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Converging environmental and genetic risk factors during embryonic development of mice cause lasting, long-term behavioural and neurobiological changes relevant to schizophrenia.

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD).

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Summary

There have been many advances in the field of neuroscience and psychiatric disorders since the first concepts of neurological involvement in the 1800s. However, a singular underlying cause of schizophrenia remains elusive to researchers. Additionally, currently available treatment options for those with schizophrenia have not changed since their discovery in the 1950s. As a result, it is important that we continue to research the development of schizophrenia as a neurological disorder to advance treatment, support and interventions.

Our current understanding characterises schizophrenia as a complex neurodevelopmental disorder which is diagnosed during adulthood upon presentation of first-episode psychosis. This occurs on appearance of positive symptoms of schizophrenia, which include hallucinations, delusions and disorganised thought patterns. Schizophrenia is also accompanied by negative symptoms (alogia, avolition, social withdrawal) which are associated with anxiety and depression, as well as cognitive symptoms (deficits in social cognition, attention, working memory and learning). Negative and cognitive symptoms can present far earlier than positive symptoms and are often traced back to childhood. Despite the debilitating and impactful nature of these symptoms, the current antipsychotic medications only show improvements in positive symptoms. These medications also cause debilitating side-effects including Parkinson's-like movement disorders, weight gain, confusion and more. This sub-standard treatment availability leaves those with schizophrenia dealing with untreated, complex psychiatric symptoms and damaging side effects. As a result, adherence to medication is low and life-time risk of suicide is much higher than the general population. New treatment options must continue to be investigated, and underlying causes researched to identify new pharmacological targets.

The current literature describes several factors which each individually contribute some risk towards development of schizophrenia. Genetically, several copy-number variants and single-nucleotide polymorphisms have been

identified which increase likelihood of schizophrenia development. Understanding the role of these genetic variants in physiology can identify molecular pathways and neurobiological components of relevance to schizophrenia. For example, these genes have highlighted the role of the immune system and GABAergic neuronal signalling pathways. One copy-number variant which carries one of the highest risks by a single genetic variant for schizophrenia, is the 16p11.2 duplication. The 16p11.2 duplication encodes genes which show deficits in key neurobiological components related to schizophrenia. These include MAPK7, TAOK2 and DOCK proteins which are involved in neuronal signalling and neuroinflammatory functions related to the pathophysiological profile of schizophrenia. Whilst this is relatively well characterised, the gene does not carry 100% risk for schizophrenia. Therefore, further research is required to understand how it may interplay with other risk factors to result in schizophrenia diagnosis in adulthood.

Other identified risk factors for the development of schizophrenia include environmental stimuli. These are environmental events which occur throughout different life-stages resulting in alterations in neurological development. Examples include early life stress, accumulating adverse childhood events or systemic, long-term stressors such as the experience of systemic racism. One developmental stage which is particularly susceptible to biological insult is embryonic development where maternal stress or maternal immune activation significantly increase risk of developing schizophrenia in offspring. In fact, there is robust data which shows maternal immune activation during the first and second trimesters is directly related to altered neuronal signalling, decreased synaptogenesis, impaired synaptic plasticity and increased neuroinflammation. As a result, the role of immune response during this key stage has been continually studied to characterise the neurobiological profile which could be involved in development of schizophrenia.

However, whilst each of these risk factors are relatively well studied on their own, it is theorised that schizophrenia develops in humans as a result of accumulating risk factors. Yet, there are currently few studies which prioritise

assessment of combined genetic and environmental risk factors together to determine how they might interact. Here, I aimed to address this by designing a dual-hit model of schizophrenia in mice which would combine multiple modalities of maternal immune activation with the high risk 16p11.2 duplication gene. I then carried out a large-scale neurobiological and behavioural profile on the mice derived from this model to determine how genes and environment interacted to alter phenotype.

To carry this out, I first made use of a common neuropharmacological model of schizophrenia – phencyclidine (PCP) administration - to assess behavioural changes that could be detected using novel behavioural testing equipment. I then carried out a behavioural analysis using these same tests to characterise phenotype of mice derived from the dual-hit, gene x environment model. Finally, I dissected the prefrontal cortex from the brains of these mice to assess for changes in expression of key neurobiological components related to neuronal signalling, neuroinflammation and microglial activation.

I found that PCP increased anxiety-related behaviours in female mice, while male mice showed a marked increase in social withdrawal with no significant effects of sex or PCP on cognitive ability. The effects on anxiety and sociability reflect data seen in humans and thus behavioural assessments used could identify changes in behaviours related to schizophrenia. These behavioural tests then formed the basis of the characterisation of behavioural phenotype in the dual-hit model. 16p11.2 duplication carrying mice showed increased anxiety-related behaviours whilst MIA induced social withdrawal. Importantly, there were also clear gene x environment interactions in both anxiety-related behaviours and in sociability whereby the combination of gene and MIA increased severity of behavioural changes. Interestingly, there were no identified alterations of neuronal signalling markers, PV and SST, as a result of gene or environment. However, MIA induced long-term changes in neuroinflammatory markers such as IL6 as well as markers of microglial activation, such as DOCK 8 and DOCK 11.

These data are significant for the area of psychiatric research as they highlight how converging risk factors can interact to induce long-term changes. In fact, this demonstrates the value of multi-modal models in psychiatric disorder research as these more accurately replicate the intrinsic development of these disorders in humans. This can provide valuable, translational data on pathophysiology and behaviour related to complex neurological disorders providing a novel method for advancing the area of neuroscience underlying psychiatric disorders.

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Author's Declaration

I declare that the work presented within this thesis is my own, except where explicit reference is made to the contribution of others. All experimental data presented in results chapters of this thesis were carried out, analysed and presented by my own hand. This thesis has not been submitted in any previous application for any other degree at the University of Glasgow or any other institution.

Signed:

Klaudia Jasionowska

October 2024

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FIGURE 5.1: NEURONAL SIGNALLING MARKERS IN THE PFC OF ADULT WT AND 16P11.2 DUPLICATION MICE FOLLOWING MIA PROTOCOLS WITH SALINE, RESIQUIMOD OR POLY IC. A) PV EXPRESSION IN PFC OF MICE SEPARATED BY MIA TREATMENT, GENOTYPE AND SEX. B) PV EXPRESSION IN PFC OF MICE SEPARATED BY MIA TREATMENT AND SEX C) SST EXPRESSION IN PFC OF MICE SEPARATED BY MIA TREATMENT, GENOTYPE AND SEX N = 41 FEMALES, 32 MALES, 39 WT, 34 DUP, 23 SALINE, 27 RESIQUIMOD, 23 POLY IC. P# INDICATES DIFFERENCES BETWEEN MEANS WHERE P < 0.05. *SIGNIFY OUTLIER DATA POINTS. 159

FIGURE 5.2: INFLAMMATORY MARKERS IN THE PFC OF ADULT WT AND 16P11.2 DUPLICATION MICE FOLLOWING MIA PROTOCOLS WITH SALINE, RESIQUIMOD OR POLY IC. A) TNFA EXPRESSION IN PFC OF MICE SEPARATED BY GROUP. B) IL6 EXPRESSION IN PFC OF MICE SEPARATED BY GROUP. C) CD68 EXPRESSION IN PFC OF MICE SEPARATED BY GROUP. N = 41 FEMALES, 32 MALES, 39 WT, 34 DUP, 23 SALINE, 27 RESIQUIMOD, 23 POLY IC. # INDICATES DIFFERENCES BETWEEN MEANS WHERE P < 0.05. *SIGNIFY OUTLIER DATA POINTS. 166

FIGURE 5.3: DOCK PROTEIN EXPRESSION IN THE PFC OF ADULT WT AND 16P11.2 DUPLICATION MICE FOLLOWING MIA PROTOCOLS WITH SALINE, RESIQUIMOD OR POLY IC. A) DOCK8 EXPRESSION IN PFC OF MICE SEPARATED BY MIA GROUP. B) DOCK11 EXPRESSION IN PFC OF MICE SEPARATED BY MIA GROUP. C) DOCK8 EXPRESSION IN PFC OF MICE SEPARATED BY SEX, GENOTYPE AND MIA TREATMENT GROUP. D) DOCK 11 EXPRESSION IN PFC OF MICE SEPARATED BY SEX, GENOTYPE AND MIA TREATMENT GROUP. N = 41 FEMALES, 32 MALES, 39 WT, 34 DUP, 23 SALINE, 27 RESIQUIMOD, 23 POLY IC. # INDICATES DIFFERENCES BETWEEN MEANS WHERE P < 0.05. *SIGNIFY OUTLIER DATA POINTS. 171

List of Abbreviations

ANOVA	**Analysis of Variance**
BDNF	Brain-derived neurotrophic factor
CCK	Cholecystokinin
CD68	Cluster of differentiation 68
cDNA	Complementary DNA
CNV	Copy Number Variant
dH2O	Distilled water
DHF	7,8 dihydroxyflavone
DNA	Deoxyribonucleic acid
DOCK	Dedicator of cytokines
DSM -5	Diagnostic and Statistical Manual of Mental Disorders 5
E/I	Excitatory-inhibitory
EEG	Electroencephaography
EPM	Elevated Plus Maze
ERK	Extracellular signal-regulated kinase
fMRI	Functional magnetic resonance imaging
GABA	Gamma aminobutyric acid
GAD	Glutamic-acid decarboxylase
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GEF	Guanine exchange factor
GWAS	Genome-wide association study
HPC	Hippocampus
HZ	Heterozygous
IL6	Interleukin 6
JNK	c-Jun-NH2-terminal kinase
LMA	locomotor Activity
MAPK	Mitogen activated protein kinase
MEG	Magnetoencephalography

MIA	Maternal Immune Activation
miRNA	microRNA
mRNA	Messenger RNA
NaCl	Sodium chloride
NOR	Novel Object Recognition
OF/OFT	Open-field test
PBS	Phosphate buffered saline
PDRL	Paired Discrimination and Reversal Learning
PET	Positronemission tomography
PFC	Prefrontal Cortex
PNN	Perineuronal Net
PPI	Prepulse inhibition
PRS	Polygenetic risk score
PTSD	Post-traumatic stress disorder
PV	Paravalbumin
RNA	Ribonucleic acid
RNA-seq	RNA Sequencing
RT-qPCR	Reverse Transcription – quantitative polymerase chain reaction
scRNA-seq	Single cell RNA sequencing
SNP	Single nucleotide polymorphism
SST	Somatostatin
SZ	Schizophrenia
TAOK2	Thousand and one amino acid kinase 2
TLR	Toll-like receptor
TBS	Tris buffered saline
TNFa	Tumour necrosis factor alpha
WT	Wild-type

1 General Introduction

1.1 Schizophrenia

1.1.1 A Historical Perspective

Throughout history mental illnesses have been stigmatised, shamed and misunderstood, perhaps none more so than schizophrenia with stigmatisation prevailing even today. However, our understanding of psychiatric illness has been progressing for hundreds of years. In the late 1700s, Philippe Pinel was the first physician who denounced claims that mental illness or psychosis was a result of demonic possession and instead posited that it could be a result of environmental stress (reviewed in Poirier et al., 2012). This began a movement in psychiatry which demanded that patients be treated fairly and humanely, and research began to investigate potential treatments. Gottlieb Burckhardt was the first psychiatrist to perform neurosurgery in the 1890s on his patients who were exhibiting symptoms of what would now be understood as schizophrenia in an attempt to understand how the physical brain and psychological disorders could be linked (reviewed in Michaleas et al., 2021).

The first instance of characterising psychiatric symptoms as distinct and complex neurological disorders was over a century ago when the German Psychiatrist Emil Kraepelin initially coined the term “Dementia Praecox” in the late 1800s. Kraepelin was a pioneer of redefining psychosis and affective disorders as distinct psychiatric illnesses which exist as either a complex neurodegenerative disorder occurring due to biological illness in the case of psychosis or as episodic events in the case of affective disorders. While the term dementia praecox was later renamed schizophrenia by Eugene Bleuler, the concepts defined by Kraepelin became the foundation on which the field of

psychiatry was built for the next hundred years (reviewed in Adityanjee et al., 1999; Jablensky, 2010; Strous, Opler and Opler, 2016)[1].

1.1.2 A Modern Perspective

Today, schizophrenia is understood as a complex neurodevelopmental disorder which is often diagnosed in late adolescence or adulthood upon the first instances of psychosis which prohibit normal social and psychological behaviours (Tandon *et al.*, 2013; Millan *et al.*, 2016). While prevalence is largely variable across geographical location and ethnic background, approximately 0.7% of the population will develop schizophrenia in their lifetime (Goldner *et al.*, 2002; Saha *et al.*, 2005). It is characterised by three symptom domains: positive symptoms (such as hallucinations or delusions), negative symptoms (such as social withdrawal or affective dysfunction) and cognitive symptoms (such as impaired working memory). Furthermore, those with schizophrenia often experience periods of anxiety and depression which are associated with the negative symptoms of schizophrenia (Waltz and Gold, 2007; Betensky *et al.*, 2008; Tandon *et al.*, 2013).

Unfortunately, prognosis for those with schizophrenia indicated that there is a higher likelihood of early death compared to the general population. In addition, it is suicide which shows the largest mortality ratio compared to the population, highlighting the severe impact schizophrenia can have on the lives of those living with the disorder (Saha *et al.*, 2005; Saha, Chant and McGrath, 2007). Whilst pharmacological and psychological interventions are available, there are high incidences of non-compliance with medication regimes due to several severe side effects as well as intellectual difficulties increasing likelihood of forgetfulness (Meepring *et al.*, 2021; Cahaya *et al.*, 2022, 2023; Li, Hsieh and Liu, 2023). Furthermore, current available medications only work

[1] While Kraepelin was a pioneer of modern psychiatric definitions he was also the prominent mentor of three noted Nazi members (Strous, Opler and Opler, 2016). In fact, Kraepelin's experimental methodology was based upon a foundation of eugenic and racist beliefs. As a result, it is important to consider how psychiatry and neuroscience have been built upon these beliefs, particularly given the racial disparities in diagnosis, support and treatment which disproportionately affect ethnic minorities (Misra *et al.*, 2022).

to reduce a subset of symptoms rather than treating the full disorder leaving those exploring treatments with few options. As a result, it is important that research continues to develop our understanding of schizophrenia development, pathophysiology and causes if we are to identify new treatment targets that will address these issues.

1.2 Clinical Background

1.2.1 Onset and Diagnosis

The diagnosis of schizophrenia is complex, with a number of psychosis disorders exhibiting similar symptomology and multiple disorders with shared characteristics. For example, episodes of psychosis which may be experienced during a period of mania in bipolar disorder can present similarly to the delusions a person with schizophrenia may experience. Equally, the definitions of schizoaffective disorder, schizotypal personality disorder and schizophrenia all involve presence of hallucinations, delusions and affective changes (Bell, 1994; Regier, Kuhl and Kupfer, 2013; American Psychiatric Association, 2022; World Health Organization, 2022).

Nevertheless, schizophrenia can be defined by the presence of “positive” symptoms and “negative” symptoms. Positive symptoms can be thought of as symptoms which occur in addition to normal behaviour and includes hallucination and delusions while negative symptoms are a loss of function in normal behaviour. In the ICD11 and DSM-5, diagnostic criteria are met if there is presence of at least two symptoms of schizophrenia, one of which must be a positive symptom which lasts for a period of at least one month and cannot be explained by any other pathology (such as brain tumour) (American Psychiatric Association, 2022; World Health Organization, 2022). This modern development has eradicated the use of subtypes within diagnosis such as “paranoid schizophrenia”, “catatonic schizophrenia” or “disorganised schizophrenia”. Instead, the DSM V now refers to “Schizophrenia Spectrum and Other Psychotic Disorder”. This acknowledges the progression of schizophrenia over time, since people with schizophrenia may present with paranoid delusions at one time in

their life and later experience catatonic behaviour. Equally this acknowledges the individual differences in symptomology which patients may experience as well as in the severity of symptom (World Health Organization, 2022; Almeida et al., 2020;Bowles, 2013; (American Psychiatric Association, 2022).

1.2.2 Symptomology

1.2.2.1 Positive symptoms

A hallucination is described as the detection of a sensory stimulus which occurs where these is none. This can manifest in visual images which do not exist, sounds or voices which are heard by a person with schizophrenia or in tactile sensations where there are none (Telles-Correia, Moreira and Gonçalves, 2015; Thompson et al., 2021). The most common hallucination is in the form of auditory hallucinations where people describe hearing voices speaking to them or other sounds as clearly as any other voice (Chaudhury, 2010). Visual hallucinations varied may include seeing intruders in the home where there are none, seeing figures which may be frightening or seeing changes in surrounding structures such as walls and floors. Tactile hallucinations are rare in schizophrenia and are more often a result of medications but the most common tactile hallucination is feeling insects crawling on the skin where there are none (Chaudhury, 2010; Thompson *et al.*, 2021).

Delusions are defined as strongly held beliefs which cannot be true. These can include delusions of grandeur, paranoid delusions or religious delusions. Delusions of grandeur include ideas of self-importance such as belief that they are the reincarnation of a religious figure or a God. Paranoid delusions may include thoughts of being spied on, watched or investigated by government bodies and can extend to beliefs of devices being implanted, being experimented on and thought insertion (a firmly held delusion that thoughts do not belong to the individual) (Kimhy *et al.*, 2005; González-Rodríguez and Seeman, 2022).

Another positive symptom includes formal thought disorder (disorganised thought patterns) which is often characterised by assessment of speech

patterns. This can be identified on discussion with patients who may be jumping quickly between topics – called flight of ideas – or sentences which do not flow together – often called "word salad" (Sass and Parnas, 2017). Other positive symptoms include feelings of passivity or control whereby one's thoughts or actions do not belong to oneself ("thought insertion"), or psychomotor dysfunction such as catatonia or physical agitation (American Psychiatric Association, 2022; World Health Organization, 2022).

