, 2005; Webster, O'Grady, Kleinman, & Weickert, 2005). Disturbances of glutamatergic and GABAergic neurotransmission is linked with schizophrenia (Poels et al., 2013) and glutamine synthase expressing astrocytes are a key part of the tripartite or multipartite system of synapses. Astrocytes also release "gliotransmitters" such as D-serine, kynurenic acid and S100B (Myint & Kim, 2014; J. Steiner et al., 2010). Changes in D-serine metabolising enzymes increase risk for schizophrenia and there is diminished D-serine concentrations in plasma and cerebrospinal fluid of patients (Caldinelli, Sacchi, Molla, Nardini, & Pollegioni, 2013; Van Horn, Sild, & Ruthazer, 2013). Cerebral and cerebrospinal kynurenic acid concentrations are elevated in patients (J. Steiner et al., 2012). Increased levels of S100B, a supposed astrocyte marker as well as other inflammatory cytokines and chemokines, have been seen in post-mortem brains of patients as well as in bodily fluids of individuals with the disorder (K. O'Connell, Thakore, & Dev, 2013; K. E. O'Connell et al., 2014; O'Connell, Thakore, & Dev, 2015; J. Steiner et al., 2012; Zhai et al., 2012). Astrocytes may also be involved in abnormal changes of dopamine as indicated by Catelchol-O-methyltransferase (COMT) being increased in astrocytes but not neurons (Brisch et al., 2009).

Importantly dopamine receptors are expressed in astrocytes of the prefrontal cortex and basal ganglia (Miyazaki, Asanuma, Diaz-Corrales, Miyoshi, & Ogawa, 2004; Mladinov et al., 2010). Antipsychotics are thought to improve disturbed metabolism in schizophrenia by binding to astrocyte dopamine receptors. Clozapine enhances the discharge of D-serine from astroglia in hippocampal cultures, while effects on other astrocyte gliotransmitters are less well explored. Also, clozapine reduces glutamate uptake in astrocytes (Vallejo-Illarramendi et al., 2005) perhaps correcting lowered glutamate levels in the CNS associated with schizophrenia. In this current study we investigate the effects of antipsychotics on toxic-induced astrocyte dysfunction, investigating cell viability and morphological changes in human astrocytes.

2. Results.

2.1. Psychosine induces toxicity and reduces cell viability in human astrocytes.

We have shown previously that the toxin psychosine reduces astrocyte numbers and cell viability in dissociated human astrocytes cultures and induces demyelination in organotypic slice cultures, where psychosine dysregulates glia cell function, inclusive of oligodendrocytes myelin stage as well as microglia and astrocyte reactivity (Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016; Velasco-Estevez et al., 2020). To confirm these effects of psychosine, human astrocytes were exposed to increasing concentrations of the toxin and cell viability was examined. MTT assays showed that psychosine significantly decreased cell viability compared to control (100+/-1.58%) at 10µM (14.13+/-1.52%, and 100µM (5.10+/-1.17%) concentrations. A positive control of 20% DMSO (4.76+/-0.89%) produced reductions in cell viability as expected (figure 3.1 - H (5) = 125.9, P<0.0001, Kruskal-Wallis test). LDH assays examined cell toxicity after exposure and showed that psychosine was significantly toxic to human astrocytes at 10µM (84.4+/-2.34%) and 100 µM (91.40+/-1.91%) concentrations compared to control (2.8+/-0.37%) (figure 3.1 – F (27, 112) = 271.4. P<0.0001, ANOVA).

2.2. Antipsychotics with primary D_2 receptor antagonism attenuate psychosine toxicity and improve astrocyte viability.

To examine whether antipsychotics with primary D_2 receptor antagonism alter cell viability, human astrocytes were treated with these drugs at 1µM and 10µM. MTT assays showed that at 1µM or 10µM concentrations of haloperidol (86.25+/-2.18%; 93.5+/-3.51%), chlorpromazine (86.36+/-2.66%; 102.30+/-3.83%) or sulpiride (84.60+/-3.23; 94.78+/-2.07%) did not alter cell viability compared to control (100+/-1.58%) (figure 3.2 - H (14) = 209.4, P>0.9999, Kruskal-Wallis test). MTT assays showed that 1µM and 10µM concentrations of haloperidol (72.55+/-4.04%; 76.40+/-4.95%), sulpiride (72.82+/-3.38; 69.86+/-6.00%) and chlorpromazine (71.35+/-3.80%; 48.15+/-7.14%) significantly prevented psychosine (10µM) induced reductions in cell viability (14.13+/-1.52%) (figure 3.2 - H (14) = 209.4, P = 0.0001, 0.0018, 0.0004, 0.0157, 0.0007, 0.0148, Kruskal-Wallis test). LDH assays showed that 1µM concentrations of haloperidol (9.60+/-1.44%), chlorpromazine (9.40+/-1.72%) or sulpiride (10.40+/-0.75%) were not toxic to cells compared to control (2.80+/-0.37%) (figure 3.2 - F (27, 112) = 271.4. P = 0.2540, 0.3088, 0.1013, ANOVA).

These LDH assays showed that 1µM concentrations of haloperidol (24.00+/-0.84%), chlorpromazine (33.00+/-1.52%) and sulpiride (30.00+/-0.84%) prevented 10µM psychosine toxicity (84.40+/-2.34%) (figure 3.2 - F (27, 112) = 271.4. P<0.0001, ANOVA).

2.3. Antipsychotics with D_2 and $5HT_2$ receptor antagonism prevented psychosine toxicity and improved astrocyte viability.

Next, the effects of antipsychotics with more D_2 and $5HT_2$ receptor influences, on psychosine-induced cell toxicity in human astrocytes was also examined. MTT assays showed that 1µM and 10µM concentrations of clozapine (87.72+/-2.24%; 93.78+/-3.50%), olanzapine (88.86+/-2.21%; 96.71+/-2.63%), amisulpride (88.57+/-1.39%; 84.96+/-3.59%), quetiapine (88.42+/-1.40%; 97.35+/-4.33%), risperidone (85.91+/-1.88%; 103.40+/-3.46%) or aripiprazole (84.95+/-2.56%; 108.1+/-4.05%) did not alter viability of human astrocytes compared with control (100.00+/-1.58%) (figure 3.3 - H (26) = 303.9, P>0.9999, Kruskal-Wallis test). MTT assays showed that 1µM and 10µM concentrations of clozapine (71.75+/-3.31%; 71.34+/-5.88%), olanzapine (72.85+/-3.25%; 73.70+/-7.02%), amisulpride (72.93+/-3.62%; 80.74+/-4.90%), quetiapine (78.88+/-2.91%; 76.06+/-8.64%), risperidone (76.30+/-3.13%; 71.31+/-7.75%) and 1µM (but not 10µM) aripiprazole (69.17+/-4.37%; 33.01+/-4.13%) significantly prevented toxicity induced by 10µM psychosine (14.13+/-1.52%) (figure 3.3 - H (26) = 303.9, P = 0.0086, 0.0316, 0.0043, 0.0059, 0.0023, 0.0005, <0.0001, 0.0001, 0.0001, 0.0047, 0.0064, >0.9999, Kruskal-Wallis test). LDH assays showed also that 1µM concentrations of clozapine (13.20+/-1.59%), olanzapine (11.00+/-1.76%), amisulpride (12.60+/-2.29%), quetiapine (10.00+/-1.00%), risperidone (12.80+/-2.31%) and aripiprazole (11.20+/-1.36%) were not toxic to human astrocytes compared to control (2.80+/-0.37%) (figure 3.3 - F (27, 112) = 271.4. P>0.9999, ANOVA). These LDH assays showed that 1µM concentrations of clozapine (24.40+/-1.50%), olanzapine (23.40+/-1.40%), amisulpride (40.40+/-1.72%), quetiapine (36.80+/-1.36%), risperidone (36.20+/-1.16%) and aripiprazole (34.60+/-1.03%) attenuated toxicity induced by 10µM psychosine (84.40+/-2.34%) (figure 3.3 - F (27, 112) = 271.4. P < 0.0001/=0.0001, ANOVA).

2.4.Selective D_2 and $5HT_{2A}$ antagonists prevented psychosine induced toxicity.

To hypothesise the receptor type involved in antipsychotics regulating astrocyte viability, a heat map of their pharmacological profile was generated (Table 1.2). All drugs were antagonists and/or inverse agonists at D_2 and $5HT_{2A}$ receptors, except aripiprazole which is a partial agonist. Therefore, a highly selective D_2 antagonist (Eticlopride) and $5HT_{2A}$ antagonist (Volinanserin) were used to determine whether antagonism of these dopamine and/or serotonin receptors was sufficient to induce protective effects against psychosine induced toxicity. MTT assays showed that at 1µM concentrations of Eticlopride (91.67+/-1.71%) or Volinanserin (86.08+/-2.11%) did not alter cell viability compared to control (100+/-1.32%). MTT assays showed that at 1µM concentrations of Eticlopride (81.40+/-2.48%) and Volinanserin (79.03+/-3.38%) significantly attenuated cell viability induced by 10µM psychosine (8.47+/-1.41%) (figure 3.4 - $H_{(6)} = 91.52$, $P = >0.9999, 0.4070, <0.0001, 0.0003$, Kruskal-Wallis test). LDH assays showed that at 1µM concentrations Eticlopride (10.20+/-1.24%) or Volinanserin (11.80+/-1.28%) were not toxic to cells compared to control (2.80+/-0.37%) and that Eticlopride (18.6+/-1.29%) and Volinanserin (44.20+/-2.29%) prevented toxicity induced by 10µM psychosine (84.40+/-2.34%) (figure 3.4 - $F_{(27, 112)} = 271.4$. $P = 0.1300, 0.1030, <0.0001$, ANOVA). We noted the D_2 receptor antagonism by Eticlopride lead to a significantly greater improvement in cell viability and reduced toxicity compared to $5HT_{2A}$ antagonism by Volinanserin ($F_{(27, 112)} = 271.4$. $P <0.0001, <0.0001$, ANOVA).

2.5.Antipsychotics attenuate psychosine induced morphological changes in human astrocytes.

Immunocytochemistry was performed to visualise morphological changes of astrocytes using expression of GFAP and Vimentin after exposure to psychosine (10µM) and treatment with antipsychotics (1µM). The average number of cellular projections beyond 50µm was assessed i.e., the number of astrocyte projections of 20-30 cells per treatment group was analysed. Image analysis was carried out using ImageJ software (https://imagej.nih.gov/ij/) (Rueden & Eliceiri, 2019; Schindelin et al., 2012) (figure 2.1). Cells under control conditions showed an average number of GFAP or Vimentin-positive projections (2.36+/-0.04; 2.23+/-0.08) with psychosine treatment causing a significant decrease (0.70+/-0.05; 0.80+/-0.06), as did 20% DMSO (0.31+/-0.03; 0.39+/-0.03) (figure 3.5 - $F_{(12, 65)} = 257.4/113.6$. $P < 0.0001$, ANOVA). None of the

antipsychotics tested haloperidol (2.24+/-0.03; 2.10+/-0.05), sulpiride (2.22+/-0.02; 2.08+/-0.04), chlorpromazine (2.24+/-0.03; 2.08+/-0.06) altered GFAP or Vimentin-positive projections compared with control (figure 3.5 - $F_{(12, 65)} = 257.4/113.6$. P = 0.6886/0.6202, 0.4862/0.3894, 0.6886/0.4439, ANOVA). Importantly, 1µM concentrations of haloperidol (2.02+/-0.05; 1.91+/-0.06), sulpiride (1.88+/-0.03; 1.90+/-0.03), and chlorpromazine (1.88+/-0.04; 1.88+/-0.05) significantly prevented the reduction in cellular projections induced by 10µM psychosine (0.70+/-0.05; 0.80+/-0.06) (figure 3.5 - $F_{(12, 65)} = 257.4/113.6$. $P<0.0001$, ANOVA). The effects of antipsychotics (1µM) on morphological changes of astrocytes induced by psychosine (10µM) was also examined (figure 3.6). Data showed that clozapine (2.33+/-0.04; 2.19+/-0.05) or olanzapine (2.26+/-0.03; 2.10+/-0.07) did not alter the number of GFAP or Vimentin-positive projections compared with control. Importantly, 1µM concentrations of clozapine (2.13+/-0.06; 2.02+/-0.05) and olanzapine (2.06+/-0.05; 1.95+/-0.03) significantly prevented the reduction in cellular projections induced by 10µM psychosine (figure 3.6 - $F_{(12, 65)} = 257.4/113.6$. $P <0.0001$, ANOVA).

2.6. Psychosine induced morphological changes in human astrocytes are attenuated by D_2 and $5HT_{2A}$ selective antagonists.

To examine further whether antagonism of dopamine and/or serotonin receptors is the mechanism by which antipsychotics protect astrocytes from psychosine (10µM) induced toxicity, the selective D_2 antagonist (Eticlopride) and $5HT_{2A}$ antagonist (Volinanserin) were used at a concentration of 1µM. Cells under control conditions showed an average number of GFAP or Vimentin-positive projections (3.20+/-0.18; 3.44+/-0.18) with psychosine treatment causing a significant decrease (0.76+/-0.06; 0.82+/-0.04), as did 20% DMSO (0.48+/-0.06; 0.64+/-0.12) (figure 3.7 - $F_{(6, 28)} = 119.6$. $P <0.0001$, ANOVA). Eticlopride (2.80+/-0.08; 2.72+/-0.05) or Volinanserin (2.40+/-0.08; 2.36+/-0.04) alone did not altered human astrocyte cell morphology compared with control, as assessed by GFAP and vimentin immunostaining (figure 3.7). In agreement with toxicity and viability studies, Eticlopride (2.64+/-0.08; 2.62+/-0.10) and Volinanserin (2.14+/-0.07; 2.18+/-0.04) significantly prevented psychosine-induced reduction in GFAP and vimentin positive projections (figure 3.7 - $F_{(6, 28)} = 119.6$. $P <0.0001$, ANOVA).

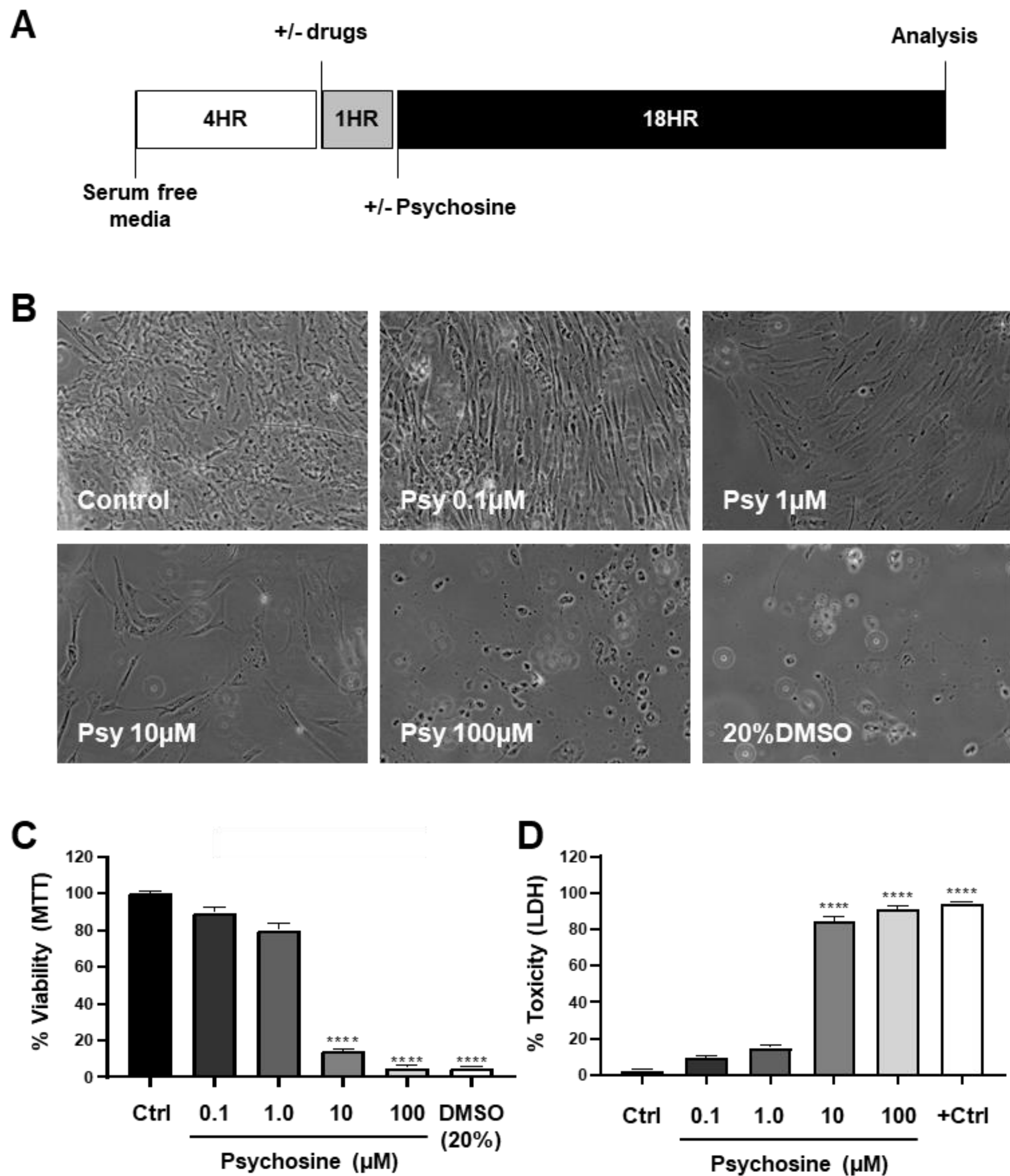


Figure 3.1 Psychosine reduces human astrocyte cell viability.
(A) Experimental schematic diagram for treatment timelines prior to analysis. **(B)** Light microscopy images of astrocytes. **(C)** MTT assays showed that psychosine decreased cell viability in a concentration dependent manner, compared to control. Positive control of 20% DMSO also impaired cell viability as expected. Kruskal-Wallis, Dunn's multiple comparison test (n=5-10). **(D)** LDH assays showed that psychosine was toxic to human astrocytes compared to control. One-way ANOVA, Tukey's multiple comparison test (n=5).

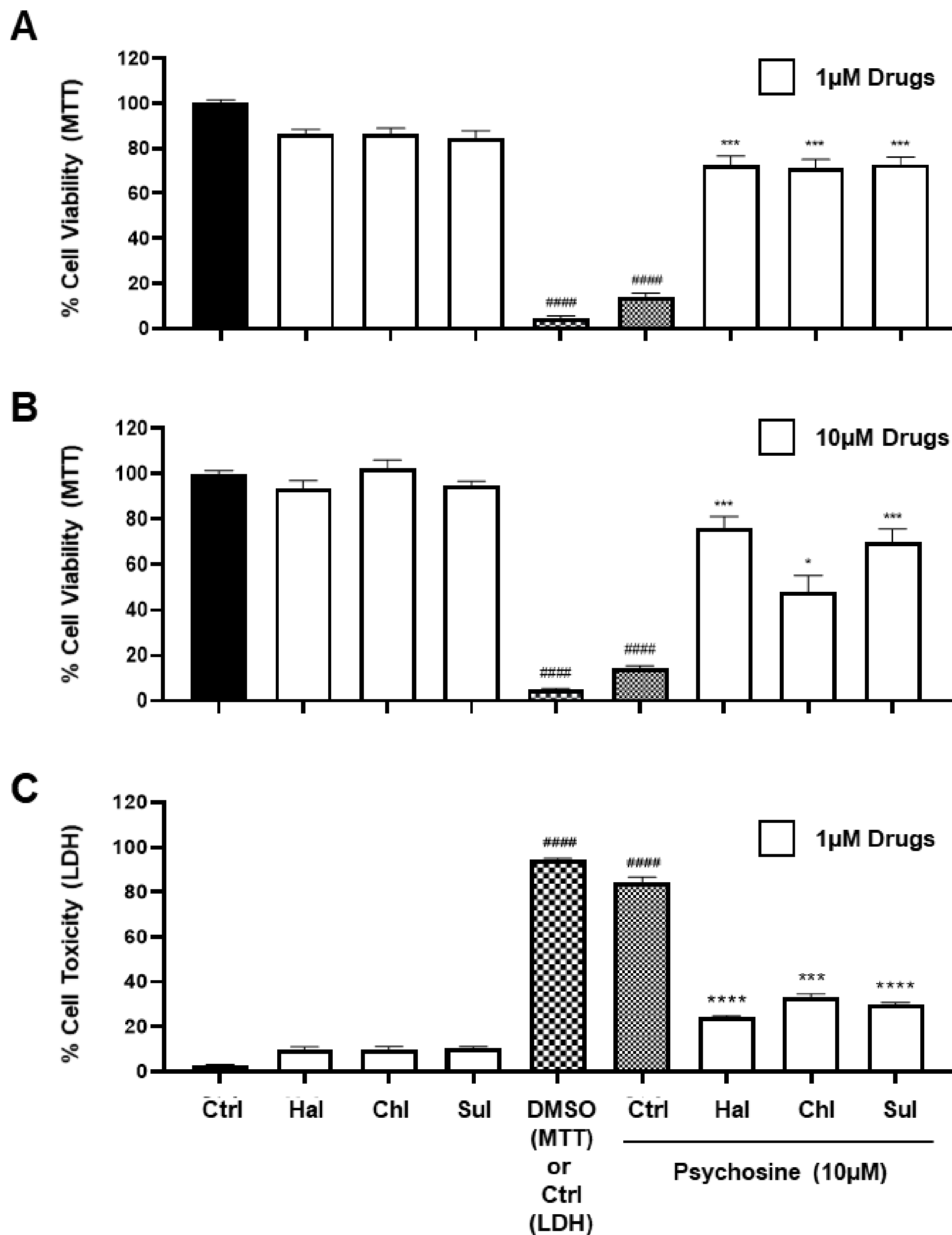


Figure 3.2. Antipsychotics with primary D_2 receptor antagonism prevented psychosine toxicity and improved astrocyte viability.

(A,B) MTT assays showed that antipsychotics haloperidol (Hal), chlorpromazine (Chl) and sulpiride (Sul) at **(A)** 1µM or **(B)** 10µM concentrations did not alter cell viability compared to control, while the positive control DMSO (20%) and psychosine (10µM) significantly reduced cell viability compared to control (####). Haloperidol (Hal), chlorpromazine (Chl) and sulpiride (Sul) at **(A)** 1µM and **(B)** 10µM concentrations significantly prevented psychosine (10µM) induced reductions in cell viability. Kruskal-Wallis, Dunn's multiple comparison test (n=5-10). **(C)** LDH assays showed that; haloperidol (Hal), chlorpromazine (Chl) and sulpiride (Sul) at 1µM were not toxic to cells compared to control. In contrast DMSO (20%) and psychosine (10µM) significantly increased cell toxicity compared to control (####). Haloperidol (Hal), chlorpromazine (Chl) and sulpiride (Sul) at 1µM prevented psychosine (10µM) toxicity. One-way ANOVA, Tukey's multiple comparison test (n=5).

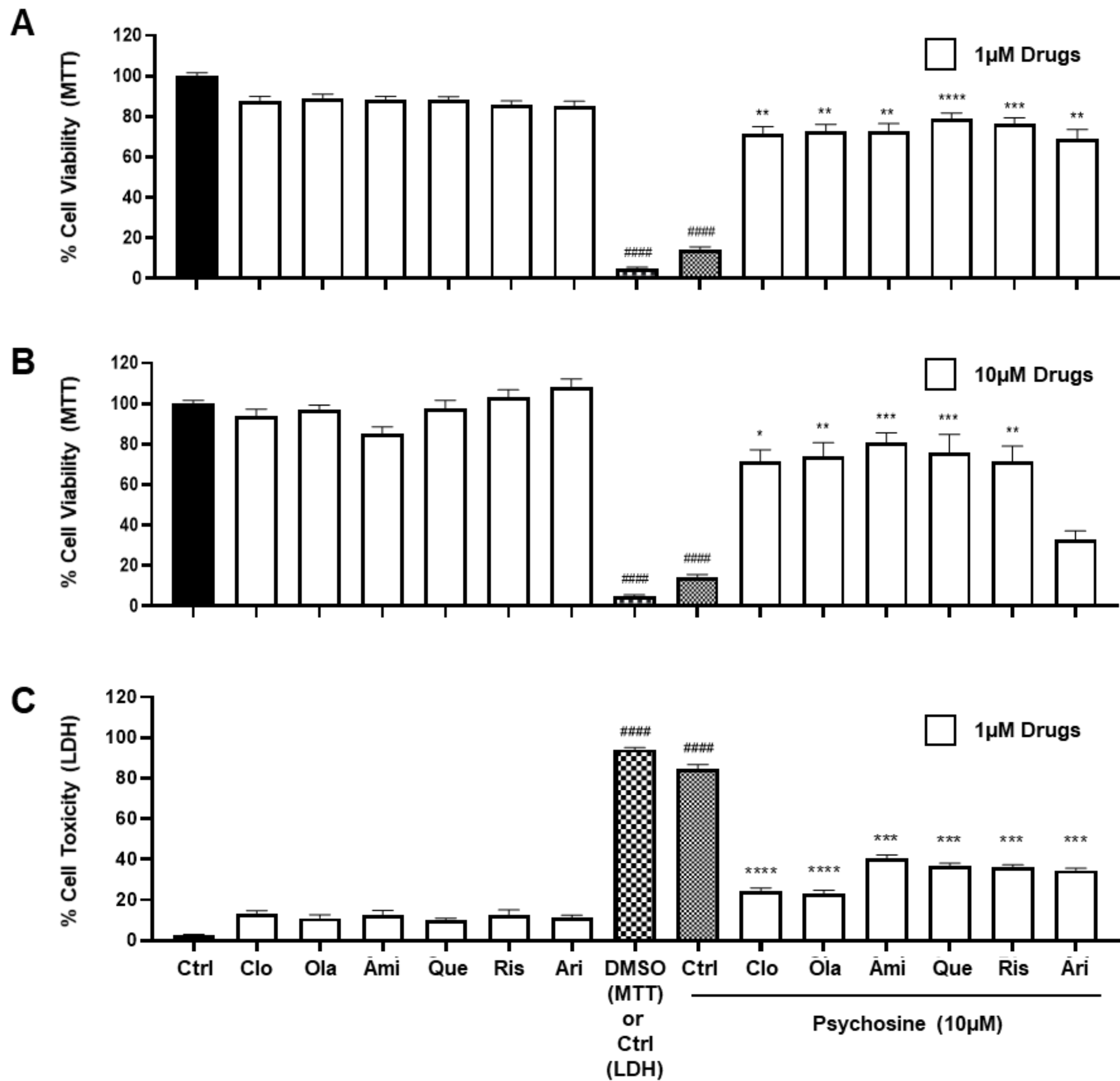


Figure 3.3. Antipsychotics with D_2 and $5HT_2$ antagonism prevented psychosine toxicity and improved astrocyte viability.

(A,B) MTT assays showed that clozapine (Clo), olanzapine (Ola), amisulpride (Ami), quetiapine (Que), risperidone (Ris) and aripiprazole (Ari) at **(A)** 1μM or **(B)** 10μM did not alter viability of human astrocytes compared with control, while DMSO (20%) and psychosine (10μM) significantly reduced cell viability compared to control (####). All antipsychotics at **(A)** 1μM or **(B)** 10μM (except aripiprazole) significantly prevented psychosine (10μM) induced reductions in cell viability. Kruskal-Wallis, Dunn's multiple comparison test (n=5-10). **(C)** LDH assays showed that clozapine (Clo), olanzapine (Ola), amisulpride (Ami), quetiapine (Que), risperidone (Ris) and aripiprazole (Ari) were not toxic to human astrocytes compared with control. In contrast DMSO (20%) and psychosine (10μM) significantly increased cell toxicity compared to control (####). All antipsychotics at 1μM attenuated psychosine (10μM) toxicity. One-way ANOVA, Tukey's multiple comparison test (n=5).

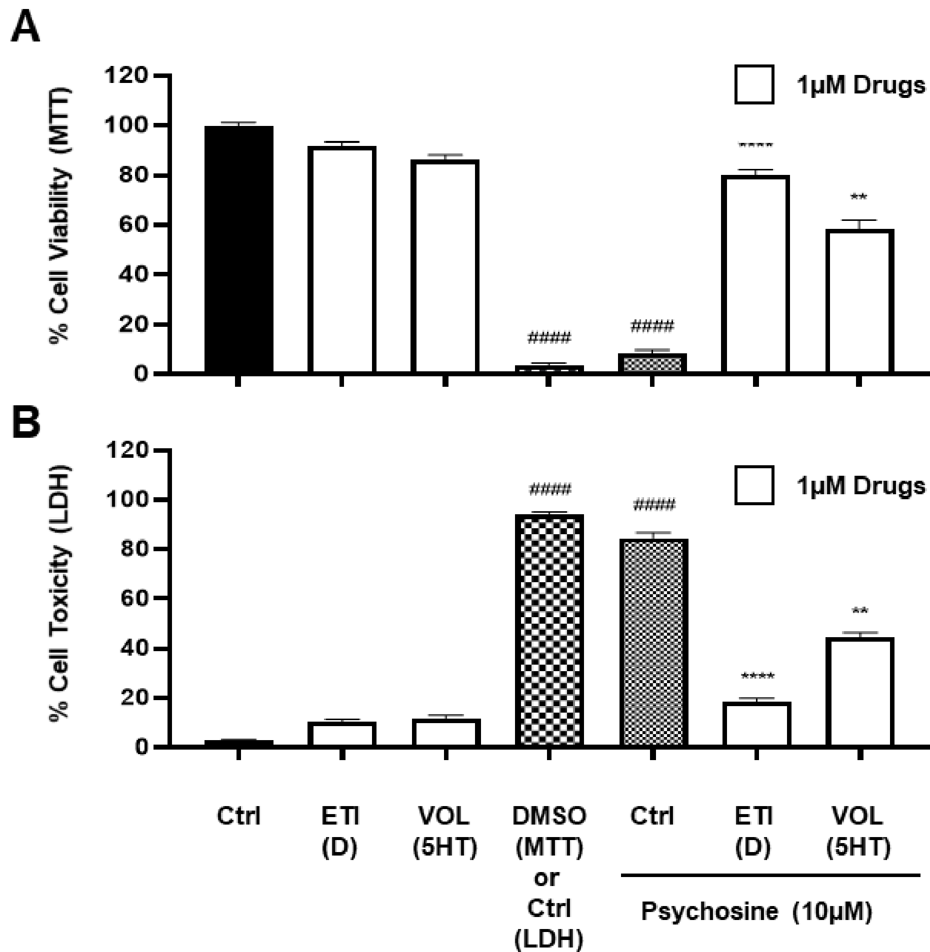


Figure 3.4. Selective D_2 and $5HT_{2A}$ antagonists prevented psychosine induced toxicity.
(A) MTT assays showed that 1µM Eticlopride (ETI) and 1µM Volinanserin (VOL) did not alter cell viability compared to control, while DMSO (20%) and psychosine (10µM) significantly reduced cell viability compared to control (####). Both 1µM Eticlopride (ETI) and 1µM Volinanserin (VOL) significantly prevented reductions in cell viability induced by 10µM psychosine Kruskal-Wallis, Dunn's multiple comparison test (n=5). **(B)** LDH assays showed that 1µM Eticlopride (ETI) and 1µM Volinanserin (VOL) did not induce cell toxicity compared to control. In contrast DMSO (20%) and psychosine (10µM) significantly increased cell toxicity compared to control (####). Both drugs significantly prevented cell toxicity induced by 10µM psychosine. One-way ANOVA, Tukey's multiple comparison test (n=5).

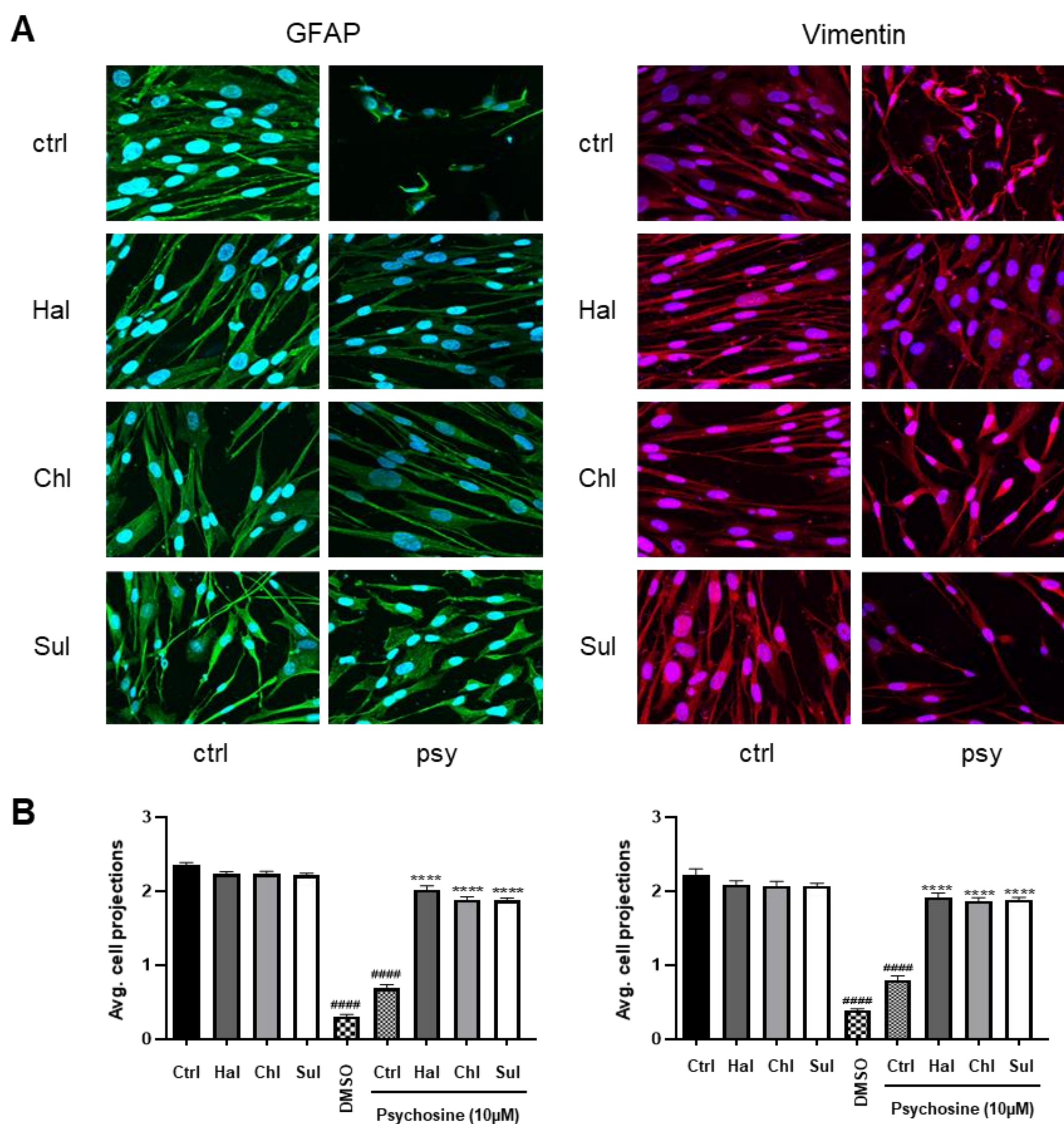


Figure 3.5. Psychosine induced morphological changes in GFAP and Vimentin were attenuated by antipsychotics with primary D_2 antagonism.

(A) Antipsychotics haloperidol (Hal), chlorpromazine (Chl) and sulpiride (Sul) at 1µM concentrations had no effect alone on astrocyte morphology compared to control, while DMSO (20%) and psychosine (10µM) reduced the number of astrocyte extensions. Haloperidol (Hal), chlorpromazine (Chl) and sulpiride (Sul) at 1µM concentrations attenuated morphological changes induced by 10µM psychosine. Representative confocal cell fluorescent images labelled for GFAP (green), Vimentin (red) and Hoescht (blue). **(B)** The number of astrocyte projections of 20-30 cells per treatment group was analysed. Images were analysed using ImageJ software. One-way ANOVA, Tukey's multiple comparison test (n=6).

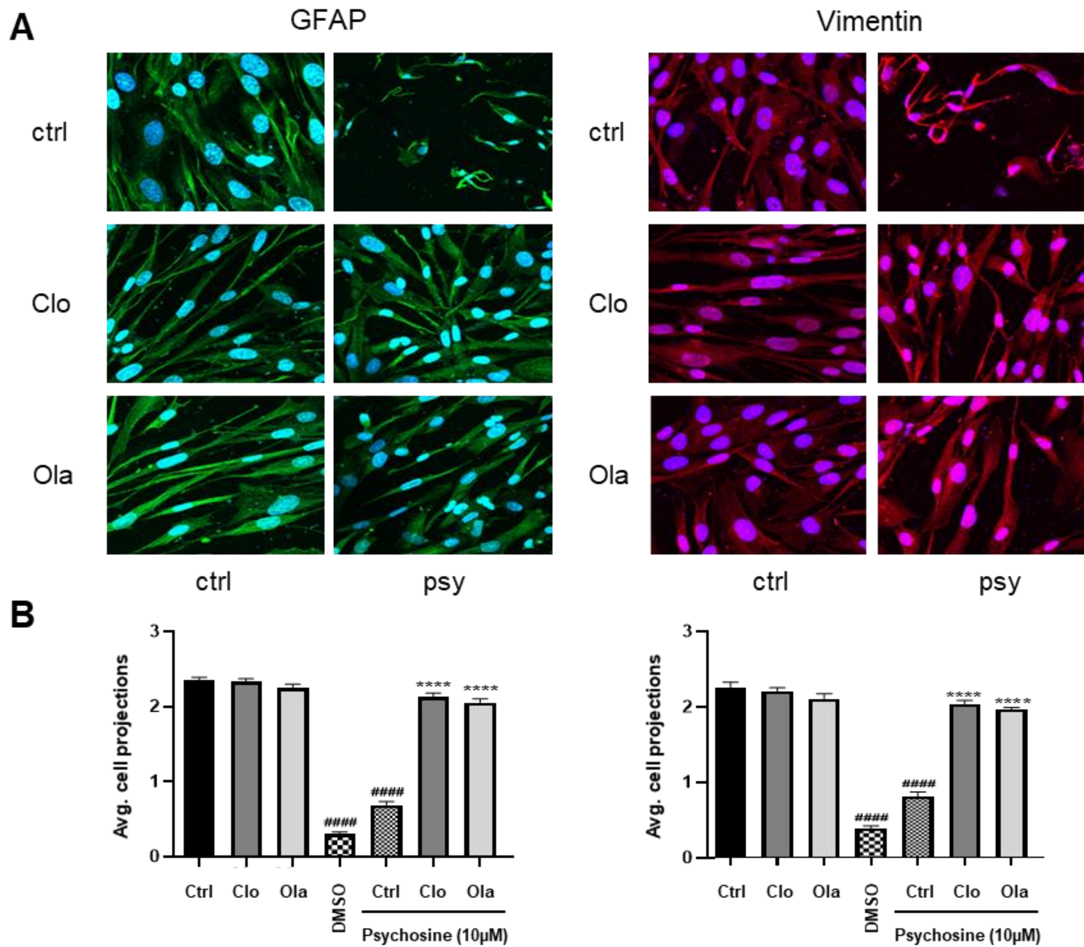


Figure 3.6. **Psychosine induced morphological changes in GFAP and Vimentin were attenuated by antipsychotics with D_2 and $5HT_2$ receptor antagonism.**

(A) Antipsychotics clozapine (Clo) and olanzapine (Ola) at 1µM concentrations had no effect alone on astrocyte morphology compared to control, while DMSO (20%) and psychosine (10µM) reduced the number of astrocyte extensions. Clozapine (Clo) and olanzapine (Ola) at 1µM concentrations attenuated morphological changes induced by 10µM psychosine. Representative confocal cell fluorescent images labelled for GFAP (green), Vimentin (red) and Hoescht (blue). **(B)** The number of astrocyte projections of 20-30 cells per treatment group was analysed. Images were analysed using ImageJ software. One-way ANOVA, Tukey's multiple comparison test (n=6).

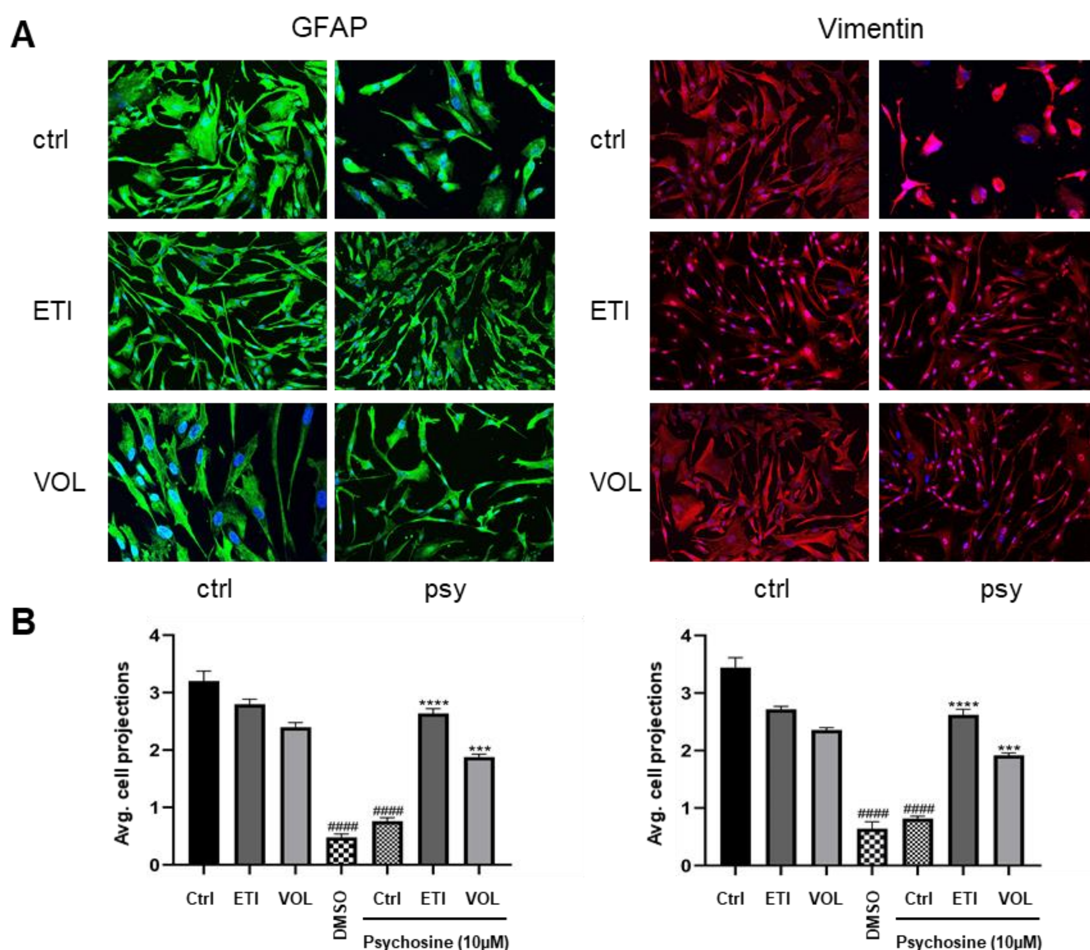


Figure 3.7. Psychosine induced reduction in GFAP and Vimentin positive projections were attenuated by selective D_2 and $5HT_{2A}$ antagonists.

(A) Selective D_2 antagonist Eticlopride (ETI) and the selective $5HT_{2A}$ antagonist Volinanserin (VOL) at 1µM concentrations had no effect alone on astrocyte morphology compared to control, while DMSO (20%) and psychosine (10µM) reduced the number of astrocyte extensions. Both selective antagonists attenuated morphological changes induced by 10µM psychosine, with some preferential effect noted with Eticlopride. Representative confocal cell fluorescent images labelled for GFAP (green), Vimentin (red) and Hoescht (blue). **(B)** The number of astrocyte projections of 20-30 cells per treatment group was analysed. Images were analysed using ImageJ software. One-way ANOVA, Tukey's multiple comparison test (n=6).

3. Discussion.

3.1. Summary of Findings.

Schizophrenia is a chronic mental illness which is also linked with significant physical morbidity and mortality (Goff et al., 2005). Glial cell dysfunction may be linked to the disorder and these cells could provide a therapeutic focus (H. G. Bernstein et al., 2015; Andrea G Dietz et al., 2019; Takahashi & Sakurai, 2013). This study aimed to investigate if antipsychotics would affect a cellular model of glial cell dysfunction, using human astrocyte cell cultures. The hypothesis was that antipsychotics would ameliorate glial cell dysfunction after exposure to a toxin, in this case the toxin psychosine. In agreement with previous data, psychosine reduced human astrocyte viability. Moreover, data showed that antipsychotics significantly improved astrocyte viability as well as reduced cell toxicity after psychosine exposure. In addition, antipsychotics attenuated morphological changes induced by psychosine, where these drugs attenuated the loss of extensions in human astrocytes. Pharmacological analysis pointed toward possible involvement of D_2 and/or $5HT_{2A}$ receptors in these effects. In agreement with this hypothesis, selective D_2 and/or $5HT_{2A}$ receptors antagonists, similar antipsychotics, significantly attenuated psychosine toxicity to human astrocytes. To our knowledge this study is the first to examine the direct effects of such a broad selection of commonly prescribed antipsychotics on human astrocytes.

3.2. Current treatments and the role of glial cells in schizophrenia.

Neurobiological research of schizophrenia has focused on neuronal abnormalities with hyperdopaminergia, hypoglutaminergia and hypoGABAergia in various neuronal pathways being linked to the positive, negative and cognitive symptoms of the condition (Lewis, Curley, Glausier, & Volk, 2012; Ross et al., 2006). However, compelling evidence accrued over the past two decades has implicated glial cell dysfunction in schizophrenia. These include structural and functional abnormalities in astrocytes, oligodendrocytes and microglia as well as glial progenitor cells (H. G. Bernstein et al., 2015; Andrea G Dietz et al., 2019; Takahashi & Sakurai, 2013). Antipsychotics are the foremost pharmacological management for schizophrenia and although efficacious, they have significant limitations. They are less effective towards negative and cognitive symptoms compared to positive, they have a range of side effects that may reduce adherence and a proportion of people remain treatment resistant (Patel et al., 2014). There is increased interest in the effects of antipsychotics as well as other compounds that effect glial

cells, in the hope of discovering novel mechanisms of action of these drugs or new compounds for treating schizophrenia (Takahashi & Sakurai, 2013). Previous studies have revealed that antipsychotics may effect glial cells, for example they are thought to improve disturbed metabolism in schizophrenia by binding to astrocyte dopamine receptors (Takahashi & Sakurai, 2013). Clozapine has shown to have direct effects on astrocytes by enhancing the release of D-serine (Tanahashi et al., 2012) and reducing glutamate uptake in these cells (Vallejo-Illarramendi et al., 2005). Contrastingly, some studies highlight the concern that chronic antipsychotic use may contribute to progressive grey matter loss perhaps by preferentially targeting astrocytes (Dorph-Petersen et al., 2005; Konopaske et al., 2008).

3.3.Psychosine toxicity as a model of glial cell dysfunction.

In this study we used psychosine (galactosylsphingosine) as a toxin to induce astrocyte dysfunction. Psychosine is a toxin that aggregates in the brains of those afflicted with the neurodegenerative disorder globoid cell leukodystrophy, Krabbe disease (KD) (Debs et al., 2012). KD is a rare condition that mainly presents during infancy, but juvenile and adult presentations are possible. Progressive demyelination likely due to the toxic effects of psychosine towards oligodendrocytes is associated with the condition. Psychosine is thought to negatively effect astrocyte viability possibly via apoptotic processes and is also proposed to be pro-inflammatory, the above are thought to lead to the clinical characteristics of the disorder (Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016; Won, Singh, & Singh, 2016). Interestingly, the association between white matter pathology including leukodystrophies and schizophrenia like psychosis has been reported although, not directly in KD (Walterfang et al., 2005). Given the association between glial cell dysfunction with schizophrenia, we utilised psychosine to induce glial cell dysfunction and to examine the possible glial protective effects of antipsychotics. This work confirmed the findings of previous studies that showed psychosine is toxic towards astrocytes (Debs et al., 2012; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016; Walterfang et al., 2005). Notably, psychosine-induced toxicity was reversed by all the antipsychotics used in this current study.

3.4. D_2 and $5HT_{2A}$ antagonism protects against psychosine induced glial cell toxicity.

Pharmacological analysis showed that most antipsychotics have a broad receptor profile, although there is general commonality to act via antagonism or inverse agonism of dopamine and/or serotonin receptors. Having demonstrated that antipsychotics reverse glial cell dysfunction induced by psychosine, we aimed to investigate if these receptor subtype(s) play a role in protection against psychosine induced astrocyte cell toxicity. To do this, the selective D_2 antagonist (Eticlopride) and $5HT_{2A}$ antagonist (Volinanserin) were used. The data showed that both D_2 antagonist (Eticlopride) and $5HT_{2A}$ antagonist (Volinanserin) reduced psychosine-induced toxicity, although D_2 antagonism appeared to have stronger efficacy compared to $5HT_{2A}$ inhibition. These findings are in agreement with previous data demonstrating the expression of D_2 and $5HT_{2A}$ receptors in astrocytes and their role in regulating intercellular signalling in these cells (J. M. Beaulieu et al., 2015; Berger et al., 2009). The results demonstrate possible utility of D_2 and $5HT_{2A}$ antagonists in rescuing astrocyte cell toxicity. While we did not set out to directly investigate the use of dopamine and serotonin receptors as drug target for KD, these findings provide early *in vitro* evidence supporting the investigation of D_2 and $5HT_{2A}$ antagonists in this disease. Possible repositioning of D_2 and $5HT_{2A}$ antagonists in KD could be supported by investigating further the effects of these drugs in psychosine-induced demyelination in organotypic slice cultures followed by testing in the twitcher mouse model of KD, as we have done previously with other marketed therapies (Béchet et al., 2020; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016).

3.5. Conclusion.

A psychosine cellular toxicity model was used to assess effects of antipsychotics on human astrocytes. It was confirmed that psychosine reduced human astrocyte viability. It was hypothesised that antipsychotics would ameliorate psychosine induced toxicity. In agreement, this current study demonstrated that a range of antipsychotics, as a broad class of medication, exert a protective effect on human astrocytes against psychosine-induced toxicity. Furthermore, it appears that D_2 and $5HT_{2A}$ antagonism is mechanistically important for this protection, with D_2 antagonism possibly being more effective. To our understanding this study is the first to examine the direct effects of such a broad selection of commonly prescribed antipsychotics on human astrocytes.

Chapter 4 – Antipsychotics attenuate psychosine-induced demyelination in organotypic cerebellar slice model.

Chapter Aims

- To confirm that psychosine induces demyelination in mouse organotypic cerebellar slice culture *ex-vivo*. Also, to verify that psychosine is toxic to astrocytes, causes axonal damage but does not effect microglia in this ex-vivo model.
- To investigate if haloperidol can prevent psychosine induced demyelination and astrocyte toxicity in mouse organotypic cerebellar slice culture *ex-vivo*.
- To investigate if clozapine can prevent psychosine induced demyelination and astrocyte toxicity in mouse organotypic cerebellar slice culture *ex-vivo*.
- To determine if haloperidol and clozapine have any effect on microglia.
- To investigate if haloperidol and clozapine prevent psychosine induced axonal damage.

Abstract

The role of altered myelin in the onset and development of schizophrenia and changes in myelin due to antipsychotics remains unclear. There exists imparity insofar as antipsychotics are D_2 receptor antagonists, yet D_2 receptor agonists increase the number of oligodendrocyte progenitors and protect against oligodendrocyte injury. There is further inconsistency over the effect of antipsychotics on oligodendrocytes, where some studies suggest these drugs promote differentiation of neural progenitors to oligodendrocyte lineage, while others report antipsychotics to inhibit proliferation and differentiation of oligodendrocyte precursor cells. Here, we investigated the effects of haloperidol and clozapine, on levels of myelin using mouse organotypic cerebellar slices treated with the demyelinating agent psychosine. Psychosine induced a concentration-dependent loss of myelin, where at 100uM there was a loss of myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) expression, with no change in the neuronal marker neurofilament H (NFH). Importantly, both haloperidol and clozapine, reduced psychosine-induced loss of MOG and MBP expression. Similarly, these drugs attenuated psychosine-induced loss of astrocyte markers glial fibrillary acidic protein (GFAP) and vimentin. As demonstrated previously, psychosine induced demyelination without involvement of microglial, showing no change in expression of Iba1 or microglia morphology, where haloperidol and clozapine had no further effect. Lastly, psychosine induced a decrease in nonphosphorylated neurofilament levels, which were restored by both haloperidol and clozapine, indicating neuroprotective and neurorestorative processes. Overall, this work points toward the protective effects of antipsychotics on oligodendrocyte toxicity and the positive effects of these drugs on myelination.

Keywords: myelination, antipsychotics, psychosine, D_2 receptors, $5HT_{2A}$ receptors

1. Introduction.

Schizophrenia is a chronic condition involving the complex interplay between genetic and environmental factors and thought to be associated with aberrant neurodevelopment (Goff et al., 2005; K. E. O'Connell et al., 2014; Schwab & Wildenauer, 2013). This illness has a global prevalence of approximately 1% and an incidence of 1-2 per 10,000 (McGrath et al., 2008), with individuals displaying positive and negative symptoms as well as cognitive decline (Patel et al., 2014). Schizophrenia is associated with abnormalities in grey and white matter as well as loss of cerebral volume and anatomical pathology (Katherine H. Karlsgodt et al., 2010; Ross et al., 2006). While neuronal dysfunction has been well studied in schizophrenia, the role of glial cells in the pathophysiology of this disease is still emerging and, in particular, the role of altered oligodendrocyte biology is less clear (H. G. Bernstein et al., 2015; Andrea G Dietz et al., 2019). While differing theories exist, there is no clear consensus to whether altered levels of myelin is linked with the onset of this illness, or if altered myelination is a cause or consequence associated with development of the condition, and/or how levels of myelin change during short and long-term use of antipsychotic treatment. There is agreement, however, that patients with schizophrenia have decreased white matter volume and integrity, where post-mortem studies demonstrate white matter pathology linked to deficits in myelin, axons and mature oligodendrocytes (Chew, Fusar-Poli, & Schmitz, 2013; Kroken et al., 2014). In addition, altered expression and risk variants of several genes playing a role in oligodendrocyte maturation and myelination have also been associated with this illness, where variants in myelin-related genes increase schizophrenia susceptibility (Balla & Frecska, 2011; McCullumsmith et al., 2007; Monji et al., 2013; Oertel-Knöchel et al., 2015; Ren, Wang, & Xiao, 2013; Samartzis et al., 2013; Thomas, 2006).

Antipsychotic treatment of patients with schizophrenia has also been suggested to directly regulate the expression of genes associated with oligodendrocyte maturation and myelination and thus influence white matter integrity (Chew et al., 2013; Kroken et al., 2014). The effects of antipsychotics are however somewhat controversial, where studies suggest these drugs either promote proliferation and differentiation of neural progenitors to oligodendrocyte lineage or instead can inhibit differentiation of oligodendrocyte precursor cells (Kroken et al., 2014; Ren et al., 2013). Antipsychotics, such as clozapine, have been demonstrated to reduce neuroinflammation and demyelination in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS) and enhance the rate of functional recovery in the

cuprizone non-immune model of demyelination (Ceylan et al., 2021; Templeton et al., 2019). When considering the pharmacology of antipsychotics regulating levels of myelin, it is known that hyper-dopaminergia in the basal ganglia is linked with schizophrenia, where traditionally "typical" antipsychotics have dopamine D_2 receptor subtype antagonism and "atypical" antipsychotics have serotonin e.g., $5HT_{2A}$ receptor antagonism (Stahl, 2008). The issues with classifying antipsychotics into "typical" and "atypical" have been discussed in section 1.3. It has been reported that oligodendrocytes express dopamine D_2 receptors, of which expression levels increase following combined oxygen and glucose deprivation (Rosin, Colombo, Calver, Bates, & Skaper, 2005). Dopamine action on D_2 receptors can affect myelin formation by regulating the development and function of oligodendrocytes (Bongarzone, Howard, Schonmann, & Campagnoni, 1998; Howard et al., 1998). In addition, dopamine receptors have been shown to play a role in development of EAE and MS (Lieberknecht et al., 2016). Paradoxically to antipsychotics that are D_2 receptor antagonists, studies show that D_2 agonism increases the number of oligodendrocyte progenitor cells and protects oligodendrocytes against oxidative injury (Rosin et al., 2005), which may explain the therapeutic effects of some antipsychotics that are partial D_2 receptor agonists. Moreover, studies suggest the protective effect of D_2 agonists can be diminished by D_2 antagonists, suggesting that D_2 receptor activation may play an important role in oligodendrocyte protection against injury, whereas antipsychotics may restrict oligodendrocyte recovery to injury. Interestingly however, haloperidol and clozapine prevented apoptotic cell death in oligodendrocytes that were cultured under glucose deprived conditions, highlighting their potential protective effects (Johann Steiner et al., 2011).

Given the uncertainty of the effects of antipsychotics on oligodendrocytes, the aim of this study was to examine the direct effects of haloperidol and clozapine, on levels of myelin in mouse organotypic cerebellar slices. Here we used the toxin psychosine, which is associated with Krabbe disease (KD) and induces demyelination in a manner that does not involve an overt inflammatory component (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016). The data here demonstrate both drugs, haloperidol and clozapine, rescue psychosine-induced demyelination, supporting the idea that antipsychotics have a positive role in myelin state.