1.2.2.2 Negative Symptoms

Negative symptoms themselves include a wide range of symptoms which comprise a loss of function. These include affective flattening, anhedonia (reduced ability in feeling pleasure), alogia (reduced speech), avolition (lack of motivation), asociality (social withdrawal) and related symptoms including anxiety and depression (American Psychiatric Association, 2022; World Health Organization, 2022). Unfortunately, these symptoms remain largely untreated with few available therapeutic options. Negative symptoms are also rarely included in research into schizophrenia, both in human and animal research. However, sex differences which have been identified in humans are mostly present within these negative symptoms while there are few identified sex differences in the positive symptoms. Males with schizophrenia generally experience more of the traditional negative symptoms such as anhedonia, avolition and particularly increased social withdrawal, whereas females have shown increased incidence of anxiety like negative symptoms (Castle and Murray, 1993; Leung and Chue, 2000; Usall *et al.*, 2002; Sommer *et al.*, 2020). However, there continues to be little investigation into underlying mechanisms beyond what is currently known about schizophrenia pathophysiology.

1.2.2.3 Cognitive symptoms

Those with schizophrenia also experience a third class of symptoms that largely affect different aspects of cognition. In fact, schizophrenia has been shown to result in deficits of attention, working memory, verbal and visual learning and memory as well as high-level thought processes such as reasoning and problem

solving (Sharma and Antonova, 2003; Bowie and Harvey, 2006; Waltz and Gold, 2007; Shipman et al., 2009). Furthermore, these symptoms can often be traced back to childhood with adults diagnosed with schizophrenia after first episode psychosis showing worse performance during school than the general population. As a result, cognitive symptoms can represent life-long difficulties that are impacted by deficits in early learning (Bowie and Harvey, 2006)(McCutcheon, Keefe and McGuire, 2023). Finally, social cognition has also been shown to be reduced, meaning emotional processing, social perception and attributional biases are all impaired. This results in inability to understand and interpret emotions of the self and others, difficulties interpreting social interaction and difficulties with relating to others (Fett *et al.*, 2011). This impacts on ability to socialise positively in early life and likely further contributes to the social withdrawal experienced in adulthood after diagnosis.

1.2.3 Treatment

From the initial definitions of dementia praecox and schizophrenia by Kraepelin and Bleuler (Adityanjee *et al.*, 1999; Jablensky, 2010; Strous, Opler and Opler, 2016) there was little advancement in the understanding of schizophrenia as a disorder until the discovery of Chlorpromazine in the 1950s, a drug which was initially tested as a potential antihistamine (Ban, 2007). Jean Delay and Pierre Deniker introduced the drug into psychiatry in 1952 after identifying a calming effect without sedation which could be employed for use in patients with psychosis. This was the first antipsychotic medication which propelled the field of psychopharmacology and gave way to the "dopamine theory" of schizophrenia (reviewed in Ban, 2007).

Since chlorpromazine is a dopamine receptor antagonist it was hypothesised that symptoms of schizophrenia were a result of excess dopamine activity in the brain. In fact, since dopamine is important in motor function, visual and auditory perception and in reward circuitry this was a viable explanation for many of the symptoms identified in people with schizophrenia (Howes and Kapur, 2009; Brisch *et al.*, 2014; Howes *et al.*, 2017; Kesby *et al.*, 2018). However, while the use of first-generation antipsychotics such as

chlorpromazine functioned in reducing many of the positive symptoms, the negative symptoms remain untreated and instead a wide range of unpleasant side effects became evident. These side effects are often severe and can include blurred vision, confusion and agitation, sexual dysfunction, affective flattening, and extra-pyramidal effects which result in Parkinson's like motor dysfunction (Gowda et al., 2017; Burschinski et al., 2023; Breitbart et al., 1996). As a result, compliance with medication is relatively low compared to other psychiatric treatments (Meepring *et al.*, 2021; Cahaya *et al.*, 2023; Li, Hsieh and Liu, 2023). While a second generation of antipsychotics was developed to have somewhat reduced extra-pyramidal side effects due to decreased specificity for D2 receptors they showed increased diversity in other side effects. Furthermore, these can be less effective in treating the positive symptoms of schizophrenia and may require a change to first-generation psychotic instead (Meepring *et al.*, 2021).

An additional treatment for schizophrenia is lithium which is often co-prescribed with antipsychotics in patients who may present with particularly difficult to manage symptoms who indicate potential harm to themselves or others (Leucht *et al.*, 2015) (Luo *et al.*, 2020). Lithium acts by preventing g-protein coupled receptor excitatory activity, affecting NMDA receptor and AMPA receptor activity as well as dopamine release in the synapse. However, lithium toxicity and effects on kidney and liver function mean this is rarely used as a long-term treatment for schizophrenia (Leucht *et al.*, 2015; Luo *et al.*, 2020). As a result, in an attempt to treat both positive and negative symptoms, a combination of antipsychotics and selective serotonin reuptake inhibitors may be prescribed. More recently, clinical trials have shown that a muscarinic receptor agonist may show promise in treating positive and negative symptoms of schizophrenia (Foster, Bryant and Conn, 2021; Dean and Scarr, 2020; Lu et al., 2024). However, research in this area is still in early clinical trials and will require further research to determine efficacy and effectiveness.

It is clear that the widely prescribed treatment options available for people with schizophrenia are not optimal, and whilst positive symptoms can be

effectively treated with antipsychotics the side effects can reduce adherence to medication. At the same time, negative and cognitive deficits remain largely untreated although cognitive behavioural therapy, family and social therapy and cognitive training have shown some promise in supporting people with schizophrenia to develop the necessary skills to manage their own symptoms (Caqueo-Urízar *et al.*, 2015; Ganguly, Soliman and Moustafa, 2018; Modesti *et al.*, 2023; Berendsen *et al.*, 2024). While some research is showing promise with the new muscarinic receptor drug treatment target, this research is still in its infancy (Dean and Scarr, 2020; Foster, Bryant and Conn, 2021; Dean *et al.*, 2023). In fact, xanomeline – an M1/M4 agonist – was recently approved for use by the FDA in September 2024 (Center for Drug Evaluation and Research, 2024). It is important that research into schizophrenia continues in an effort to further understand the underlying neurobiological and physiological causes that result in the complex disorder.

1.3 Psychopharmacology

1.3.1 Dopamine Theory

The dopamine theory of schizophrenia has functioned as the prevailing hypothesis for the underlying neurobiological cause since the discovery of chlorpromazine in the 1950s (Howes, McGuire and Kapur, 2008; Howes and Kapur, 2009). It was clear that dopamine receptor antagonism could effectively reduce the positive symptoms of schizophrenia that are associated with psychosis episodes. Studies further identified that drugs which showed specificity for D2 receptor antagonism improved clinical efficacy for those who were being treated with these antipsychotics (Seeman and Lee, 1975). With dopamine receptor antagonists the only treatment which had shown specificity for treatment of psychosis, the dopamine theory of schizophrenia became the topic of research on schizophrenia (van Rossum, 1966). Studies investigated how healthy controls would respond to dopamine receptor agonism and showed that psychosis could be induced in healthy individuals (Perry and Juhl, 1977; Bramness and Rognli, 2016). Post-mortem studies showed that the brains of

those who had been diagnosed with schizophrenia had elevated levels of dopamine receptors and specifically showed increased D2 receptor expression in subcortical areas (Howes, McGuire and Kapur, 2008). More recently, PET scans and neuroimaging, have shown increased dopaminergic activity in those with schizophrenia (Okubo et al., 1997; Meyer-Lindenberg et al., 2005; Howes, McGuire and Kapur, 2008; Howes and Kapur, 2009; Slifstein et al., 2015; Rao et al., 2019). However, these studies also showed that the prefrontal cortex (PFC) had reduced expression of D1 receptors (Okubo *et al.*, 1997; Howes, McGuire and Kapur, 2008) (Pycock, Kerwin and Carter, 1980). This could seemingly be explained by the presence of positive and negative symptoms with an understanding of dopaminergic function in the brain.

There are individual dopaminergic signalling neural circuits in the brain which are functionally and structurally unique. Neural projections from the subcortical structure of the ventral tegmental area project to limbic areas of the brain as well as to the cortex to form the mesocorticolimbic pathway which has been identified as the primary mechanism underlying reward seeking and motivation. The neural projections which extend from the substantia nigra to the striatum (forming the Nigrostriatal pathway) on the other hand have been identified as greatly important in motor function as well as in learning and memory (Klein *et al.*, 2019). The dopaminergic signalling of the PFC, an area of the brain important in cognition, high-level thought processes and reasoning is mainly modulated by D1 receptor activity. At the same time, the nigrostriatal pathway which is important in motivation, signalling to other subcortical regions as well as learning and memory is mostly modulated by D2 receptor activity (Xu and Yang, 2022). Relating these neurobiological differences back to symptomology, reduced D1 receptor activity, particularly in the PFC has been hypothesised to be the cause of negative and cognitive symptoms of schizophrenia due to dysfunction in mesocorticolimbic pathways. At the same time, overexpression and overactivity of D2 receptors in the nigrostriatal pathway has been identified as the potential mechanism by which positive symptoms may occur (Desbonnet, Waddington and O'Tuathaigh, 2009).

1.3.2 NMDA Receptor Theory

A second theory of schizophrenia has arisen more recently after phencyclidine (PCP, AKA phenylcyclohexyl piperidine) was shown to cause psychosis in individuals that was similar to the psychosis seen in those with schizophrenia (Luby *et al.*, 1959; Allen and Young, 1978). PCP functions as a non-competitive antagonist of the NMDA receptor and so when it was identified that PCP could induce psychosis in healthy individuals as well as exacerbate psychosis in those already diagnosed with schizophrenia, researchers began to research how NMDA receptors may be involved (Luby *et al.*, 1959; Allen and Young, 1978). Furthermore, the chronic use of PCP has been associated with anxiety, depression and cognitive deficits in patients who have become addicted to the drug (Pradhan, 1984) which could suggest presence of schizophrenia-like negative symptoms. However, it is impossible to determine if this is in fact due to long-term PCP use or due to social factors which predispose individuals to drug addiction.

Animal studies have utilised PCP as a model for schizophrenia and identified a number of effects which mimic schizophrenia related behavioural changes (Javitt *et al.*, 2012; Grayson *et al.*, 2016; Moghadam *et al.*, 2021). Cognitive deficits in working memory, attention and visual learning have been identified (Andersen and Pouzet, 2004; Egerton et al., 2005; Fellini et al., 2014) as well as behaviours related to negative symptoms (Noda *et al.*, 2000; Lee *et al.*, 2005; Turgeon, Anderson and O'Loughlin, 2010; Turgeon *et al.*, 2011). Low-doses of PCP have also been shown to result in decreased activity in the PFC as can be seen in patients with schizophrenia (Morris, Cochran and Pratt, 2005). Interestingly, there is evidence that D1 receptor agonism can reverse effects of PCP-induced cognitive deficits (McLean *et al.*, 2009). This suggests that NMDA receptor hypofunction can be mediated by dopaminergic activity. As a result, it is possible that the effects of antipsychotic treatments could module behaviour by knock-on effects on NMDA receptors rather than through dopamine receptors alone. Finally, PCP had been shown to reduce Parvalbumin (PV) expressing neuronal expression as well as function (Cochran *et al.*, 2003;

Nakazawa *et al.*, 2012) similarly to data seen in post-mortem studies of patients with schizophrenia (Chung, Fish and Lewis, 2016; Kaar *et al.*, 2019).

Further evidence supports the NMDA receptor hypofunction theory of schizophrenia with other NMDA receptor antagonists also inducing behavioural and structural changes comparable to schizophrenia-like phenotypes. Ketamine (a PCP derivative) has been shown to induce cognitive deficits and social deficits as well as disruption to memory and learning (Krystal *et al.*, 1994; Fellini *et al.*, 2014). Ketamine has also shown to impair GABAergic signalling through altered expression of PV interneurons (Sabbagh *et al.*, 2013; Barrutieta-Arberas *et al.*, 2023). MK-801, another NMDA receptor antagonist has also shown similar results including reduced PV expression in the PFC (Löscher and Hönack, 1992; Xi et al., 2009; Kumar et al., 2018; Toader et al., 2020; Janus, Lustyk and Pytka, 2023).

Finally, further evidence to support the NMDA receptor theory of schizophrenia is through analysis of a different disorder: anti-NMDA receptor encephalitis, an autoimmune disorder which results in reduced expression of NMDA receptors throughout the brain (Barry *et al.*, 2015; Ford, McDonald and Srinivasan, 2019; Staley *et al.*, 2019). Patients with anti-NMDA receptor encephalitis portray several psychiatric symptoms which mimic those found in schizophrenia including not only in the presence of positive symptoms but also in anxiety, depression and cognitive deficits (Barry *et al.*, 2015; D. *et al.*, 2019; Endres *et al.*, 2019; Gurrera, 2019; Tamayo-Medina *et al.*, 2023).

Overall, there is a large body of evidence which suggests that NMDA receptor hypofunction is a more likely underlying mechanism for schizophrenia given that relatively crude antagonism appears to induce a wide spectrum of schizophrenia-like symptoms. Furthermore, there is evidence that this NMDA receptor hypofunction could affect dopaminergic signalling as a secondary effect. As a result, it is not possible to say that dopaminergic signalling could be the sole underlying mechanism of schizophrenia as was previously thought. Instead, the NMDA receptor hypofunction theory could be responsible for symptomology as well as altered dopaminergic signalling.

1.4 Pathophysiology

With the understanding of schizophrenia as a pathophysiological disorder developing through the discovery of first-generation antipsychotics, research began to investigate the underlying biological and structural changes that were evident in those diagnosed. Post-mortem research has revealed that the brains of those with schizophrenia show gross structural changes as well as underlying neurobiological differences. These pathophysiological findings have resulted in a number of theories around the mechanisms by which schizophrenia can result in symptomology seen in humans.

1.4.1 Underlying Neurobiology

Understanding the neural circuitry that is involved in schizophrenia has been key to developing a deeper clarity on the underlying neurobiology. With two competing theories for the development of schizophrenia, each which largely impacts complex neural circuits, research has focused on how excitatory and inhibitory circuits function in the brains of those with schizophrenia. The mammalian cortical signalling structures are based upon a foundation and balance of glutamatergic excitatory signalling and GABAergic inhibitory interneurons which each modulate activity in cortical structures as well as cross-structural connectivity (Hendry *et al.*, 1987; Tamamaki *et al.*, 2003; Sherwood *et al.*, 2010). Whilst the population of GABAergic inhibitory interneurons is a small proportion of glutamatergic excitatory neurons, it has been posited that the importance of these interneurons is in the fine balance of cortical network signalling. This subtle complexity provides the basis for higher-order cognitive abilities and behaviours which the mammalian brain is capable of (Tremblay, Lee and Rudy, 2016).

GABAergic inhibitory neurons can be divided into subtypes through the analysis of neurochemical expression. Specifically, the lack of co-expression of three main markers provides the basis for these subtypes into; paravalbumin (PV) expressing neurons, somatostatin (SST) expressing neurons and 5HT3aR expressing neurons (Rudy *et al.*, 2011; Tremblay, Lee and Rudy, 2016; Lim *et*

al., 2018). Furthermore, these inhibitory interneuron populations have shown aberrant expression and function in schizophrenia studies.

1.4.1.1 Paravalbumin Expressing Inhibitory Interneurons

Paravalbumin is a calcium binding protein which functions to modulate calcium activity and therefore neuronal cell activation (Schwaller, Meyer and Schiffmann, 2002; Arif, 2009). PV-expressing interneurons form the largest population of interneurons in the cortex and can be characterised by fast-spiking activity patterns which function to inhibit the excitatory transmission of cortical pyramidal neurons through modulating AMPA receptor activity (Goldberg *et al.*, 2008; Pfeffer *et al.*, 2013; Tremblay, Lee and Rudy, 2016; Rupert and Shea, 2022). PV interneurons are also highly implicated in processes of synaptic plasticity through their associated perineuronal net structures(Wang and Fawcett, 2012; Gonzalez *et al.*, 2022; Willis, Pratt and Morris, 2022).

In schizophrenia, PV interneurons have shown reduced expression within inhibitory neurons as identified through post-mortem studies particularly in the PFC of those with schizophrenia (Hashimoto *et al.*, 2003, 2008; Morris, Hashimoto and Lewis, 2008; Curley *et al.*, 2011). Further, not only is expression of PV reduced within inhibitory neuron populations, but density of PV-expressing neurons is also reduced(Kaar *et al.*, 2019). Additionally, the density of excitatory neuron synapses with PV expressing neurons is also reduced (Chang *et al.*, 2016). Furthermore, expression of glutamic acid decarboxylase (GAD), an enzyme crucial to the synthesis of GABA and therefore inhibitory activity in the brain, shows reduced expression specifically in PV-expressing inhibitory interneurons (Curley *et al.*, 2011; Curley and Lewis, 2012; Lewis *et al.*, 2012). This suggests that the deficits seen in PV expressing neurons in schizophrenia are having direct effects on the functionality of inhibitory interneuron activity. In fact, PV-expressing neurons have been shown to underpin the gamma oscillations which functionally affect behavioural abilities in attention, social cognition and working memory(Fries *et al.*, 2001; Sohal *et al.*, 2009; Williams and Boksa, 2010; González-Rodríguez and Seeman, 2022).

Given PV neurons form the largest subtype of inhibitory interneuron it is likely that the excitatory-inhibitory balance in cortical neural circuits is grossly affected. This pathological reduction in PV-expression is also specific to schizophrenia rather than other psychiatric conditions (Lewis *et al.*, 2001). Therefore, the wide range of deficits seen in PV-expressing interneurons of humans with schizophrenia shows pathological specificity of schizophrenia diagnosis. As a result, any research which investigates schizophrenia, particularly in animals, must replicate this finding if it is to provide reliable construct validity.

1.4.1.2 Somatostatin Expressing Inhibitory Interneurons

The second largest population of inhibitory interneurons can be characterised by the expression of somatostatin. SST is a neuropeptide which is released in the presynaptic terminal of inhibitory interneurons which modulates activity of excitatory pyramidal cells in the cortex. SST expressing interneurons, contrastingly to the PV population, exhibit slow-spiking activity patterns through function of G-protein coupled receptor activity (Song, Yoon and Lee, 2021).

SST-expressing interneurons have also shown deficits in schizophrenia post-mortem research (Morris, Hashimoto and Lewis, 2008; Konradi *et al.*, 2011; de Jonge *et al.*, 2017). However, while there is robust evidence on the role of PV-expressing interneuron deficits in schizophrenia, there is little on the role of SST-expressing neurons beyond identification of reduced mRNA expression of SST (Hashimoto *et al.*, 2008; Morris, Hashimoto and Lewis, 2008; Konradi *et al.*, 2011; Fung *et al.*, 2014; de Jonge *et al.*, 2017). Animal research has however identified that this reduced expression may be specific to the hippocampus rather that the PFC as is seen in the PV-expressing interneurons (Murueta-Goyena *et al.*, 2020).

1.4.1.3 5HT3aR Expressing Inhibitory Interneurons

Inhibitory interneurons expressing 5HT3 receptors are subdivided into two major classes: CCK-expressing interneurons due to the large subpopulations

which show co-expression with cholecystokinin (CCK) and VIP-expressing interneurons which express vasoactive intestinal peptide (VIP) (Ferezou et al,. 2002). VIP interneurons function primarily by the disinhibition of other interneurons, such as PV and SST expression interneurons through serotonergic signalling (Melchitzky and Lewis., 2008; Pi et al., 2013; Letzkus et al., 2015; William et al., 2019; Georgiou., et al 2022). In particular, they have been shown to be a modulator of synaptic plasticity of PV-interneurons (Donator et al., 2013; Dienel and Lewis., 2019). However, there is little evidence as to the direct involvement of VIP interneurons in schizophrenia pathophysiology (McCutcheon et al., 2023).

CCK-expressing interneurons, form the smallest subpopulation of inhibitory interneurons (Tremblay, Lee and Rudy, 2016). There is also large variability within this population in activation patterns (Kawaguchi and Kubota, 1996; Karube, Kubota and Kawaguchi, 2004; Kubota-Sakashita *et al.*, 2014; Tremblay, Lee and Rudy, 2016). CCK-expressing interneurons primarily function through serotonergic signalling, however due to the complexity around this subpopulation little information has been identified around their role in neural circuitry population (Bartos and Elgueta, 2012; Nguyen *et al.*, 2020). Furthermore, there is little evidence on the role of CCK-expressing neurons in schizophrenia pathology, although reduced expression of CCK has been identified in some studies (Gabriel *et al.*, 1996; Hashimoto *et al.*, 2008). Despite this, there has been little further research into CCK-inhibitory interneurons in schizophrenia.

1.4.2 Underlying Structural Changes

Alongside changes in cellular composition of cortical regions and altered neurotransmission, schizophrenia pathology can be seen on a wider scale through gross-structural changes in the brain. Post-mortem and neuroimaging studies in particular have identified key regions of the brain associated with schizophrenia pathology including the PFC, the hippocampus, and subcortical regions such as the thalamus (Meyer-Lindenberg *et al.*, 2005; Dawson, Morris and Pratt, 2015; Upthegrove and Khandaker, 2020; Lizano *et al.*, 2021). If

assessing the translational potential for animal research on schizophrenia, changes in these regions replicating data found in post-mortem and neuroimaging research would support the adequate construct validity of any animal model with similar effects.

One structure which is at the centre of gross-structural changes seen in schizophrenia is the PFC. The PFC is a large cortical structure which functions to allow for high-order cognitive processes in humans. These cognitive processes include working memory, attention and executive functioning particularly in relation to the dorsolateral regions of the PFC (Funahashi, 2017; Ott and Nieder, 2019). Unsurprisingly, the PFC shows high interconnectivity with the hippocampus which is structurally important in the formation of long-term memory and learning processes. This interconnectivity with the hippocampus is particularly important in memory retrieval, working memory and anxiety (Sigurdsson and Duvarci, 2016). Further, interconnectivity has been identified with the thalamus, a structure important for sensory processing and motor signalling (Pratt, Morris and Dawson, 2018; Torrico and Munakomi, 2019). Furthermore, the ventromedial PFC is also highly involved with emotional regulation and emotional processing, with incoming projections from structures in the limbic system including the amygdala (Sigurdsson and Duvarci, 2016). It is clear therefore, that the PFC itself is functionally relevant to cognitive and emotional processes which are often seen as dysfunctional in schizophrenia. Furthermore, the complex interconnectivity with other key-regions highlights the gross effects that could occur throughout other neural structures should the PFC become dysfunctional.

In fact, this is a key area of pathology in schizophrenia. Post-mortem and neuroimaging research has identified that the PFC shows reduced cortical size and grey and white matter volume in schizophrenia (Weinberger, 1988; Gur *et al.*, 2000; Wible *et al.*, 2001). However, dysfunctionality of the PFC in schizophrenia goes beyond simple volume and extends to connectivity and activity. Neuroimaging studies have shown that hypofunction of the PFC is a key marker or schizophrenia (Wible *et al.*, 2001; Fusar-Poli *et al.*, 2007; Arnsten,

2011; Dawson, Morris and Pratt, 2015). With key roles in attention, working memory, social cognition and complex emotional processing hypofunction in this region could be highly related to the cognitive and negative symptoms seen in those diagnosed. Furthermore, the PFC has shown reduced connectivity with the thalamus (Pratt *et al.*, 2012; Dawson *et al.*, 2014; Dawson, Morris and Pratt, 2015) and the hippocampus (Meyer-Lindenberg *et al.*, 2005). Given the importance of the thalamus in sensory processing it stands to reason that disrupted connectivity could be a key factor in the development in hallucinations as a positive symptom of schizophrenia. Furthermore, with the role of the hippocampus in working memory and further in limbic activity, disruption to this neural pathway likely represents impairments in emotional processing, anxiety, depression and learning and memory processes which underly cognition. In fact, whilst the hippocampus shows reduced grey matter volume (Arnold *et al.*, 2015; Duan *et al.*, 2021; Brosch *et al.*, 2022) and the thalamus shows further abnormal structure (Csernansky *et al.*, 2004; Harms *et al.*, 2007; Byne *et al.*, 2009) the role of connectivity to the PFC of both key structures perhaps indicates the PFC as a central point of pathophysiological significance in schizophrenia.

This is further supported by the robust neurobiological findings which show that dysfunction in PV-expressing interneurons is found primarily within the PFC (Hashimoto *et al.*, 2003, 2008; Curley and Lewis, 2012). At the same time, PV-expressing interneurons have shown deficits in response to prolonged administration of PCP, an NMDA receptor antagonist (Cochran *et al.*, 2003; Morris, Cochran and Pratt, 2005; Nakazawa *et al.*, 2012) and D1 receptors are shown in post-mortem studies to be reduced in schizophrenia (McLean *et al.*, 2009, 2017). Overall, this links the PFC to both dominating theories of underlying mechanisms which could be responsible for the pathophysiology of schizophrenia. Furthermore, these neurobiological changes can also be directly associated to the deficits seen in all three symptom classes, with the PFC itself related to cognitive process and higher order function, connectivity with the limbic system and hippocampus related to negative symptoms and working memory. Finally, functionality with the thalamus can be associated with the

positive symptoms and even the motor phenotype often seen in those with schizophrenia. It is clear therefor that the PFC should continue to be a key region of interest in schizophrenia research. Furthermore, in studies which aim to model schizophrenia, it would be important to replicate altered PFC function if relating the model to human schizophrenia pathophysiology.

1.5 Developmental Origins

The discovery of an underlying cause for any disorder or singular factor which is sufficiently influential that it may equate to a cause is perhaps the primary goal in research. In schizophrenia, understanding the developmental origins of the disorder is particularly complex. This is firstly the case due to the relative naiveté of research in this field compared to other diseases or disorders. Secondly, the latency of the diagnosis in relation to influential factors that can occur decades prior mean that the nature of schizophrenia has long been misunderstood. Historically, schizophrenia has been considered an adulthood disorder, with diagnosis occurring in late adolescence to early adulthood for men and generally in early adulthood for women or in some cases during later-adulthood (Tandon *et al.*, 2013; Millan *et al.*, 2016). This is due to the diagnostic process which occurs upon presentation of first-episode psychosis whereby positive symptoms of schizophrenia become so incompatible with society and/or life, that seeking diagnosis through medical care becomes urgently essential (Tandon *et al.*, 2013; Millan *et al.*, 2016). However, this requires hallucinations, delusions and or disordered thought patterns to become so severe that clinical support is inevitable. It is now clear though, that schizophrenia is a complex disorder which has symptomology which extends far beyond the positive symptoms seen in a psychosis episode.

It has only been recent research which has propelled our understanding of schizophrenia as a neurodevelopmental disorder which shows pathological and clinical signs throughout adolescence and childhood (Baum and Walker, 1995; Cannon *et al.*, 2000). Genetic architecture of those with schizophrenia was quickly determined to be of importance with clear data to show familial

inheritability of schizophrenia (Cardno and Gottesman, 2000; Sullivan, Kendler and Neale, 2003). Studies that began to look at genetic expression in those diagnosed have identified a clear genetic component with numerous mutations carrying varying levels of risk (Winchester, Pratt and Morris, 2014; Henriksen, Nordgaard and Jansson, 2017; Trubetskoy *et al.*, 2022). However, despite some relatively high-risk genes, no single genetic mutation carries 100% risk for schizophrenia development. In fact, even in studies on monozygotic twins who share 100% of the same DNA, there is only approximately 50% concordance rate. That is, if one twin is diagnosed, there is a 50% chance that the second twin will also be diagnosed (Gottesman, 1991; Cardno and Gottesman, 2000; Sulivan et al, 2003). It is clear therefore that there must be an environmental element to the development of schizophrenia which perhaps accumulates or interacts with genetic components to enhance risk.

Research which has collated epidemiological data from those diagnosed has been able to trace a number of common events and environmental factors which appear to carry risk for schizophrenia development. These environmental insults primarily occur during early childhood, postnatally and even during pre and peri-natal periods although some events such as trauma during adolescence can also pose an element of risk (Brown *et al.*, 2004; Brown and Derkits, 2010; Brown, 2011, 2012; Cheslack-Postava and Brown, 2022). However, how these environmental factors may interplay with genetic architecture to trigger or exacerbate symptoms of schizophrenia, should now be the topic of future research.

1.5.1 Genetic Influences

Our current understanding of the genetic architecture associated with schizophrenia is based upon a body of research which employs numerous methodological techniques. These studies include genome-wide association studies (GWAS), copy-number variant (CNV) studies as well as exome sequencing (Winchester, Pratt and Morris, 2014; Henriksen, Nordgaard and Jansson, 2017; Trubetskoy *et al.*, 2022). These studies have highlighted a number of genes of interest however as previously discussed, no single gene

carries 100% risk for development. Instead, GWAS studies have identified genetic variants which may be more common in the schizophrenia population compared to the general population, although they are unlikely to individually carry high risk for development (Trubetskoy *et al.*, 2022). On the other hand, exome and CNV studies have identified genetic variants which are rare, even amongst the schizophrenia population, but instead carry a high risk for development (Williams *et al.*, 2011; Henriksen, Nordgaard and Jansson, 2017). Interestingly, there is some evidence that there is an accumulative effect of genes which carry individually low risk, but multiple co-expressions can increase risk of development as well as severity of symptoms (Purcell *et al.*, 2014; Jonas *et al.*, 2019). This accumulative effect is primarily seen in the combination of multiple single nucleotide polymorphisms (SNPs) while CNV studies have identified genes which perhaps carry higher risk for development.

1.5.1.1 Single Nucleotide Polymorphisms

SNPs are genetic variants in which there is an individual nucleotide alteration at a specific locus. SNPs are part of natural variation in the human genome, however there are some SNPs which are particularly common in the schizophrenia population (Ripke *et al.*, 2014; Henriksen, Nordgaard and Jansson, 2017). Ripke et al conducted a GWAS in which a number of SNPs were associated with schizophrenia. These SNPs were shown to be related to NMDA receptor signalling, synaptic plasticity and immune function (Sullivan, 2012; Morris and Pratt, 2014; Winchester, Pratt and Morris, 2014). This has been supported by the most recent GWAS study in schizophrenia (Trubetskoy *et al.*, 2022), which identified 287 genetic loci contributing risk for the disease. The relevance of SNPs related to NMDA receptor signalling and synaptic plasticity is perhaps not unsurprising given the robust data identifying dysfunctional pathophysiology in NMDA receptor function and related neurotransmission. Furthermore, data shows PV neuron associated perineuronal nets which are highly correlated with synaptic plasticity are dysfunctional in schizophrenia models (Willis, Pratt and Morris, 2022). SNPs such as those related to Map2K7 are likely to be related to these functions with knock-on effects of Map2K7

impacting TAOK2 signalling as well as BDNF expression all of which are relevant to cellular transmission through JNK/ERK modulated activity (Morris and Pratt, 2014; Winchester, Pratt and Morris, 2014).

Ripke et al also identified a SNP related to schizophrenia which was associated with DOCK 1, a member of the dedicator of cytokine family (DOCKs) which function as RhoGTPase regulators as guanine nucleotide exchange factors (GEFs). DOCK proteins have been implicated further in schizophrenia through CNV and exome studies which have identified other genetic variants members of the family, such as DOCK 4 (Shi, 2013; Samani *et al.*, 2022). In fact, a number of DOCK proteins have been implicated in schizophrenia and other psychiatric conditions (Namekata *et al.*, 2012, 2014, 2019, 2020; Guo *et al.*, 2021; Umehara *et al.*, 2021; Samani *et al.*, 2022). Despite this, there is little research into direct effects or their relevance in animal models of schizophrenia.

Overall, SNPs on their own do not necessarily convey high-risk for schizophrenia however accumulation of numerous co-expressions can increase risk and symptom severity (Purcell *et al.*, 2009, 2014). Perhaps more interesting however, is the research which analyses the functionality of these SNPs. Some SNPs have shown relevance to NMDA receptor function and synaptic plasticity as well as in the DOCK family of GEFs (Morris and Pratt, 2014). This research provides the basis for further investigation into underlying neurobiological mechanisms which may be related to schizophrenia pathophysiology.

1.5.1.2 Copy-number variations

Copy number variants are structurally larger the SNPs and usually show abnormalities in gene loci which can span a number of different genes. There are several CNVs which have been associated with numerous neuropsychiatric and neurodevelopmental conditions including autism, ADHD and other intellectual disabilities (Weiss *et al.*, 2008; McCarthy *et al.*, 2009; Chang *et al.*, 2017). Moreover, there is evidence to support strong links with schizophrenia for 8 different CNVs with the most common being the 16p11.2 duplication, the 15q13.3 deletion and the 22q11.2 deletion (Marshall *et al.*, 2017). While the

22q11.2 deletion carries high-risk of developing psychosis at approximately 30% likelihood, this CNV is also associated with facial deformity and cardiovascular defects (Walsh *et al.*, 2008; Morris and Pratt, 2014). On the other hand, the 16p11.2 duplication shows relative specificity of a schizophrenia phenotype with a high penetrance of up to 14% (Kirov *et al.*, 2014; Marshall *et al.*, 2017).

The 16p11.2 genomic locus is interesting due to its strong relationship with neuropsychiatric conditions. In fact, deletion at this locus has been strongly associated with autism and ADHD, (Weiss *et al.*, 2008; Kushima *et al.*, 2018; Niarchou *et al.*, 2019; Jutla, Foss-Feig and Veenstra-VanderWeele, 2022). However, of relevance to this thesis is the 16p11.2 duplication, a CNV which carries one of the highest risks for schizophrenia development of any genetic factors (Kirov *et al.*, 2014; Marshall *et al.*, 2017). Whilst the 22q11.2 duplication does show increased penetrance for psychosis compared to the 16p11.2 duplication, there are numerous additional abnormalities unrelated to psychiatric conditions (Williams, O'Donovan and Owen, 2006). On the other hand, those with the 16p11.2 duplication show high specificity for schizophrenia diagnosis (Giaroli *et al.*, 2014; Kirov *et al.*, 2014; Rees *et al.*, 2014).

The 16p11.2 duplication CNV itself encodes approximately 29 individual genes (Horev *et al.*, 2011; Macé *et al.*, 2017). Interestingly, this includes the genes which encode TAOK2 and MAPK3 (ERK1) both of which have been shown to be important in schizophrenia neuropathology. Specifically, these genes are both related to modulation of neurotransmission through Erk signalling cascades pathology (De Anda *et al.*, 2012; Blizinsky *et al.*, 2016; Richter *et al.*, 2019). TAOK2 was also implicated as a SNP associated with schizophrenia further highlighting how the 16p11.2 duplication is highly likely to be involved mechanistically in the schizophrenia pathophysiology.

In order to research the 16p11.2 duplication and its relation to schizophrenia, genetic mouse models were developed containing the CNV (Horev *et al.*, 2011; Arbogast *et al.*, 2016). These genetic mouse lines have shown a number of schizophrenia related phenotypes in pathophysiology as well as behaviourally. Behaviourally, these mice have shown deficits in social behaviours, cognitive

function via working memory and an increase in anxiety (Arbogast *et al.*, 2016; Bristow *et al.*, 2020; Rein *et al.*, 2021). Furthermore, neurobiological profile of these mice has also shown similar deficits as would be expected of a schizophrenia phenotype including reduced volume (Horev *et al.*, 2011; Bristow *et al.*, 2020), deficits in PV and SST expression (Bristow *et al.*, 2020) altered regulation of GABAergic interneuron function through PNN structure abnormalities (Willis, Pratt and Morris, 2022) and dysfunctional GABAergic signalling in the PFC (Rein *et al.*, 2021).