2. Results.

2.1. Haloperidol and clozapine attenuate psychosine induced demyelination in cultured cerebellar slices.

Studies have shown that psychosine causes demyelination in cultured cerebellar slices (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016). Here we induced demyelination in slices prepared from 10-day old (P10) C57BL/6J mice, cultured for 12 DIV and treated with or without psychosine in the presence or absence of haloperidol or clozapine, with analysis conducted at 14 DIV (figure 4.1). Slices were treated with 10nM, 100nM, 1000nM psychosine, which demonstrated a concentration dependent decrease, in both myelin markers MOG (93.29 +/- 4.26%, 35.89 +/- 1.23%, 13.74 +/- 0.77%) (figure 4.1 – H(3) = 169.6, P = >0.9999, <0.0001, <0.0001 Kruskal-Wallis test) and MBP (118.00 +/- 4.67%, 51.26 +/- 2.11%, 45.00 +/- 1.75%) (figure 4.1 – H(3) = 151.5, P = 0.6253, <0.0001, <0.0001), comparative to NFH (92.31 +/- 25.44%, 89.94 +/- 22.77%; 46.71 +/- 14.88%) (figure 4.1 – H(3) = 104.6 P = 0.4629, 0.4699, <0.0001) relative to control. To examine whether haloperidol 10µM (figure 4.2) or clozapine 10µM (figure 4.3) prevented psychosine induced demyelination, cerebellar slices were treated with these drugs in the presence and absence of psychosine. Neither haloperidol 10µM (MOG: 91.67 +/- 2.731%; MBP: 98.11 +/- 3.14%) (figure 4.2 – H (3) = 129.2/120.7, P = 0.0580, > 0.9999, Kruskal-Wallis test) or clozapine 10µM (MOG: 111.9 +/- 4.23%; MBP: 100.60 +/- 2.72%) (figure 4.3 - H(3) = 113.7/133.5, P = 0.8785, >0.9999, Kruskal-Wallis test) individually caused significant change in MOG or MBP fluorescence compared to control. Mean NFH fluorescence was also not significantly affected by psychosine 100nM (89.94 +/- 22.77%), haloperidol 10µM (99.32 +/- 18.21%) or clozapine 10µM (96.38 +/- 18.65%). Importantly, psychosine 100nM induced decreased in mean MOG and MBP fluorescence, which was attenuated by haloperidol 10µM (MOG: 45.44 +/- 1.49% vs 83.84 +/- 2.36%; MBP: 41.82 +/- 1.60% vs 89.16 +/- 2.32%) (figure 4.2 - H(3) = 129.2/120.7, P = <0.0001, 0.0001, Kruskal-Wallis test) and clozapine 10µM (MOG: 46.06 +/- 2.06% vs 100.6 +/- 3.27%; MBP: 56.27 +/- 1.73% vs 72.80 +/- 2.16%) (figure 4.3 - H(3) = 113.7/133.5, P = <0.0001, 0.0044, Kruskal-Wallis test). These data cumulatively suggest that haloperidol and clozapine exert some myelin protective effect against psychosine induced demyelination.

2.2. Haloperidol and clozapine reduce psychosine induced loss of GFAP and vimentin expression.

Psychosine is toxic to astrocytes derived from multiple species including humans and mice (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015). Here, mouse organotypic cerebellar slices exposed to increasing concentrations of psychosine (10nM, 100nM and 1000nM) showed a concentration-dependent decrease in mean fluorescence of GFAP (105.8 +/- 3.29%, 45.46 +/- 1.89%, 32.30 +/- 1.94%) and vimentin (93.88 +/- 9.70%, 30.12 +/- 9.66%, 21.86 +/- 4.39%) (figure 4.4 - H(3) = 155.1/159.9, P = >0.9999, <0.0001, <0.0001, 0.3326, <0.0001, 0.0001, Kruskal-Wallis test). Neither haloperidol 10µM or clozapine 10µM individually had a significant effect on mean fluorescence of GFAP (Hal: 94.15 +/- 14.17%, Clo: 102.4 +/- 11.65%) or vimentin (Hal: 95.45 +/- 1.71%, Clo: 90.67 +/- 2.17%). In this set of experiments, the decrease in GFAP and vimentin florescence induced by 100nM psychosine was attenuated by haloperidol 10µM (GFAP: 31.19 +/- 7.64% vs 90.97 +/- 11.16%, vimentin: 23.81 +/- 0.83% vs 88.88 +/- 1.45%) (figure 4.4 - H(3) = 126.3/133.9, P = <0.0001, 0.0001 Kruskal-Wallis test) and clozapine 10µM (GFAP: 42.25 +/- 9.76% vs 74.04 +/- 7.88%, vimentin: 26.54 +/- 0.64% vs 60.71 +/- 1.44%) (figure 4.4 - H(3) = 169/168.9, P = <0.0001, 0.0040, Kruskal-Wallis test). These data illustrate that haloperidol and clozapine prevented psychosine astrocyte toxicity in organotypic cerebellar slice cultures.

2.3. Haloperidol and clozapine attenuate psychosine-induced demyelination independent of changes to microglia.

Psychosine-induced demyelination in organotypic cerebellar slices occurs without change in microglia activation and morphology (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016). Therefore, we examined if alterations in microglia morphology or microglia activation influenced the protective influence of haloperidol and clozapine on psychosine induced demyelination. No significant change in mean Iba1 fluorescence was observed in white matter tracts (WM) or across whole cerebellar slices (whole) in psychosine 100nM (WM: 100.10 +/- 3.26%, whole: 98.84 +/- 2.76%), haloperidol 10µM (WM: 113.10 +/- 3.83%, whole: 93.59 +/- 2.51%) and psychosine plus haloperidol (WM: 101.7 +/- 4.05%, whole: 92.16 +/- 2.99%) groups relative to control (figure 4.5 - H(3) = 8.45, P = 0.7122, >0.9999, >0.9999, Kruskal-Wallis test). No significant change in mean Iba1 fluorescence was observed in white matter tracts (WM) or across whole cerebellar slices

(whole) in psychosine 100nM (WM: 94.66 +/- 2.29%, whole: 98.09 +/- 1.97%), clozapine 10µM (WM: 107.6 +/- 2.73%, whole: 95.50 +/- 2.35%) and psychosine plus clozapine (WM: 98.41 +/- 2.78%, whole: 99.59 +/- 1.94%) groups compared to control (figure 4.5 - H(3) = 11.39, P = 0.9721, 0.4306, >0.9999, Kruskal-Wallis test). These data re-confirm the finding that psychosine toxicity in organotypic cerebellar slices occurs independently to microglia activation/morphology. They also suggest that the protective effects of haloperidol and clozapine in attenuating the toxicity of psychosine also occur independently to changes in microglia activation and morphology.

2.4. Haloperidol and clozapine prevented psychosine induced axonal damage in white matter tracts of cultured cerebellar slices.

Previous studies have shown that psychosine causes axonal damage in organotypic cerebellar slices demonstrated by an increase in mean SMI-32 fluorescence within white matter tracts (Clementino et al., 2021; Misslin et al., 2017). In agreement, here we found that psychosine induced a concentration-dependent change in mean SMI-32 fluorescence in white matter tracts of cerebellar slices compared to control, 10nM (103.7 +/- 5.24%), 100nM (208.20 +/- 8.80%) and 1000nM (325.70 +/- 13.78%) (figure 4.6 - H(3) = 156., P = >0.9999, <0.0001, <0.0001, Kruskal-Wallis test). No change, relative to control, was seen in mean SMI-32 fluorescence at psychosine 10nM (110.60 +/- 4.69%), 100nM (116.30 +/- 4.48%) or 1000nM (111.8 +/- 3.90%) when the whole cerebellar slice was examined (figure 4.6 - H(3) = 156., P = >0.9999, <0.0568, , 0.5255, Kruskal-Wallis test). To examine whether haloperidol or clozapine attenuated psychosine induced axonal damage in white matter tracts of organotypic cerebellar slices, slices were treated with these drugs with and without psychosine. In this case, the psychosine 100nM induced increase in mean SMI-32 fluorescence in white matter tracts of cerebellar slices was attenuated by haloperidol 10µM (167.9 +/- 3.72% vs 71.52 +/- 2.99%) (figure 4.6 - H(3) = 157.7, P = <0.0001, Kruskal-Wallis test) and clozapine 10µM (214.30 +/- 9.68% vs 136.4 9 +/- 6.67%) (figure 4.6 - H(3) = 105.8, P = <0.0001, Kruskal-Wallis test). No change was observed across the whole cerebellar slice. These data confirm that psychosine causes axonal damage in white matter tracts of organotypic cerebellar slices. They also suggest that haloperidol and clozapine attenuate this axonal damage.

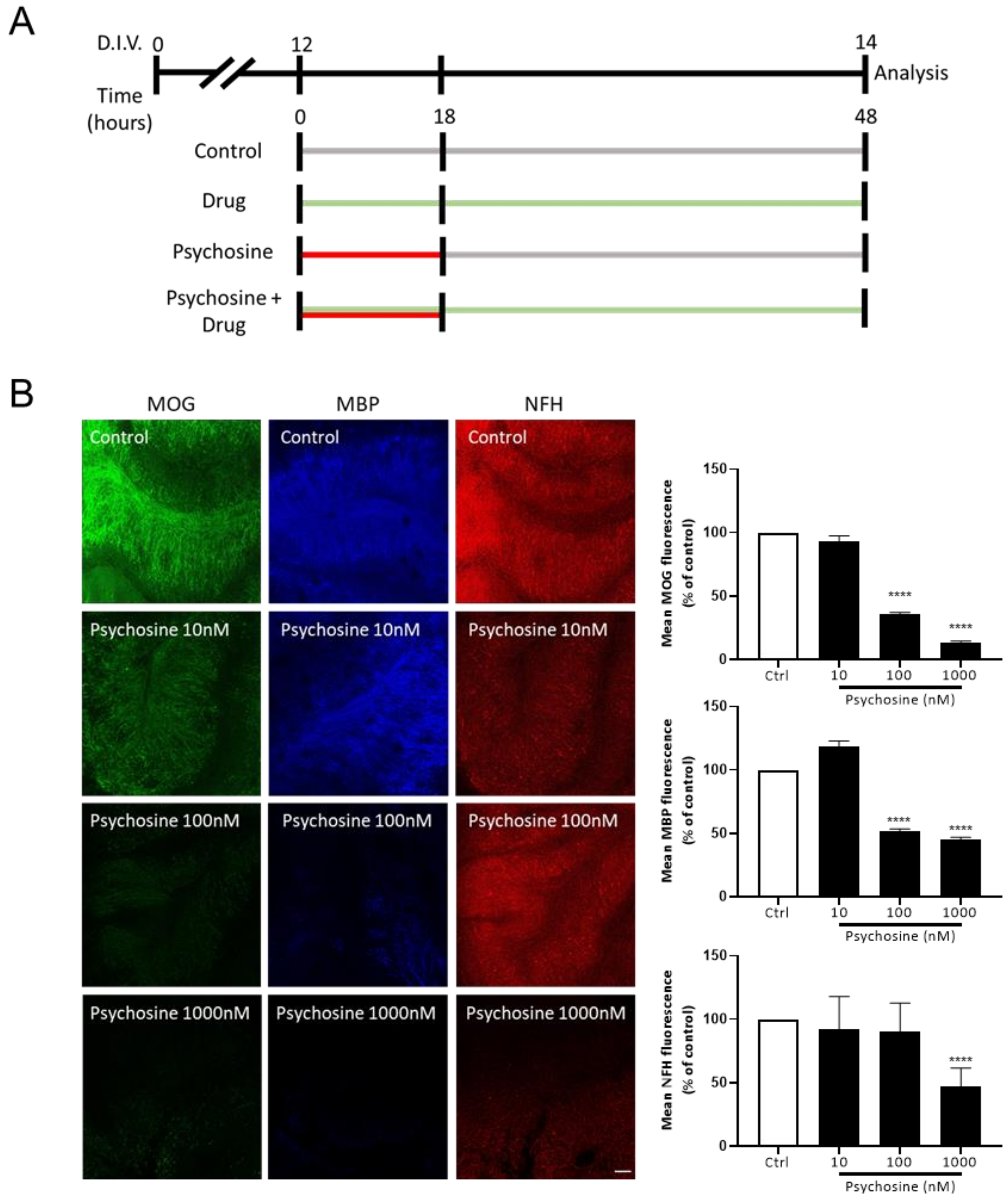


Figure 4.1. Psychosine induces demyelination in organotypic cerebellar slices.
(A) Experimental design schematic, cerebellar slices from 10-day old C57BL/6J mice were cultured 12 days in-vitro (DIV) before treatment with or without psychosine and/or antipsychotics. Immunohistochemical analysis was performed at 14 DIV. **(B)** Decrease in MOG and MBP fluorescence was seen at 100nM and 1000nM concentrations of psychosine. NFH fluorescence was reduce at 1000nM, but not 100nM psychosine. Confocal images at 10 X magnification, scale bar 100µm. Data are shown as mean +/- SEM, Kruskal-Wallis test, Dunn's multiple comparisons test, ****p<0.0001 (n=5-10).

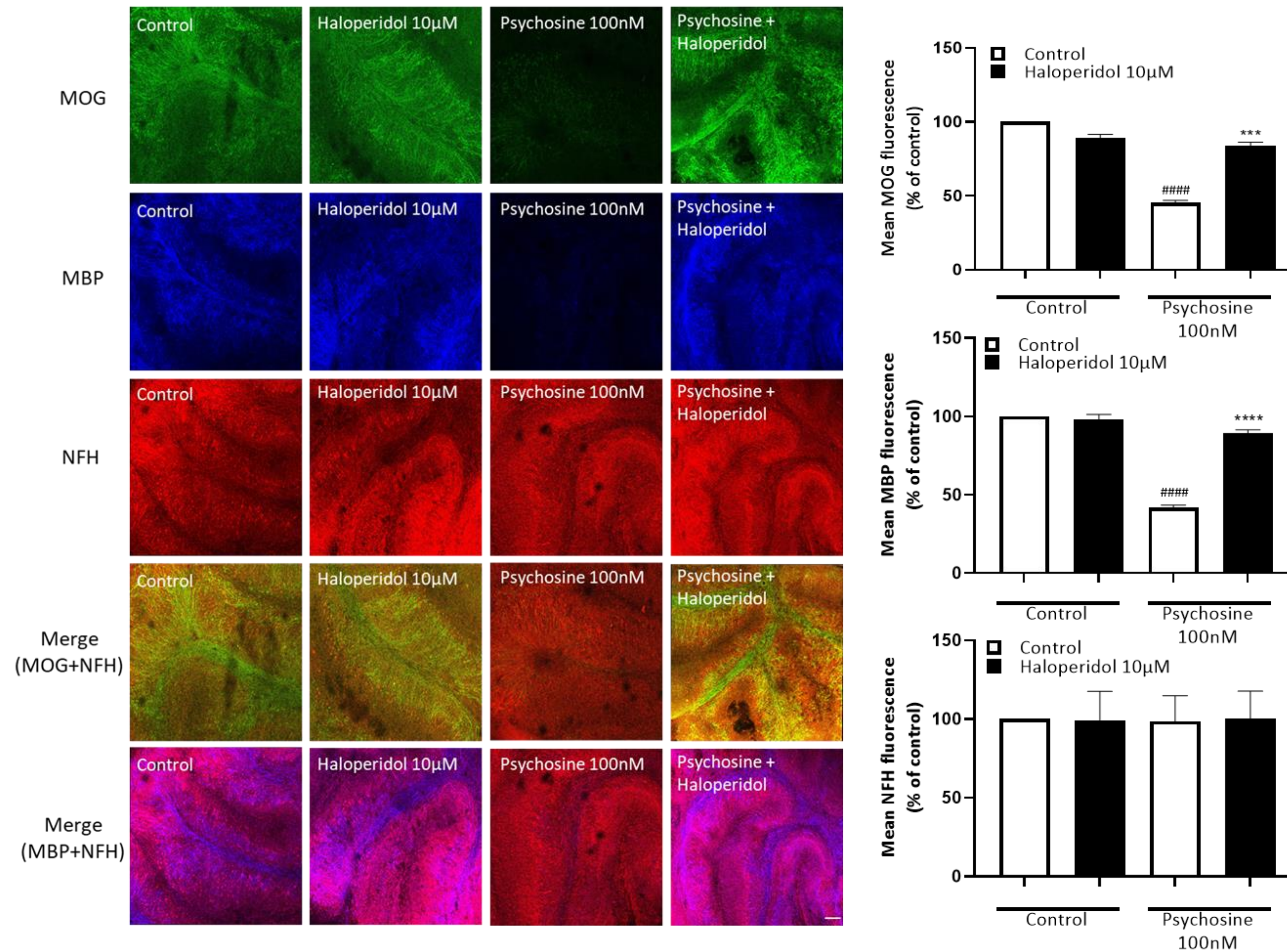


Figure 4.2. Haloperidol attenuated psychosine induced demyelination in cerebellar slices.
Psychosine 100nM decreased MOG and MBP, but not NFH fluorescence compared to control that was attenuated by haloperidol 10µM. Representative confocal images at 10 X magnification, scale bar 100µm. Data are shown as mean +/- SEM, Kruskal-Wallis test, Dunn's multiple comparisons test, $^{####}p<0.0001$, $^{****}p<0.0001$ (n=5-10).

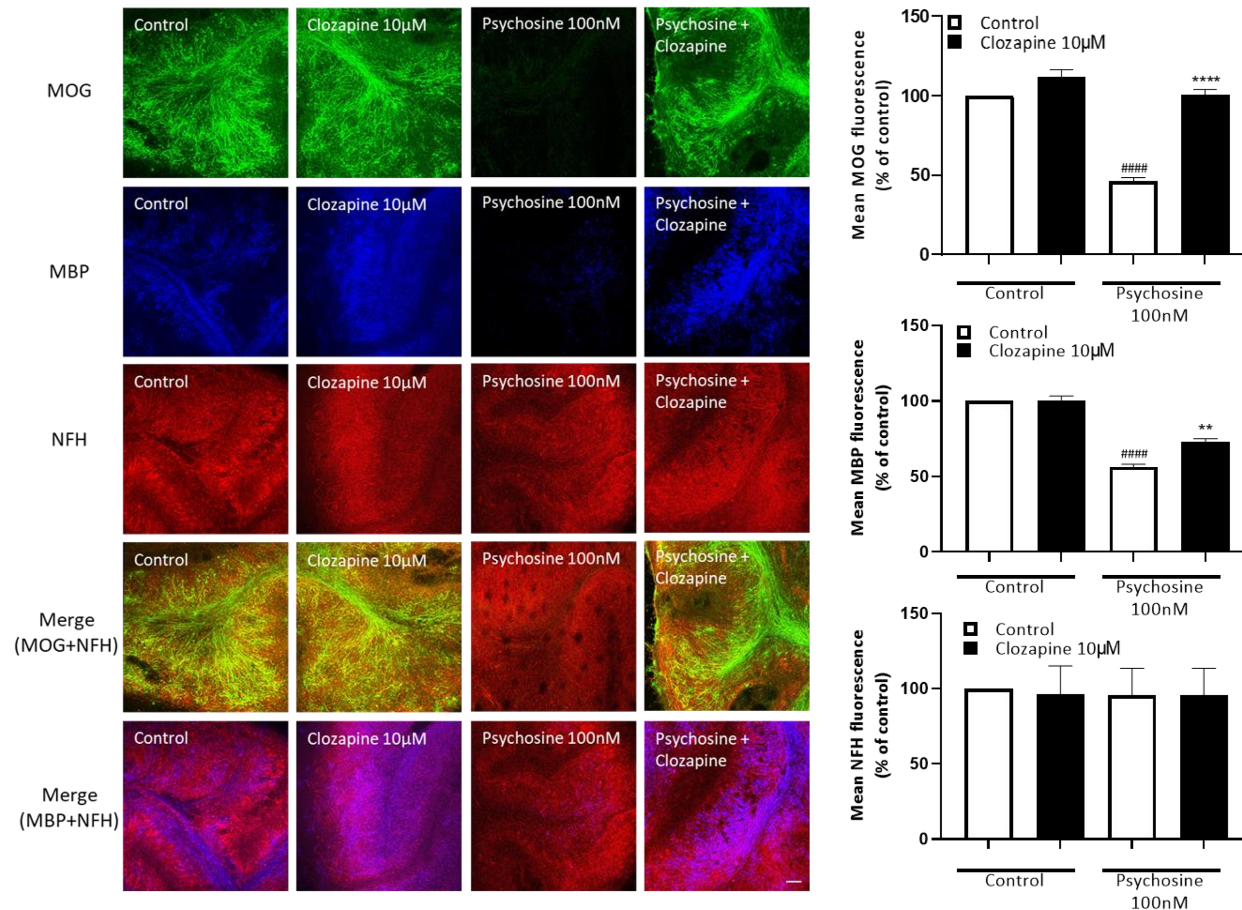


Figure 4.3. Clozapine attenuated psychosine induced demyelination in cerebellar slices.
Psychosine 100nM decreased MOG and MBP, but not NFH fluorescence compared to control that was attenuated by clozapine 10µM. Representative confocal images at 10 X magnification, scale bar 100µm. Data are shown as mean +/- SEM, Kruskal-Wallis test, Dunn's multiple comparisons test, $^{\#\#\#\#}p<0.0001$, $^{****}p<0.0001$ (n=5-10).

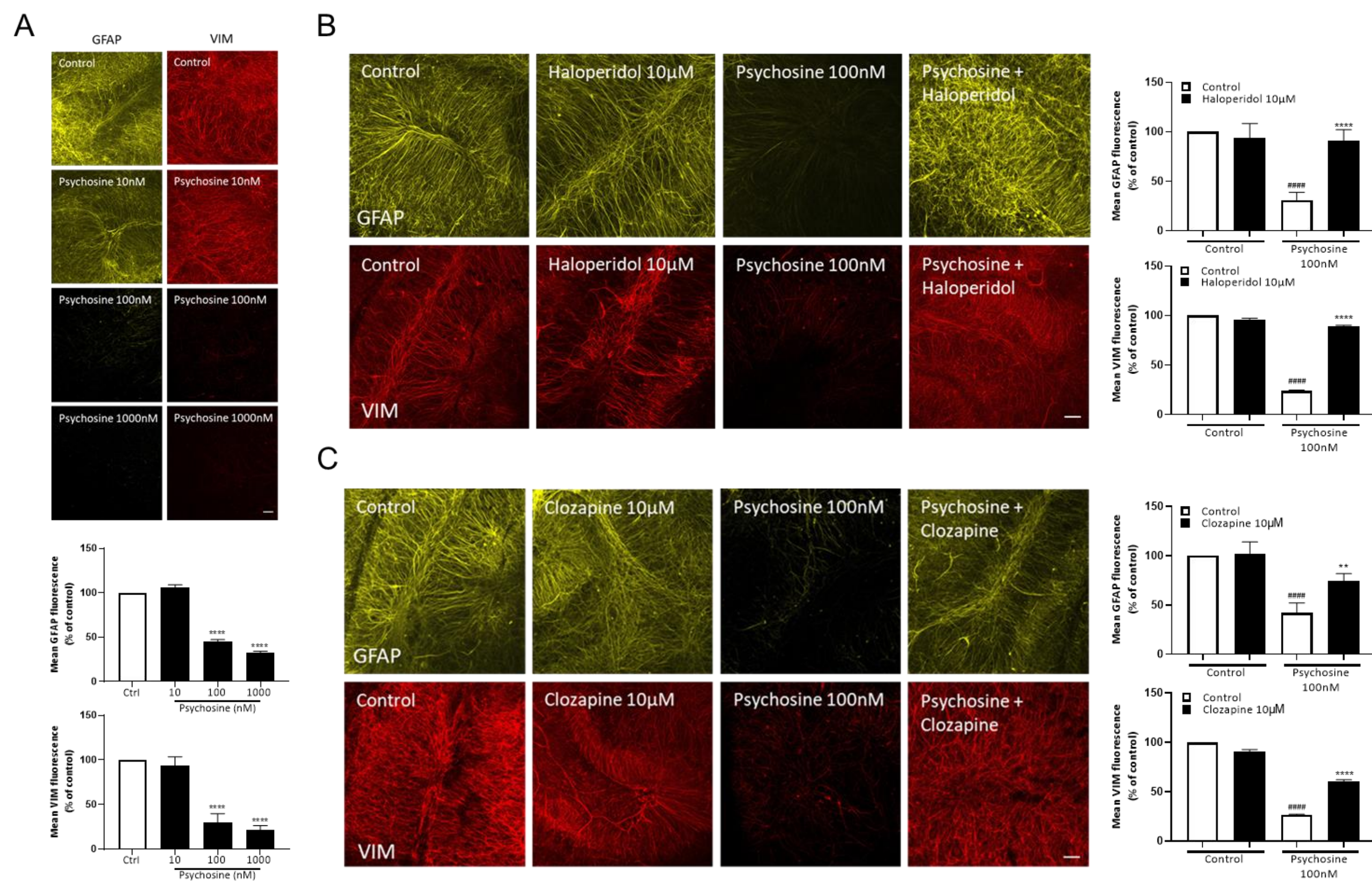


Figure 4.4. Haloperidol and Clozapine reduce astrocyte marker loss caused by psychosine.
(A) Decrease in GFAP and Vimentin fluorescence at 100nM and 1000nM psychosine. Decrease in GFAP and Vimentin fluorescence at 100nM psychosine were attenuated with **(B)** haloperidol 10µM and **(C)** clozapine 10µM. Confocal images at 10 X magnification, scale bar 100µm. Data are shown as mean +/- SEM, Kruskal-Wallis test, Dunn's multiple comparisons test, $^{####}p<0.0001$, $^{****}p<0.0001$ (n=5).