Whilst the 16p11.2 duplication is evidently closely related to schizophrenia pathophysiology and is relatively well characterised as a mouse model, there is little evidence which investigates how it may interact with other risk factors. In fact, there are numerous environmental insults which convey risk for schizophrenia. Yet there is little research which investigates these factors together.

1.5.2 Environmental Influences

It is now well accepted throughout research and psychiatry that environmental risk factors exist for the development of schizophrenia. In fact, there is a large body of research which is focussed on understanding in depth and characterising these environmental factors. It has become evident through this research that key neurodevelopmental stages during embryonic development, perinatally and during early childhood are particularly vulnerable to environmental insult. For example, childhood and adolescent stressors such as exposure to urban lifestyle, immigration and experience of systemic racism all convey risk for schizophrenia (Cantor-Graae and Selten, 2005; Wicks *et al.*, 2005). Early childhood stress through psychological trauma or accumulating numbers of adverse childhood events (ACEs) also carries risk for schizophrenia development (Bebbington *et al.*, 2004; Spataro *et al.*, 2004; Spauwen *et al.*, 2006; Morgan and Fisher, 2007). Furthermore, embryonic neural development is another key stage which is susceptible to environmental risk development. In fact, factors which influence mothers during pregnancy can have lasting effects on embryonic neurodevelopment resulting in increased risk for schizophrenia.

Maternal stress, such as due to the result of paternal death, has been highly associated with psychiatric conditions in offspring including schizophrenia (Huttunen and Niskanen, 1978; Khashan *et al.*, 2008). Finally, maternal immune activation (MIA), particularly during the first and second trimesters, through viral infection – has been highly correlated with development of schizophrenia in the offspring (Brown, 2006, 2012; Brown and Derkits, 2010; Canetta and Brown, 2012; Janoutová *et al.*, 2016; Aguilar-Valles, Rodrigue and Matta-Camacho, 2020).

1.5.2.1 Maternal Immune Activation

Epidemiological studies identified maternal immune activation during the first two trimesters of pregnancy as a risk factor for schizophrenia and other psychiatric conditions firstly through longitudinal analysis following viral epidemics of influenza and rubella. Researchers identified that, decades later, regions that had experienced these epidemics showed increased rates of diagnosis (Brown, 2011, 2012). Retrospective epidemiological studies then identified through tracing of medical records that those diagnosed with schizophrenia had higher rates of infection during embryonic development compared to the general population. These studies highlighted that infection during embryonic development can have 2-7 fold increase in risk of developing schizophrenia. This risk can be modulated by a number of factors from severity of the immune response to infection during key stages of embryonic neuronal development (infection during the second trimester has higher risk). (Brown and Derkits, 2010; Brown, 2011, 2012; Canetta and Brown, 2012; Janoutová *et al.*, 2016; Aguilar-Valles, Rodrigue and Matta-Camacho, 2020). With this evidence increasingly widely accepted, researchers began to investigate underlying mechanisms through creation of animal models.

Several studies began to artificially activate the immune systems of pregnant rodent through the use of immunogenic agents such as LPS or Poly I:C. These agents function by the activation of toll-like receptor (TLR) 4 and TLR3 respectively, as would occur during infection by bacteria or double-stranded RNA viruses. These studies have investigated behavioural changes as a result of

MIA in the adult offspring and identified deficits in social behaviours (Schwartzer et al., 2013; Gzielo et al., 2021; Kwon et al., 2021; Vitor-Vieira, Vilela and Giusti-Paiva, 2021; Carbone et al., 2023, cognitive ability (Ito *et al.*, 2010; Simões *et al.*, 2018; Sun *et al.*, 2022; Woods *et al.*, 2023) and anxiety-related behaviours (Babri, Doosti and Salari, 2014; Depino, 2015). These studies highlight that MIA can indeed impact on long-term behavioural outputs that last through to adulthood.

Furthermore, the biological impact of maternal immune activation in animals has also been studied and showed long term changes in inflammatory cytokine expression (Hemmi *et al.*, 2002; Isobe *et al.*, 2006; Hameete *et al.*, 2021; Kwon *et al.*, 2021). This is directly relevant to the neuroinflammatory theory of schizophrenia. This theory postulates that overactivity in immune regulation during key stages of neuronal development is a primary mechanism by which schizophrenia occurs (Meyer, 2013; Müller *et al.*, 2015; Aricioglu *et al.*, 2016; Watkins and Andrews, 2016; Trovão *et al.*, 2019). Further research has suggested this could be related to the process of synaptic pruning where synapse generation and growth is heavily modulated by neurological immune function (Brown, 2011; Millan *et al.*, 2016; Sellgren *et al.*, 2019; Chini, Pfeffer and Hanganu-Opatz, 2022). Synaptic pruning begins in early childhood and continues into late adolescence and is heavily regulated through microglial activity and has been linked with overactivity of microglia during embryonic development (Sellgren *et al.*, 2019). Theoretically this would suggest that upregulated microglial immune function occurring as early as embryonic development could have sustained and consistent changes which are upheld through to adulthood.

There is further evidence to support this theory of schizophrenia development. Firstly, inflammatory markers such as TNFa and IL6 have been shown to be increased in people with schizophrenia (Fillman *et al.*, 2013; Dimitrov *et al.*, 2014; Trépanier *et al.*, 2016; Hoseth *et al.*, 2017; Miller and Goldsmith, 2019; Lizano *et al.*, 2021). Furthermore, a microglial cell activation marker has also shown increased expression in schizophrenia (De Picker *et al.*, 2021; Snijders

et al., 2021; Jenkins, Lewis and Volk, 2023). Finally, 2 DOCK proteins, DOCK8 and DOCK11 have been shown to be upregulated directly as a result of maternal immune activation (Oskvig *et al.*, 2012; Namekata *et al.*, 2014). As previously mentioned, DOCK proteins are GEFs which modulate RhoGTPase activity and have roles in intracellular signalling. DOCK 1 and DOCK 4 have both been genetically linked to schizophrenia (Shi, 2013; Morris and Pratt, 2014; Samani *et al.*, 2022). In this case DOCK8 and DOCK11 are of relevance due to their specific roles with microglial cell activity (Mortazavi *et al.*, 2008). DOCK 8 is associated with microglial activation and function during neuroinflammation. On the other hand, whilst DOCK 11 is highly expressed in microglial cells, it is not clear how functionally relevant it is to microglial activation or function (Namekata *et al.*, 2014, 2019, 2020). Nevertheless, MIA studies have shown both short- and long-term increased expression of both DOCK proteins in mice (Oskvig *et al.*, 2012; Namekata *et al.*, 2020). Long-term increased expression of DOCK 8 could further indicate that even a single instance of MIA can result in altered microglial activation through to adulthood which would certainly support the theory that synaptic pruning could be affected in childhood as a result of embryonic MIA (Sellgren *et al.*, 2019) (Sellgren et al, 2009).

Overall, there are several environmental risk factors which can increase risk of schizophrenia development in humans. One such environmental insult is that of MIA during pregnancy. MIA has been shown to result in prolonged inflammatory response which is also seen in humans with schizophrenia. Furthermore, MIA has been shown to affect expression of 2 members in the DOCK protein family. Given the genetic associations of DOCK proteins and schizophrenia as well as the altered expression following MIA and their relevance to microglial activity, this appears to be a family of proteins which should be of further interest to schizophrenia research.

1.6 Modelling Schizophrenia

Whilst an enhanced understanding of the underlying pathophysiology, psychopharmacology and developmental origins of schizophrenia has been established through extensive epidemiological and clinical studies, research has also utilised the development of animal models in order to assess biological underpinnings of the disorder (Pratt et al., 2012). However, the use of animal models must be considered alongside analysis of face, construct and predictive validity of each model if research is to truly advance (Young et al., 2010; Pratt et al., 2012; Silverman et al., 2020). That is, it is important that any animal model is a valid representation of the disorder across all facets of the human disorder. Face validity refers to the evidence of a phenotype which is similar or matches aspects of the human phenotype. This is important when carrying out research in human disorders on animals as the animal model must sufficiently represent the effects of the disorder (Young et al., 2010; Pratt et al., 2012; Silverman et al., 2020). However, this is not the strongest method of assessing validity of a model since behavioural or physiological phenotypes in animals may not represent the same effects as they do in humans. Furthermore, in behavioural models, the interpretation of data can often be subjective.

On the other hand, construct validity is a fundamental aspect of animal research which should be met where possible. In this case, construct validity refers to a model which shows the same underlying mechanisms as the human disorder and therefore shows the relevant pathophysiology. In studies where research on the underlying mechanisms of a disorder form the basis of investigation, construct validity is crucial (Young et al., 2010; Pratt et al., 2012; Silverman et al., 2020). Finally, research which focusses on testing therapeutic effects of different treatments such as new pharmacological interventions will focus more prominently on predictive validity. In this instance, predictive validity refers to the strength of predicting outcomes in the human population based on results from the animal model. Interestingly, good predictive validity does not always concur with strong face or construct validity. This is the case in the studies of anti-depressant effects on forced-swim tests. Studies show

relatively strong predictive validity, with successfully prescribed antidepressants also showing positive results in the forced swim tests (Petit-Demouliere., et al 2005). However, the construct validity and face validity of the forced-swim test as a model of depression is not high given it is an acute behaviour which does not accurately represent the human experience (Petit-Demouliere., et al 2005; Kara et al., 2018).

In the case of schizophrenia research, animal models exist that focus on different aspects of the disorder. Pharmacological models which aim to replicate the underlying psychopharmacology are often used to assess both the biological mechanisms as well as response to new therapeutics (Pratt et al., 2012). Genetic mouse models have been created to replicate the human genetic correlates (Pratt et al., 2012). Environmental models of schizophrenia aim to mimic the environmental risk factors that increase risk of diagnosis in humans (Pratt et al., 2012). And finally, dual-hit models have been created which have begun to look at how multiple risk factors might increase the risk of development compared to singular risk factors alone.

1.6.1 Pharmacological Models of Schizophrenia

A number of pharmacological animal models of schizophrenia exist which primarily aim to mimic either the dopamine or the NMDA receptor theory of schizophrenia. Studies have used amphetamines in animal models to assess the efficacy of antipsychotic medications (Stockton and Rassmussen, 1996; Idris et al., 2005) in an effort to replicate the dopamine theory of schizophrenia. The predictive validity of such studies could be argued since these antipsychotics do have successful outcomes on the modulation of positive symptoms of schizophrenia in humans, suggesting good predictive validity. However, these antipsychotics do not provide relief from negative symptoms and so only one aspect of schizophrenia is being treated (Gowda et al., 2017; Burschinski et al., 2023; Breitbart et al., 1996). Additionally, with further knowledge of the potential role of the NMDA receptor hypofunction theory, it would suggest the construct validity of amphetamines as a model of schizophrenia is also low.

On the other hand, the use of NMDA receptor antagonist such as PCP or ketamine as a model of schizophrenia shows good face validity and to an extent good construct validity which perhaps encompasses a more accurate reflection of the underlying mechanisms of schizophrenia (Abdul-Monim, Reynolds and Neill, 2006; Didriksen, Skarsfeldt and Arnt, 2007; Llado-Pelfort et al., 2016). However, it could be argued that a short-term acute effect cannot accurately represent the underlying mechanisms of a complex, long-term disorder. Additionally, the predictive validity is similar to that of amphetamines in that antipsychotics may reverse effects of NMDA receptor antagonists in tested behaviours, but the full repertoire of human symptomology is not successfully treated (Abdul-Monim, Reynolds and Neill, 2006; Didriksen, Skarsfeldt and Arnt, 2007; Llado-Pelfort et al., 2016).

Overall, the pharmacological models may offer strong face validity given similar schizophrenia-like psychosis presenting in humans after administration of these drugs (Luby *et al.*, 1959; Allen and Young, 1978). Additionally, construct validity may be addressed in some aspects with these drugs replicating the underlying psychopharmacology of schizophrenia. However, predictive validity in these cases are often not addressed with a large proportion of symptoms experienced by those with schizophrenia showing no improvements despite animal studies showing reversal of behaviours modulated by these drugs.

1.6.2 Environmental Models of Schizophrenia

With several environmental factors that have been shown to increase risk of schizophrenia (as described in section 1.5.2) studies have looked at animal models which replicate these factors. Maternal immune activation is often used as a model of schizophrenia to mimic the increased risk of schizophrenia development as a result of maternal infection during embryonic development (Brown, 2006, 2012; Brown and Derkits, 2010; Canetta and Brown, 2012; Aguilar-Valles, Rodrigue and Matta-Camacho, 2020). These studies have shown strong face validity with effects seen in cognitive abilities, anxiety-related behaviour and sociability that can often be seen in people with schizophrenia (Schwartzer et al., 2013; Gzielo et al., 2021; Carbone et al., 2023; Ito *et al.*,

2010; Simões *et al.*, 2018; Sun *et al.*, 2022; Woods *et al.*, 2023; Babri, Doosti and Salari, 2014; Depino, 2015). Likewise, construct validity is strong since these studies successfully mimic the biological effects of maternal immune activation which leads to increased risk of schizophrenia. Additionally, there are currently early studies which research the predictive validity of maternal immune activation (Chamera et al., 2022; Scarborough et al., 2021; MacDowell et al., 2021). These studies have shown that antipsychotics may reduce the effects which have been induced by MIA, however further research must continue to determine whether MIA can be used as a predictive measure of successful therapeutics.

Other studies aim to replicate the social risk factors that can lead to increased risk of schizophrenia development such as childhood distress (Bebbington *et al.*, 2004; Spataro *et al.*, 2004; Spauwen *et al.*, 2006; Morgan and Fisher, 2007) or other prolonged stress (Cantor-Graae and Selten, 2005; Wicks *et al.*, 2005). To assess these factors, models which induce early life stress such as removal of the mother from the home-cage in the first few days after birth, social isolation or utilisation of light to induce stress (Oliver et al., 2012; Jiang et al., 2013; Bhardwaj et al., 2015; Powell and Swerdlow, 2023; Oginga and Mpofana, 2023). These studies focus on the construct validity of animal models with research focussed on understanding the underlying mechanisms related to these environmental stimuli and how they map on to the underlying biology of people with schizophrenia.

1.6.3 Genetic Models of Schizophrenia

There have been several lines of genetically modified mice which have been altered to contain genes of interest in human populations of schizophrenia. Several of these genes have been identified through GWAS, CNV and exome studies and have led to the development of animal models which aim to assess the effects of these genetic variations (described in section 1.5.1).

The 22q11.2 deletion is a genetic variant which shows one of the highest risks for development of schizophrenia in humans. Mice containing this genetic-

variant have shown behavioural changes related to schizophrenia phenotypes such as hyperactivity, deficits in working and spatial memory and decreased sociability (Earls et al., 2011; Piskorowski et al., 2016; Stark et al., 2008). Additionally, these mice have shown biological changes which replicate findings from human studies including altered connectivity between the hippocampus and PFC and decreased PV cell expression (Earls *et al.*, 2011; Piskorowski *et al.*, 2016; Sigurdsson *et al.*, 2010).

Similarly, mice containing the 16p11.2 duplication, which also carries high-risk for schizophrenia development, have also shown relevant behavioural and biological changes concurrent with the human schizophrenia phenotype. 16p11.2 duplication containing mice have shown deficits in sociability, cognitive function and increased anxiety related behaviours (Arbogast *et al.*, 2016; Bristow *et al.*, 2020; Rein *et al.*, 2021). Additionally, neurobiologically these mice have shown reduced brain volume, reduced PV and SST expression, alterations to PNN structures and dysfunctional signalling in the PFC (Horev *et al.*, 2011; Bristow *et al.*, 2020; Willis, Pratt and Morris, 2022; Rein *et al.*, 2021).

Finally, the disrupted in schizophrenia -1 (DISC1) variant has shown relevant behavioural and biological phenotype in DISC1-containing mice. These mice have shown deficits in working memory and reduced sociability (Koike *et al.*, 2006; Brandon & Sawa, 2011; Kuroda *et al.*, 2011; Lipina *et al.*, 2013). Biologically there is evidence that DISC1 mice show deficits in development of GABAergic interneurons (Lee *et al.*, 2013).

Overall, it is clear that genetic mouse lines produce strong face and construct validity with mice that present behavioural changes that mimic symptomology of humans with schizophrenia as well as underlying biological changes that can be found in human pathophysiology.

1.6.4 Dual-hit Models of Schizophrenia

Whilst each of the models discussed throughout section 1.6 may show different types of validity in relation to how accurately they represent the human disorder, no single model covers every aspect of schizophrenia. Environmental

and pharmacological models may show strong behavioural phenotype whilst genetic models show the strongest biological phenotype. However, to build an animal model which truly addresses face, construct and predictive validity, research should move to combine multiple risk factors into a single model. With evidence that accumulating factors can increase risk of diagnosis this would result in a more translatable model which could lead to novel findings in the development of schizophrenia.

There has been some research which has utilised this type of dual hit model. Several studies in DISC1 containing mice who were exposed to maternal immune activation have shown that schizophrenia-related behavioural phenotype can be exacerbated (Abazyan et al., 2010; Ibi et al., 2010; Lipina et al., 2013). Another study showed that biological changes in hippocampal signalling were exacerbated by early life stress models in DISC1 containing mice (Brydges et al., 2014). In relation to the 22q11.2 deletion, one study investigated the effects of social isolation on mice containing the 22q11.2 deletion before administration of antipsychotic drugs to determine whether medications would alleviate changes induced through the combined genetic variant and environmental stimuli. The study showed combined risk factors resulted in both behavioural deficits in working memory as well as biological changes in decreased PV neuron expression and decreased plasticity in the hippocampus and PFC (Tripathi et al., 2020).

Whilst these studies provide some evidence that dual-hit models may provide stronger face, construct and even predictive validity in future there is still very few completed investigations. As a result, expanding the animal models in schizophrenia research to include dual and triple-hit models would advance understanding of schizophrenia.

1.7 Thesis Outline

1.7.1 Summary and Aims

Schizophrenia has for a long time been a misunderstood disorder which was historically associated with dangerous individuals resulting in negligent and damaging treatment of those who live with the disorder. However, as research began to understand the neurobiological underpinnings of psychiatric disorders, our ability to treat individuals began to take precedence over confinement. Despite this, the current pharmacological treatment options are largely the same as those that were initially discovered over 70 years ago (Ban, 2007). These treatments are often accompanied by severe and unacceptable side effects which leaves those seeking interventions with few viable options (Breitbart *et al.*, 1996; Gowda *et al.*, 2017; Meepring *et al.*, 2021; Burschinski *et al.*, 2023). Unfortunately, this results in a dangerously high mortality rate with suicide the leading cause of death amongst those diagnosed (Saha *et al.*, 2005; Saha, Chant and McGrath, 2007; McGrath *et al.*, 2008).

It is imperative that research continues to better understand schizophrenia and the neurobiological underpinnings which cause its development if we are to improve the quality of life that those diagnosed can experience. There has been some advance with the recent approval of muscarinic receptor agonists which may function to alleviate both positive and negative symptoms (Dean and Scarr, 2020; Foster, Bryant and Conn, 2021; Lu *et al.*, 2024). However, this research is still in its infancy. Further, cognitive and social deficits are evident throughout childhood and can therefore result in life-long impacts prior to first-episode psychosis and eventual diagnosis(Baum and Walker, 1995; Cannon *et al.*, 2000; Bowie and Harvey, 2006; Fett *et al.*, 2011; McCutcheon, Keefe and McGuire, 2023). We must strive therefore to understand in depth the developmental origins of the disorder if we are to identify early treatment interventions or diagnostic tools.

Our current understanding of schizophrenia stems from two prevailing theories which are posited as the underlying mechanism: the dopamine theory and the

NMDA receptor hypofunction theory. Whilst the dopamine theory can explain a large number of the symptoms seen in schizophrenia (van Rossum, 1966; Howes, McGuire and Kapur, 2008; Howes and Kapur, 2009), research on the NMDA receptor hypofunction appears to show a similar repertoire of behavioural changes as well as robust post-mortem, pathophysiological and pharmacological data supporting its likely relevance as a mechanism (Luby *et al.*, 1959; Andersen and Pouzet, 2004; Morris, Cochran and Pratt, 2005; Turgeon, Anderson and O'Loughlin, 2010; Turgeon *et al.*, 2011; Javitt *et al.*, 2012; McLean *et al.*, 2017). Furthermore, the NMDA receptor theory can be directly related to other underlying pathologies such as PV-expressing interneuron deficits, PFC dysfunction and PFC connectivity deficits (Löscher and Hönack, 1992; Cochran *et al.*, 2003; Barry *et al.*, 2015). However, a common model for schizophrenia which is based upon the NDMA receptor theory – PCP use in animals (Morris, Cochran and Pratt, 2005) – could still be further investigated particularly in more complex behavioural domains. Specifically, novel methodologies allow researchers to investigate complex behaviours using more translational approached than was previously available. As a result, it is important that we continue to assess the construct validity of such models by their application to these novel methods if we are to continue to develop our understanding of the underlying neurobiology in schizophrenia.

Whilst pharmacological interventions are a useful method of understanding underlying neurobiology, this cannot replicate the nature of schizophrenia development. As a result, further research must continue to investigate how the disorder develops if we are to identify underlying causes. It is clear that there is a genetic component to schizophrenia with GWAS, CNV and exome studies all identifying genes which carry various levels of risk for schizophrenia development (Winchester, Pratt and Morris, 2014; Henriksen, Nordgaard and Jansson, 2017; Trubetskoy *et al.*, 2022). The highest risk CNV which shows the clearest specificity for schizophrenia phenotype and fewer co-morbidities is the 16p11.2 duplication. The locus is responsible for a number of individual genes some of which are important in cortical neurotransmission. Furthermore, animal studies have identified similar deficits in PV and SST expression as can

be seen in the human neurobiology (Horev *et al.*, 2011; Kirov *et al.*, 2014; Macé *et al.*, 2017; Marshall *et al.*, 2017; Richter *et al.*, 2019; Bristow *et al.*, 2020; Mitchell, Thomson, *et al.*, 2020; Openshaw *et al.*, 2023). However, research must continue if we are to understand how each individual gene at the 16p11.2 locus could be involved in schizophrenia and how it may convey this risk for development.

On the other hand, whilst genetics play a role in development, no single gene carries 100% risk for development. As a result, environmental factors must be at play alongside potential genetic involvement. In fact, research has shown a number of environmental factors which all carry increased risk of developing schizophrenia. These environmental factors occur through psychological trauma with early life stress, physiological effects of stress from mothers as well as instances of sustained inflammation at different life stages including during embryonic development, childhood and adolescence (Bebbington *et al.*, 2004; Spauwen *et al.*, 2006; Khashan *et al.*, 2008; Brown, 2011; Müller *et al.*, 2015; Kose *et al.*, 2021). Investigations into these individual risk factors have revealed that maternal immune activation (MIA) not only carries risk in humans as can be seen through epidemiological studies, but this effect can also be replicated in animals resulting in complex behavioural changes in offspring (Brown, 2006; Oskvig *et al.*, 2012; Brown and Meyer, 2018; Simões *et al.*, 2018; Choudhury and Lennox, 2021; Carbone *et al.*, 2023). Furthermore, long term neurobiological changes have also been seen following artificial MIA protocols. This strong schizophrenia-like phenotype indicates that MIA and inflammation may be highly relevant to schizophrenia development.

1.7.1.1 Thesis Aims

Whilst there is robust data around these genetic and environmental risk factors individually, there is less research into how these risks may converge or interact. This is despite clear evidence that no single risk factor is causative and those diagnosed can often identify a number of combining factors that have been experienced throughout their lifetime. Theoretically, it appears that genes and environment interact and accumulate to result in individuals who

become predisposed to schizophrenia which then becomes evident upon first-episode psychosis in adulthood. To scientifically assess whether this theory is true, it is necessary for research to begin utilising multi-modal and dual-hit models which can actively assess numerous risk factors, their interactions and the resulting behavioural and biological phenotypes.

On this basis, this thesis aims to address three main outstanding questions:

1. How does neuropharmacological intervention which mimics neural activation patterns related to the NMDA receptor hypofunction theory of schizophrenia affect complex behaviours in mice in novel experimental methodologies?
2. Can the use of dual-hit models for schizophrenia which utilise genetic and environmental risk factor interventions portray the full repertoire of behavioural outcomes seen in neuropharmacological models?
3. Can the use of dual-hit models for schizophrenia which utilise genetic and environmental risk factor interventions portray the full repertoire of neurobiological changes that are seen in the underlying pathophysiology of humans with schizophrenia?

1.7.2 Behavioural repertoire of acute, low-dose PCP

To assess the first aim, PCP was administered to wild-type mice in order to assess how NMDA receptor hypofunction could affect behavioural output. A series of behavioural tests were used to assess cognition, sociability and anxiety-related symptoms in novel test environments. These experimental environments were chosen due to the translatability of the methodology to innate circumstances in humans. It was important in this experiment to assess the validity of each behavioural test as a method for assessing schizophrenia-related phenotype in mice. Since PCP has been used extensively in schizophrenia research, with high face-validity to the human disorder, the use of the NMDA receptor antagonist model could determine validity of novel behavioural tests in schizophrenia. In this case, if PCP induced a strong behavioural phenotype which represented similar behavioural output to that

which is seen in humans, it would be pertinent to use similar behavioural tests to assess a more elaborate animal model. Changes in locomotor activity, thigmotaxis behaviours and exploration of the elevated plus maze would provide information on anxiety-related behaviours. Home-cage monitoring would provide detailed information about sociability of the animals and novel object recognition and paired discrimination and reversal learning tasks would determine any changes in cognitive function. Since in humans, schizophrenia has shown increased anxiety, reduced sociability and increased cognitive deficits, these studies would assess a wide repertoire of behavioural phenotypes related to humans.

Based on research in other animal models which shows reduced cognitive abilities, increased anxiety and decrease sociability, it was hypothesised that similar results would be seen here. Furthermore, with the identification of sex-differences particularly in anxiety and social withdrawal in humans, it was hypothesised the female mice would exhibit stronger anxiety-related phenotype compared to males whilst male mice would exhibit increased social withdrawal compared to females. The results of this investigation are detailed in Chapter 3 of this thesis.

1.7.3 A dual-hit model of schizophrenia; assessing genetic and environmental risk factor interactions

The next step in this thesis was to create a dual-hit model which combines environmental and genetic risk for schizophrenia and assess how interactions may affect behavioural and biological phenotype in mice. This was done through the use of a genetic mouse line containing 16p11.2 duplication given this CNV has been strongly and specifically associated with schizophrenia and schizophrenia-like behavioural changes in animals. Secondly, there is robust and consistent behavioural and biological effects seen in animals after MIA during embryonic development. This is associated with the increased risk of schizophrenia in humans as a result of maternal infection during pregnancy. To replicate this environmental risk factor in this dual-hit model, artificial MIA was

induced through multiple methods in mice carrying 16p11.2 duplication. To do this, a commonly used immunogenic agent, Poly IC was used. However, since Poly IC induced immune response through activation of TLR3 receptors replicating infection by double stranded viruses, another immunogenic was used (Kwon *et al.*, 2021). This is due to the epidemiological studies which show primarily single-stranded viruses, such as rubella or influenza, are those which cause increased risk of schizophrenia. Here, we included immune activation through Resiquimod, a TLR7 receptor agonist which mimics infection by single stranded viruses(Kwon *et al.*, 2021). This resulted in both wildtype and 16p11.2 duplication carrying mice who had either experience MIA or not allowing for comparison of gene vs environment as well as assessing gene x environment interactions.

1.7.3.1 Behavioural repertoire of a dual-hit model

A number of behavioural assessments were carried out on mice derived from the dual-hit model for schizophrenia which utilised MIA and 16p11.2 duplication to assess genetic and environmental risk factors. Behavioural tests assess cognition, anxiety and sociability in mice in order to test for presence of cognitive and negative symptoms of schizophrenia. Furthermore, both male and female mice were used in these experiments in order to assess for sex differences as can be seen in humans. It was hypothesised that there would be individual effects of gene and MIA each behaviour with presence of cognitive deficit, reduced sociability and increased anxiety. Furthermore, there would be sex-specific effects with female mice showing increased anxiety compared to male mice and male mice showing reduced sociability compared to female mice. Finally, it was hypothesised that there would be a gene x environment interaction which would result in the combination of gene and MIA showing increased severity in behavioural deficits compared to gene or MIA alone. Results of this experiment are detailed in Chapter 4.

1.7.3.2 Neurobiological repertoire of a dual-hit model

Finally, after completion of behavioural tests, the brains from the mice of the dual-hit model were removed and the prefrontal cortex was dissected. Given the highly relevant pathophysiology that can be seen in the prefrontal cortex in humans with schizophrenia as well as in several animal schizophrenia models, an assessment of mRNA expression of this region was carried out. Assessing mRNA expression of a number of markers would elicit information on neuronal transmission and signalling (by assessing PV and SST expression (Hashimoto *et al.*, 2008; Nakazawa *et al.*, 2012)), long term neuroinflammation (by assessing TNFa, CD68 and IL6 expression (Miller and Goldsmith, 2019; Upthegrove and Khandaker, 2020)) and microglial activation patterns (by assessing DOCK 8 and DOCK11 expression (Namekata *et al.*, 2014, 2019, 2020)). It was hypothesised the 16p11.2 duplication would reduce expression of PV and SST and MIA would increase expression of TNFa, IL6, CD68, DOCK 8 and DOCK11. Furthermore, it was hypothesised that there would be gene x environment interactions in relation to expression of PV and SST given the role inflammation and microglial activity can have on neurotransmission. Results of this experiment are detailed in Chapter 5.

1.7.4 Gene x Environment interactions as a model for schizophrenia

Finally, a comprehensive assessment of each individual investigation as well as how these results relate to one another is detailed in Chapter 6 of this thesis. The validity of dual hit models will be assessed in relation to the behavioural and neurobiological profile and its relevance to the schizophrenia phenotype in humans. At the same time, this will be compared to the more commonly used model of NMDA receptor hypofunction to determine if any behavioural effects are not replicated in the novel model. It was hypothesised that the dual-hit model used here would replicate similar behaviour output as the neuropharmacological model with both studies showing cognitive deficits, social withdrawal and increased anxiety. Furthermore, behavioural and biological analysis of the dual-hit model would show mirroring effects in gene x environment interaction as well as in severity of effect. In this case, it was

hypothesised that genes and environment would have individual effects on behaviour and neurobiological marker expression and that these effects would increase in severity as a result of combined gene x environment interaction.

The overarching aim of this thesis is therefore to determine how converging environmental and genetic risk factors interact to increase likelihood of schizophrenia and whether it is possible to replicate this in an animal model. If so, this has implications for future schizophrenia research and methodology that is used to assess complex neurobiological and behavioural disorders.

2 General Methods

2.1 Animals

All animals were maintained in accordance with the Animals (Scientific Procedures) Act, 1986 with further efforts made to address the "3R's": Replacement, reduction and refinement in animal research (Home Office, 2014; MacArthur Clark, 2018; du Sert *et al.*, 2020). This was done by; reducing the number of animals bred to the minimum required to maintain the colony, utilising as many animals as possible which were bred in experiments, using experimental animals in a number of experiments, and finally ensuring sample size was sufficient to provide meaningful data. Minimum sample sizes of 5 mice per group were chosen based on previous experiments carried out within the lab. However, due to the nature of breeding protocols and fecundity as well as requirement for distinct genotypes, any available animals were used. Furthermore, since experiments measuring social behaviours could be influenced by cage-mate dynamic it was important to include a minimum of 3 cages in each group. This means sample sizes in some groups are higher than in others. Single housed animals which were not suitable for experiments were culled to prevent long term single housing. All animals were provided with adequate housing and enrichment and monitored for signs of stress including overgrooming and fighting with cage mates.

2.1.1 16p11.2 Duplication Mouse-Line

The animals used throughout these experiments were 16p11.2 duplication mice who were maintained congenic on a background of C57Bl5/J mice (Horev *et al.*, 2011; Arbogast *et al.*, 2016). The human 16p11.2 duplication is mapped onto mouse chromosome 7F3. Mice were originally sourced from the Jackson Lab and are described in Horev et al, 2011 but were maintained and bred in house throughout the experiment.

2.1.2 Colony Maintenance Breeding

For colony maintenance, WT females were paired with HZ 16p11.2 males and litters weaned at 3 weeks old into the general colony. The strain showed relatively low fecundity with litters not readily available and several weeks delay between pairing before pregnancy was detected.

2.1.3 Maternal Immune Activation and Timed Breeding

WT males and HZ 16p11.2 duplication females were paired and kept single housed between breeding. Once a week, breeding pairs were placed together at 4pm and left overnight to breed. At 9am the next morning, females were checked for plugs and males removed. The data and weight of females was assessed. 7 days later, at embryonic development day 7 from timed mating (E7) the weight of animals was checked and then again at day E12.5. If the weight of the female had increased by a minimum of 2g since the day of breeding and a plug was detected the female was judged to pregnant and injected subcutaneously with either of 0.2-0.3ml of sterile, 0.9% sodium chloride solution (saline), 2mg/kg of Resiquimod or 20mg/kg of Poly I:C. If an injection was made, weight was checked 24 hours later and recorded as an indicator of immune activation. Pups born were then genotyped as described in section before being implemented into experiments.

2.1.4 Experimental Animals

All experimental animals were moved to a reversed light cycle room where the dark cycle began at 10am and ended at 10pm. This was to make sure all behavioural experiments were completed within the waking period of the animals and to prevent disturbance to sleeping patterns which could confound results of behavioural analyses. Power analyses completed previously within the lab by Ashleigh Willis indicated that a minimum of 5 animals per group would provide sufficient statistical power (Willis, PhD Thesis, 2019). However, in the case of the maternal immune activation and timed matings, this was not reached for all groups. This is primarily due to low fecundity in the 16p11.2 population. At the same time, occasionally there was an excess of animals

within a group due to chance breeding. These mice were included in the experiment in order to avoid these experimental animals having been bred unnecessarily.

2.1.4.1 PCP Experimental Animals

A total of 26 WT mice from 8 litters underwent behavioural experiments following injection of PCP. These experiments were completely in 3 distinct groups, primarily due to different restrictions for laboratory access during the Covid19 pandemic. The first group of 9 females completed the open-field test, the paired discrimination test and the home-cage monitoring test as well as 2 males which completed the open-field test. The second group included 9 males and 6 females. The 9 males completed the home-cage after which one mouse unfortunately suffered an infection which was deemed unrelated to these experiments by the vet at the Strathclyde University Behavioural Animal Unit and was culled. The remaining 8 males completed the novel object recognition test and elevated plus maze. The 6 females completed only the novel object recognition and elevated plus maze tests. Animals were between 8 and 16 weeks old before beginning experiments. One female WT mouse was included at 46 weeks old due to animal availability. The results of all behavioural tests were not affected by this animal which was not deemed an outlier and so results were included in the groups. In each experiment every mouse received both saline vehicle and PCP in a within-subjects model. Given potential extended effects of even a low, acute dose of PCP behavioural, experiments were carried out at least a week apart to ensure any PCP effects did not confound effects of saline vehicle experiments. Details of total numbers of animals for each behavioural test are shown in Table 2.1.

Experimental Group	Male WT	Female WT
Open Field	11	9
Paired Discrimination	0	9
Novel Object Recognition	8	6
Elevated Plus Maze	8	6
Home Cage	9	9
Total	11	15

Table 2-1: Table depicting total number of animals that underwent behavioural testing with PCP.