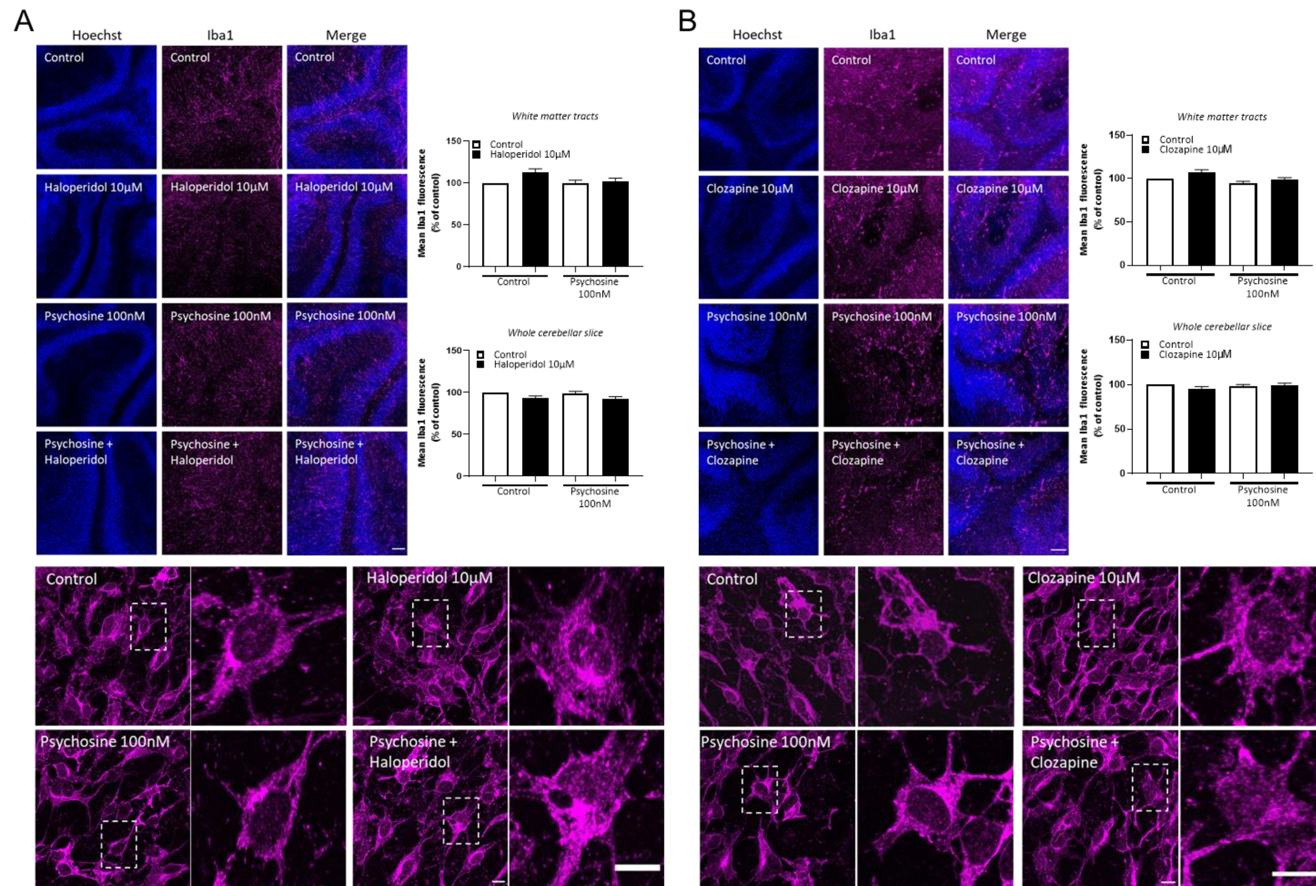


Figure 4.5. Psychosine, haloperidol and clozapine effects were independent of changes in microglia.
Treatment of cerebellar slices with psychosine in the presence or absence of **(A)** haloperidol or **(B)** clozapine did not alter microglia morphology or Iba1 fluorescence in white matter tracts or whole cerebellar slice areas. Confocal images at 10 X and 20 X magnification, scale bar 100µm and 10µm respectively. Data are shown as mean +/- SEM, Kruskal-Wallis test, Dunn’s multiple comparisons test (n=5)

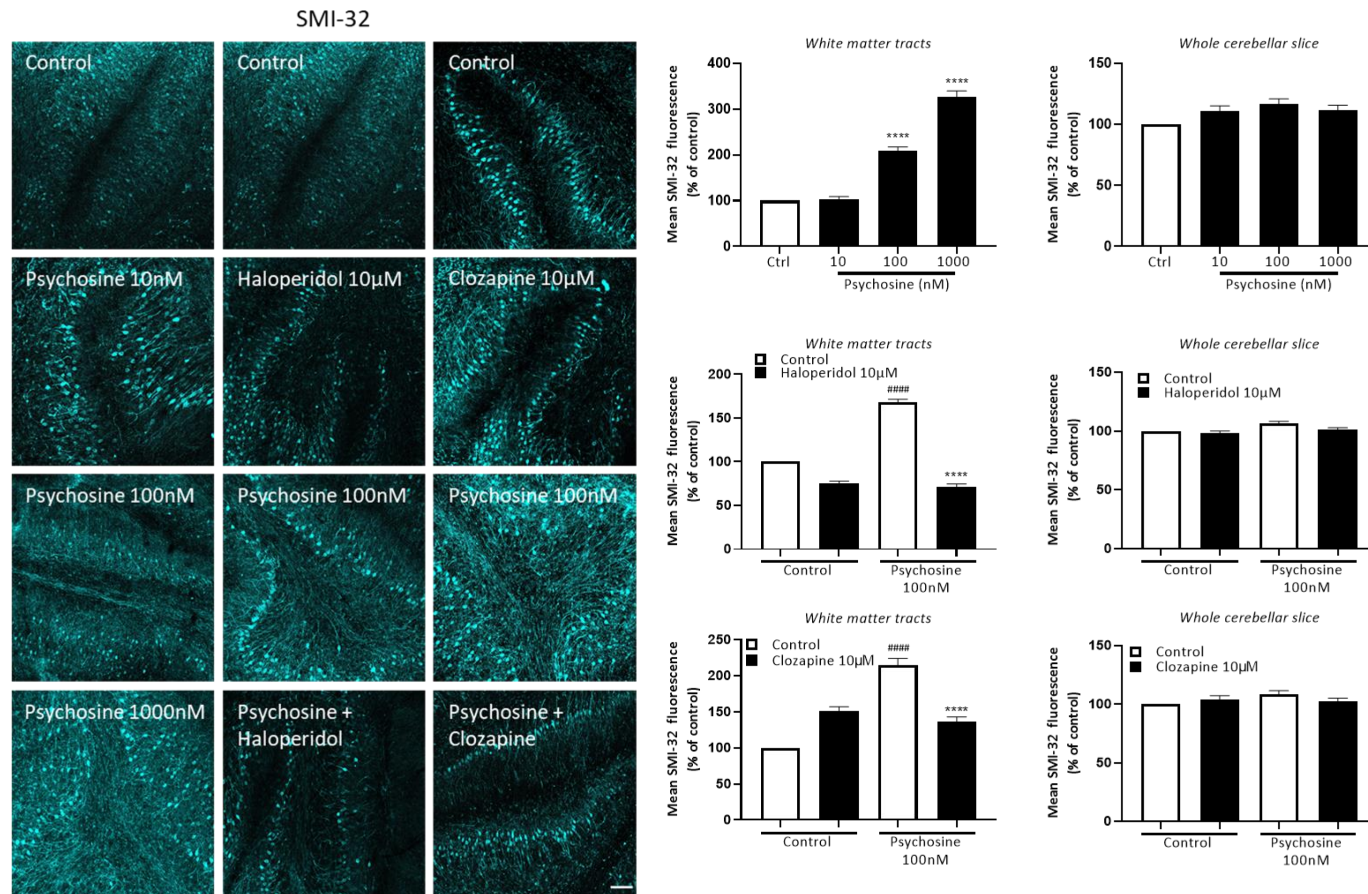


Figure 4.6. Psychosine induced increase in SMI-32 fluorescence is attenuated by haloperidol and clozapine.
Increase in SMI-32 fluorescence at 100nM and 1000nM psychosine in white matter tracts of cerebellar slices, without change in whole cerebellar slice areas. Increase in SMI-32 fluorescence at 100nM psychosine in white matter tracts of cerebellar slices were attenuated by haloperidol 10µM and clozapine 10µM. Confocal images at 10 X magnification, scale bar 100µm. Data are shown as mean +/- SEM, Kruskal-Wallis test, Dunn's multiple comparisons test, ****p<0.0001, $^{\#\#\#\#}$p<0.0001, **p<0.01 (n=5).

3. Discussion.

3.1.Summary of findings.

Schizophrenia is a severe and chronic mental illness associated with significant disability, increased morbidity, and mortality, with those affected dying on average 10-15 years sooner than those without schizophrenia (McCutcheon et al., 2020). There is evidence that glial cells are implicated in the neuropathophysiology of schizophrenia as well as growing data that antipsychotics influence glial cells (H. G. Bernstein et al., 2015; Andrea G Dietz et al., 2019). This study aimed to examine whether haloperidol and clozapine, attenuated psychosine induced toxicity in mixed glial organotypic cerebellar slice culture. We found both these antipsychotics attenuated psychosine induced demyelination in cerebellar slices and prevented psychosine associated astrocyte toxicity suggesting these agents exert a protective influence on glial cells. As previously shown (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016) and confirmed here psychosine did not alter microglial markers, and interestingly neither did haloperidol or clozapine. Importantly, both haloperidol and clozapine also attenuated psychosine induced axonal damage in white matter areas of cerebellar slices. Taken together these data support that antipsychotics play a constructive role in regulating myelin state, by influencing oligodendrocytes and astrocytes. These data imply antipsychotics are myelin protective and highlight the need for further research into glial cell targets for antipsychotic drug development. Also, this data suggests that antipsychotics or agents with similar pharmacology, namely D_2 receptor antagonists, may be a potential treatment for KD, a leukodystrophy that leads to the accumulation of the toxin psychosine.

3.2.Mechanisms of psychosine induced demyelination.

The mechanisms by which psychosine induces demyelination remains unclear, however may include: (i) psychosine-induced apoptotic processes and caspase dependent pathway activation (Shailendra Giri, Khan, Nath, Singh, & Singh, 2008; S. Giri et al., 2006; Haq et al., 2003; Tohyama, Matsuda, & Suzuki, 2001; Zaka & Wenger, 2004) (ii) preferential accumulation of psychosine in lipid rafts, associated with regional cholesterol increases and inhibition of PKC (protein kinase c) activity (Davenport et al., 2011; Hannun & Bell, 1987; White et al., 2011; Yamada, Martin, & Suzuki, 1996) (iii) psychosine-induced generation of LPC and arachidonic acid with regulation of secreted phospholipase A2 (sPLA2) (S. Giri et al., 2006), and/or (iv) induced phosphorylation of

neurofilament proteins, resulting in reduced radial growth of axons, axonal defects and neuronal cell death (Cantuti-Castelvetri et al., 2012; Castelvetri et al., 2011). Studies show that oligodendrocytes express dopamine D_2 receptors (Rosin et al., 2005) and that dopamine can regulate myelin formation and the development and function of oligodendrocytes (Bongarzone et al., 1998; Howard et al., 1998). Studies also show that agonists of D_2 receptors increases the number of oligodendrocyte progenitor cells and protect oligodendrocytes against oxidative injury (Rosin et al., 2005), which may explain the therapeutic effects of some antipsychotics that are partial D_2 receptor agonists. D_2 antagonists, such as haloperidol and clozapine are protective in the inflammatory demyelinating EAE model of MS and the non-immune cuprizone model of demyelination (Ceylan et al., 2021; Templeton et al., 2019). Also, haloperidol and clozapine prevented apoptotic cell death in oligodendrocytes that were cultured under glucose deprived conditions (Johann Steiner et al., 2011). Our findings show that haloperidol and clozapine attenuate psychosine induced demyelination in organotypic cerebellar slices, and thus support the studies suggesting protective effects of antipsychotics on myelin state.

3.3. Psychosine deregulates astrocytes, which is attenuated by haloperidol and clozapine.

While the damaging effects of psychosine on oligodendrocyte survival and function, and subsequent myelination is well established, the effect of psychosine on astrocytes and their role in KD is less well studied (Suzuki, 1998; D. Wenger, 2001). The sole attribution of KD to oligodendrocyte dysfunction is questionable given that oligodendrocytes from twitcher mice (twi/twi - a naturally occurring mouse model of KD) transplanted into the shiverer mouse model of demyelination can myelinate the shiverer axons indicating twi/twi oligodendrocytes may function normally (Kondo, Wenger, Gallo, & Duncan, 2005). It is possible that astrocytic reactivity in KD may represent a secondary response to demyelination or may even be a primary response to psychosine where astrocytes contribute a central role to the pathogenesis of KD (Claycomb, Johnson, Winokur, Sacino, & Crocker, 2013). We have demonstrated that psychosine can directly dysregulate astrocytes (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016) and in agreement with these previous studies, we show here that psychosine reduces the expression of two astrocyte markers, namely GFAP and vimentin. Importantly, we find that both haloperidol and clozapine attenuated the psychosine-induced decrease in expression of GFAP and vimentin. The data supports the idea that astrocyte function is altered by psychosine and that astrocytes may be

potential cellular drug targets for antipsychotic drugs such as haloperidol and clozapine. This data adds to the currently small number of studies examining the direct effects of antipsychotics on astrocyte biology. Some recent works suggest that antipsychotics may regulate specific astrocytes subtypes (Akkouh et al., 2022), enhance astroglial glutamatergic transmission (K. Fukuyama & M. Okada, 2021), regulate cytokine expression (Okazaki et al., 2021), activate Cx43 channel activity (Kouji Fukuyama, Okubo, Murata, Shiroyama, & Okada, 2020) and alter a number of other signalling pathways in astrocytes, which may contribute to the clinical efficacy of these drugs.

3.4. Microglia independent demyelination is regulated by haloperidol and clozapine.

Using organotypic cerebellar slices, we have demonstrated that psychosine does not alter ionized calcium binding adaptor molecule 1 (Iba1) microglia staining, although we note Iba1 is not a specific marker of altered microglia reactivity (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016). In addition, we have reported that psychosine does not alter pro-inflammatory cytokine release in BV2 microglia cells (Catherine O'Sullivan & Kumlesh K Dev, 2015). These results suggest that, in organotypic cerebellar slices, psychosine-induced demyelination occurs via a mechanism that may be independent of overt microglia activation and not dependent on increased levels of pro-inflammatory cytokines. In contrast to this data derived from organotypic cerebellar slice models, KD (both patient and animal models) is associated with neuroinflammation, microglial activation and phagocytic multinucleated globoid cells (Potter & Petryniak, 2016). Studies ascribe the early demise in KD to neuroinflammation (Nicaise, Bongarzone, & Crocker, 2016), with astrocyte and microglia activation being important in disease progression (Ijichi et al., 2013; Mohri et al., 2006). More recently, we confirm that a significant increase in Iba1 immunostaining in twitcher mice, with Iba1 immunostaining showing amoeboid morphology in these animals (Béchet et al., 2020). With a prevailing focus to data derived from *in vivo* studies, altered microglia and inflammatory mechanisms are highly likely to play an important role in KD. These studies collectively point toward a limitation in use of organotypic cerebellar slices as a test model. Despite having significant advantages over dissociated cell culture, and although having an intact cytoarchitecture with markers for neurons, oligodendrocytes, microglia, and astrocytes, CD25-positive T and B lymphocytes and CD68/CD163-positive markers of macrophages, monocytes, and dendritic cells, these brain slice cultures nevertheless lack

interaction with peripheral systems, which are likely to be important when experimentally modelling multiorgan diseases such as KD (Sheridan & Dev, 2014). While taken all this into account, the current study still nevertheless suggests strongly that haloperidol and clozapine can regulate myelination state in this apparent non-inflammatory paradigm.

3.5.Axonal damage associated with demyelination.

Axonal damage can occur before or after demyelination, in two distinct hypothetical models, namely inside-out or outside-in demyelination (Tsunoda & Fujinami, 2002; Zipp & Aktas, 2006). Neurofilament H is a structural cytoskeletal protein specific to neurons comprising phosphorylated and non-phosphorylated epitopes (M.-E. Lin, Rivera, & Chun, 2012; Ohsawa, Miyabe, Katsu, Yamamoto, & Ono, 2013). Non-phosphorylated NFH epitopes, such as SMI-32, are expressed by purkinje and neuronal cell bodies under physiological conditions, with minimal expression in the axonal tracts (Bymaster, 2001; Kinloch & Cox, 2005). In multiple sclerosis, aberrant axonal transport, demyelination and neuronal damage is associated with increased expression of SMI-32 in the axonal tracts (Bymaster, 2001; Truini, Barbanti, Pozzilli, & Cruccu, 2013). In our previous study, we observed an increased expression of SMI32 in twitcher animals, with a level of variance in the expression of SMI-32 more so in the twitcher animals compared with wild-type controls (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016). In agreement with our previous studies using organotypic slice cultures, we found that the total levels of NFH were not changed at psychosine concentrations (100nM) used to induce demyelination. These previous studies also demonstrated that psychosine increased the expression of SMI-32 in the white matter axonal tracts of the *arbor vitae*, collectively suggestive of axonal damage related and likely subsequent to demyelination. Importantly, in the current study, haloperidol and clozapine treatment of organotypic slices prevented the axonal expression of SMI-32. These results support our hypothesis that haloperidol and clozapine are able to prevent psychosine-induced pathology, as observed in our *ex-vivo* experiments.

3.6.Conclusion.

Glial cells are implicated in the neuropathophysiology of schizophrenia and may be potential targets for antipsychotic medications. This study examined whether antipsychotics attenuated psychosine induced demyelination in organotypic cerebellar slice culture. Haloperidol and

clozapine attenuated psychosine induced demyelination and prevented astrocyte toxicity, independent of microglial activation. These data suggest that antipsychotics are myelin protective and highlights the potential of glial cell targets for antipsychotic drug development as well as antipsychotics or agents with similar pharmacology being a potential treatment for KD.

Chapter 5 – The antipsychotic haloperidol improves survival, locomotion, and behaviour in twitcher mice – preclinical evidence for a potential novel treatment for Krabbe disease.

Chapter Aims

- To determine if haloperidol treated twitcher mice would have increased survival compared to those untreated.
- To investigate if body weight would differ significantly between haloperidol treated twitcher mice and those untreated.
- To observe whether twitching scores and immobility scores would differ significantly between haloperidol treated twitcher mice and those untreated.
- To determine if subtle behavioural metrics on open field testing would differ between haloperidol treated twitcher mice and those untreated.

Abstract

Krabbe disease (KD) is a rare autosomal recessive disorder associated with the accumulation of psychosine which causes demyelination in the central and peripheral nervous system. There is no cure for the disorder, clinical interventions involve screening for the disease and providing general symptomatic support. Treatment with haematopoietic stem cell transplantation (HSCT) increases longevity but KD is still associated with severe morbidity and a greatly reduced life expectancy. Twitcher mice are an established murine model for the study of KD and potential treatments. Psychosine is toxic to human astrocytes *in-vitro* and causes demyelination in mouse organotypic cerebellar slice cultures *ex-vivo*. Antipsychotics prevent psychosine toxicity in these *in-vitro* and *ex-vivo* cultures. This study aimed to examine if these findings would translate into improved survival, mobility, and behavioural metrics in an *in-vivo* model of psychosine toxicity i.e., the twitcher mouse model of KD. Haloperidol increased survival, improved mobility, and positively influenced behaviours in twitcher mice. This suggests that antipsychotics or agents with similar pharmacology, namely D_2 receptor antagonists, may be a potential novel treatment for KD, a leukodystrophy that leads to the accumulation of the toxin psychosine. The mechanism by which this occurs requires further investigation, but we propose that antipsychotics may interact with glial cells and exert a myelin protective effect.

Keywords: haloperidol, antipsychotics, twitcher mice, psychosine, Krabbe disease

1. Introduction.

Krabbe disease (KD) also known as globoid cell leukodystrophy is a rare autosomal recessive disease caused by mutations in the *GALC* gene which codes for the enzyme galactosylceramidase, it has an incidence of 1 per 100,000 births and therefore is designated an orphan disease for drug development research. The genetic abnormalities associated with the disorder lead to the accumulation of lipids, including psychosine, which is toxic and causes demyelination in the central and peripheral nervous system (Béchet et al., 2020). This pathology leads to those afflicted with infantile forms of the disease manifesting with seizures (including febrile seizures), muscle frailty, irritability, vomiting, feeding difficulties with eventual mortality usually prior to two years of age. A minority have a slower disease progression or later-onset of the disease and can present with delayed developmental milestones. There is no cure for the disorder, clinical interventions focus on screening for the disease in those deemed to be at risk, providing general symptomatic support and treatment with haematopoietic stem cell transplantation (HSCT). HSCT has shown to increase longevity in those with the disease but those with KD still have serious mobility and speech deficits as well as a much-reduced life expectancy (D. A. Wenger et al., 2021). Therefore, there is a need for continued research for novel therapies for KD and murine models provide a useful tool for investigating potential therapies. Twitcher mice are a naturally occurring mouse mutant that contain a mutation in the *GALC* gene, which leads to the accumulation of psychosine in homozygous mice and associated toxicity centrally and peripherally. The genetic pathology in twitcher mice approximates to human KD and they are an established murine model for the study of KD pathology and potential treatments (D. A. Wenger et al., 2016).

Psychosine, which accumulates abnormally in those with KD, has been shown to be toxic to oligodendrocytes and cause demyelination by various proposed mechanisms. These include activating apoptotic processes, inhibiting protein kinase c activity, forming lysophosphatidylcholine resulting in cytotoxicity and causing phosphorylation leading to cell death (Cantuti-Castelvetri et al., 2012; Davenport et al., 2011; S. Giri et al., 2006; Haq et al., 2003). We have shown previously that psychosine causes demyelination in mouse organotypic cerebellar slice cultures and that psychosine causes toxicity in mouse and human astrocytes (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016; S. A. O'Sullivan, Velasco-Estevez, & Dev, 2017). Haloperidol is a potent dopamine-2 receptor (D_2) antagonist and is mainly used in the treatment of schizophrenia and

other neuropsychiatric disorders. Antipsychotics may be classified into “typical” (first generation) and “atypical” (second generation) compounds, but this method is limited and largely based upon chronology i.e., the time they were marketed. A more scientifically informed method of classifying antipsychotics is by using neuroscience-based nomenclature (NbN) which incorporates up to date neuroscientific knowledge and classifies antipsychotics as A) D_2 antagonists B) D_2 and $5HT_2$ antagonists C) D_2 and $5HT_{1A}$ partial agonists D) D_2, $5HT_2$ and $NE_{\alpha 2}$ antagonists and E) D_2, $5HT_2$ antagonists and NE reuptake inhibitors (Zohar & Kasper, 2016). There is increasing evidence that antipsychotic medication effect the structure and function of different glial cells (H. G. Bernstein et al., 2015; Andrea G Dietz et al., 2019; Liu et al., 2022). Interestingly, chapters 3 and 4 have shown that antipsychotics including haloperidol prevented psychosine induced toxicity in human astrocytes *in-vitro* and prevented psychosine related demyelination in mouse organotypic cerebellar slice cultures *ex-vivo*. Moreover, chapter 3 highlighted that the selective D_2 antagonist eticlopride preferentially prevented psychosine induced astrocyte toxicity relative to the selective $5HT_{2A}$ antagonist volinanserin. Given these findings this study aimed to examine if these results translated into improved survival, mobility, and behavioural metrics in an *in-vivo* model of psychosine toxicity i.e., the twitcher mouse model of KD specifically using the potent D_2 antagonist antipsychotic haloperidol.

2. Results.

2.1. Haloperidol ameliorated weight loss, improved twitching scores and immobility scores in twitcher mice.

Chapter 3 and chapter 4 have shown that haloperidol ameliorated psychosine glial cell toxicity *in-vitro* and improved myelination in organotypic cerebellar slices *ex-vivo*. Therefore, it was hypothesised that these drugs may improve clinical outcomes in twitcher mice, an *in-vivo* model of psychosine toxicity. Haloperidol was administered to twitcher mice and their weight (figure 5.1), twitching severity scores and immobility severity scores (figure 5.2) were recorded. Haloperidol treated twitcher mice had significantly increased body weight compared to untreated twitcher mice during the experiment (figure 5.1 - $F_{(60, 560)} = 26.41$. $P = 0.0013$, ANOVA), twitching severity scores improved with administration of haloperidol showing a significantly slower increase over the course of the experiment (figure 5.2A - $F_{(60, 560)} = 72.43$. $P = 0.0009$, ANOVA). Also, mobility scores deteriorated significantly less rapidly in haloperidol treated twitcher mice compared to untreated twitcher mice during the experimental period (figure 5.2B - $F_{(60, 560)} = 54.43$. $P = 0.01$, ANOVA). As expected, no significant differences in twitching scores, mobility scores and body weight were observed between treated and untreated wild-type animals.

2.2. Haloperidol improved behavioural metrics in twitcher mice.

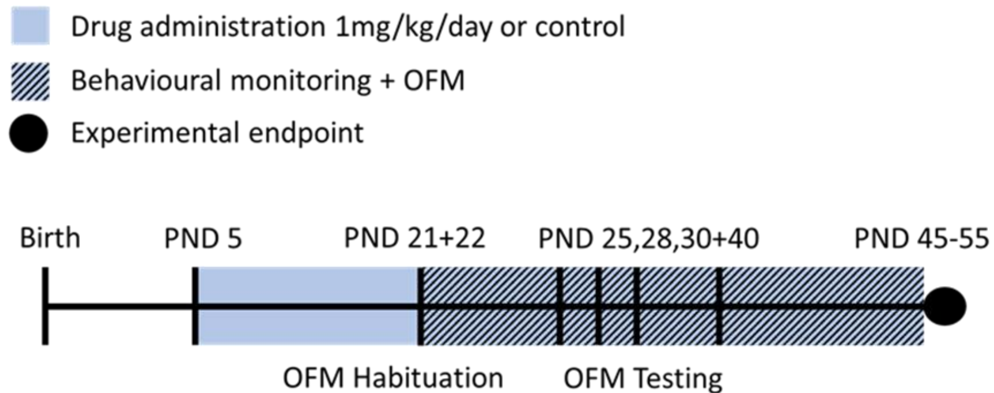
To assess behavioural outcomes in addition to weight, twitching and mobility scores an open field maze test was conducted on PND 25,28,30 (when twitching and immobility scores were expected to be mild to moderate). A computer video tracking system (ANYmaze - Stoelting) was utilised to record the following metrics: distance (m); mean speed (m/s or cm/s); max speed (m/s or cm/s); time mobile (%); centre entries and corner time (%) (figure 5.3). On PND 25 no significant differences were observed in any measurement between twitcher mice treated with haloperidol and those untreated. On PND 28 and 30 subtle but significant improvements in distance (m); mean speed (m/s or cm/s); time mobile (%); centre entries and corner time (%) but not max speed (m/s or cm/s) were observed between haloperidol treated twitcher mice and untreated twitcher mice. No significant differences were observed between treated and untreated wild-type mice and significant differences were noted between wild-type (treated

and untreated) and twitcher (treated and untreated) mice in several metrics. Details of comparisons are fully outlined in tables 5.1, 5.2 and 5.3.

2.3. Haloperidol improved survival in twitcher mice.

A Kaplan-Meier survival curve was used to assess whether twitcher mice treated with haloperidol had increased survival compared to those untreated with the drug. Haloperidol treated twitcher mice showed increased lifespan when compared with vehicle control treated twitcher mice (figure 5.4) (Log rank Mantel-Cox test **p=0.0041, Median survival: TWI_{Hal} 42 days; TWI_{Ctrl} 36.5 days, n=8 per group). There was no difference in lifespan between wild-type mice who were treated and untreated with haloperidol. Cumulatively, these data suggest that ameliorations observed by antipsychotics in *in-vitro* and *ex-vivo* models of psychosine toxicity translate to improved clinical outcomes, twitching scores, immobility scores, behaviour, and survival in an *in-vivo* model of psychosine toxicity i.e., twitcher mice. Furthermore, they suggest that subtle but statistically significant behavioural improvements may emerge early in the life of twitcher mice treated with haloperidol compared to those untreated.

A



PND = Post Natal Day
OFM = Open Field Maze

B

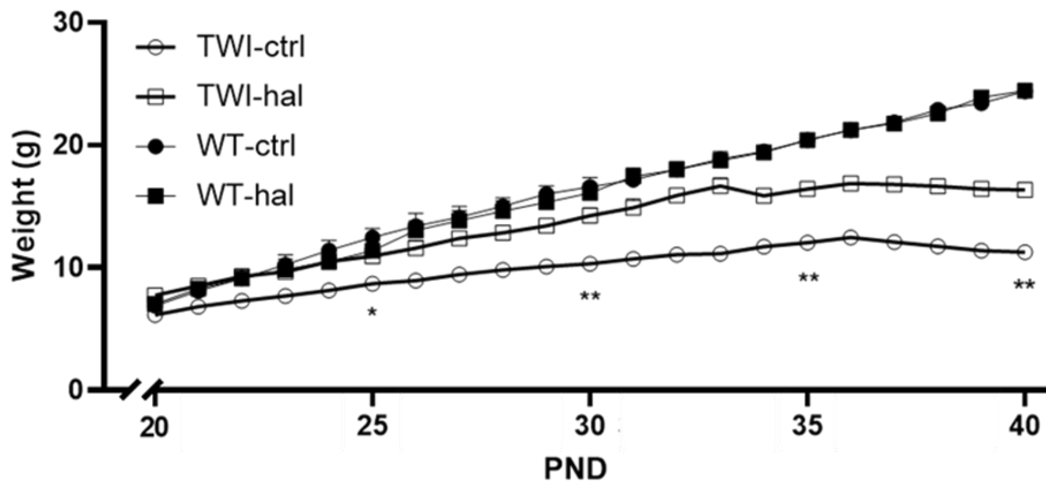


Figure 5.1 Haloperidol ameliorated weight loss in twitcher mice.
(A) Experimental design schematic, Homozygous mice and their wild-type litter mates were treated with haloperidol at a dose of 1mg/kg/day in drinking water or just water control via suckling pipette. **(B)** Weight for different treatment groups of wild-type and twitcher mice. Twitcher mice treated with haloperidol had significantly higher body weight at different time points compared to twitcher mice not treated with the drug. Two-way ANOVA followed by Tukey's multiple comparisons analysis demonstrated significant differences in weight between haloperidol treated and untreated twitcher mice. (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n=8 per group). Graphs show data as mean ± SEM.

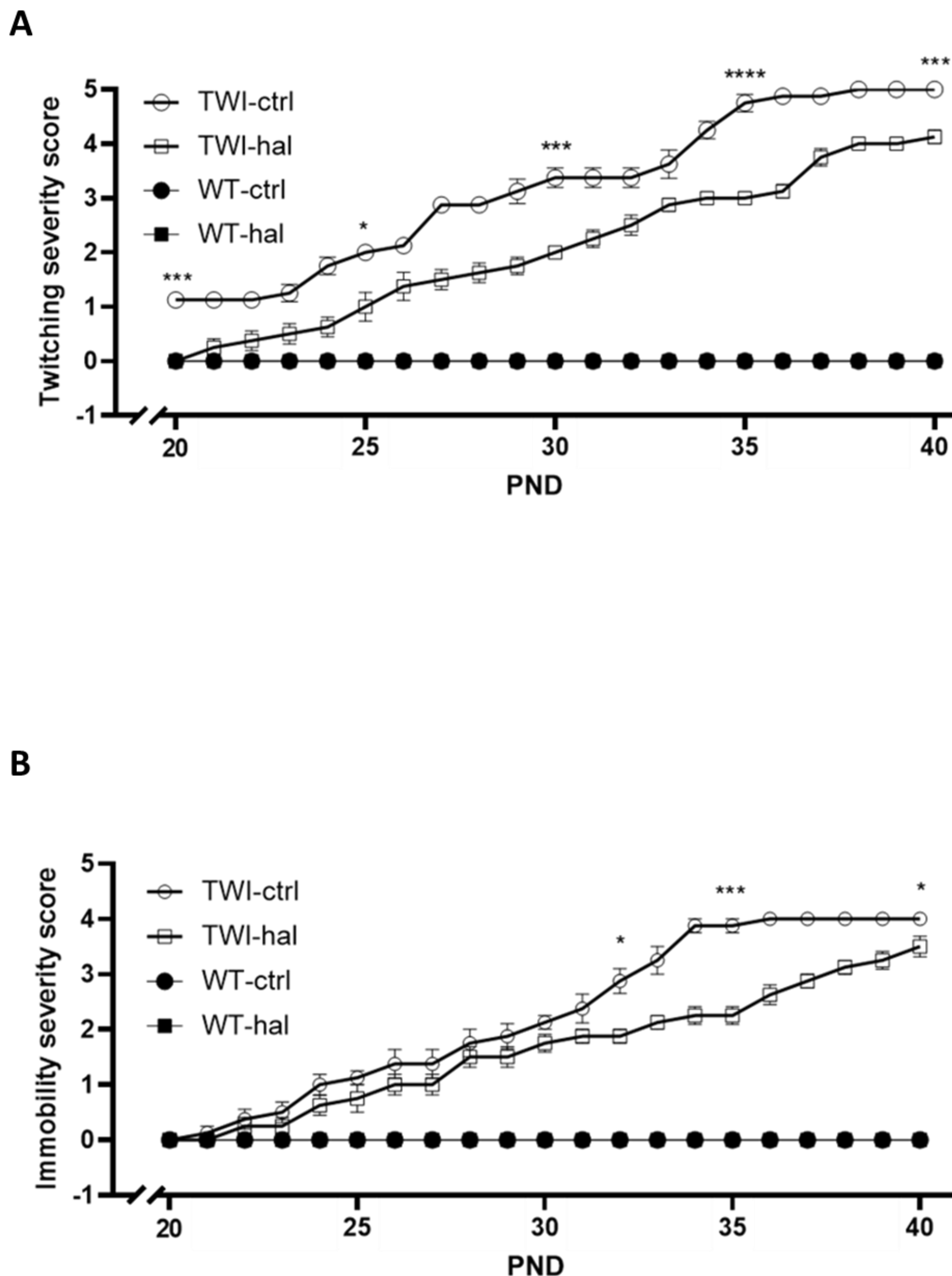


Figure 5.2. Haloperidol improved twitching and immobility scores in twitcher mice.
(A) Twitching severity and **(B)** immobility severity scores for different treatment groups of wild-type and twitcher mice. Twitcher mice treated with haloperidol had significantly improved twitching and immobility at different time points compared to twitcher mice not treated with the drug. Two-way ANOVA followed by Tukey's multiple comparisons analysis demonstrated significant differences in twitching score and immobility score between haloperidol treated and untreated twitcher mice. (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n=8 per group). Graphs show data as mean ± SEM.

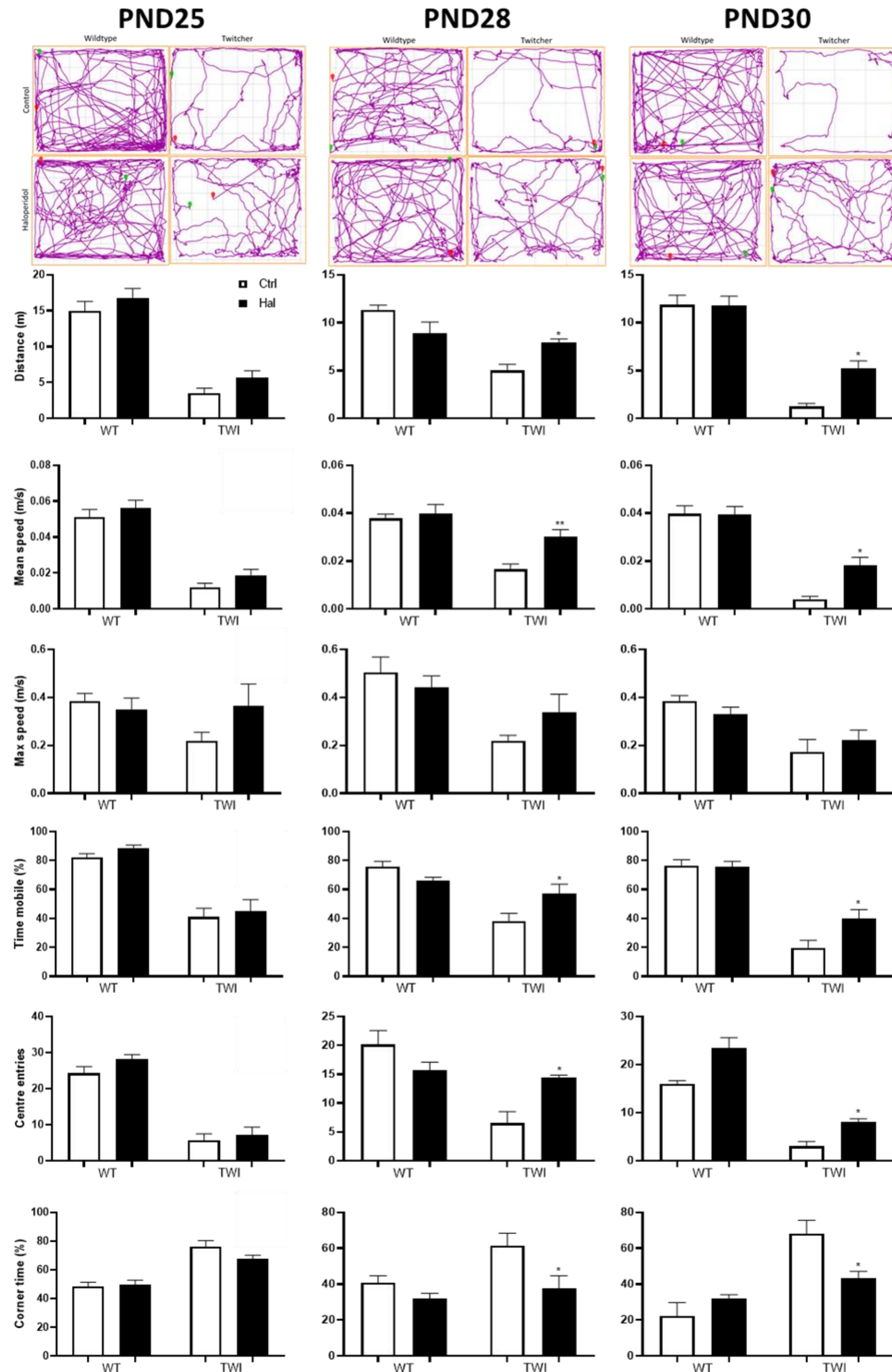


Figure 5.3. Haloperidol caused significant behavioural differences between treated and untreated twitcher mice.

(A) Representative image of open field maze test carried out at PND 25, 28 and 30. **(B)** Bar graphs for distance (m), mean speed (m/s), max speed (m/s), time mobile (%), centre entries and corner time (%). No significant difference was observed between haloperidol treated twitcher mice and untreated twitcher mice in any behavioural metric at PND 25. Subtle but significant differences were observed in distance, mean speed, time mobile, centre entries, corner time but not max speed at PND 28 and 30 between haloperidol treated twitcher mice and untreated twitcher mice. No significant difference was observed in haloperidol treated and untreated wild-type mice. Significant differences were observed between twitcher mice and wild-type littermates (tables 5.1, 5.2 and 5.3). One-way ANOVA followed by Tukey's multiple comparisons analysis (n=8 per group). Graphs show data as mean ± SEM.

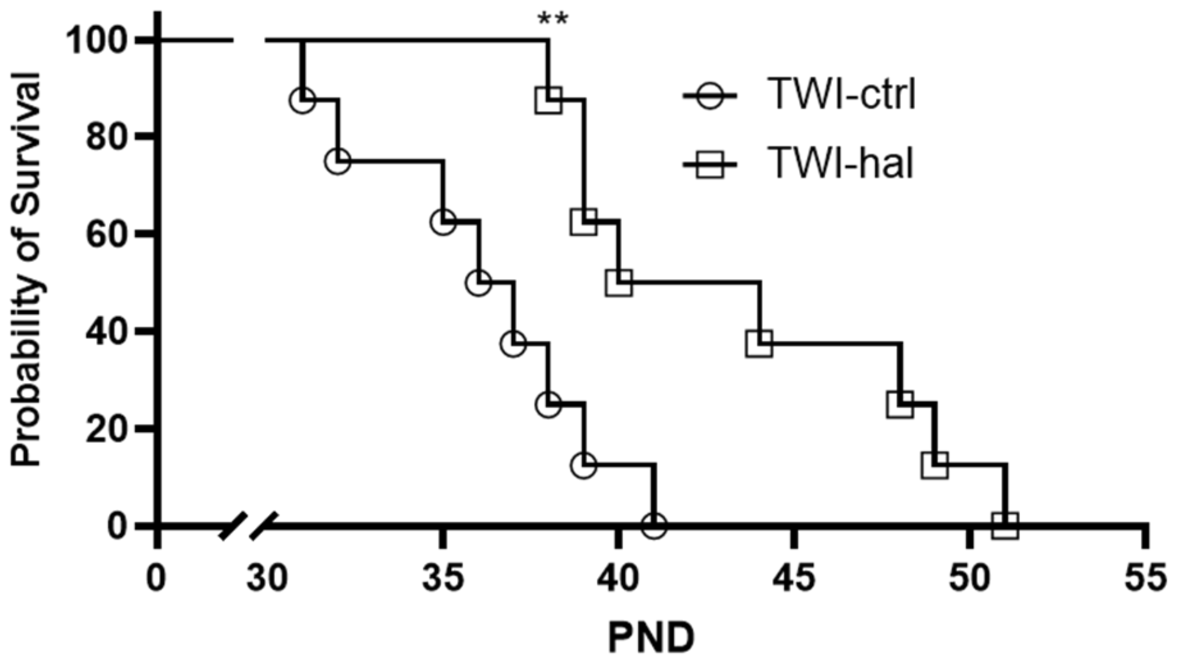


Figure 5.4. Haloperidol improved survival in twitcher mice.
Survival analysis by Kaplan-Meier curve comparing haloperidol treated twitcher mice versus untreated twitcher mice. A log rank Mantel-Cox test showed that haloperidol treated twitcher mice had a significantly increased lifespan compared to untreated twitcher mice (**p<0.01, n=8 per group).

	WT^{Ctrl}	WT^{Hal}	TWI^{Ctrl}	TWI^{Hal}	WT^{Ctr} vs. WT^{Hal}	WT^{Ctr} vs. TWI^{Ctrl}	WT^{Ctr} vs. TWI^{Hal}	WT^{Hal} vs. TWI^{Ctrl}	WT^{Hal} vs. TWI^{Hal}	TWI^{Ctrl}vs. TWI^{Hal}
Metric	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM						
No. of animals	8	8	8	8						
Distance (m)	15.00±1.3	16.81±1.29	3.54±0.70	5.67±0.97	ns	$^{****}p<0.0001$	$^{****}p<0.0001$	$^{****}p<0.0001$	$^{****}p<0.0001$	ns
Mean speed (cm/s)	5.11±0.43	5.61±0.49	1.19±0.24	1.89±0.32	ns	$^{****}p<0.0001$	$^{****}p<0.0001$	$^{****}p<0.0001$	$^{****}p<0.0001$	ns
Max speed (cm/s)	38.53±3.06	35.04±4.67	21.88±3.53	36.65±8.90	ns	ns	ns	ns	ns	ns
Time mobile (%)	82.15±2.67	88.33±2.35	40.95±6.07	45.08±7.87	ns	$^{****}p<0.0001$	$^{****}p=0.0002$	$^{****}p<0.0001$	$^{****}p<0.0001$	ns
Centre entries	24.25±1.88	28.25±1.19	5.63±1.82	7.25±2.05	ns	$^{****}p<0.0001$	$^{****}p<0.0001$	$^{****}p<0.0001$	$^{****}p<0.0001$	ns
Corner time (%)	48.48±2.86	49.95±2.83	76.03±4.28	67.60±2.57	ns	$^{****}p<0.0001$	$^{***}p=0.0013$	$^{****}p<0.0001$	$^{*}p=0.003$	ns

Table 5.1 Behavioural metrics for each treatment group for PND 25.
No significant difference was observed between haloperidol treated twitcher mice and untreated twitcher mice in distance, mean speed, max speed, time mobile, centre entries or corner time at PND 25 also no significant difference was observed in haloperidol treated and untreated wild-type mice. Significant differences were observed between twitcher mice and wild-type littermates. One-way ANOVA followed by Tukey's multiple comparisons analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n=8 per group).

	WT^{Ctrl}	WT^{Hal}	TWI^{Ctrl}	TWI^{Hal}	WT^{Ctr} vs. WT^{Hal}	WT^{Ctr} vs. TWI^{Ctrl}	WT^{Ctr} vs. TWI^{Hal}	WT^{Hal} vs. TWI^{Ctrl}	WT^{Hal} vs. TWI^{Hal}	TWI^{Ctrl} vs. TWI^{Hal}
Metric	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM						
No. of animals	8	8	8	8						
Distance (m)	11.31±0.53	8.92±1.14	5.01±0.65	7.93±0.38	ns	****p<0.0001	*p=0.0146	**p=0.004	ns	*p=0.0412
Mean speed (cm/s)	3.78±0.18	3.99±0.38	1.66±0.22	3.01±0.30	ns	****p<0.0001	ns	****p<0.0001	ns	***p=0.0099
Max speed (cm/s)	50.41±6.43	44.26±4.74	21.86±2.38	33.86±7.51	ns	**p=0.0063	ns	*p=0.0407	ns	ns
Time mobile (%)	75.68±3.88	65.89±2.53	38.16±5.27	57.14±6.45	ns	****p<0.0001	p=0.0477	**p=0.0017	ns	*p=0.0412
Centre entries	20.13±2.43	15.63±1.46	6.50±2.02	14.38±0.46	ns	****p<0.0001	ns	**p=0.0052	ns	*p=0.0181
Corner time (%)	40.81±3.99	32.06±2.93	61.56±6.87	37.53±7.24	ns	ns	ns	**p=0.0043	ns	*p=0.0241

Table 5.2. Behavioural metrics for each treatment group for PND 28.
Significant differences were observed between haloperidol treated twitcher mice and untreated twitcher mice in distance, mean speed, time mobile, centre entries or corner time at PND 28. No significant difference was observed for max speed between haloperidol treated and untreated twitcher mice. No significant difference was observed in haloperidol treated and untreated wild-type mice. Significant differences were observed between twitcher mice and wild-type littermates. One-way ANOVA followed by Tukey's multiple comparisons analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n=8 per group).

	WT^{Ctrl}	**WT^{Hal}**	**TWI^{Ctrl}**	**TWI^{Hal}**	**WT^{Ctr} vs. WT^{Hal}**	**WT^{Ctr} vs. TWI^{Ctrl}**	**WT^{Ctr} vs. TWI^{Hal}**	**WT^{Hal} vs. TWI^{Ctrl}**	**WT^{Hal} vs. TWI^{Hal}**	**TWI^{Ctrl} vs. TWI^{Hal}**
Metric	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM						
No. of animals	8	8	8	8						
Distance (m)	11.87±1.00	11.77±0.10	1.26±0.32	5.22±0.80	ns	****P<0.0001	****P<0.0001	****P<0.0001	****P<0.0001	*P=0.0107
Mean speed (cm/s)	3.98±0.33	3.95±0.33	0.41±0.11	1.81±0.33	ns	****p<0.0001	****p<0.0001	****p<0.0001	***p=0.0001	*p=0.0114
Max speed (cm/s)	38.45±2.32	33.09±2.83	17.18±5.25	22.28±4.09	ns	**p=0.0025	*p=0.0264	*p=0.0296	ns	ns
Time mobile (%)	76.66±3.98	75.72±3.81	19.76±5.11	39.77±6.25	ns	****p<0.0001	****p<0.0001	****p<0.0001	****p<0.0001	*p=0.0346
Centre entries	16.00±0.66	23.50±2.10	3.00±1.02	8.00±0.73	ns	****p<0.0001	***p=0.0007	****p<0.0001	****p<0.0001	*p=0.0439
Corner time (%)	22.24±7.52	32.22±1.92	68.09±7.49	43.58±3.56	ns	****p<0.0001	ns	***p=0.0007	ns	*p=0.0241

Table 5.3. Behavioural metrics for each treatment group for PND 30.
Significant differences were observed between haloperidol treated twitcher mice and untreated twitcher mice in distance, mean speed, time mobile, centre entries or corner time at PND 30. No significant difference was observed for max speed between haloperidol treated and untreated twitcher mice. No significant difference was observed in haloperidol treated and untreated wild-type mice. Significant differences were observed between twitcher mice and wild-type littermates. One-way ANOVA followed by Tukey's multiple comparisons analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, n=8 per group)

3. Discussion.

3.1. Summary of findings Haloperidol improved survival, mobility and behaviour in twitcher mice.

KD is a rare autosomal recessive disorder that usually leads to mortality before two years of age in untreated individuals. Psychosine accumulates abnormally in the condition and leads to demyelination centrally and peripherally (D. Wenger, 2001). We have shown that psychosine is toxic to human astrocytes and causes demyelination in mouse organotypic cerebellar slices (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016) and in chapters 3 and 4 this psychosine-induced toxicity was shown to be reversed *in-vitro* and *ex-vivo* by haloperidol and other antipsychotics. The aim of this study was to examine if these findings translated to improved survival and behaviour in an animal model of psychosine toxicity namely twitcher mice. We found that the haloperidol increased survival in twitcher mice, improved mobility scores and had a subtle but significant positive effect on behaviour compared to mice untreated with the drug. These data suggest that antipsychotics or agents with similar pharmacology, namely D_2 receptor antagonists, may be a potential treatment for KD, a leukodystrophy that leads to the accumulation of the toxin psychosine. The mechanism by which antipsychotics lead to this improvement requires further investigation but we hypothesise that antipsychotics may positively influence glial cells which may lead to improved demyelination in twitcher mice treated with haloperidol. There is increasing evidence that antipsychotics influence the structure and function of glial cells which is summarised in the sections below.

3.2. Antipsychotics influence oligodendrocyte and oligodendrocyte progenitor cells.

Many in-vitro studies have shown the influence of antipsychotics on oligodendrocytes and have proposed that fixing damaged myelin by revitalising these cells may be one of the mechanisms by which the drugs exert a neuroprotective effect (A. T. Chen & Nasrallah, 2019). Quetiapine and clozapine but not olanzapine, were shown to reverse the negative effects of cuprizone on oligodendrocyte progenitor cells development to mature oligodendrocytes *in-vitro* (Xu et al., 2014). A comprehensive proteomic analysis of developing oligodendrocytes treated with

chlorpromazine, haloperidol, quetiapine and risperidone implicated numerous cellular biological systems that the drugs may influence including but not limited to, cell communication, protein metabolism and cell growth or maintenance (Brandão-Teles et al., 2019). These pathway links were also confirmed in mature oligodendrocytes in a proteomic analysis by (Seabra et al., 2020) using haloperidol and clozapine. Haloperidol and clozapine prevented apoptotic cell death in oligodendrocytes that were cultured under glucose deprived conditions, highlighting their potential protective effects (Johann Steiner et al., 2011). Studies have shown that haloperidol (Niu et al., 2010) and olanzapine (Kimoto et al., 2011) are able to induce propagation of oligodendrocytes progenitor cells while both did not aid in the development of these cells into mature oligodendrocytes in primary culture of rat cells.

In-vivo studies have shown the potential benefits of antipsychotics on oligodendrocytes and myelin. Quetiapine halted cuprizone induced demyelination in mice and positively influenced their behaviour on Y-maze tasks, by increasing propagation of precursor cells (Xiao et al., 2008). This finding has been replicated and it has been suggested that quetiapine exerts such benefits via the notch signalling pathway which is involved in oligodendrocyte development and myelin formation (H.-n. Wang et al., 2016). Further, quetiapine was shown to directly improve the developmental morphology of oligodendrocytes towards myelination, by increasing the activity of OLIG1 (oligodendrocyte transcription factor 1 – promotes maturation) and decreasing the activity of GPR17 (OLIG1 regulated G protein coupled receptor 17 -inhibits maturation) in mouse oligodendrocytes (X. Wang et al., 2021). Quetiapine also prevented demyelination in an immune mouse model for multiple sclerosis via downregulation of T-cell propagation implicating immune interactions of antipsychotic may also lead oligodendrocyte improvements (Mei et al., 2012). Olanzapine increased the amount of precursor cells in the hypothalamus of mice and clozapine aided the recovery of mice exposed to the demyelinating toxin cuprizone (Templeton et al., 2019; Yamauchi et al., 2010). Haloperidol was noted to increase the amount of oligodendrocyte precursor cells in the corpus callosum, hippocampus and cerebral cortex of mouse brains (H. Wang et al., 2010). Olanzapine was associated with more retained brain volume in those with an initial episode of psychosis compared to the haloperidol (Lieberman et al., 2005). Those prescribed clozapine were shown to have more peripheral BDNF (brain derived neurotrophic factor – involved in many CNS pathways) compared to those prescribed "typical" agents (Pedrini et al., 2011). While antipsychotic treatment especially with "typical" agents or a combination of "typical" and "atypical" drugs appears to promote grey matter loss in imaging studies (Vita et al., 2015), the medication seem to also correct white matter changes including

those that occur during initial presentations of schizophrenia in drug naïve individuals (Sagarwala & Nasrallah, 2021). This may indicate a preferential effect of these medications on oligodendrocytes and myelin relative to other glial cells. Interestingly, clozapine and haloperidol were shown to prevent autophagy and mitophagy in multiple sclerosis in a study by (Patergnani et al., 2021) using human cerebrospinal fluid samples as well as animal *in-vitro*, *ex-vivo* and *in-vivo* experiments. This highlights the possible benefits of such medication in this condition as well as illuminating how they may function to improve other leukodystrophies. Cumulatively, this evidence suggests that antipsychotics act, in part, by influencing oligodendrocytes and their precursor cells.

3.3. Antipsychotics effects on astrocytes.

D-serine is an amino acid gliotransmitter secreted by astrocytes, that is involved in interactions between neurons and astrocytes by being a co-agonist at N-methyl-D-aspartate (NMDA) receptors. Clozapine but not haloperidol was shown to promote d-serine release in rat cortical astrocytes suggesting an interaction between clozapine and astrocyte-neuron signalling specifically via NMDA receptor stimulation (Tanahashi et al., 2012). D-serine as an augmentation to antipsychotic treatments was shown to be beneficial in a recent systematic review and meta-analysis by (Goh et al., 2021) as well as at high doses in a clinical trial by (Kantrowitz et al., 2010). The suggestion is that d-serine works along with antipsychotic treatment to correct low levels of glutamate seen in schizophrenia, by influencing factors such as glutamate uptake in astrocytes via decreasing glutamate transporter (GLT-1) expression. The ability of antipsychotics like clozapine to reduce GLT-1 expression has been confirmed *in-vitro* using rat cortical astrocytes (Vallejo-Illarramendi et al., 2005) and *in-vivo* using adult wistar rats (da Rocha et al., 2021).

In concordance with such evidence zotepine as well as clozapine and quetiapine (Kouji Fukuyama & Motohiro Okada, 2021; K. Fukuyama & M. Okada, 2021) have been shown to increase release of another astrocyte linked transmitter L-glutamate by activating astrocytic hemichannels. Also, a collection of agents including clozapine, olanzapine, aripiprazole, risperidone, haloperidol, and chlorpromazine have also been shown to stimulate the release of macrophage migration inhibitory factor (MIF), a cytokine expressed in astrocytes, neurons, and immune cells in primary cultured mouse astrocytes (Okazaki et al., 2021). This suggests that mechanistically antipsychotics may function in part by influence astrocyte expression on MIF. A

study also suggests that haloperidol and risperidone may act differentially, the former promoting the release of inflammatory cytokines while the latter suppressing them compared to controls in primary astrocyte culture (Bobermin et al., 2018). Animal studies using macaque monkeys treated long-term with haloperidol or olanzapine demonstrated reduced brain volume (Dorph-Petersen et al., 2005). Interestingly, it was observed that this treatment led to reduced astrocyte amounts in the parietal cortex as well as reduced volume in that area (Konopaske et al., 2008).

There are few human CNS studies that examine effects of antipsychotics on astrocytes. Human imaging studies have shown grey and white matter reductions in those prescribed antipsychotic treatment. In a longitudinal imaging study of patients presenting with first-episode schizophrenia and treated "as usual" with antipsychotics, longer use of these medications was associated with deficits in grey and white matter (Ho et al., 2011). Another study observed that haloperidol but not olanzapine was associated with reduced grey matter in those with first episode psychosis (Lieberman et al., 2005). It has been suggested that such deficits may be because of the effects of antipsychotics on astrocytes as well as other glial cells (X. Zhang, Alnafisah, Hamoud, Shukla, McCullumsmith, et al., 2021; X. Zhang, Alnafisah, Hamoud, Shukla, Wen, et al., 2021) but further research especially using human samples is required to confirm this. Collectively, these data suggest that antipsychotic treatment effects may be explained mechanistically via astrocyte interaction. It also highlights the importance of further research in this area for potential drug development.

3.4. Antipsychotics and microglia.

It has been proposed that antipsychotics may act in part, by having an anti-inflammatory effect with agents decreasing the expression of inflammatory cytokines and influencing microglia response *in-vitro* (A Kato et al., 2011). Cellular studies for example in cultured rat microglia, that were immune activated by exposure to lipopolysaccharide (LPS), drugs like flupentixol (Kowalski et al., 2003) and chlorpromazine (Labuzek et al., 2005) reduced tumour necrosis factor α (TNF-α), nitric oxide (NO) and interleukin (IL-1) and IL-2 release respectively. Similar effects in reducing expression of NO and inflammatory cytokines in immune induced microglia was seen with drugs like olanzapine (Hou et al., 2006), risperidone (Takahiro Kato et al., 2007), quetiapine (Bian et al., 2008) and aripiprazole (T. Kato et al., 2008). It is hypothesised that this may occur by the influence of antipsychotics on calcium signalling as well as mitogen-activated protein

kinase (MAPK), protein kinase C (PKC) and NF-KB pathways within microglia (A Kato et al., 2011; Mizoguchi et al., 2014). Animal studies have shown that rats treated with haloperidol and olanzapine show increased IBA1 expression in certain areas like the striatum, somatosensory cortex, and hippocampus (Cotel et al., 2015) while another study showed that clozapine reversed microglial activation in the striatum and hippocampus (Ribeiro et al., 2013). Finally, the tetracycline antibiotic minocycline in known to be an inhibitor of microglia and exert neuroprotective effects (Domercq & Matute, 2004). Its use has been proposed as a novel antipsychotic as well as an augmentation to antipsychotic medications. So too has the use of anti-inflammatory agents like cyclooxygenase-2 (COX-2) inhibitors and these agents have been shown to be effective as an augmentation strategy for schizophrenia in a recent systematic review and meta-analysis (Jeppesen et al., 2020). Taken together the above evidence highlights the effects that antipsychotic medications may have via microglia and suggests further study in this area for novel mechanistic insights or drug development.

3.5. Conclusion.

KD is a rare disorder which leads to the abnormal accumulation of the toxin psychosine and associated demyelination centrally and peripherally. This study demonstrated that the antipsychotic haloperidol increased survival, improved mobility, and positively affected behaviours in twitcher mice a naturally occurring murine model of psychosine toxicity. This strengthens findings we have shown in chapter 3 and 4 demonstrating *in-vitro* and *ex-vivo* studies showing that antipsychotics prevent psychosine induced glial cell toxicity. Although the exact mechanism for this requires further investigation, we propose that antipsychotics may interact with glial cells and exert myelin protective effect. This study prompts further research in this area examining the potential for antipsychotics or agents with similar pharmacology, especially D_2 receptor antagonists, in the treatment for KD, a leukodystrophy that leads to the accumulation of the toxin psychosine. Furthermore, pre-clinical models of schizophrenia are utilised to examine the neuro-developmental aetiology of schizophrenia and there is also a great deal of evidence linking glial cell pathology to schizophrenia (Andrea G Dietz et al., 2019; Koszła et al., 2020). The build-up of psychosine in twitcher mice leads to glial cell pathology which may mimic that found in schizophrenia for experimental purposes. Therefore, demonstrating that the potent D_2 antagonist haloperidol improves survival and behavioural metrics in twitcher mice highlights the utility of this model as a method of investigating the possible glial cell effects of antipsychotic medications, potentially identifying future glial cell specific targets for treating

schizophrenia which is an active area of research (Abdelfattah et al., 2023; Xiaolu Zhang et al., 2022).

Chapter 6 – Discussion.

1. Study background.

Schizophrenia is a chronic mental illness associated with positive (delusions and hallucinations), negative (apathy and avolition), and cognitive symptoms (working memory deficits) as well as difficulties in social and occupational functioning. It is ranked among the top 25 leading causes of disability worldwide, has a lifetime prevalence close to 1% and an incidence of approximately 15.2 per 100,000 per annum (McCutcheon et al., 2020). Estimated annual direct and indirect healthcare costs associated with the disorder per country range from $94 million to $102 billion (Chong et al., 2016). Those suffering from schizophrenia die on average 10-15 years sooner than those without schizophrenia and have up to a ten percent chance of dying by suicide (McCutcheon et al., 2020). The main biological treatment for schizophrenia is antipsychotic medication and these drugs act by antagonising dopamine and serotonin receptors in the CNS. The neuronal pathology and dopamine hypothesis of schizophrenia has been well outlined but there has been growing interest in the influence of various glial cells in the neuropathophysiology of the disorder. There has also been much interest in the effects antipsychotics have on glial cells (H. G. Bernstein et al., 2015; Andrea G Dietz et al., 2019; Toda & Abi-Dargham, 2007). This work used a psychosine toxicity model to examine if antipsychotics exerted a protective effect on human astrocytes *in-vitro* and on a mouse mixed glial organotypic cerebellar slice culture *ex-vivo*. Finally, a murine model of psychosine toxicity i.e., twitcher mice was used to examine if the haloperidol improved survival *in-vivo*.