2.1.4.2 Maternal Immune Activation Animals

Over the course of a year, a total of 73 animals from 14 litters were weaned from dams who had underwent timed mating and were subsequently immune activated at E12.5. Where possible weaned animals were housed in cages with a minimum of 3 animals and a maximum of 4 per cage in order to complete home-cage monitoring which requires a minimum of three animals per cage whilst more than 4 indicates overcrowding. However, given a relatively low fecundity with the time-matings it was not always possible to have 3 in a cage. In cases where there was only 2 animals in a cage, it was not possible to complete home-cage analysis with these mice. However, these mice still completed all other behavioural analyses in the open field, elevated plus maze and novel object recognition tasks. Details of total animals per group for all behavioural analyses are shown in section 4.2.1. After behavioural tests, animals were culled and prefrontal cortex, hippocampus and spleen were dissected and stored at -80°C in preparation for RT-qPCR.

2.1.5 Genotyping

Genotyping of animals was completed by laboratory technicians at the University of Strathclyde and the University of Glasgow. Lee Wheeler was the primary technician to take ear clipping samples from mice upon weaning. Ear

clips were then transferred to the University of Glasgow where they were then genotyped by John Craig using the following protocols.

2.1.5.1 DNA Extraction

Upon date of weaning, ears were clipped using a 2mm ear punch and stored in a -20°C freezer. To extract DNA from ear clippings, each clipping was submerged in 40µl of DirectPCR and 0.8µl of Proteinase K. They were then placed in a PCR machine for two cycles at 55°C, the optimal lysis temperature, and then one cycle of 30 minutes at 85°C to deactivate the Proteinase K. Alternatively, samples could be left overnight with 12 cycles of 60 minutes at 55°C for lysis before 1 30-minute cycle at 85°C to deactivate Proteinase K. The samples were then placed in a centrifuge and supernatant collected in preparation for PCR.

2.1.5.2 Polymerase Chain Reaction

DreamTaq PCR Master mix was prepared with wild type primer mix or 16p11.2 duplication master mix (sequences in Table ?) and 1µl of the collected supernatant added. Samples then underwent PCR with an initial 3-minute denaturation cycle at 95°C before 37 cycles of 95°C for 30 seconds, 64°C for 30 seconds and then 72°C for one minute. Then there was one final cycle of 7 minutes at 72°C before samples were cooled to 4°C for collection.

2.1.5.3 Gel Electrophoresis

1.2g of Agarose was added to 120ml of 0.5X TBE Buffer and microwaved for 20 second increments until all agarose was dissolved. 1µl of ethidium bromide was added and then the solution slowly poured into a gel mould. Gel was set after 30-45 minutes. The gel tank was then filled with 0.5X TBE Buffer until gel was fully covered. 5µl of a 100bp DNA ladder was pipetted into the first lane with 14µl of samples pipetted in each subsequent lane. Gel was run for 1.5 – 4 hours at 52 volts 200mA and stopped once the green guide dye from the DreamTaq Hotstart was in line with the lower band of the 100bp ladder. A Sony camera was then used to visualise bands and determine presence of 16p11.2

duplication. The bands would be visualised as shown in Figure 2.3 with WT bands showing at 370bp and duplication bands showing at 800bp.

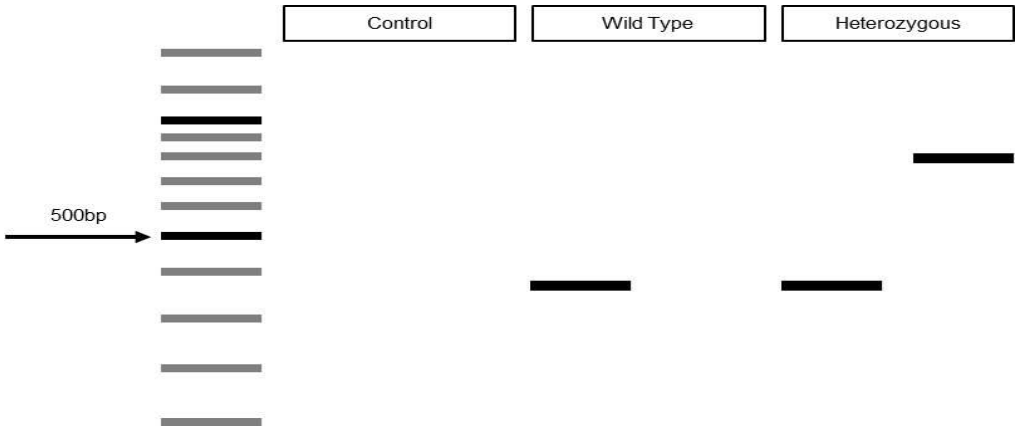

Figure 2.1: Diagram depicting banding which would be visible for wildtype or 16p11.2 duplication carrying mice on gel electrophoresis.

2.2 Behavioural Tests

2.2.1 Home cage Monitoring

Radio-frequency identification (RFID) microchips (Biomark, Idaho, USA) were injected via pre-loaded syringes subcutaneously in the lower abdominal quadrant between the midline and hindlimb, under 4% isofluorane anaesthesia. The injection site was sealed using VetBond Surgical glue to prevent removal of microchip before animals were placed in a recovery cage until fully awake from anaesthesia before being returned to home-cage. All animals within a single cage underwent this procedure within an hour of one another to prevent disruption to cage hierarchy. After a minimum of 24 hours of recovery, animals were placed into clear IVC cages and placed in the Actual HCA^{TM} system (Actual Analytics Ltd, UK). Recording was started at 10am – the beginning of the dark cycle and was stopped at 10am on the desired day. The location of the RFID microchip in relation to the antenna in the Actual

HCA system was recorded every 4ms for the duration of the recording. Data collected reveals locomotor activity over time as well as proximity of microchips which can indicate time spent in isolation or distance of separation between cage-mates.

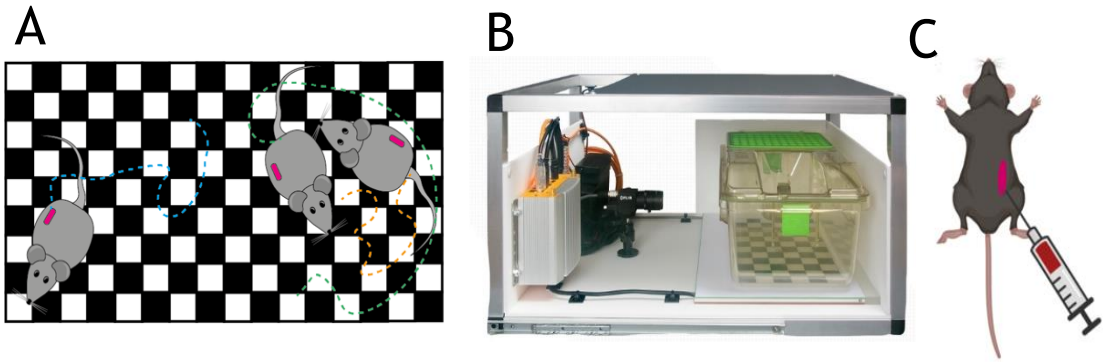

Figure 2.2: Diagram of home-cage continuous monitoring equipment. A) Depiction of RFID detecting antenna grid. B) photograph of recording aparatus. C) Depiciton of RFID chip injection, subcutaneously into abdomen.

2.2.2 Open-field Test

A 40x40cm open-field was set up with Noldus Ethovision recording software and an infra-red movement tracking camera (Figure 2.5). Open-fields were placed in the centre of a room and had black walls and floors. During recording, lights were switched off and recording occurred in an adjacent room to minimise light and sound disturbance to the mice. The mouse was placed into the open field and recording started in the adjacent room. Noldus Ethovision software tracked velocity and distance travelled of the mouse as well as the location within the open-field. Once recording was completed after 2 hours, animals were placed back in their home-cage. Further details are described in the corresponding methods and materials sections for Chapter 3 and 4.

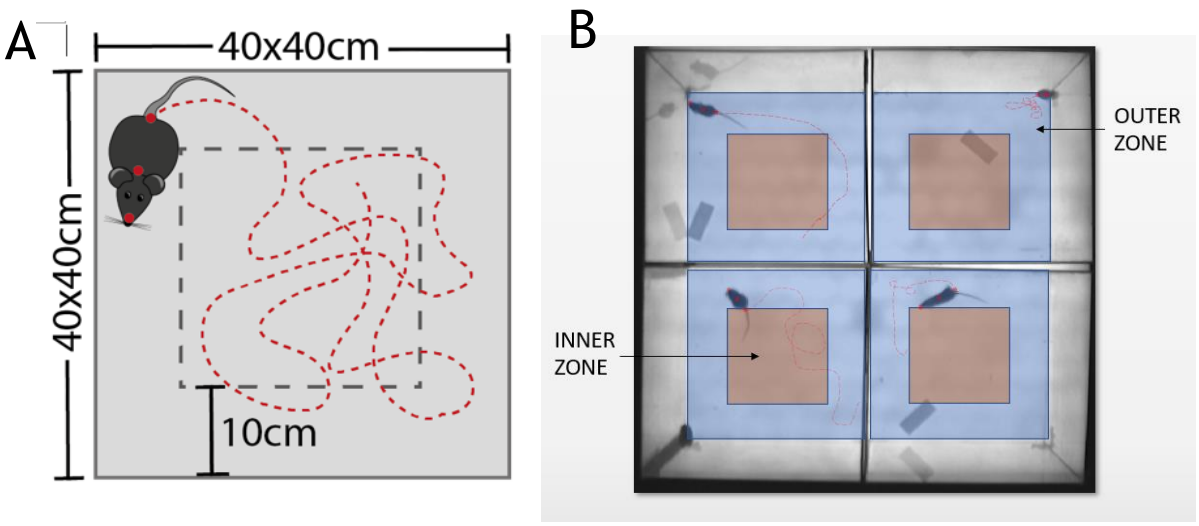

Figure 2.3: Diagram of open-field test apparatus. A) diagram of open-field with inner and outer zones highlighted. B) photograph from Noldus Ethovision Infrared camera depicting open-field recording equipment.

2.2.3 Elevated Plus Maze

An EPM with 20cm arms was set up in the centre of a room with an infra-red camera directly overhead. Lights were switched off in the room and a mouse was placed in the EPM before recording was started in an adjacent room to prevent light and noise disturbance. Noldus Ethovision tracking software detected velocity, distance travelled and movement between open arms, closed arms, and the centre zone of the EPM. Entrance into the final third of the open-arms was also tracked. Recording lasted 15 minutes before animals were removed and returned to their home-cage. Further details are described in the corresponding methods and materials sections for Chapter 3 and 4.

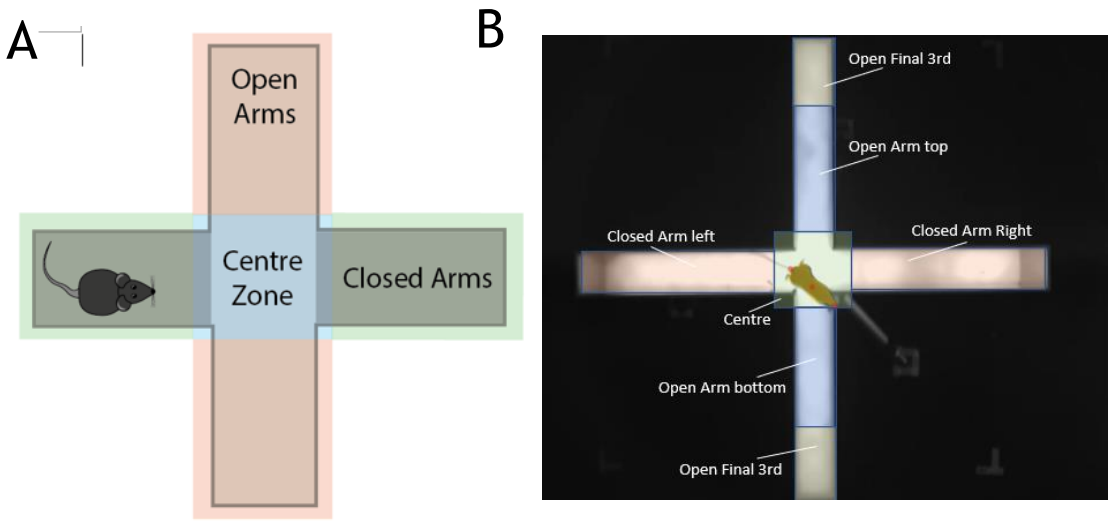

Figure 2.4: Diagram of elevated plus maze apparatus. A) diagram of the elevated plus maze with different zones highlighted. B) photograph from Noldus Ethovision Infrared camera depicting elevated plus maze recording equipment.

2.2.4 Novel Object Recognition

2 sets of paired objects were selected based on previous experiments by Emma Mitchell who piloted different objects and identified the objects which had equal levels of interest by mice. Objects chosen were a short plastic cylinder, paired with a brass doorknob and a brass ring paired with a wooden cube. The object which was novel and the location of the novel object was counter balanced across the mice. Mice were then placed in an open-field (Figure 2.

7) and habituated for 5 minutes with the lights off and no one in the room before being returned to the home-cage. The following day mice were placed in an open-field and recording via Noldus Ethovision was started in an adjacent room. The animal was temporarily removed and two identical objects were then placed in the lower two corners of the open-field 10cm from adjacent walls. The animal was then returned to the open-field closest to the centre of the opposite wall from the objects. Recording was started. Once the animal had explored both objects for a total of 15 seconds or was in the open-field for a maximum of 5 minutes the recording was stopped and the animal removed temporarily. One of the objects was then replaced by a second, novel object and the moused placed back in the centre of the opposite wall. Once the mouse explored both objects for a total of 30 seconds or was in the open-field for a maximum of 5 minutes recording was stopped and mouses returned to the home-cage. Further details are described in the corresponding methods and materials sections for Chapter 3 and 4.

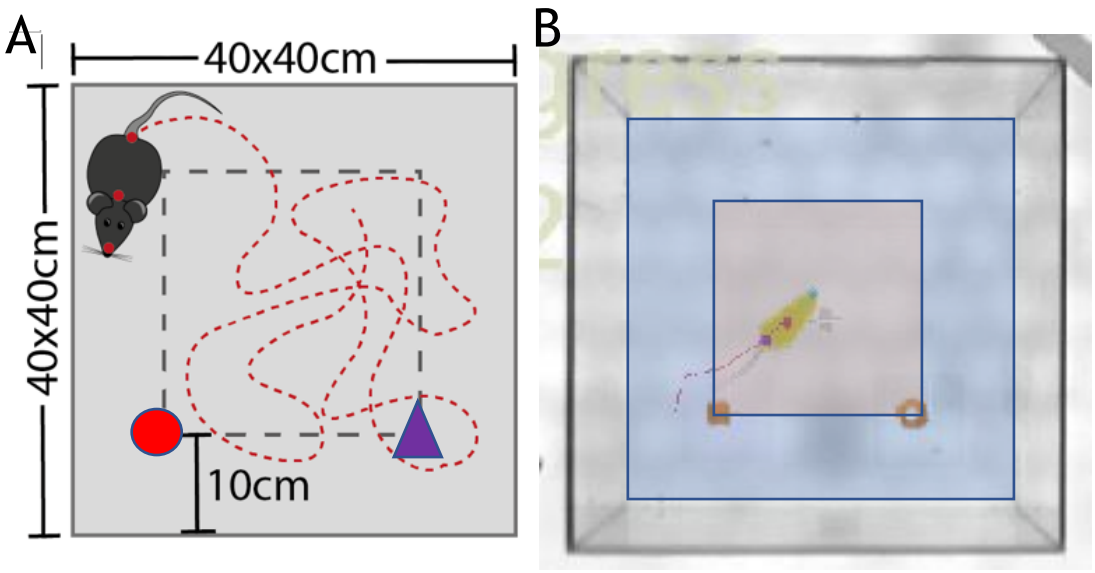

Figure 2.5: Diagram of Novel Object Recognition apparatus. A) diagram of the novel object recognition apparatus with locations of objects highlighted. B) photograph from Noldus Ethovision Infrared camera novel object recognition task equipment.

2.2.5 Paired Discrimination

Animals were trained on a Cambridge instruments Paired discrimination system. All mice were first habituated and trained to use the equipment as indicated in figure 2.8. Once trained animals entered the Acquisition Phase of the experiment where they learned to touch one of two images on the touch screen to receive a Yazoo™ strawberry milkshake reward. Once accuracy reached more than 75% on 3 consecutive days, they entered the Reversal Learning phase where the correct image was the image which was initially correct in the Acquisition phase. Once accuracy was reached the mice were then entered into a second reversal learning phase. On day 3-6 of reversal phases animals received either 0.2-0.3ml of sterile, 0.9% sodium chloride solution (saline) or 2.5mg/kg PCP solution in saline 15 minutes prior to beginning a session. Some mice received saline during reversal phase 1 while some had been given PCP. The rest of the animals received PCP on reversal phase 1 and saline on the second reversal phase to counterbalance treatment with ability in the paired discrimination experiment. Further details are described in the corresponding methods and materials sections for Chapter 3.

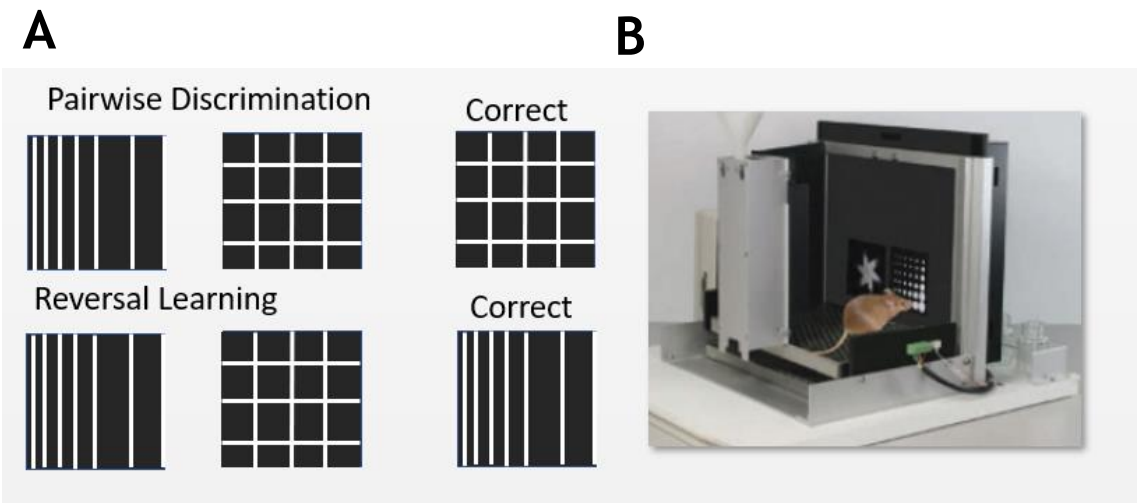

Figure 2.6: Diagram of paired discrimination apparatus. A) Diagram showing images on touch-screen which correlate to pairwise discrimination and reversal learning phases. B) Photograph of paired discrimination apparatus.