2. Summary of findings.

This work examined the effects of antipsychotics on glial cells as follows:

In the first study (chapter 3) we investigated whether a range of antipsychotics prevented psychosine induced toxicity in human astrocytes *in-vitro*. Human astrocytes were cultured and treated with antipsychotics in the presence and absence of psychosine. MTT assays and LDH assays were then done to establish cell viability and toxicity respectively. Furthermore, immunocytochemistry and fluorescent microscopy was done to determine morphological changes in human astrocytes. In keeping with previous studies (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016), we found that psychosine was toxic to human astrocytes. Importantly antipsychotics attenuated psychosine toxicity and improved astrocyte viability. We also showed that antipsychotics

attenuated psychosine induced morphological changes in GFAP and Vimentin expression in human astrocytes. Finally, highly selective D_2 and $5HT_{2A}$ receptor antagonists were used to determine whether antagonism of these receptors was sufficient to induce the protective effects observed. It was shown that the highly selective D_2 antagonist eticlopride and $5HT_{2A}$ antagonist volinanserin prevented psychosine induced toxicity and morphological changes in human astrocytes. This implicated D_2 and $5HT_{2A}$ antagonism as being mechanistically important for the protective effects observed and taken together these data suggested that antipsychotics prevented psychosine toxicity in human astrocytes by their D_2 and $5HT_{2A}$ receptor antagonism.

Having shown that antipsychotics prevented psychosine toxicity in human astrocytes *in-vitro*, the second study (chapter 4) aimed to investigate the possible effects of antipsychotics on a mixed glial mouse organotypic cerebellar slice culture *ex-vivo*. Given the results of the previous study (chapter 3) we hypothesised that antipsychotics may influence other macroglia i.e., oligodendrocytes and possibly microglia. Mouse organotypic cerebellar slices were cultured and treated with the antipsychotics haloperidol and clozapine in the presence and absence of psychosine. Slices were immunohistochemically stained for the expression oligodendrocyte, astrocyte and microglia markers and were imaged using confocal fluorescent microscopy. In concordance with other studies psychosine caused demyelination, was toxic to astrocytes but did not influence microglia in mouse organotypic cerebellar slices (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016). Interestingly, both haloperidol and clozapine attenuated psychosine induced demyelination, prevented psychosine induced astrocyte toxicity and did not alter microglial expression in cultured cerebellar slices. The antipsychotics also prevented psychosine induced axonal damage in white matter tracts of cultured cerebellar slices. Together, these data suggested that antipsychotics may play a constructive role in regulating myelin state, by influencing oligodendrocytes and astrocytes. These data imply antipsychotics are myelin protective and they or agents with similar pharmacology may be a potential treatment for KD.

Having shown that antipsychotics including haloperidol prevented psychosine induced toxicity in human astrocytes *in-vitro* in chapter 3 and prevented psychosine related demyelination in mouse organotypic cerebellar slice cultures *ex-vivo* in chapter 4 the third study (chapter 5) aimed to determine if these results translated into improved survival, mobility, and behavioural metrics in an *in-vivo* model of psychosine toxicity i.e., the twitcher mouse model of KD. Twitcher mice, a naturally occurring murine model of KD/psychosine toxicity were treated with the

haloperidol or vehicle control. Their weight, twitching scores, immobility scores, a range of behavioural metrics on open field maze testing and survival time were recorded. Haloperidol ameliorated weight loss as well as improved twitching scores and immobility scores in twitcher mice. Subtle but significant improvements in various behavioural metrics on open field testing were also observed in twitcher mice treated with haloperidol. Also, twitcher mice treated with haloperidol showed improved survival compared to those not treated. Cumulatively these data strengthen the findings shown in the previous two studies showing that antipsychotics prevent psychosine induced glial cell toxicity. Although the exact mechanism for this requires further investigation, antipsychotics may interact with glial cells and exert a myelin protective effect. This work prompts further research in this area examining the potential for antipsychotics or agents with similar pharmacology, namely $D_2/5HT_{2A}$ receptor antagonists, in the treatment for KD, a leukodystrophy that leads to the accumulation of the toxin psychosine.

3. The role of astrocytes in schizophrenia and the effects of antipsychotics.

Many studies have implicated astrocyte dysfunction in the pathology of neuropsychiatric disorders including schizophrenia as reviewed in (Andrea G Dietz et al., 2019; Verkhratsky, Rodríguez, & Steardo, 2014). A GWAS (Goudriaan et al., 2014) showed that astrocyte related gene sets for synaptic signalling, glial development and epigenetic factors are connected to a higher risk of schizophrenia a finding confirmed by other genetic studies (Gandal et al., 2018; González-Peñas et al., 2019; Ramaker et al., 2017; Toker et al., 2018) (Gandal et al., 2018). There is some heterogeneity when different brain regions of patients with schizophrenia are examined post-mortem for various astrocyte markers like GFAP, S100β, EAAT and ALDH. This may be attributable to numerous confounding factors but there is ample evidence to implicate astrocytes pathology in schizophrenia from such studies (X. Zhang, Alnafisah, Hamoud, Shukla, McCullumsmith, et al., 2021). Animal experiments also provide evidence for abnormalities in astrocytes being connected to schizophrenia with impairments in astrocyte structure and number leading to schizophrenia like phenotypes in animals (H. S. Lee et al., 2014; Lima et al., 2014; Nishiyama et al., 2002; Roder et al., 1996). Antipsychotics have been shown to stimulate release of various astrocyte linked transmitters or “gliotransmitters” like D-serine, L-glutamate, and MIF (Kouji Fukuyama & Motohiro Okada, 2021; K. Fukuyama & M. Okada, 2021; Tanahashi et al., 2012) with D-serine showing promise as an augmentation strategy in the treatment of schizophrenia (Goh et al., 2021). Human imaging studies have shown alterations in grey matter in various CNS areas in those treated with antipsychotics and it has been suggested that such

deficits may be to the effects of antipsychotics on astrocytes (X. Zhang, Alnafisah, Hamoud, Shukla, Wen, et al., 2021).

The data from our studies agree with research that shows that antipsychotics exert a protective effect on astrocytes. We demonstrated that a range of agents prevented psychosine toxicity in human astrocytes *in-vitro* likely via D_2 and $5HT_{2A}$ antagonism. This finding was also confirmed in a mouse organotypic cerebellar slice culture *ex-vivo* using haloperidol and clozapine. To our understanding this study is the first to examine the direct effects of such a broad selection of commonly prescribed antipsychotics on human astrocytes. Astrocytes and astrocyte related cellular pathways may provide novel avenues in elucidating the mechanism of action or side effect profile of antipsychotic medications. To illustrate, clozapine related metabolic side effects may be due in part to increased AMPK signalling as demonstrated in astrocyte cultures from rats treated with the drug (K. Fukuyama, Motomura, & Okada, 2023). Amisulpride and/or aripiprazole may benefit cognitive dysfunction associated with schizophrenia in part by ameliorating inflammation by effecting the sonic hedgehog pathway in astrocytes (Abdelfattah et al., 2023). Finally, the various subtypes of astrocytes and their influences on schizophrenia as well as the effects of antipsychotics medications mediated via astrocytes is an active area of investigation (Xiaolu Zhang et al., 2022). The astrocyte related data in this thesis adds incrementally to the growing knowledge of the importance of this cell type in the pathology and the treatment of schizophrenia. Future work is needed to further examine cellular specific pathways to eventually improve the efficacy or reduce the side effects of antipsychotics in the treatment of schizophrenia.

4. Oligodendrocyte pathology in schizophrenia and myelin protective effects of antipsychotics.

Numerous studies have implicated oligodendrocytes and oligodendrocyte precursor cells in the pathology of schizophrenia (H. G. Bernstein et al., 2015; Andrea G Dietz et al., 2019). Genetic studies provide robust evidence for oligodendrocyte pathology being linked to schizophrenia with numerous gene sets being found to be associated with the disorder (Goudriaan et al., 2014). A good tabular summary of studies implicating oligodendrocyte gene related abnormalities being associated with schizophrenia can be found in reviews by (H. G. Bernstein et al., 2015; Liu et al., 2022). Post-mortem studies showed decreases in oligodendrocyte associated markers in patients with schizophrenia and most of the studies reviewed noted

alterations in oligodendrocyte density (Liu et al., 2022). Various animal models exposed to toxins that cause demyelination have shown schizophrenia like behaviours including prepulse inhibition, increased activity as well as showing movement and working memory deficits (Winship et al., 2019). Imaging studies using diffusion tensor technology have quantified changes in white matter that have been noted to develop prior to symptoms and develop along with the clinical course of the illness (Podwalski et al., 2021; Samartzis, Dima, Fusar-Poli, & Kyriakopoulos, 2014). Also, symptoms of schizophrenia can manifest in diseases with white matter pathologies such as metachromatic leukodystrophy, Niemann Pick's disease and multiple sclerosis (Walterfang et al., 2005). Given the association between oligodendrocyte dysfunction and schizophrenia the effects of antipsychotics on these cells may explain their therapeutic effect and highlight further areas for drug development. Antipsychotics can prevent the deleterious effects of cuprizone (Xu et al., 2014) on oligodendrocytes as well as correcting cuprizone induced demyelination (Xiao et al., 2008). They prevent apoptotic cell death in oligodendrocytes that were cultured under glucose deprived conditions (Johann Steiner et al., 2011). Antipsychotics have been shown to induce propagation of oligodendrocytes progenitor cells (Kimoto et al., 2011; Niu et al., 2010) and increase the number of precursor cells in the hypothalamus, corpus callosum, hippocampus and cerebral cortex (Templeton et al., 2019; H. Wang et al., 2010; Yamauchi et al., 2010). Antipsychotic medication appears to correct white matter changes including those that occur during initial presentations of schizophrenia in drug naïve individuals (Sagarwala & Nasrallah, 2021). But the effects of antipsychotics are somewhat controversial, with some studies suggesting these drugs can inhibit differentiation of oligodendrocyte precursor cells (Kroken et al., 2014; Ren et al., 2013).

Our data supports research that suggests that antipsychotics are myelin protective, we have shown that haloperidol and clozapine prevented psychosine induced demyelination in mouse organotypic cerebellar slice culture. Furthermore, we demonstrated that these drugs prevented psychosine induced axonal damage in slices. These data highlight the potential of glial cell targets for antipsychotic drug development as well as antipsychotics or agents with similar pharmacology being a potential treatment for KD. Altogether, there is clear evidence that oligodendrocytes and myelin play a part in the pathology of schizophrenia and oligodendrocytes may offer a therapeutic target for novel antipsychotic agents. Better understanding molecular pathways involved in antipsychotic and oligodendrocyte interactions would also elucidate current drug mechanisms. This work also adds to the suggestion that some antipsychotics exert their effect via improving myelination and other such agents may have utility in the treatment

of schizophrenia. Further research in this area especially examining short and long term use of antipsychotics on myelination is required to better understand oligodendrocyte pathways that may explain the therapeutic or side effect profile of antipsychotics as well as identify novel avenues for future treatments in schizophrenia (Gouvêa-Junqueira et al., 2020).

5. Antipsychotics as a potential novel therapy for Krabbe disease.

KD is a rare autosomal recessive leukodystrophy associated with the accumulation of psychosine. There is no cure for the disorder, clinical interventions focus on screening for the disease, providing general symptomatic support and treatment with haematopoietic stem cell transplantation (HSCT). HSCT has shown to increase longevity in those with the disease but those with KD still have serious mobility and speech deficits as well as a much-reduced life expectancy (D. A. Wenger et al., 2021). Current experimental treatments include gene therapy (D. Lin et al., 2005; Rafi, Rao, Luzi, Curtis, & Wenger, 2012), substrate decreasing therapy (LeVine, Pedchenko, Bronshteyn, & Pinson, 2000) and enzyme substitution therapy (W. C. Lee et al., 2007). However, no one treatment has been shown to fully ameliorate the clinical symptoms of the disease and it is thought that a combination of therapies are required to fully address the symptoms of the disorder (Li & Sands, 2014; Mikulka & Sands, 2016). The findings from our *in-vitro* and *ex-vivo* studies provided evidence supporting the investigation of D_2 and $5HT_{2A}$ antagonists in this disease and to our knowledge this was the first-time antipsychotics were used for such an investigation. For the first time we demonstrated that haloperidol increased the lifespan of twitcher mice and improved various behavioural metrics.

These data suggested that antipsychotics or agents with similar pharmacology, namely D_2/$5HT_{2A}$ receptor antagonists, may be a potential treatment for KD. The mechanism by which antipsychotics lead to this improvement requires further investigation but we hypothesise, given the evidence summarised above that antipsychotics influence the structure and function of glial cells, that these drugs may positively influence glial cells which may lead to improved demyelination in twitcher mice treated with haloperidol.

6. Dopamine antagonism as a possible mechanism for the protective effects of antipsychotics on psychosine induced glial cell toxicity.

Having demonstrated that antipsychotics prevented psychosine induced glial cell toxicity, the exact mechanism by which these drugs prevent cell toxicity requires further investigation. Psychosine has been shown to be toxic to oligodendrocytes and cause demyelination by various proposed mechanisms. These include activating apoptotic processes, inhibiting protein kinase c activity, forming lysophosphatidylcholine resulting in cytotoxicity and causing phosphorylation leading to cell death (Cantuti-Castelvetri et al., 2012; Davenport et al., 2011; S. Giri et al., 2006; Haq et al., 2003). One suggestion is that psychosine may lead to glial cell death by altering mitochondrial function for example by down regulation of the NF-κB pathway (Haq et al., 2003). MTT data from this study supports mitochondrial dysfunction being linked to cell toxicity however, this may not completely explain psychosine's toxic effects as down regulation of AMP-activated protein kinase (AMPK) by psychosine has also been implicated in astrocytes and oligodendrocytes (Shailendra Giri et al., 2008).

Notably, D_1 type receptors upregulate while D_2 type receptors downregulate cAMP (figure 1.2). It has been shown antipsychotics may exert metabolic side effects by interacting with the AMPK pathway (Souza et al., 2012), also mTOR (inhibited by AMPK) and NF-κB systems are believed to be linked with antipsychotic induced extrapyramidal side effects (Mas, Gassó, Parellada, Bernardo, & Lafuente, 2015), these or other yet unidentified pathways may link dopamine and/or serotonin antagonism with the reversal of psychosine toxicity. Interestingly, it has been shown that stimuli that increase cAMP also increase the activity of AMPK in primary cultured adipocytes (Omar, Zmuda-Trzebiatowska, Manganiello, Göransson, & Degerman, 2009; Yin, Mu, & Birnbaum, 2003). Given this, one potential mechanism may be that D_2 antagonism by antipsychotics reverses psychosine downregulation of AMPK thus preventing cell toxicity in glia cells (figure 6.1).

7. Limitations and Future Directions.

This work examined whether antipsychotic medications prevented psychosine induced toxicity in human astrocytes *in-vitro*, in a mixed glial organotypic cerebellar slice culture *ex-vivo* and whether haloperidol improved survival in a murine model of psychosine toxicity i.e., twitcher mice. We showed that a range of antipsychotics prevented psychosine toxicity *in-vitro*, that

haloperidol and clozapine prevented psychosine induced demyelination *ex-vivo* and that haloperidol increased survival in twitcher mice. This study suggested the potential positive effects of antipsychotics, or agents with similar pharmacology, towards glial cells and as a potential novel therapy in the treatment of KD.

However, this work did have some limitations. Firstly, the *in-vitro* studies relied on MTT assays of cell viability and there are confounding factors that can influence assays such as MTT concentration, cell number used, incubation time and optical wavelength measured (Ghasemi, Turnbull, Sebastian, & Kempson, 2021). Potential issues were addressed by carrying out additional LDH assays of cell toxicity to improve the robustness of this data. Previously published standard protocols were utilised for each experiment (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016). Selective dopamine and serotonin antagonists were used to determine whether one receptor pathway is more implicated than the other however no other potential mechanistic pathway was investigated in this work. Future studies could use other compounds to further examine the potential mechanisms by which antipsychotics might exert the protective effects seen e.g., the use of pertussis toxin, selective protein kinase B (AKT) and extracellular signal regulated kinase (ERK) inhibitors could be utilised to further analyse the potential downstream signalling effects of antagonism of these receptors on psychosine induced toxicity in human astrocytes. Also, examining for the expression of AMPK in treated astrocytes would investigate if antipsychotic cAMP regulation of this pathway is important.

Secondly in *ex-vivo* experiments the strength of various fluorescent markers were utilised to quantify the expression of various glial cell related antibodies. These markers can be associated with glial cell types at various stages of development and act as a proxy marker for such cells. Where possible two markers were utilised per glial cell type to improve the robustness of the data. Cerebellar slices were used as they are rich in white matter, myelin producing oligodendrocytes and psychosine has been shown to be toxic in this organotypic model in previous studies (Clementino et al., 2021; Misslin et al., 2017; Catherine O'Sullivan & Kumlesh K Dev, 2015; C. O'Sullivan et al., 2016). In future studies, the use of other brain regions more directly linked to neuropathology of schizophrenia would be interesting. Also, psychosine is a toxin associated with the leukodystrophy KD and not directly related to the pathology of schizophrenia. However, it has been shown to be toxic to glial cells and symptoms of schizophrenia are noted in other leukodystrophies.

Finally, the *in-vivo* experiments involved the use of observer scores and blinding was not fully possible as phenotypic changes between twitcher and wild-type mice became observable over time, therefore mice genotypes became identifiable. The experimenter also prepared the treatments, so no blinding was possible at the drug treatment phase. However, data were pseudo-anonymised by using animal numbers and data analysis was carried out blinded to genotype and treatment. Experimental data is limited to survival, weight and behavioural outcomes in this study and future work could carry out further immunohistochemical assays looking at changes in specific glial cell markers within the CNS and PNS of twitcher mice treated with antipsychotics. Also, this study only used haloperidol future studies could use other antipsychotics which could add weight to the suggestion here that antipsychotics may promote survival in twitcher mice and be a potential novel therapy for KD.

8. Closing remarks.

This work employed an *in-vitro* psychosine toxicity model to examine whether antipsychotics prevented human astrocyte toxicity (chapter 3). An *ex-vivo* organotypic cerebellar slice model was used to examine if haloperidol and clozapine prevented psychosine glia cell toxicity (chapter 4). Finally, the twitcher mouse model was used to examine if the *in-vitro* and *ex-vivo* findings translated into increased survival in an *in-vivo* model of psychosine toxicity (chapter 5).

Chapter 3 confirmed that psychosine reduced human astrocyte viability and demonstrated that a range of antipsychotics protected against psychosine-induced toxicity. Furthermore, it showed that D_2 and $5HT_{2A}$ antagonism is mechanistically important for this protection. Chapter 4 examined whether antipsychotics attenuated psychosine induced demyelination in organotypic cerebellar slice culture. Haloperidol and clozapine attenuated psychosine induced demyelination and prevented astrocyte toxicity, independent of microglial activation. Chapter 5 demonstrated that the antipsychotic haloperidol increased survival, improved mobility, and positively affected behaviours in twitcher mice a naturally occurring murine model of psychosine toxicity.

To our understanding this work is the first to examine the direct effects of such a broad selection of commonly prescribed antipsychotics on psychosine induced glial cell toxicity. We propose that antipsychotics may interact with glial cells and exert protective effects e.g., be protective

of myelin state. This work highlights the potential of glial cell targets for antipsychotic drug development as well as antipsychotics or agents with similar pharmacology being a potential novel treatment for KD a rare leukodystrophy which leads to the abnormal accumulation of the toxin psychosine and associated demyelination centrally and peripherally.

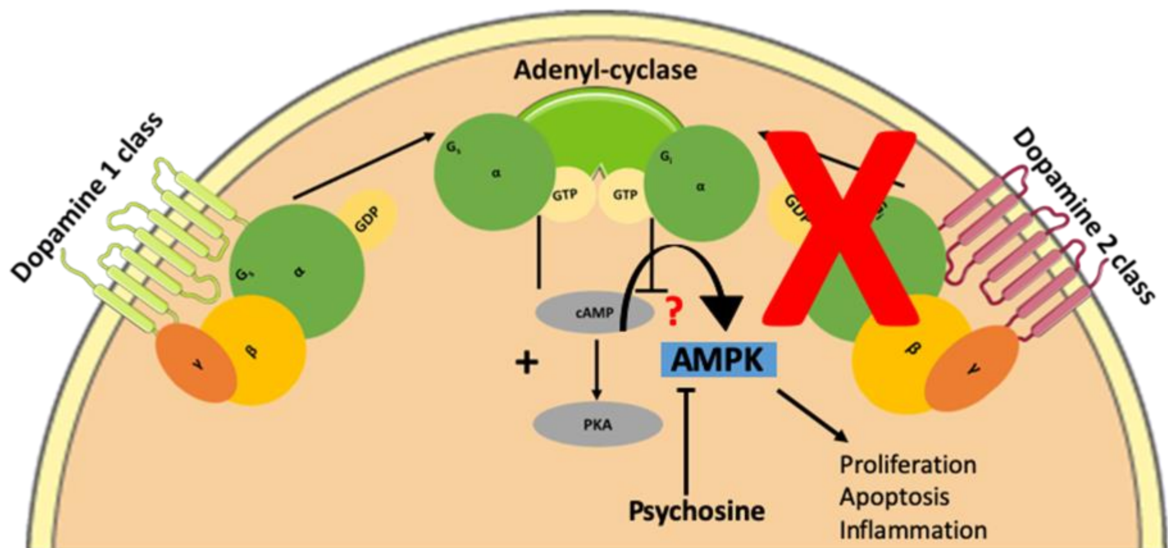


Figure 6.1 Hypothesised proposed mechanism for antipsychotic reversal of psychosine induced toxicity (?).

Psychosine downregulates AMPK activity in rat astrocytes and oligodendrocytes (Shailendra Giri et al., 2008), stimuli that increase cAMP also increase the activity of AMPK in primary cultured adipocytes (Omar et al., 2009; Yin et al., 2003). Given this, a potential mechanism may be that D2 antagonism or partial agonism by antipsychotics reverses psychosine downregulation of AMPK thus preventing cell toxicity.

References

A Kato, T., Monji, A., Mizoguchi, Y., Hashioka, S., Horikawa, H., Seki, Y., . . . Kanba, S. (2011). Anti-Inflammatory properties of antipsychotics via microglia modulations: are antipsychotics a ‘fire extinguisher’in the brain of schizophrenia? *Mini reviews in medicinal chemistry, 11*(7), 565-574.

Abbott, N. J., Rönnbäck, L., & Hansson, E. (2006). Astrocyte–endothelial interactions at the blood–brain barrier. *Nature reviews neuroscience, 7*(1), 41-53.

Abdelfattah, A. M., Abuelezz, S. A., Hendawy, N., Negm, E. A., Nawishy, S. A. E. K., & Khalil, A. M. M. (2023). Sonic hedgehog pathway as a new target of atypical antipsychotics: Revisiting of amisulpride and aripiprazole effects in a rat model of schizophrenia. *Life Sciences*, 121366.

Akhondzadeh, S., Tabatabaee, M., Amini, H., Abhari, S. A. A., Abbasi, S. H., & Behnam, B. (2007). Celecoxib as adjunctive therapy in schizophrenia: a double-blind, randomized and placebo-controlled trial. *Schizophrenia research, 90*(1-3), 179-185.

Akkouh, I. A., Hribkova, H., Grabiec, M., Budinska, E., Szabo, A., Kasparek, T., . . . Djurovic, S. (2022). Derivation and Molecular Characterization of a Morphological Subpopulation of Human iPSC Astrocytes Reveal a Potential Role in Schizophrenia and Clozapine Response. *Schizophr Bull, 48*(1), 190-198. doi:10.1093/schbul/sbab092

Araque, A., Parpura, V., Sanzgiri, R. P., & Haydon, P. G. (1999). Tripartite synapses: glia, the unacknowledged partner. *Trends in neurosciences, 22*(5), 208-215.

Association, A. P. (2013). *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)*: American Psychiatric Publishing.

Balla, P., & Frecska, E. (2011). Changes in the brain with schizophrenia: postmortem findings. *Neuropsychopharmacologia Hungarica: a Magyar Pszichofarmakologiai Egyesulet Lapja= Official Journal of the Hungarian Association of Psychopharmacology, 13*(4), 219-227.

Ban, T. A. (2007). Fifty years chlorpromazine: a historical perspective. *Neuropsychiatr Dis Treat, 3*(4), 495-500.

Beaulieu, J.-M., & Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological reviews, 63*(1), 182-217.

Beaulieu, J. M., Espinoza, S., & Gainetdinov, R. R. (2015). Dopamine receptors–IUPHAR R eview 13. *British journal of pharmacology, 172*(1), 1-23.

Béchet, S., O'Sullivan, S. A., Yssel, J., Fagan, S. G., & Dev, K. K. (2020). Fingolimod Rescues Demyelination in a Mouse Model of Krabbe's Disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 40*(15), 3104-3118. doi:10.1523/JNEUROSCI.2346-19.2020

Berger, M., Gray, J. A., & Roth, B. L. (2009). The expanded biology of serotonin. *Annual review of medicine, 60*, 355-366.

Bernstein, H.-G., Steiner, J., Guest, P. C., Dobrowolny, H., & Bogerts, B. (2015). Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. *Schizophrenia research, 161*(1), 4-18.

Bernstein, H. G., Steiner, J., Guest, P. C., Dobrowolny, H., & Bogerts, B. (2015). Glial cells as key players in schizophrenia pathology: recent insights and concepts of therapy. *Schizophr Res, 161*(1), 4-18. doi:10.1016/j.schres.2014.03.035

Bian, Q., Kato, T., Monji, A., Hashioka, S., Mizoguchi, Y., Horikawa, H., & Kanba, S. (2008). The effect of atypical antipsychotics, perospirone, ziprasidone and quetiapine on microglial activation induced by interferon-γ. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 32*(1), 42-48.

Bobermin, L. D., da Silva, A., Souza, D. O., & Quincozes-Santos, A. (2018). Differential effects of typical and atypical antipsychotics on astroglial cells in vitro. *International Journal of Developmental Neuroscience, 69*, 1-9.

Bongarzone, E. R., Howard, S. G., Schonmann, V., & Campagnoni, A. T. (1998). Identification of the dopamine D3 receptor in oligodendrocyte precursors: potential role in regulating differentiation and myelin formation. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 18*(14), 5344-5353. doi:10.1523/JNEUROSCI.18-14-05344.1998

Brandão-Teles, C., De Almeida, V., Cassoli, J. S., & Martins-de-Souza, D. (2019). Biochemical pathways triggered by antipsychotics in human oligodendrocytes: potential of discovering new treatment targets. *Frontiers in Pharmacology, 10*, 186.

Brisch, R., Bernstein, H.-G., Krell, D., Dobrowolny, H., Bielau, H., Steiner, J., . . . Bogerts, B. (2009). Dopamine–glutamate abnormalities in the frontal cortex associated with the catechol-O-methyltransferase (COMT) in schizophrenia. *Brain Research, 1269*, 166-175. doi:10.1016/j.brainres.2009.02.039

Bruneau, E. G., McCullumsmith, R. E., Haroutunian, V., Davis, K. L., & Meador-Woodruff, J. H. (2005). Increased expression of glutaminase and glutamine synthetase mRNA in the

thalamus in schizophrenia. *Schizophrenia research, 75*(1), 27-34. doi:10.1016/j.schres.2004.12.012

Button, K. S., Ioannidis, J., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S., & Munafò, M. R. (2013). Power failure: why small sample size undermines the reliability of neuroscience. *Nature reviews neuroscience, 14*(5), 365-376.

Bymaster, F. (2001). Comparative Affinity of Duloxetine and Venlafaxine for Serotonin and Norepinephrine Transporters in vitro and in vivo, Human Serotonin Receptor Subtypes, and Other Neuronal Receptors. *Neuropsychopharmacology, 25*(6), 871-880. doi:10.1016/s0893-133x(01)00298-6

Caldinelli, L., Sacchi, S., Molla, G., Nardini, M., & Pollegioni, L. (2013). Characterization of human DAAO variants potentially related to an increased risk of schizophrenia. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, 1832*(3), 400-410. doi:10.1016/j.bbadis.2012.11.019

Cantuti-Castelvetri, L., Zhu, H., Givogri, M. I., Chidavaenzi, R. L., Lopez-Rosas, A., & Bongarzone, E. R. (2012). Psychosine induces the dephosphorylation of neurofilaments by deregulation of PP1 and PP2A phosphatases. *Neurobiology of disease, 46*(2), 325-335. doi:10.1016/j.nbd.2012.01.013

Castelvetri, L. C., Givogri, M. I., Zhu, H., Smith, B., Lopez-Rosas, A., Qiu, X., . . . Bongarzone, E. R. (2011). Axonopathy is a compounding factor in the pathogenesis of Krabbe disease. *Acta neuropathologica, 122*(1), 35-48. doi:10.1007/s00401-011-0814-2

Ceylan, U., Haupeltshofer, S., Kämper, L., Dann, J., Ambrosius, B., Gold, R., & Faissner, S. (2021). Clozapine Regulates Microglia and Is Effective in Chronic Experimental Autoimmune Encephalomyelitis. *Frontiers in immunology, 12*, 656941-656941. doi:10.3389/fimmu.2021.656941

Chen, A. T., & Nasrallah, H. A. (2019). Neuroprotective effects of the second generation antipsychotics. *Schizophrenia research, 208*, 1-7.