Phase	Protocol
Habituation: Day 1	20 minutes in the test box.
Habituation: Day 2	40 minutes in the test box.
Initial Touch Training: Day 3 and 4	60 minutes. Animals rewarded for touching anywhere on the screen. A tone and reward light indicates Yazoo™ Strawberry milkshake has been dispensed. Reward light switches off on nose-poke. 30 trials maximum.
Must Touch Training: Day 5 and 6	60 minutes. Animals rewarded for touching the image on the screen only. A tone and reward light indicates Yazoo™ Strawberry milkshake has been dispensed. Reward light switches off on nose-poke. There is no response to touching the screen where no image is present. 30 trials maximum.
Punish Incorrect: Day 6 and 7	60 minutes. Animals rewarded for touching the image on the screen only. A tone and reward light indicates Yazoo™ Strawberry milkshake has been dispensed. Reward light switches off on nose-poke. If the screen is touched in a location where there is no image the house light is switched on for 10 seconds. 30 trials maximum.
Acquisition Phase: Day 7 - 17	60 minutes. Animals rewarded for touching the “Correct” image on the screen only. A tone and reward light indicates Yazoo™ Strawberry milkshake has been dispensed. Reward light switches off on nose-poke. If the “incorrect” image is touched the house light is switched on for 10 seconds. 30 trials

	maximum. Once animals reach 75% accuracy for 3 consecutive days they qualify to Reversal Learning Phase.
Reversal Learning Phase 1: Day 17-27	60 minutes. The correct and incorrect images from the Acquisition Phase are reversed. Animals rewarded for touching the "Correct" image on the screen only. A tone and reward light indicates Yazoo™ Strawberry milkshake has been dispensed. Reward light switches off on nose-poke. If the "incorrect" image is touched the house light is switched on for 10 seconds. 30 trials maximum.
Reversal Learning Phase 2: Day 27-37	60 minutes. The correct and incorrect images are reversed back to the same combination as in the first Acquisition Phase. Animals rewarded for touching the "Correct" image on the screen only. A tone and reward light indicates Yazoo™ Strawberry milkshake has been dispensed. Reward light switches off on nose-poke. If the "incorrect" image is touched the house light is switched on for 10 seconds. 30 trials maximum.

Table 2-2: Table describing phases of training for pairwise discrimination and reversal learning protocols.

2.3 Biological Experiments

2.3.1 Dissection

Animals were culled by cervical dislocation on completion of experiments. First spleen was removed before removal of the brain. Left and right PFC and hippocampus of the mouse brain was dissected and all samples placed in individual Eppendorf tubes before storage on dry ice. Samples were transferred on dry ice from the University of Strathclyde to the University of Glasgow where they were stored at -80°C.

2.3.2 cDNA Extraction

2.3.2.1 RNA Isolation

Samples were removed from the -80°C freezer and placed on ice. 10µl of ß-mercaptoethanol was added to each 1ml of Buffer RLT with enough solution made for the number of samples. Samples which weighed less than 20mg were placed into Lysis Matrix tubes containing 1.4mm Zirconium Silicate Spheres with 350µl ß-mercaptoethanol and Buffer RLT solution. If the sample weighed between 20mg and 30mg, 600µl ß-mercaptoethanol and Buffer RLT solution was added instead. If the sample weighed more than 30g it was sliced to a smaller size and excess sample stored at -80°C.

Tubes were then placed into a Hybaid Ribolyser for 60 seconds in 20 second increments before being centrifuged. Supernatant was collected and centrifuged again before being added to a 1:1 equivalent volume of 70% ethanol. This was then loaded onto RNeasy minicolumns with a maximum of 700µl loaded at a time. Minicolumns were centrifuged and flowthrough discarded before loading any remaining sample.

Once all samples were loaded onto RNeasy minicolumns, 350µl of buffer RW1 was added and centrifuges with flowthrough discarded. Then 80µl of DNase incubation mix was added and left at room temperature for 15 minutes. A second load of 350µl of buffer RW1 was added and centrifuged with flowthrough discarded. 2 cycles of loading 500µl Buffer RPE and centrifuging before

discarding flowthrough were completed. Finally, 50µl of RNase free water were added to the column and left at room temperature for 5 minutes. After centrifuging the sample of isolated RNA was stored at -80°C or if possible immediately used to synthesize cDNA.

2.3.2.2 cDNA Synthetics from isolated RNA

First the quantity of RNA in each sample was measured using a nanodrop. The volume of sample which contained 0.2µg of RNA was calculated and combined with RNAase free water to bring the total volume to 9µl. This was mixed with 10µl of RT Buffer and 1µl of 20X high capacity enzyme mix. Samples were then placed into a thermocycler for 60 minute at 37°C then 5 minutes at 95°C to deactivate enzymes before being cooled to 4°C until collected. 80µl of nuclease free water was added to dilute the samples before storage at -20°C.

2.3.3 Reverse Transcription Quantitative Polymerase Chain Reaction (RT qPCR)

A master-mix containing; 5µl 2X Fast SYBR Green Master Mix, 0.3µl of the relevant forward and reverse primer, 25µl of NFW and 0.15µl of SYBR Dye was made for the appropriate number of samples. 9µl of this master-mix and 1µl of cDNA was then pipetted into 10µl wells of a 48-well plate before the plate was sealed. The plate was first shaken for 30 seconds before being centrifuged to ensure no bubbles were present in the wells. The RT qPCR was then started with 1 cycle of 2 minutes at 50°C followed by 1 cycle at 95°C for 2 minutes. There were then 40 cycles of 10 seconds at 95°C followed by 30 seconds at 60°C. Once completed the cycle number at which the sample reached the fluorescence threshold of SYBR Green (Ct) is collected and can be directly related to the expression of cDNA. Primer efficiencies were previously calculated within the lab group and determined to be within 90%-110% efficiency.

2.4 Data Analyses and Visualisation

2.4.1 Data Wrangling

Data exported from Noldus Ethovision software or Actual HCA systems in the form of CSV or XLSX files were loaded and wrangled using R version 4.4.1 within RStudio (RStudio Team., 2021; R Core Team, 2023), utilizing the packages tidyverse (Wickham et al., 2019), dplyr (Wickham et al., 2023), and readxl (Wickham & Bryan, 2023) in order to collate data of all mice into a single file as well as organise data by mouse ID and include other relevant grouping information such as treatment, sex, time. Once a file including all relevant information was generated it was exported as a CSV file.

2.4.2 Statistical Analyses

For each experiment a CSV file which had been collated as detailed in section (data wrangling) was imported into MiniTab® version 20.3. Normality was assessed using the Kolomogorov-Smirnov test which showed that data was not-normally distributed. Whilst ANOVA does make an assumption of normality, there is data which suggests ANOVA maintain reliable results in not-normally distributed data particularly in respect to repeated-measures ANOVA (Blanca et al., 2023). As a result, it was decided that the best method to assess variance amongst groups with several independent and dependent variables with repeated measures over time would be through use of multi-factorial repeated-measures ANOVA. Multi-factorial ANOVA highlights significant effects of each individual independent variable on the dependent variable but also shows whether there is significant interactions between independent variables which can explain the change in the dependent variable (Blanca et al., 2023). This was used to assess relationship between multiple independent variables such as treatment, genotype and sex on the dependent variables relevant to each experiment as detailed in Chapter 3, 4 and 5. In addition, nesting was used within the multi-factorial analyses in order to assess effects of other factors which cannot be independent of the variables being tested (Hamilton et al., 1994). In this case, mouse ID or cage number are factors which can influence

the dependent variables since a single animal can alter the mean of the dependent variable or complex social hierarchies in a cage can alter behaviour within one cage. Including these as nested factors within the ANOVA allowed the differences in variance between each group to more accurately represent the variance due to the independent variable rather than due to potentially confounding factors (Hamilton et al,. 1994). For example, effects of genotype on distance travelled will not be attributed incorrectly to the behaviour of a single mouse. At the same time, the use of nesting can therefore account for variance related to outliers. Additionally, there was no biologically relevant reason to remove outliers given the occasional outlier could have been a result of normal variation in the population. In fact, given some groups were potentially underpowered it could not be clear whether an outlier, such as a more hyperactive mouse, was an outlier or this was simply due to normal variance in the population. Overall, the multi-factorial, nested ANOVA provides more accurate measures of significance between independent variables since analyses include not only analysis of variance of an independent variable in a dependent variable, but also potential confounding factors which could influence results as well as interactions between multiple independent variables which cannot be separated.

Once multi-factorial, nested ANOVA was completed, Tukey's Pairwise comparisons was used for post-hoc testing of significant differences of all individual factors. This would allow for comparison between groups compared to controls as well as to determine if there were differences between the groups compared to each other. Tukey's grouping method was then used to clearly show which groups were significantly different from each other. All individual pairwise comparisons can be found in the supplementary materials for each experiment.

2.4.3 Visualisation

Data was visualised through a number of routes which were used to best depict effects identified in statistical analyses. To generate line, interval plots or boxplots, data was loaded into Minitab® version 20.3 and plots generated for

relevant data showing the mean for each group with error bars depicting the standard error of the mean. For generating heatmaps a kernel density estimation was calculated and plotted in R version 4.4.1 using libraries “ggplot2”, “RColorBrewer”, “MASS” and “rasterVis”. Kernel density estimation is a form of bivariate distribution and plots the histogram of one value (x) by the histogram of a second value (y). Areas where the two overlapping histograms have a higher density of data points can then be visualised through a 3D map. Finally, levels of density can be binned and assigned and a colour associated with each density level to depict this bivariate distribution in a 2D format. Each colour therefore indicates the density of points at each (x, y) co-ordinate and can depict areas which show the highest occurrence. Throughout this thesis higher density areas were assigned red and lower density areas were assigned blue to highlight areas on a map which show the highest occurrence of (x, y) co-ordinate. In real terms, this indicates the locations on the map in which mice appeared most often. Images which were generated to depict methodology were drawn and created using Adobe: Illustrator™. In some cases, pre-illustrated images were used through use of BioRender™. Further details for each experiment can be found in the corresponding methods or results section for each chapter.

3 Results: Behavioural profile of a psychopharmacological model of schizophrenia.

3.1 Introduction

Schizophrenia is a psychiatric condition which is most often diagnosed in adulthood as a result of the occurrence of positive symptoms such as delusions and hallucinations (Regier, Kuhl and Kupfer, 2013). However, schizophrenia is also comprised of a series of cognitive (Waltz and Gold, 2007) and negative (Betensky *et al.*, 2008) symptoms which can include anhedonia, avolition, apathy or other secondary negative symptoms such as depression and anxiety (Regier, Kuhl and Kupfer, 2013). Research in animals has focussed on creating useful models which replicate schizophrenic symptoms, in order to further assess the impact on neurological, behavioural and biological changes (Winship *et al.*, 2019). Whilst the dopamine theory of schizophrenia serves as an adequate explanation of presentation of positive symptoms, antipsychotic medications which act as dopamine receptor antagonists only alleviate these positive symptoms with no real effect on negative symptoms (Carpenter, 2012; Howes *et al.*, 2017). On the other hand, the NMDA receptor hypofunction theory has been theorised to result in both the positive and negative symptoms of schizophrenia and post-mortem, brain studies of those diagnosed with schizophrenia has identified transcription of NMDA receptors subunits to be reduced in the prefrontal cortex of those with schizophrenia (Weickert *et al.*, 2013; Nakazawa and Sapkota, 2020), while genetic studies strongly implicate NMDA receptor dysfunction in schizophrenia aetiology (Trubetskoy *et al.*, 2022). As such, phenylcyclohexyl piperidine (PCP, aka phencyclidine) has been used to mimic NMDA receptor antagonism to determine if the negative symptoms of schizophrenia can also be modelled with PCP (Morris, Cochran and Pratt, 2005).

3.1.1 The PCP animal model of schizophrenia

In humans, PCP causes a schizophrenic-like psychosis state and in long-term users can result in PCP induced psychosis very similar to schizophrenia (Allen and Young, 1978). Chronic abusers of PCP have additionally reported anxiety, depression and other affective symptoms alongside psychosis related symptoms. As a result, PCP has often been used in animal models as a method to induce a schizophrenia phenotype in order to examine changes in behaviour and biology (Javitt *et al.*, 2012; Grayson *et al.*, 2016; Moghadam *et al.*, 2021). In rodents, high doses of PCP have shown increases in the early firing in prefrontal cortex. However, interestingly whilst this is initially replicated with lower doses, the low dose PCP additionally shows a delayed response in the PFC of decreased firing which replicates activity seen in humans with schizophrenia (Morris, Cochran and Pratt, 2005). This delayed decrease in activity mimics the prefrontal cortex reduction in activity found in humans with schizophrenia suggesting the low-dose PCP model can replicate the neurobiological changes that occur in schizophrenia (Morris, Cochran and Pratt, 2005). Although it is not truly possible to assess presence of positive symptoms in rodents, behaviourally, PCP has shown decreased cognitive abilities in spatial working memory, attentional set shifting tasks and visual discrimination memory and learning task (Andersen and Pouzet, 2004; Egerton *et al.*, 2005; Talpos *et al.*, 2009, 2014). Additionally, studies have shown, deficits in sociability using novel social interaction tests, anxiety and depression in elevated plus mazes (EPM) and forced-swim tests indicating that PCP can mimic a wide range of the schizophrenia phenotype (Noda *et al.*, 2000; Lee *et al.*, 2005; Turgeon, Anderson and O'Loughlin, 2010; Turgeon *et al.*, 2011). However, these tests are carried out in novel environments after removal from home-cage and handling by researchers. This can result in an increased anxiety level in animals which could elicit artificially altered results simply due to the experimental environment. Alternative, longitudinal methods of behavioural assessment such as home-cage behaviour monitoring could provide a clearer picture of changes in anxiety as well as more detailed information about social behaviours in

rodents as is detailed in Thomson et al., 2021. The study utilises home-cage monitoring to assess these complex behaviours with more nuanced data around behavioural changes in an animal model of psychiatric disorders.

An additional caveat to the use of PCP, is the effect on locomotor activity. Contrasting results show that PCP can cause motor impairment, however some studies indicate hyperactivity while others show motor dysfunction (Toth and Lajtha, 1986; Steinpreis and Salamone, 1993; Redmond *et al.*, 1999) and this could affect the outcome of tests, particularly those which rely on movement such as the forced swim test, spatial memory tasks and even novel sociability paradigms. To mitigate this, some studies utilise a sub-chronic administration of PCP where PCP is given every day for a week or two-weeks in some cases before behavioural experiments begin during the washout phase. Although studies have shown continuous administration of PCP can cause long term effects which imitate the schizophrenia phenotype, this omits the initial phase of PCP activity in which here are clear schizophrenia-like behaviours in humans (Castañé, Santana and Artigas, 2015).

3.1.2 Sex Differences in Schizophrenia

There are notable differences in the presentation of schizophrenia between males and females. There is a higher incidence of diagnosis in men than in woman and it is well established that men are often diagnosed earlier than woman (Li *et al.*, 2016). Although there appears no clear difference in positive symptoms, men in general have a higher rate and severity of negative symptoms (Leung and Chue, 2000), and have an increased social function impairment compared to females (Usall *et al.*, 2002). Conversely, women are more likely to experience affective dysfunction, namely anxiety or depressive symptoms (Castle and Murray, 1993; Leung and Chue, 2000; Sommer *et al.*, 2020). However, there does not appear to be any difference in cognitive abilities between males and females with schizophrenia, although any advantage in cognitive tests found in healthy females appears to be lost in females with schizophrenia (Shipman *et al.*, 2009). These differences are also evident in

treatment response since females with schizophrenia show a better response to antipsychotic medications (Grossman *et al.*, 2006).

Despite these clear differences between the sexes in schizophrenia phenotype, there are a limited number of animal studies investigating sex differences. One study suggested possible behavioural differences to PCP in relation to spatial memory with only male rats showing impairment (Andersen and Pouzet, 2004) and another study suggested that females rats showed decreased anxiety after PCP compared to males (Turgeon, Anderson and O'Loughlin, 2010). Studies have also shown altered cognitive abilities as a result of PCP in attentional set shifting tasks and paired discrimination and reversal learning tasks and (Egerton *et al.*, 2005; Brigman *et al.*, 2009). However, further research must be completed in order to truly determine the different effects PCP may have between the sexes. If PCP can mimic these sex differences found in humans this further solidifies the use of PCP as a model relevant to schizophrenia and strengthens the NMDAR receptor hypofunction theory of schizophrenia.

3.1.3 Aims

Here, I aimed to examine the effect of an acute, low-dose of PCP in male and female mice in various behavioural paradigms and environmental contexts that span a wide repertoire of potential behaviours that can inform our understanding of schizophrenia. These include traditional tests such as open-field tests for assessment of locomotor effects whilst thigmotaxis in the open-field and the EPM were used to assess anxiety related behaviours. We also utilised a novel method of behavioural analysis, home-cage continuous monitoring, which has been shown to provide information about natural cage-mate sociability and longer-term activity patterns (Bristow *et al.*, 2020; Mitchell, Thomson, *et al.*, 2020; Thomson *et al.*, 2021). With comparison to the EPM and open-field this can be used to assess whether changes in anxiety related behaviours are due to increased basal anxiety levels, or if they are rather due to an altered anxiety response to a stressful stimuli in the experimental environment. Finally, I aimed to assess cognitive changes as a result of PCP with an initial investigation using a visual paired discrimination

and reversal learning task, followed by novel object recognition tasks. Following analysis of previous human and rodent research we hypothesise that females will depict increased anxiety related behaviours in response to PCP compared to males but fewer sociability deficits than male mice. We also hypothesise there will be no difference in cognitive abilities between male and female mice but that there will be reduction in cognitive abilities as a result of PCP as depicted in literature of humans with schizophrenia and the currently limited animal research.