Chen, X., Liu, H., Gan, J., Wang, X., Yu, G., Li, T., . . . Xiao, L. (2020). Quetiapine modulates histone methylation status in oligodendroglia and rescues adolescent behavioral alterations of socially isolated mice. *Frontiers in Psychiatry*, 984.

Chew, L.-J., Fusar-Poli, P., & Schmitz, T. (2013). Oligodendroglial alterations and the role of microglia in white matter injury: relevance to schizophrenia. *Developmental neuroscience, 35*(2-3), 102-129. doi:10.1159/000346157

Claycomb, K. I., Johnson, K. M., Winokur, P. N., Sacino, A. V., & Crocker, S. J. (2013). Astrocyte regulation of CNS inflammation and remyelination. *Brain sciences, 3*(3), 1109-1127. doi:10.3390/brainsci3031109

Clementino, A., Velasco-Estevez, M., Buttini, F., Sonvico, F., & Dev, K. K. (2021). Hybrid nanoparticles as a novel tool for regulating psychosine-induced neuroinflammation and demyelination in vitro and ex vivo. *Neurotherapeutics*, 1-15.

Connell, P. H. (1957). Amphetamine psychosis. *British medical journal, 1*(5018), 582.

Cotel, M.-C., Lenartowicz, E. M., Natesan, S., Modo, M. M., Cooper, J. D., Williams, S. C., . . . Vernon, A. C. (2015). Microglial activation in the rat brain following chronic antipsychotic treatment at clinically relevant doses. *European neuropsychopharmacology, 25*(11), 2098-2107.

Cunningham, C., & Peters, K. (2014). Aetiology of Schizophrenia and Implications for Nursing Practice: A Literature Review. *Issues in Mental Health Nursing, 35*(10), 732-738. doi:10.3109/01612840.2014.908441

da Rocha, A. S., Bellaver, B., Souza, D. G., Schu, G., Fontana, I. C., Venturin, G. T., . . . Machado, L. S. (2021). Clozapine Induces Astrocyte-Dependent FDG-PET Hypometabolism.

Davenport, A., Williamson, P., & Taylor, R. (2011). Pathophysiology of Krabbe disease. *Orbit: University of Sydney Undergraduate Research Journal, 2*(1).

de Hoz, L., & Simons, M. (2014). The emerging functions of oligodendrocytes in regulating neuronal network behaviour. *BioEssays, 37*(1), 60-69. doi:10.1002/bies.201400127

De Pittà, M., Brunel, N., & Volterra, A. (2016). Astrocytes: Orchestrating synaptic plasticity? *Neuroscience, 323*, 43-61.

Debs, R., Froissart, R., Aubourg, P., Papeix, C., Douillard, C., Degos, B., . . . Sedel, F. (2012). Krabbe disease in adults: phenotypic and genotypic update from a series of 11 cases and a review. *Journal of Inherited Metabolic Disease, 36*(5), 859-868. doi:10.1007/s10545-012-9560-4

Dennison, C. A., Legge, S. E., Pardinas, A. F., & Walters, J. T. (2020). Genome-wide association studies in schizophrenia: Recent advances, challenges and future perspective. *Schizophrenia research, 217*, 4-12.

Dietz, A. G., Goldman, S. A., & Nedergaard, M. (2019). Glial cells in schizophrenia: a unified hypothesis. *The Lancet Psychiatry*.

Dietz, A. G., Goldman, S. A., & Nedergaard, M. (2020). Glial cells in schizophrenia: a unified hypothesis. *Lancet Psychiatry, 7*(3), 272-281. doi:10.1016/s2215-0366(19)30302-5

Domercq, M., & Matute, C. (2004). Neuroprotection by tetracyclines. *Trends in pharmacological sciences, 25*(12), 609-612.

Dorph-Petersen, K.-A., Pierri, J. N., Perel, J. M., Sun, Z., Sampson, A. R., & Lewis, D. A. (2005). The influence of chronic exposure to antipsychotic medications on brain size before and after tissue fixation: a comparison of haloperidol and olanzapine in macaque monkeys. *Neuropsychopharmacology, 30*(9), 1649-1661.

Duchen, L., Eicher, E., Jacobs, J. M., Scaravilli, F., & Teixeira, F. (1980). Hereditary leucodystrophy in the mouse: the new mutant twitcher. *Brain: a journal of neurology, 103*(3), 695-710.

Elain, G., Jeanneau, K., Rutkowska, A., Mir, A. K., & Dev, K. K. (2014). The selective anti-IL17A monoclonal antibody secukinumab (AIN457) attenuates IL17A-induced levels of IL6 in human astrocytes. *Glia, 62*(5), 725-735. doi:10.1002/glia.22637

Figley, C. R. (2011). Lactate transport and metabolism in the human brain: implications for the astrocyte-neuron lactate shuttle hypothesis. *Journal of Neuroscience, 31*(13), 4768-4770.

Fukuyama, K., Motomura, E., & Okada, M. (2023). Opposing effects of clozapine and brexpiprazole on β-aminoisobutyric acid: Pathophysiology of antipsychotics-induced weight gain. *Schizophrenia (Heidelb), 9*(1), 8. doi:10.1038/s41537-023-00336-1

Fukuyama, K., & Okada, M. (2021). Effects of an Atypical Antipsychotic, Zotepine, on Astroglial L-Glutamate Release through Hemichannels: Exploring the Mechanism of Mood-Stabilising Antipsychotic Actions and Antipsychotic-Induced Convulsion. *Pharmaceuticals, 14*(11), 1116.

Fukuyama, K., & Okada, M. (2021). Effects of Atypical Antipsychotics, Clozapine, Quetiapine and Brexpiprazole on Astroglial Transmission Associated with Connexin43. *Int J Mol Sci, 22*(11). doi:10.3390/ijms22115623

Fukuyama, K., Okubo, R., Murata, M., Shiroyama, T., & Okada, M. (2020). Activation of Astroglial Connexin is Involved in Concentration-Dependent Double-Edged Sword Clinical Action of Clozapine. *Cells, 9*(2), 414. doi:10.3390/cells9020414

Gaebel, W., & Zielasek, J. (2015). Schizophrenia in 2020: Trends in diagnosis and therapy. *Psychiatry and Clinical Neurosciences, 69*(11), 661-673. doi:10.1111/pcn.12322

Gandal, M. J., Zhang, P., Hadjimichael, E., Walker, R. L., Chen, C., Liu, S., . . . Wang, Y. (2018). Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. *Science, 362*(6420), eaat8127.

Georgieva, L., Moskvina, V., Peirce, T., Norton, N., Bray, N. J., Jones, L., . . . Wilkinson, J. (2006). Convergent evidence that oligodendrocyte lineage transcription factor 2 (OLIG2) and

interacting genes influence susceptibility to schizophrenia. *Proceedings of the National Academy of Sciences, 103*(33), 12469-12474.

Ghasemi, M., Turnbull, T., Sebastian, S., & Kempson, I. (2021). The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. *International Journal of Molecular Sciences, 22*(23), 12827.

Gibson, E. M., Purger, D., Mount, C. W., Goldstein, A. K., Lin, G. L., Wood, L. S., . . . Zuchero, J. B. (2014). Neuronal activity promotes oligodendrogenesis and adaptive myelination in the mammalian brain. *Science, 344*(6183), 1252304.

Giri, S., Khan, M., Nath, N., Singh, I., & Singh, A. K. (2008). The role of AMPK in psychosine mediated effects on oligodendrocytes and astrocytes: implication for Krabbe disease. *Journal of neurochemistry, 105*(5), 1820-1833.

Giri, S., Khan, M., Rattan, R., Singh, I., & Singh, A. K. (2006). Krabbe disease: psychosine-mediated activation of phospholipase A2 in oligodendrocyte cell death. *Journal of Lipid Research, 47*(7), 1478-1492. doi:10.1194/jlr.m600084-jlr200

Goff, D. C., Cather, C., Evins, A. E., Henderson, D. C., Freudenreich, O., Copeland, P. M., . . . Sacks, F. M. (2005). Medical Morbidity and Mortality in Schizophrenia. *The Journal of Clinical Psychiatry, 66*(02), 183-194. doi:10.4088/jcp.v66n0205

Goh, K. K., Wu, T.-H., Chen, C.-H., & Lu, M.-L. (2021). Efficacy of N-methyl-D-aspartate receptor modulator augmentation in schizophrenia: a meta-analysis of randomised, placebo-controlled trials. *Journal of psychopharmacology, 35*(3), 236-252.

González-Peñas, J., Costas, J., Villamayor, M. J. G., & Xu, B. (2019). Enrichment of rare genetic variants in astrocyte gene enriched co-expression modules altered in postmortem brain samples of schizophrenia. *Neurobiol Dis, 121*, 305-314. doi:10.1016/j.nbd.2018.10.013

Goudriaan, A., de Leeuw, C., Ripke, S., Hultman, C. M., Sklar, P., Sullivan, P. F., . . . Verheijen, M. H. (2014). Specific glial functions contribute to schizophrenia susceptibility. *Schizophr Bull, 40*(4), 925-935. doi:10.1093/schbul/sbt109

Gouvêa-Junqueira, D., Falvella, A. C. B., Antunes, A. S. L. M., Seabra, G., Brandão-Teles, C., Martins-de-Souza, D., & Crunfli, F. (2020). Novel treatment strategies targeting myelin and oligodendrocyte dysfunction in schizophrenia. *Frontiers in Psychiatry, 11*, 379.

Hablitz, L. M., Plá, V., Giannetto, M., Vinitsky, H. S., Stæger, F. F., Metcalfe, T., . . . Nedergaard, M. (2020). Circadian control of brain glymphatic and lymphatic fluid flow. *Nature communications, 11*(1), 1-11.

Han, X., Chen, M., Wang, F., Windrem, M., Wang, S., Shanz, S., . . . Betstadt, S. (2013). Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. *Cell stem cell, 12*(3), 342-353.

Hannun, Y. A., & Bell, R. M. (1987). Lysosphingolipids Inhibit Protein Kinase C: Implications for the Sphingolipidoses. *Science, 235*(4789), 670-674. doi:10.1126/science.3101176

Haq, E., Giri, S., Singh, I., & Singh, A. K. (2003). Molecular mechanism of psychosine-induced cell death in human oligodendrocyte cell line. *Journal of neurochemistry, 86*(6), 1428-1440. doi:10.1046/j.1471-4159.2003.01941.x

Harrison, P. J., & Weinberger, D. R. (2005). Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Molecular psychiatry, 10*(1), 40-68.

Hill, R. A., & Nishiyama, A. (2014). NG2 cells (polydendrocytes): Listeners to the neural network with diverse properties. *Glia, 62*(8), 1195-1210. doi:10.1002/glia.22664

Ho, B.-C., Andreasen, N. C., Ziebell, S., Pierson, R., & Magnotta, V. (2011). Long-term antipsychotic treatment and brain volumes: a longitudinal study of first-episode schizophrenia. *Archives of General Psychiatry, 68*(2), 128-137.

Hopper, K., Harrison, G., Janca, A., & Sartorius, N. (2007). *Recovery from schizophrenia: An international perspective: A report from the WHO Collaborative Project, the international study of schizophrenia*: Oxford University Press.

Hou, Y., Wu, C. F., Yang, J. Y., He, X., Bi, X. L., Yu, L., & Guo, T. (2006). Effects of clozapine, olanzapine and haloperidol on nitric oxide production by lipopolysaccharide-activated N9 cells. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 30*(8), 1523-1528.

Howard, S., Landry, C., Fisher, R., Bezouglaia, O., Handley, V., & Campagnoni, A. (1998). Postnatal localization and morphogenesis of cells expressing the dopaminergic D2 receptor gene in rat brain: Expression in non-neuronal cells. *The Journal of Comparative Neurology, 391*(1), 87-98. doi:10.1002/(sici)1096-9861(19980202)391:1<87::aid-cne8>3.0.co;2-n

Howes, O., & McCutcheon, R. (2017). Inflammation and the neural diathesis-stress hypothesis of schizophrenia: a reconceptualization. *Translational psychiatry, 7*(2), e1024-e1024.

Hughes, E. G., Kang, S. H., Fukaya, M., & Bergles, D. E. (2013). Oligodendrocyte progenitors balance growth with self-repulsion to achieve homeostasis in the adult brain. *Nature neuroscience, 16*(6), 668-676.

Ijichi, K., Brown, G. D., Moore, C. S., Lee, J. P., Winokur, P. N., Pagarigan, R., . . . Crocker, S. J. (2013). MMP-3 mediates psychosine-induced globoid cell formation: Implications for leukodystrophy pathology. *Glia, 61*(5), 765-777.

Jeppesen, R., Christensen, R. H., Pedersen, E. M., Nordentoft, M., Hjorthøj, C., Köhler-Forsberg, O., & Benros, M. E. (2020). Efficacy and safety of anti-inflammatory agents in treatment of psychotic disorders–A comprehensive systematic review and meta-analysis. *Brain, behavior, and immunity, 90*, 364-380.

Jones, C., Watson, D., & Fone, K. (2011). Animal models of schizophrenia. *British journal of pharmacology, 164*(4), 1162-1194.

Juckel, G., Manitz, M. P., Brüne, M., Friebe, A., Heneka, M. T., & Wolf, R. J. (2011). Microglial activation in a neuroinflammational animal model of schizophrenia—a pilot study. *Schizophrenia research, 131*(1-3), 96-100.

Kahn, R. S., & Keefe, R. S. (2013). Schizophrenia is a cognitive illness: time for a change in focus. *JAMA Psychiatry, 70*(10), 1107-1112.

Kambeitz, J., Kambeitz-Ilankovic, L., Cabral, C., Dwyer, D. B., Calhoun, V. D., Van Den Heuvel, M. P., . . . Malchow, B. (2016). Aberrant functional whole-brain network architecture in patients with schizophrenia: a meta-analysis. *Schizophrenia bulletin, 42*(suppl_1), S13-S21.

Kantrowitz, J. T., Malhotra, A. K., Cornblatt, B., Silipo, G., Balla, A., Suckow, R. F., . . . Javitt, D. C. (2010). High dose D-serine in the treatment of schizophrenia. *Schizophrenia research, 121*(1-3), 125-130.

Karlsgodt, K. H., Sun, D., & Cannon, T. D. (2010). Structural and Functional Brain Abnormalities in Schizophrenia. *Current directions in psychological science, 19*(4), 226-231. doi:10.1177/0963721410377601

Karlsgodt, K. H., van Erp, T. G., Poldrack, R. A., Bearden, C. E., Nuechterlein, K. H., & Cannon, T. D. (2008). Diffusion tensor imaging of the superior longitudinal fasciculus and working memory in recent-onset schizophrenia. *Biological psychiatry, 63*(5), 512-518.

Kato, T., Mizoguchi, Y., Monji, A., Horikawa, H., Suzuki, S. O., Seki, Y., . . . Kanba, S. (2008). Inhibitory effects of aripiprazole on interferon-gamma-induced microglial activation via intracellular Ca2+ regulation in vitro. *J Neurochem, 106*(2), 815-825. doi:10.1111/j.1471-4159.2008.05435.x

Kato, T., Monji, A., Hashioka, S., & Kanba, S. (2007). Risperidone significantly inhibits interferon-γ-induced microglial activation in vitro. *Schizophrenia research, 92*(1-3), 108-115.

Katsel, P., Davis, K. L., Li, C., Tan, W., Greenstein, E., Kleiner Hoffman, L. B., & Haroutunian, V. (2008). Abnormal indices of cell cycle activity in schizophrenia and their potential association with oligodendrocytes. *Neuropsychopharmacology, 33*(12), 2993-3009.

Kimoto, S., Okuda, A., Toritsuka, M., Yamauchi, T., Makinodan, M., Okuda, H., . . . Kishimoto, T. (2011). Olanzapine stimulates proliferation but inhibits differentiation in rat oligodendrocyte precursor cell cultures. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 35*(8), 1950-1956.

Kinloch, R. A., & Cox, P. J. (2005). New targets for neuropathic pain therapeutics. *Expert Opinion on Therapeutic Targets, 9*(4), 685-698. doi:10.1517/14728222.9.4.685

Kondo, Y., Wenger, D. A., Gallo, V., & Duncan, I. D. (2005). Galactocerebrosidase-deficient oligodendrocytes maintain stable central myelin by exogenous replacement of the missing enzyme in mice. *Proceedings of the National Academy of Sciences of the United States of America, 102*(51), 18670-18675. doi:10.1073/pnas.0506473102

Konopaske, G. T., Dorph-Petersen, K. A., Sweet, R. A., Pierri, J. N., Zhang, W., Sampson, A. R., & Lewis, D. A. (2008). Effect of chronic antipsychotic exposure on astrocyte and oligodendrocyte numbers in macaque monkeys. *Biol Psychiatry, 63*(8), 759-765. doi:10.1016/j.biopsych.2007.08.018

Koszła, O., Targowska-Duda, K. M., Kędzierska, E., & Kaczor, A. A. (2020). In Vitro and In Vivo Models for the Investigation of Potential Drugs Against Schizophrenia. *Biomolecules, 10*(1), 160.

Kowalski, J., Labuzek, K., & Herman, Z. S. (2003). Flupentixol and trifluperidol reduce secretion of tumor necrosis factor-α and nitric oxide by rat microglial cells. *Neurochemistry international, 43*(2), 173-178.

Kroken, R. A., Løberg, E.-M., Drønen, T., Grüner, R., Hugdahl, K., Kompus, K., . . . Johnsen, E. (2014). A critical review of pro-cognitive drug targets in psychosis: convergence on myelination and inflammation. *Frontiers in Psychiatry, 5*, 11-11. doi:10.3389/fpsyt.2014.00011

Kuhn, S., Gritti, L., Crooks, D., & Dombrowski, Y. (2019). Oligodendrocytes in development, myelin generation and beyond. *Cells, 8*(11), 1424.

Labuzek, K., Kowalski, J., Gabryel, B., & Herman, Z. S. (2005). Chlorpromazine and loxapine reduce interleukin-1β and interleukin-2 release by rat mixed glial and microglial cell cultures. *European neuropsychopharmacology, 15*(1), 23-30.

Lee, H. S., Ghetti, A., Pinto-Duarte, A., Wang, X., Dziewczapolski, G., Galimi, F., . . . Verma, I. M. (2014). Astrocytes contribute to gamma oscillations and recognition memory. *Proceedings of the National Academy of Sciences, 111*(32), E3343-E3352.

Lee, W. C., Tsoi, Y. K., Troendle, F. J., DeLucia, M. W., Ahmed, Z., Dicky, C. A., . . . Eckman, C. B. (2007). Single-dose intracerebroventricular administration of galactocerebrosidase improves survival in a mouse model of globoid cell leukodystrophy. *The FASEB Journal, 21*(10), 2520-2527.

LeVine, S. M., Pedchenko, T. V., Bronshteyn, I. G., & Pinson, D. M. (2000). L-cycloserine slows the clinical and pathological course in mice with globoid cell leukodystrophy (twitcher mice). *Journal of neuroscience research, 60*(2), 231-236.

Lewis, D. A., Curley, A. A., Glausier, J. R., & Volk, D. W. (2012). Cortical parvalbumin interneurons and cognitive dysfunction in schizophrenia. *Trends in neurosciences, 35*(1), 57-67. doi:10.1016/j.tins.2011.10.004

Li, Y., & Sands, M. S. (2014). Experimental therapies in the murine model of globoid cell leukodystrophy. *Pediatric neurology, 51*(5), 600-606.

Lieberknecht, V., Junqueira, S. C., Cunha, M. P., Barbosa, T. A., de Souza, L. F., Coelho, I. S., . . . Dutra, R. C. (2016). Pramipexole, a Dopamine D2/D3 Receptor-Preferring Agonist, Prevents Experimental Autoimmune Encephalomyelitis Development in Mice. *Molecular neurobiology, 54*(2), 1033-1045. doi:10.1007/s12035-016-9717-5

Lieberman, J. A., Tollefson, G. D., Charles, C., Zipursky, R., Sharma, T., Kahn, R. S., . . . McEvoy, J. (2005). Antipsychotic drug effects on brain morphology in first-episode psychosis. *Archives of General Psychiatry, 62*(4), 361-370.

Lim, S., & Kaldis, P. (2013). Cdks, cyclins and CKIs: roles beyond cell cycle regulation. *Development, 140*(15), 3079-3093.

Lima, A., Sardinha, V. M., Oliveira, A., Reis, M., Mota, C., Silva, M., . . . Sousa, N. (2014). Astrocyte pathology in the prefrontal cortex impairs the cognitive function of rats. *Molecular psychiatry, 19*(7), 834-841.

Lin, D., Fantz, C. R., Levy, B., Rafi, M. A., Vogler, C., Wenger, D. A., & Sands, M. S. (2005). AAV2/5 vector expressing galactocerebrosidase ameliorates CNS disease in the murine model of globoid-cell leukodystrophy more efficiently than AAV2. *Molecular Therapy, 12*(3), 422-430.

Lin, M.-E., Rivera, R. R., & Chun, J. (2012). Targeted deletion of LPA5 identifies novel roles for lysophosphatidic acid signaling in development of neuropathic pain. *The Journal of biological chemistry, 287*(21), 17608-17617. doi:10.1074/jbc.M111.330183

Liu, S. H., Du, Y., Chen, L., & Cheng, Y. (2022). Glial Cell Abnormalities in Major Psychiatric Diseases: A Systematic Review of Postmortem Brain Studies. *Mol Neurobiol*. doi:10.1007/s12035-021-02672-8

Manitz, M. P., Esslinger, M., Wachholz, S., Plümper, J., Friebe, A., Juckel, G., & Wolf, R. (2013). The role of microglia during life span in neuropsychiatric disease—An animal study.

Martins-de-Souza, D., Gattaz, W. F., Schmitt, A., Maccarrone, G., Hunyadi-Gulyás, E., Eberlin, M. N., . . . Turck, C. W. (2009). Proteomic analysis of dorsolateral prefrontal cortex indicates the involvement of cytoskeleton, oligodendrocyte, energy metabolism and new potential markers in schizophrenia. *Journal of psychiatric research, 43*(11), 978-986.

Marui, T., Torii, Y., Iritani, S., Sekiguchi, H., Habuchi, C., Fujishiro, H., . . . Masaki, K. (2018). The neuropathological study of myelin oligodendrocyte glycoprotein in the temporal lobe of schizophrenia patients. *Acta neuropsychiatrica, 30*(4), 232-240.

Mas, S., Gassó, P., Parellada, E., Bernardo, M., & Lafuente, A. (2015). Network analysis of gene expression in peripheral blood identifies mTOR and NF-κB pathways involved in antipsychotic-induced extrapyramidal symptoms. *The Pharmacogenomics Journal, 15*(5), 452-460.

McCullumsmith, R. E., Gupta, D., Beneyto, M., Kreger, E., Haroutunian, V., Davis, K. L., & Meador-Woodruff, J. H. (2007). Expression of transcripts for myelination-related genes in the anterior cingulate cortex in schizophrenia. *Schizophrenia research, 90*(1-3), 15-27. doi:10.1016/j.schres.2006.11.017

McCutcheon, R. A., Reis Marques, T., & Howes, O. D. (2020). Schizophrenia-An Overview. *JAMA Psychiatry, 77*(2), 201-210. doi:10.1001/jamapsychiatry.2019.3360

McGrath, J., Saha, S., Chant, D., & Welham, J. (2008). Schizophrenia: a concise overview of incidence, prevalence, and mortality. *Epidemiologic reviews, 30*(1), 67-76.

Mei, F., Guo, S., He, Y., Wang, L., Wang, H., Niu, J., . . . Xiao, L. (2012). Quetiapine, an atypical antipsychotic, is protective against autoimmune-mediated demyelination by inhibiting effector T cell proliferation.

Meltzer, H. Y., & Gadaleta, E. (2021). Contrasting typical and atypical antipsychotic drugs. *Focus, 19*(1), 3-13.

Merenlender-Wagner, A., Malishkevich, A., Shemer, Z., Udawela, M., Gibbons, A., Scarr, E., . . . Gozes, I. (2015). Autophagy has a key role in the pathophysiology of schizophrenia. *Molecular psychiatry, 20*(1), 126-132. doi:10.1038/mp.2013.174

Michalski, J.-P., & Kothary, R. (2015). Oligodendrocytes in a nutshell. *Frontiers in cellular neuroscience, 9*, 340.

Mikulka, C. R., & Sands, M. S. (2016). Treatment for Krabbe's disease: finding the combination. *Journal of neuroscience research, 94*(11), 1126-1137.

Misslin, C., Velasco-Estevez, M., Albert, M., O'Sullivan, S. A., & Dev, K. K. (2017). Phospholipase A2 is involved in galactosylsphingosine-induced astrocyte toxicity, neuronal damage and demyelination. *PLoS One, 12*(11), e0187217.

Miyazaki, I., Asanuma, M., Diaz-Corrales, F. J., Miyoshi, K., & Ogawa, N. (2004). Direct evidence for expression of dopamine receptors in astrocytes from basal ganglia. *Brain Research, 1029*(1), 120-123. doi:10.1016/j.brainres.2004.09.014

Mizoguchi, Y., Kato, T. A., Horikawa, H., & Monji, A. (2014). Microglial intracellular Ca2+ signaling as a target of antipsychotic actions for the treatment of schizophrenia. *Frontiers in cellular neuroscience, 8*, 370.

Mladinov, M., Mayer, D., Brčić, L., Wolstencroft, E., Man, N., Holt, I., . . . Šimić, G. (2010). Astrocyte expression of D2-like dopamine receptors in the prefrontal cortex. *Translational Neuroscience, 1*(3). doi:10.2478/v10134-010-0035-6

Mohri, I., Taniike, M., Taniguchi, H., Kanekiyo, T., Aritake, K., Inui, T., . . . Sasai, H. (2006). Prostaglandin D2-mediated microglia/astrocyte interaction enhances astrogliosis and demyelination in twitcher. *Journal of Neuroscience, 26*(16), 4383-4393.

Monji, A., Kato, T. A., Mizoguchi, Y., Horikawa, H., Seki, Y., Kasai, M., . . . Kanba, S. (2013). Neuroinflammation in schizophrenia especially focused on the role of microglia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 42*, 115-121. doi:10.1016/j.pnpbp.2011.12.002

Moore, S., Meschkat, M., Ruhwedel, T., Trevisiol, A., Tzvetanova, I. D., Battefeld, A., . . . Möbius, W. (2020). A role of oligodendrocytes in information processing. *Nature communications, 11*(1), 1-15.

Muoio, V., Persson, P., & Sendeski, M. (2014). The neurovascular unit–concept review. *Acta physiologica, 210*(4), 790-798.

Myint, A.-M., & Kim, Y.-K. (2014). Network beyond IDO in psychiatric disorders: Revisiting neurodegeneration hypothesis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 48*, 304-313. doi:10.1016/j.pnpbp.2013.08.008

Nayak, D., Roth, T. L., & McGavern, D. B. (2014). Microglia development and function. *Annual review of immunology, 32*, 367-402.

Nicaise, A. M., Bongarzone, E. R., & Crocker, S. J. (2016). A microglial hypothesis of globoid cell leukodystrophy pathology. *Journal of neuroscience research, 94*(11), 1049-1061.

Nishiyama, H., Knöpfel, T., Endo, S., & Itohara, S. (2002). Glial protein S100B modulates long-term neuronal synaptic plasticity. *Proceedings of the National Academy of Sciences, 99*(6), 4037-4042.

Niu, J., Mei, F., Li, N., Wang, H., Li, X., Kong, J., & Xiao, L. (2010). Haloperidol promotes proliferation but inhibits differentiation in rat oligodendrocyte progenitor cell cultures. *Biochemistry and cell biology, 88*(4), 611-620.

Norman, R., Lecomte, T., Addington, D., & Anderson, E. (2017). Canadian Treatment Guidelines on Psychosocial Treatment of Schizophrenia in Adults. *Can J Psychiatry, 62*(9), 617-623. doi:10.1177/0706743717719894

Notter, T. (2021). Astrocytes in schizophrenia. *Brain and Neuroscience Advances, 5*, 23982128211009148.

O'Connell, K., Thakore, J., & Dev, K. K. (2013). Levels of S100B are raised in female patients with schizophrenia. *BMC psychiatry, 13*, 146-146. doi:10.1186/1471-244X-13-146

O'Connell, K. E., Thakore, J., & Dev, K. K. (2014). Pro-inflammatory cytokine levels are raised in female schizophrenia patients treated with clozapine. *Schizophrenia research, 156*(1), 1-8. doi:10.1016/j.schres.2014.03.020

O'Sullivan, C., & Dev, K. K. (2015). Galactosylsphingosine (psychosine)-induced demyelination is attenuated by sphingosine 1-phosphate signalling. *Journal of cell science, 128*(21), 3878-3887.

O'Sullivan, C., & Dev, K. K. (2015). Galactosylsphingosine (psychosine) induced demyelination is attenuated by sphingosine 1-phosphate signalling. *Journal of cell science*. doi:10.1242/jcs.169342

O'Sullivan, C., Schubart, A., Mir, A. K., & Dev, K. K. (2016). The dual S1PR1/S1PR5 drug BAF312 (Siponimod) attenuates demyelination in organotypic slice cultures. *Journal of neuroinflammation, 13*, 31-31. doi:10.1186/s12974-016-0494-x

O'Sullivan, S. A., & Dev, K. K. (2017). The chemokine fractalkine (CX3CL1) attenuates H(2)O(2)-induced demyelination in cerebellar slices. *Journal of neuroinflammation, 14*(1), 159-159. doi:10.1186/s12974-017-0932-4

O'Sullivan, S. A., Velasco-Estevez, M., & Dev, K. K. (2017). Demyelination induced by oxidative stress is regulated by sphingosine 1-phosphate receptors. *Glia, 65*(7), 1119-1136. doi:10.1002/glia.23148

O'Connell, K. E., Thakore, J., & Dev, K. K. (2015). Increased interleukin 23 (IL23) levels in schizophrenia patients treated with depot antipsychotic medication. *Cytokine, 73*(1), 196-198. doi:10.1016/j.cyto.2014.11.003

Oertel-Knöchel, V., Lancaster, T. M., Knöchel, C., Stäblein, M., Storchak, H., Reinke, B., . . . Linden, D. E. J. (2015). Schizophrenia risk variants modulate white matter volume across the psychosis spectrum: evidence from two independent cohorts. *NeuroImage. Clinical, 7*, 764-770. doi:10.1016/j.nicl.2015.03.005

Ohsawa, M., Miyabe, Y., Katsu, H., Yamamoto, S., & Ono, H. (2013). Identification of the sensory nerve fiber responsible for lysophosphatidic acid-induced allodynia in mice. *Neuroscience, 247*, 65-74. doi:10.1016/j.neuroscience.2013.05.014

Okazaki, S., Boku, S., Otsuka, I., Horai, T., Kimura, A., Shimmyo, N., . . . Hishimoto, A. (2021). Clozapine increases macrophage migration inhibitory factor (MIF) expression via increasing histone acetylation of MIF promoter in astrocytes. *J Psychiatr Res, 135*, 237-242. doi:10.1016/j.jpsychires.2021.01.033

Omar, B., Zmuda-Trzebiatowska, E., Manganiello, V., Göransson, O., & Degerman, E. (2009). Regulation of AMP-activated protein kinase by cAMP in adipocytes: roles for phosphodiesterases, protein kinase B, protein kinase A, Epac and lipolysis. *Cellular signalling, 21*(5), 760-766.

Organization, W. H. (2019). International Statistical Classification of Diseases and Related Health Problems (11th ed.). Retrieved from https://icd.who.int/

Patel, K. R., Cherian, J., Gohil, K., & Atkinson, D. (2014). Schizophrenia: overview and treatment options. *Pharmacy and Therapeutics, 39*(9), 638.

Patergnani, S., Bonora, M., Ingusci, S., Previati, M., Marchi, S., Zucchini, S., . . . Pugliatti, M. (2021). Antipsychotic drugs counteract autophagy and mitophagy in multiple sclerosis. *Proceedings of the National Academy of Sciences, 118*(24).

Pedrini, M., Chendo, I., Grande, I., Lobato, M. I., Belmonte-de-Abreu, P. S., Lersch, C., . . . Gama, C. S. (2011). Serum brain-derived neurotrophic factor and clozapine daily dose in patients with schizophrenia: a positive correlation. *Neuroscience Letters, 491*(3), 207-210.

Petrasch-Parwez, E., Schöbel, A., Benali, A., Moinfar, Z., Förster, E., Brüne, M., & Juckel, G. (2020). Lateralization of increased density of Iba1-immunopositive microglial cells in the anterior midcingulate cortex of schizophrenia and bipolar disorder. *European Archives of Psychiatry and Clinical Neuroscience, 270*(7), 819-828.

Podwalski, P., Szczygieł, K., Tyburski, E., Sagan, L., Misiak, B., & Samochowiec, J. (2021). Magnetic resonance diffusion tensor imaging in psychiatry: A narrative review of its potential role in diagnosis. *Pharmacological Reports, 73*(1), 43-56.

Poels, E. M. P., Kegeles, L. S., Kantrowitz, J. T., Slifstein, M., Javitt, D. C., Lieberman, J. A., . . . Girgis, R. R. (2013). Imaging glutamate in schizophrenia: review of findings and implications for drug discovery. *Molecular psychiatry, 19*(1), 20-29. doi:10.1038/mp.2013.136

Potter, G. B., & Petryniak, M. A. (2016). Neuroimmune mechanisms in K rabbe's disease. *Journal of neuroscience research, 94*(11), 1341-1348.

Prata, D. P., Kanaan, R. A., Barker, G. J., Shergill, S., Woolley, J., Georgieva, L., . . . Allin, M. (2013). Risk variant of oligodendrocyte lineage transcription factor 2 is associated with reduced white matter integrity. *Human brain mapping, 34*(9), 2025-2031.

Prinz, M., Jung, S., & Priller, J. (2019). Microglia biology: one century of evolving concepts. *Cell, 179*(2), 292-311.

Rafi, M. A., Rao, H. Z., Luzi, P., Curtis, M. T., & Wenger, D. A. (2012). Extended normal life after AAVrh10-mediated gene therapy in the mouse model of Krabbe disease. *Molecular Therapy, 20*(11), 2031-2042.

Ramaker, R. C., Bowling, K. M., Lasseigne, B. N., Hagenauer, M. H., Hardigan, A. A., Davis, N. S., . . . Vawter, M. P. (2017). Post-mortem molecular profiling of three psychiatric disorders. *Genome medicine, 9*(1), 1-12.

Rang, H. P., Dale, M. M., Ritter, J. M., Flower, R. J., & Henderson, G. (2011). *Rang & Dale's Pharmacology*: Elsevier Health Sciences UK.

Rapaport, M. H., Delrahim, K. K., Bresee, C. J., Maddux, R. E., Ahmadpour, O., & Dolnak, D. (2005). Celecoxib augmentation of continuously ill patients with schizophrenia. *Biological psychiatry, 57*(12), 1594-1596.

Ren, Y., Wang, H., & Xiao, L. (2013). Improving myelin/oligodendrocyte-related dysfunction: a new mechanism of antipsychotics in the treatment of schizophrenia? *International Journal of Neuropsychopharmacology, 16*(3), 691-700. doi:10.1017/s1461145712001095

Ribeiro, B. M., do Carmo, M. R., Freire, R. S., Rocha, N. F., Borella, V. C., de Menezes, A. T., . . . Macêdo, D. (2013). Evidences for a progressive microglial activation and increase in iNOS expression in rats submitted to a neurodevelopmental model of schizophrenia: reversal by clozapine. *Schizophr Res, 151*(1-3), 12-19. doi:10.1016/j.schres.2013.10.040

Robinson, D. G., Woerner, M. G., McMeniman, M., Mendelowitz, A., & Bilder, R. M. (2004). Symptomatic and functional recovery from a first episode of schizophrenia or schizoaffective disorder. *Am J Psychiatry, 161*(3), 473-479. doi:10.1176/appi.ajp.161.3.473

Roder, J. K., Roder, J. C., & Gerlai, R. (1996). Conspecific exploration in the T-maze: Abnormalities in S100β transgenic mice. *Physiology & behavior, 60*(1), 31-36.

Rosin, C., Colombo, S., Calver, A. A., Bates, T. E., & Skaper, S. D. (2005). Dopamine D2 and D3 receptor agonists limit oligodendrocyte injury caused by glutamate oxidative stress and oxygen/glucose deprivation. *Glia, 52*(4), 336-343. doi:10.1002/glia.20250

Ross, C. A., Margolis, R. L., Reading, S. A. J., Pletnikov, M., & Coyle, J. T. (2006). Neurobiology of Schizophrenia. *Neuron, 52*(1), 139-153. doi:10.1016/j.neuron.2006.09.015

Rueden, C. T., & Eliceiri, K. W. (2019). ImageJ for the next generation of scientific image data. *Microscopy and microanalysis, 25*(S2), 142-143.

Rutkowska, A., Preuss, I., Gessier, F., Sailer, A. W., & Dev, K. K. (2014). EBI2 regulates intracellular signaling and migration in human astrocyte. *Glia, 63*(2), 341-351. doi:10.1002/glia.22757

Sagarwala, R., & Nasrallah, H. A. (2021). The effect of antipsychotic medications on white matter integrity in first-episode drug-naive patients with psychosis: A review of DTI studies. *Asian Journal of Psychiatry, 61*, 102688.

Samartzis, L., Dima, D., Fusar-Poli, P., & Kyriakopoulos, M. (2013). White Matter Alterations in Early Stages of Schizophrenia: A Systematic Review of Diffusion Tensor Imaging Studies. *Journal of neuroimaging, 24*(2), 101-110. doi:10.1111/j.1552-6569.2012.00779.x

Samartzis, L., Dima, D., Fusar-Poli, P., & Kyriakopoulos, M. (2014). White matter alterations in early stages of schizophrenia: a systematic review of diffusion tensor imaging studies. *Journal of neuroimaging, 24*(2), 101-110.

Sarnyai, Z., Alsaif, M., Bahn, S., Ernst, A., Guest, P. C., Hradetzky, E., . . . Wesseling, H. (2011). Behavioral and Molecular Biomarkers in Translational Animal Models for Neuropsychiatric Disorders. In *International Review of Neurobiology* (pp. 203-238): Elsevier.

Schafer, Dorothy P., Lehrman, Emily K., Kautzman, Amanda G., Koyama, R., Mardinly, Alan R., Yamasaki, R., . . . Stevens, B. (2012). Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner. *Neuron, 74*(4), 691-705. doi:10.1016/j.neuron.2012.03.026

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., . . . Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. *Nature methods, 9*(7), 676-682.

Schwab, S. G., & Wildenauer, D. B. (2013). Genetics of psychiatric disorders in the GWAS era: an update on schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience, 263*(S2), 147-154. doi:10.1007/s00406-013-0450-z

Seabra, G., de Almeida, V., Reis-de-Oliveira, G., Crunfli, F., Antunes, A. S. L. M., & Martins-de-Souza, D. (2020). Ubiquitin–proteasome system, lipid metabolism and DNA damage repair are triggered by antipsychotic medication in human oligodendrocytes: implications in schizophrenia. *Scientific reports, 10*(1), 1-15.

Servier. Servier Medical Art (smart.servier.com)

Retrieved from smart.servier.com

Shergill, S. S., Kanaan, R. A., Chitnis, X. A., O'Daly, O., Jones, D. K., Frangou, S., . . . Murray, R. M. (2007). A diffusion tensor imaging study of fasciculi in schizophrenia. *American Journal of Psychiatry, 164*(3), 467-473.

Sheridan, G. K., & Dev, K. K. (2014). Targeting S1P receptors in experimental autoimmune encephalomyelitis in mice improves early deficits in locomotor activity and increases ultrasonic vocalisations. *Scientific reports, 4*(1), 1-6.

Siafis, S., Tzachanis, D., Samara, M., & Papazisis, G. (2018). Antipsychotic drugs: from receptor-binding profiles to metabolic side effects. *Current neuropharmacology, 16*(8), 1210-1223.

Smolders, S., Smolders, S. M., Swinnen, N., Gärtner, A., Rigo, J.-M., Legendre, P., & Brône, B. (2015). Maternal immune activation evoked by polyinosinic: polycytidylic acid does not evoke microglial cell activation in the embryo. *Frontiers in cellular neuroscience, 9*, 301.

Sneeboer, M. A., van der Doef, T., Litjens, M., Psy, N., Melief, J., Hol, E. M., . . . de Witte, L. D. (2020). Microglial activation in schizophrenia: Is translocator 18 kDa protein (TSPO) the right marker? *Schizophrenia research, 215*, 167-172.

Snijders, G. J., van Zuiden, W., Sneeboer, M. A., Berdenis van Berlekom, A., van der Geest, A. T., Schnieder, T., . . . de Witte, L. D. (2021). A loss of mature microglial markers without immune activation in schizophrenia. *Glia, 69*(5), 1251-1267.

Souza, R. P., Tiwari, A. K., Chowdhury, N. I., Ceddia, R. B., Lieberman, J. A., Meltzer, H. Y., . . . Müller, D. J. (2012). Association study between variants of AMP-activated protein kinase catalytic and regulatory subunit genes with antipsychotic-induced weight gain. *Journal of psychiatric research, 46*(4), 462-468.

Stahl, S. M. (2008). *Antipsychotics and Mood Stabilizers: Stahl's Essential Psychopharmacology, 3rd Edition*: Cambridge University Press.

Stefansson, H., Ophoff, R. A., Steinberg, S., Andreassen, O. A., Cichon, S., Rujescu, D., . . . Mortensen, P. B. (2009). Common variants conferring risk of schizophrenia. *Nature, 460*(7256), 744-747.

Steiner, J., Myint, A. M., Schiltz, K., Westphal, S., Bernstein, H. G., Walter, M., . . . Bogerts, B. (2010). S100B Serum Levels in Schizophrenia Are Presumably Related to Visceral Obesity and Insulin Resistance. *Cardiovasc Psychiatry Neurol, 2010*, 480707. doi:10.1155/2010/480707

Steiner, J., Sarnyai, Z., Westphal, S., Gos, T., Bernstein, H.-G., Bogerts, B., & Keilhoff, G. (2011). Protective effects of haloperidol and clozapine on energy-deprived OLN-93 oligodendrocytes. *European Archives of Psychiatry and Clinical Neuroscience, 261*(7), 477-482.

Steiner, J., Westphal, S., Schroeter, M. L., Schiltz, K., Jordan, W., Müller, U. J., . . . Jacobs, R. (2012). Increased S100B+ NK cell counts in acutely ill schizophrenia patients are correlated with the free cortisol index, but not with S100B serum levels. *Brain Behav Immun, 26*(4), 564-567. doi:10.1016/j.bbi.2012.01.018

Stilo, S. A., & Murray, R. M. (2019). Non-genetic factors in schizophrenia. *Current psychiatry reports, 21*(10), 1-10.

Stone, J. M., Morrison, P. D., & Pilowsky, L. S. (2007). Glutamate and dopamine dysregulation in schizophrenia—a synthesis and selective review. *Journal of psychopharmacology, 21*(4), 440-452.

Sullivan, P. F., Kendler, K. S., & Neale, M. C. (2003). Schizophrenia as a Complex Trait. *Archives of General Psychiatry, 60*(12), 1187. doi:10.1001/archpsyc.60.12.1187

Suzuki, K. (1998). Twenty five years of the "psychosine hypothesis": a personal perspective of its history and present status. *Neurochemical Research, 23*(3), 251-259.

Takahashi, N., & Sakurai, T. (2013). Roles of glial cells in schizophrenia: possible targets for therapeutic approaches. *Neurobiology of disease, 53*, 49-60.

Takahashi, N., Sakurai, T., Davis, K. L., & Buxbaum, J. D. (2011). Linking oligodendrocyte and myelin dysfunction to neurocircuitry abnormalities in schizophrenia. *Progress in neurobiology, 93*(1), 13-24.

Tanahashi, S., Yamamura, S., Nakagawa, M., Motomura, E., & Okada, M. (2012). Clozapine, but not haloperidol, enhances glial D-serine and L-glutamate release in rat frontal cortex and primary cultured astrocytes. *British journal of pharmacology, 165*(5), 1543-1555.

Tarasov, V. V., Svistunov, A. A., Chubarev, V. N., Sologova, S. S., Mukhortova, P., Levushkin, D., . . . Aliev, G. (2019). Alterations of Astrocytes in the Context of Schizophrenic Dementia. *Front Pharmacol, 10*, 1612. doi:10.3389/fphar.2019.01612

Templeton, N., Kivell, B., McCaughey-Chapman, A., Connor, B., & La Flamme, A. C. (2019). Clozapine administration enhanced functional recovery after cuprizone demyelination. *PLoS One, 14*(5), e0216113.

Terrillion, C. E., Abazyan, B., Yang, Z., Crawford, J., Shevelkin, A. V., Jouroukhin, Y., . . . Snyder, S. H. (2017). DISC1 in astrocytes influences adult neurogenesis and hippocampus-dependent behaviors in mice. *Neuropsychopharmacology, 42*(11), 2242-2251.

Thomas, E. A. (2006). Molecular Profiling of Antipsychotic Drug Function: Convergent Mechanisms in the Pathology and Treatment of Psychiatric Disorders. *Molecular neurobiology, 34*(2), 109-128. doi:10.1385/mn:34:2:109

Toda, M., & Abi-Dargham, A. (2007). Dopamine hypothesis of schizophrenia: making sense of it all. *Current psychiatry reports, 9*(4), 329-336.

Tohyama, J., Matsuda, J., & Suzuki, K. (2001). *Neurochemical Research, 26*(6), 667-671. doi:10.1023/a:1010991420942

Toker, L., Mancarci, B. O., Tripathy, S., & Pavlidis, P. (2018). Transcriptomic Evidence for Alterations in Astrocytes and Parvalbumin Interneurons in Subjects With Bipolar Disorder and Schizophrenia. *Biol Psychiatry, 84*(11), 787-796. doi:10.1016/j.biopsych.2018.07.010

Trépanier, M., Hopperton, K., Mizrahi, R., Mechawar, N., & Bazinet, R. (2016). Postmortem evidence of cerebral inflammation in schizophrenia: a systematic review. *Molecular psychiatry, 21*(8), 1009-1026.

Truini, A., Barbanti, P., Pozzilli, C., & Cruccu, G. (2013). A mechanism-based classification of pain in multiple sclerosis. *Journal of neurology, 260*(2), 351-367. doi:10.1007/s00415-012-6579-2

Tsoi, D. T.-Y., Hunter, M. D., & Woodruff, P. W. (2008). History, aetiology, and symptomatology of schizophrenia. *Psychiatry, 7*(10), 404-409.

Tsunoda, I., & Fujinami, R. S. (2002). Inside-Out versus Outside-In models for virus induced demyelination: axonal damage triggering demyelination. *Springer seminars in immunopathology, 24*(2), 105-125. doi:10.1007/s00281-002-0105-z

Uhlhaas, P. J., & Singer, W. (2010). Abnormal neural oscillations and synchrony in schizophrenia. *Nature reviews neuroscience, 11*(2), 100-113.

Uranova, N. A., Vikhreva, O. V., Rakhmanova, V. I., & Orlovskaya, D. D. (2018). Ultrastructural pathology of oligodendrocytes adjacent to microglia in prefrontal white matter in schizophrenia. *npj Schizophrenia, 4*(1), 1-10.

Uranova, N. A., Vikhreva, O. V., Rakhmanova, V. I., & Orlovskaya, D. D. (2020). Dystrophy of oligodendrocytes and adjacent microglia in prefrontal gray matter in schizophrenia. *Frontiers in Psychiatry, 11*, 204.

Vallejo-Illarramendi, A., Torres-Ramos, M., Melone, M., Conti, F., & Matute, C. (2005). Clozapine reduces GLT-1 expression and glutamate uptake in astrocyte cultures. *Glia, 50*(3), 276-279. doi:10.1002/glia.20172

Van Horn, M. R., Sild, M., & Ruthazer, E. S. (2013). D-serine as a gliotransmitter and its roles in brain development and disease. *Front Cell Neurosci, 7*, 39. doi:10.3389/fncel.2013.00039

Van Kesteren, C., Gremmels, H., De Witte, L., Hol, E., Van Gool, A., Falkai, P., . . . Sommer, I. (2017). Immune involvement in the pathogenesis of schizophrenia: a meta-analysis on postmortem brain studies. *Translational psychiatry, 7*(3), e1075-e1075.

Vasile, F., Dossi, E., & Rouach, N. (2017). Human astrocytes: structure and functions in the healthy brain. *Brain Struct Funct, 222*(5), 2017-2029. doi:10.1007/s00429-017-1383-5

Velasco-Estevez, M., Gadalla, K. K., Liñan-Barba, N., Cobb, S., Dev, K. K., & Sheridan, G. K. (2020). Inhibition of Piezo1 attenuates demyelination in the central nervous system. *Glia, 68*(2), 356-375.

Verkhratsky, A., & Nedergaard, M. (2014). Astroglial cradle in the life of the synapse. *Philosophical Transactions of the Royal Society B: Biological Sciences, 369*(1654), 20130595.

Verkhratsky, A., & Nedergaard, M. (2018). Physiology of Astroglia. *Physiological Reviews, 98*(1), 239-389. doi:10.1152/physrev.00042.2016

Verkhratsky, A., Rodríguez, J. J., & Steardo, L. (2014). Astrogliopathology: a central element of neuropsychiatric diseases? *The Neuroscientist, 20*(6), 576-588.

Vidal, P. M., & Pacheco, R. (2020). The cross-talk between the dopaminergic and the immune system involved in schizophrenia. *Frontiers in Pharmacology, 11*, 394.

Vikhreva, O., Rakhmanova, V., Orlovskaya, D., & Uranova, N. (2016). Ultrastructural alterations of oligodendrocytes in prefrontal white matter in schizophrenia: a post-mortem morphometric study. *Schizophrenia research, 177*(1-3), 28-36.

Vita, A., De Peri, L., Deste, G., Barlati, S., & Sacchetti, E. (2015). The effect of antipsychotic treatment on cortical gray matter changes in schizophrenia: does the class matter? A

meta-analysis and meta-regression of longitudinal magnetic resonance imaging studies. *Biological psychiatry, 78*(6), 403-412.

Walterfang, M., Wood, S. J., Velakoulis, D., Copolov, D., & Pantelis, C. (2005). Diseases of white matter and schizophrenia-like psychosis. *Australian & New Zealand Journal of Psychiatry, 39*(9), 746-756.

Wang, C., Aleksic, B., & Ozaki, N. (2015). Glia-related genes and their contribution to schizophrenia. *Psychiatry and Clinical Neurosciences, 69*(8), 448-461.

Wang, H.-n., Liu, G.-h., Zhang, R.-g., Xue, F., Wu, D., Chen, Y.-c., . . . Tan, Q.-r. (2016). Quetiapine ameliorates schizophrenia-like behaviors and protects myelin integrity in cuprizone intoxicated mice: the involvement of notch signaling pathway. *International Journal of Neuropsychopharmacology, 19*(2).

Wang, H., Xu, H., Niu, J., Mei, F., Li, X., Kong, J., . . . Xiao, L. (2010). Haloperidol activates quiescent oligodendroglia precursor cells in the adult mouse brain. *Schizophrenia research, 119*(1-3), 164-174.

Wang, X., Su, Y., Li, T., Yu, G., Wang, Y., Chen, X., . . . Xiao, L. (2021). Quetiapine promotes oligodendroglial process outgrowth and membrane expansion by orchestrating the effects of Olig1. *Glia, 69*(7), 1709-1722.

Webster, M. J., O'Grady, J., Kleinman, J. E., & Weickert, C. S. (2005). Glial fibrillary acidic protein mRNA levels in the cingulate cortex of individuals with depression, bipolar disorder and schizophrenia. *Neuroscience, 133*(2), 453-461. doi:10.1016/j.neuroscience.2005.02.037

Wegener, A., Deboux, C., Bachelin, C., Frah, M., Kerninon, C., Seilhean, D., . . . Nait-Oumesmar, B. (2015). Gain of Olig2 function in oligodendrocyte progenitors promotes remyelination. *Brain, 138*(1), 120-135.

Wenger, D. (2001). Galactosyl-ceramide lipidosis: globoid cell leukodystrophy (Krabbe disease). *The metabolic and molecular bases of inherited disease*.

Wenger, D. A., Luzi, P., & Rafi, M. A. (2021). Advances in the Diagnosis and Treatment of Krabbe Disease. *Int J Neonatal Screen, 7*(3). doi:10.3390/ijns7030057

Wenger, D. A., Rafi, M. A., & Luzi, P. (2016). Krabbe disease: One Hundred years from the bedside to the bench to the bedside. *J Neurosci Res, 94*(11), 982-989. doi:10.1002/jnr.23743

White, A. B., Galbiati, F., Givogri, M. I., Lopez Rosas, A., Qiu, X., van Breemen, R., & Bongarzone, E. R. (2011). Persistence of psychosine in brain lipid rafts is a limiting factor in the therapeutic recovery of a mouse model for Krabbe disease. *Journal of neuroscience research, 89*(3), 352-364. doi:10.1002/jnr.22564

Wicks, S. E., Londot, H., Zhang, B., Dowden, J., Klopf-Eiermann, J., Fisher-Perkins, J. M., . . . Gimble, J. M. (2011). Effect of intrastriatal mesenchymal stromal cell injection on progression of a murine model of Krabbe disease. *Behavioural brain research, 225*(2), 415-425.

Williams, M., Marsh, R., Macdonald, C., Jain, J., Pearce, R., Hirsch, S., . . . Maier, M. (2013). Neuropathological changes in the nucleus basalis in schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience, 263*(6), 485-495.

Williams, M. R., Hampton, T., Pearce, R. K., Hirsch, S. R., Ansorge, O., Thom, M., & Maier, M. (2013). Astrocyte decrease in the subgenual cingulate and callosal genu in schizophrenia. *Eur Arch Psychiatry Clin Neurosci, 263*(1), 41-52. doi:10.1007/s00406-012-0328-5

Windrem, M. S., Osipovitch, M., Liu, Z., Bates, J., Chandler-Militello, D., Zou, L., . . . Miller, R. H. (2017). Human iPSC glial mouse chimeras reveal glial contributions to schizophrenia. *Cell stem cell, 21*(2), 195-208. e196.

Winship, I. R., Dursun, S. M., Baker, G. B., Balista, P. A., Kandratavicius, L., Maia-de-Oliveira, J. P., . . . Howland, J. G. (2019). An overview of animal models related to schizophrenia. *The Canadian Journal of Psychiatry, 64*(1), 5-17.

Won, J.-S., Singh, A. K., & Singh, I. (2016). Biochemical, cell biological, pathological, and therapeutic aspects of Krabbe's disease. *Journal of neuroscience research, 94*(11), 990-1006. doi:10.1002/jnr.23873

Wright, P., Nimgaonkar, V. L., Donaldson, P. T., & Murray, R. M. (2001). Schizophrenia and HLA: a review. *Schizophrenia research, 47*(1), 1-12.

Xiao, L., Xu, H., Zhang, Y., Wei, Z., He, J., Jiang, W., . . . Deng, Y. (2008). Quetiapine facilitates oligodendrocyte development and prevents mice from myelin breakdown and behavioral changes. *Molecular psychiatry, 13*(7), 697-708.

Xu, H., Yang, H.-J., & Li, X.-M. (2014). Differential effects of antipsychotics on the development of rat oligodendrocyte precursor cells exposed to cuprizone. *European Archives of Psychiatry and Clinical Neuroscience, 264*(2), 121-129.

Yamada, H., Martin, P., & Suzuki, K. (1996). Impairment of protein kinase C activity in twitcher Schwann cells in vitro. *Brain Research, 718*(1-2), 138-144. doi:10.1016/0006-8993(96)00098-4

Yamauchi, T., Tatsumi, K., Makinodan, M., Kimoto, S., Toritsuka, M., Okuda, H., . . . Wanaka, A. (2010). Olanzapine increases cell mitotic activity and oligodendrocyte-lineage cells in the hypothalamus. *Neurochemistry international, 57*(5), 565-571.

Yin, W., Mu, J., & Birnbaum, M. J. (2003). Role of AMP-activated protein kinase in cyclic AMP-dependent lipolysis In 3T3-L1 adipocytes. *Journal of Biological Chemistry, 278*(44), 43074-43080.

Zaka, M., & Wenger, D. A. (2004). Psychosine-induced apoptosis in a mouse oligodendrocyte progenitor cell line is mediated by caspase activation. *Neuroscience Letters, 358*(3), 205-209. doi:10.1016/j.neulet.2003.12.126

Zhai, J., Cheng, L., Dong, J., Shen, Q., Zhang, Q., Chen, M., . . . Chen, C. (2012). S100B gene polymorphisms predict prefrontal spatial function in both schizophrenia patients and healthy individuals. *Schizophrenia research, 134*(1), 89-94. doi:10.1016/j.schres.2011.09.029

Zhang, X., Alnafisah, R. S., Hamoud, A. A., Shukla, R., McCullumsmith, R. E., & O'Donovan, S. M. (2021). Astrocytes in Neuropsychiatric Disorders: A Review of Postmortem Evidence. *Adv Neurobiol, 26*, 153-172. doi:10.1007/978-3-030-77375-5_8

Zhang, X., Alnafisah, R. S., Hamoud, A. A., Shukla, R., Wen, Z., McCullumsmith, R. E., & O'Donovan, S. M. (2021). Role of Astrocytes in Major Neuropsychiatric Disorders. *Neurochem Res, 46*(10), 2715-2730. doi:10.1007/s11064-020-03212-x

Zhang, X., Wolfinger, A., Wu, X., Alnafisah, R., Imami, A., Hamoud, A.-r., . . . Shukla, R. (2022). Gene Enrichment Analysis of Astrocyte Subtypes in Psychiatric Disorders and Psychotropic Medication Datasets. *Cells, 11*(20), 3315.

Zipp, F., & Aktas, O. (2006). The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. *Trends in neurosciences, 29*(9), 518-527. doi:10.1016/j.tins.2006.07.006

Zohar, J., & Kasper, S. (2016). Neuroscience-based Nomenclature (NbN): A call for action. *The World Journal of Biological Psychiatry, 17*(5), 318-320.