3.2 Methods

3.2.1 Animals

All animals were housed in social groups in a temperature-controlled room with light cycle reversed. Lights were switched off at 10am and switched on at 10pm and all experiments were done between the hours of 10am and 6pm. In total, 15 female and 11 males were used and were between 8 and 16 weeks old except a single female who was 46 weeks old. With no indication that behavioural outcomes in this mouse were an outlier there was no reason to exclude this mouse on age alone. Experiments were carried out in 2 groups, the first group included 9 females and 2 males. The 9 females completed the home-cage monitoring, open field and paired discrimination behaviours. The two males completed the open-field test. There was a pause in experiments due to covid 19 lockdown in which period these animals had to be culled. The experiments restarted with a new group of 6 females and 9 males who completed novel object recognition and elevated plus maze tasks. This second group of 9 males also completed home-cage monitoring and open field tests. PCP or vehicle (saline) was administered by the i.p. route in all behavioural tasks. Each mouse completed each behavioural experiment twice, once with PCP and once with vehicle in a within-subjects design. Experiments were carried out at least 1 week apart between saline and PCP injections to account for washing out of PCP effects and there was at least 2 weeks between different behavioural tests to ensure that individual doses did not continue to have effects of an acute dose as this would move to a a sub-chronic model which could occur with repetitive doses in close succession. For each behavioural test, approximately half of the animals were given PCP first with the other half being given saline first with the exception of the home-cage monitoring test which occurred as a single 6 day recording: 2 days of habituation to the room, saline injection on day 3 and PCP injection on day 5.

Experiment Group (Round 1)	**Male WT**	**Female WT**
Open Field	2	9
Paired Discrimination	0	9
Novel Object Recognition	0	0
Elevated Plus Maze	0	0
Home Cage	0	9
Round 1: Total	2	9
Experimental Group (Round 2)	**Male WT**	**Female WT**
Open Field	9	0
Paired Discrimination	0	0
Novel Object Recognition	8	6
Elevated Plus Maze	8	6
Home Cage	9	0
Round 2: Total	9	6
Final Total	11	15

Table 3-1: Table depicting animals used in behavioural tests with PCP. *Experiments occurred over 2 distinct rounds, animals used in each round are indicated for clarity.*

3.2.2 Home-cage behavioural monitoring

Mice were monitored in their home cage for a variety of behaviours such as movement, social interaction and time spent in isolation. To do this, mice were microchipped and placed into Actual Analytics home-cage continuous monitoring equipment and microchips were tracked for movement across a 9x16 grid pattern of antennas as described in section 2.2.1.

Mice were first placed into clear IVC cages in the Actual HCA™ system (Actual Analytics Ltd, UK) and recording was started at 10am. Recording continued for 48 hours to habituate to the home-cage monitoring equipment before being given an injection of 0.2-0.3ml of sterile, 0.9% sodium chloride solution (saline)

at 10am – the beginning of the dark cycle. 48 hours later mice were given an i.p injection of 2.5mg/kg PCP solution (approximately 0.2-0.3ml of 0.025mg/ml stock solution). Recording continued for a further 48 hours.

3.2.3 Open-field Test

To monitor the effect of PCP on locomotor activity, mice were placed into a 40x40cm open-field and their movement tracked using an infra-red camera as described in section 2.2.2. Mice were habituated to the open-field environment for 45 minutes before briefly removing the animal to be injected with either saline vehicle or 2.5mg/kg PCP. Animals were then placed into the open-field and recording commenced and continued for 2 hours. The centre-point of the animal was tracked using Noldus Ethovision Infrared tracking software which also identified placement of the animal in inner and outer zones of the open-field arena. Once completed, the animal repeated this experiment a minimum of 1 week later with the opposite injection of either saline vehicle or 2.4mg/kg PCP.

3.2.4 Elevated Plus Maze

To monitor anxiety behaviours, mice were placed into an EPM 15 to 20 minutes after i.p. injection of either saline vehicle or 2.5mg/kg PCP. Recording lasted 15 minutes and the centre point of the animal was tracked using Noldus Ethovision Infrared tracking software which assessed movement between open and closed arms as well as the centre zone of the EPM. Movement into the outer thirds of the open arm were also assessed. Once completed, the animal repeated this experiment a minimum of 1 week later with the opposite injection of either saline vehicle or 2.4mg/kg PCP.

3.2.5 Novel Object Recognition

Cognitive abilities were measured by assessment of novel object recognition. Mice were first placed in the open-field to habituate 24 hours prior to test day. 15 minutes prior to testing mice received i.p. injection of either saline vehicle or 2.5mg/kg PCP before being placed into an empty open-field for 5 minutes of

recording. Animals were briefly removed from the open-field while two identical objects were placed into the open field in locations indicated in figure 2.7. Once animals had explored both objects for a total time of 15 second or had been in the open-field for 5 minutes they were briefly removed and one of the objects replaced with a novel object. Recording continued for 5 minutes or until both objects had been explored for up to 30 seconds. Once completed, the animal repeated this experiment a minimum of 1 week later with the opposite injection of either saline vehicle or 2.4mg/kg PCP.

3.2.6 Paired Discrimination and Reversal Learning

Animals were trained on a touchscreen paired discrimination task (See methods section 2.2.5). Following acquisiton of the task they entered a reversal learning phase. Once accuracy was reached (>75% on 3 consecutive days) they entered a second reversal phase. On days 3-6 mice received an injection of either saline vehicle or 2.5mg/kg PCP. Reversal Learning then continued until mice qualified to the next reversal learning phase. In the second reversal learning phases mice received the opposite injection to what they had received in the first phase. Mice who received PCP first and those who received saline first was balanced amongst the group. The reversal learning continued until completion.

3.2.7 Data Analyses

Data were analysed using Minitab, and all behavioural experiments were statistically tested using Two-way multi-factorial repeated-measures ANOVAs. The independent variables in these experiments were treatment (two levels; PCP and vehicle), sex (two levels; male and female) and time. Mouse ID and cage number were also included as nested factors within the independent variables of treatment and sex. The dependent variables which were analysed were distance travelled (for open field test, elevated plus maze and home-cage monitoring), time spent in the outer areas of the arena (for thigmotaxis measures in the open-field and home-cage), ratios of time spent in different zones of the EPM, mean distance to the nearest cage mate (during home-cage monitoring) ratio of time spent exploring the novel object (during novel object

recognition) and accuracy during the paired discrimination and reversal learning tasks. Additionally, interactions between the independent variables of sex, treatment and time were also measured as part of the multi-factorial ANOVA. Tukey post-hoc tests were used to determine differences between individual means and significantly different groups are shown through Tukey's method grouping with full lists of pairwise comparisons provided in the supplementary materials. Tukey's method of grouping puts all groups which are not statistically significantly different from one another into a single group which is assigned a letter (e.g group A). Groups which are significantly different from each other are assigned a separate letter (e.g. group B). Some groups can be part of both group A and group B which means there is not significant difference in the means from both groups. However, groups which are only in Group A are significantly different to those in Group B.

3.3 Results

3.3.1 Anxiety related behaviours in the Open-Field

After tracking movement in a 40x40cm open-field, the distance travelled and location of the animal in the home-cage was assessed to determine whether the animal was displaying changes in locomotor activity or thigmotaxis, a behaviour which has been linked to anxiety in mice in the open-field. Two-way multifactorial ANOVAs showed that in the open-field there was no overall effect of PCP (2.5 mg. kg^{-1} i.p.) over time, nor was there a treatment by sex interaction. However female animals were significantly more active in the open-field than male animals ($p < 0.01 \times 10^{-15}$) (Figure 3.1b, Supplementary table 1). The open-field was then divided into an inner and outer zone (Figure 3.1a) to determine whether animals spent more time near the edges of the open-field (thigmotaxis) or the centre. Across all animals there appeared to be no overall effect of PCP ($p = 0.107$) (Figure 3.1d), although there was a significant effect of sex overall ($p = 1.3 \times 10^{-5}$.) and a treatment by sex interaction ($p < 0.01 \times 10^{-15}$). When analysing the sexes separately, females showed a significant increase in thigmotaxic behaviour after PCP ($p = 2.9 \times 10^{-15}$) (Figure 3.1d and f) where males showed a significant decrease in thigmotaxis ($p = 5.5 \times 10^{-15}$) (Figure 3.1c and e).

Comparison	Locomotor Activity		Thigmotaxis	
	F-value	p-value	F-value	p-value
Time	3.10	5.09×10^{-4}	1.12	0.34
Sex	329.3	$< 1 \times 10^{-15}$	19.44	1.3×10^{-5}
Treatment	0.06	0.808	2.61	0.107
Treatment*Sex	1.04	0.309	77,79	$< 1 \times 10^{-15}$

Table 3-2: Results of Multi-factorial, nested ANOVA indicating relevant statistical results.

		Time spent in Outer Zone (s)			Total Distance Travelled (mm)		
Group	N	Mean	Standard Error	Standard Deviation	Mean	Standard Error	Standard Deviation
PCP (Males)	8	8094	295	3237	1878	64	699
PCP (Females)	6	11459	215	2238	3259	200	2080
Saline (Males)	8	9879	405	4657	1711	58	663
Saline (Females)	6	8722	239	2485	3323	97	1003

Table 3-3: Summary data including the mean time spent in the outer zone of the open field (s) as well as the mean distance travelled (mm) for each group. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group (Time Spent in Outer Zone)	Tukey Method Grouping		
Female PCP	A		
Male Saline		B	
Female Saline			C
Male PCP			C

Table 3-4: The Tukey Method Grouping for differences in means of the time spent in the outer-zones of the open field. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

Group (Total Distance Travelled)	Tukey Method Grouping	
Female Saline	A	
Female PCP	A	
Male PCP		B
Male Saline		B

Table 3-5: The Tukey Method Grouping for differences in mean distance travelled in the open field. Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.

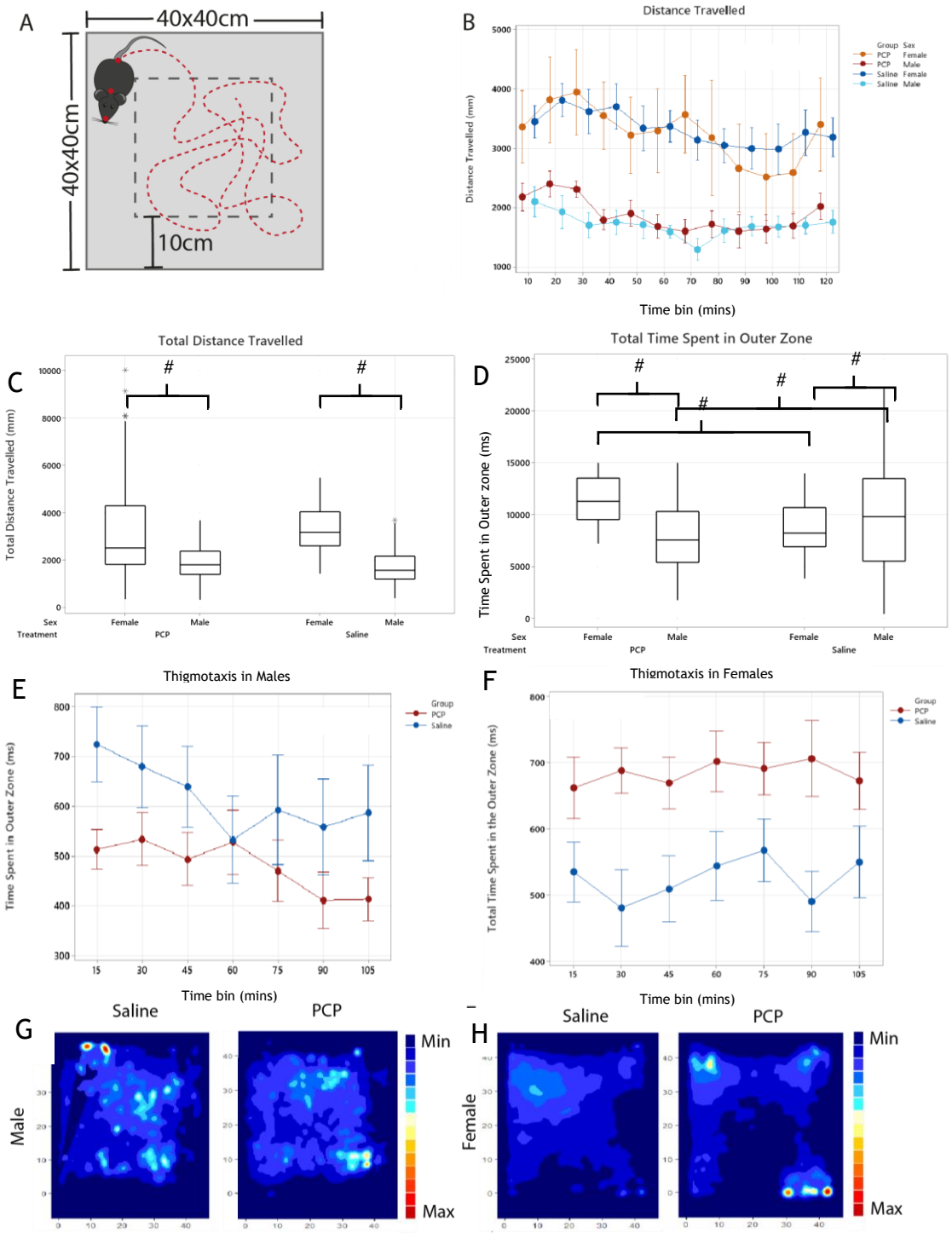

Figure 3.1: Acute, low-dose PCP increases anxiety behaviours in female mice but not males. A) diagram of open-field with inner and outer zones identified. B) Activity of all animals by measure of distance travelled over time and grouped by sex and treatment. C) Activity of all animals by measure of overall distance travelled and grouped by sex and treatment. D) Thigmotaxis of all animals by measure of time spent in outer zones of the open-field and grouped by sex and treatment. E) thigmotaxis in male animals. F) thigmotaxis in female animals over time. G) Kernel-density estimation plot of male animals in the open-field. H) Kernel-density estimation plot of female animals in the open-field. *N = 9 females, 11 males. # indicates significant difference where p < 0.05.*

3.3.2 Anxiety related behaviours in the Elevated-Plus-Maze

Activity of animals was tracked in an EPM where two arms of the maze were enclosed by 15cm high walls, and two arms of the maze had no walls. Movement of the animals into different key zones of the EPM were monitored following acute PCP administration (2.5mg/kg) (Figure 3.2a). A ratio of time spent in the closed arms, open arms and centre zones vs the other regions of the EPM were calculated as an indicator of time spent in different zones and therefore changes in anxiety. Supplementary table 1 details significant results and interactions. The open arm ratio showed no effect of PCP ($p = 0.943$) (Figure 3.2b), and there was no effect of PCP within the individual sexes (females; $p = 0.270$, males; $p = 0.580$). However, females in general spent significantly less time in the open arms than the males ($p = 6.45 \times 10^{-5}$) (Fig 3.2f).

There was a significant overall effect of drug on time spent in the centre zone ($p = 1.66 \times 10^{-4}$) with males ($p = 0.015$) and females ($p = 9.03 \times 10^{-5}$) spending less time in the centre zone following administration of PCP (Figure 3.2c). There was also an overall effect of time ($p = 2.19 \times 10^{-10}$) with time spent in the centre zone decreasing over time.

There was seemingly no overall effect of drug on time spent in the closed arms ($p = 0.765$) although there was an overall effect of time ($p = 6.96$ x 10-8) (Figure 3.2d). However, separating the sexes showed a significantly increased time spent in the closed arms in females after PCP ($p = 6.13 \times 10^{-4}$) (Figure 3.2e) while males showed a significant decrease in time spent in the closed arms compared to females ($p = 0.015$) (Figure 3.2e).

There was a significant overall effect of treatment ($p = 0.003$) and time ($p =$ 9.73 x 10-7) (Fig 3.2d) in the closed arm ratio, with the ratio of time spent in the closed arm increasing over time. There were also clear sex differences and the response to treatment was affected by sex (treatment x sex interaction $p = 0.014$). When analysing the sexes separately, there was a significant increase in the ratio of time spent in the closed arms after PCP ($p = 6.13$ x 10-4) (Fig 3.2e). Whilst males did not show an altered response to PCP, they

showed a significant decrease in time spent overall in the closed arms compared to females (p=0.015) (Fig 3.2e).

Comparison	Time Spent in Open Arms of EPM	
	F-Value	P-Value
Time	0.951	0.500
Sex	27.95	2.07×10^{-7}
Treatment	0.005	0.943
Sex*Treatment	1.59	0.208

Table 3-6: Results of Multi-factorial, nested ANOVA indicating relevant statistical results.

Group	N	Mean (Open-Arm Ratio)	Standard Deviation (Open-Arm Ratio)
PCP (Males)	8	0.09	0.12
PCP (Females)	6	0.03	0.06
Saline (Males)	8	0.08	0.10
Saline (Females)	6	0.04	0.08

Table 3-7: Summary data including the mean time spent in the open arm of the EPM for each group. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Time Spent in the Open Arms of the EPM		
Group	Tukey Method Grouping	
Male PCP	A	
Male Saline	A	
Female Saline		B
Female PCP		B

Table 3-8: The Tukey Method Grouping for differences in ratio of time spent in the open arms of the EPM. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another*.

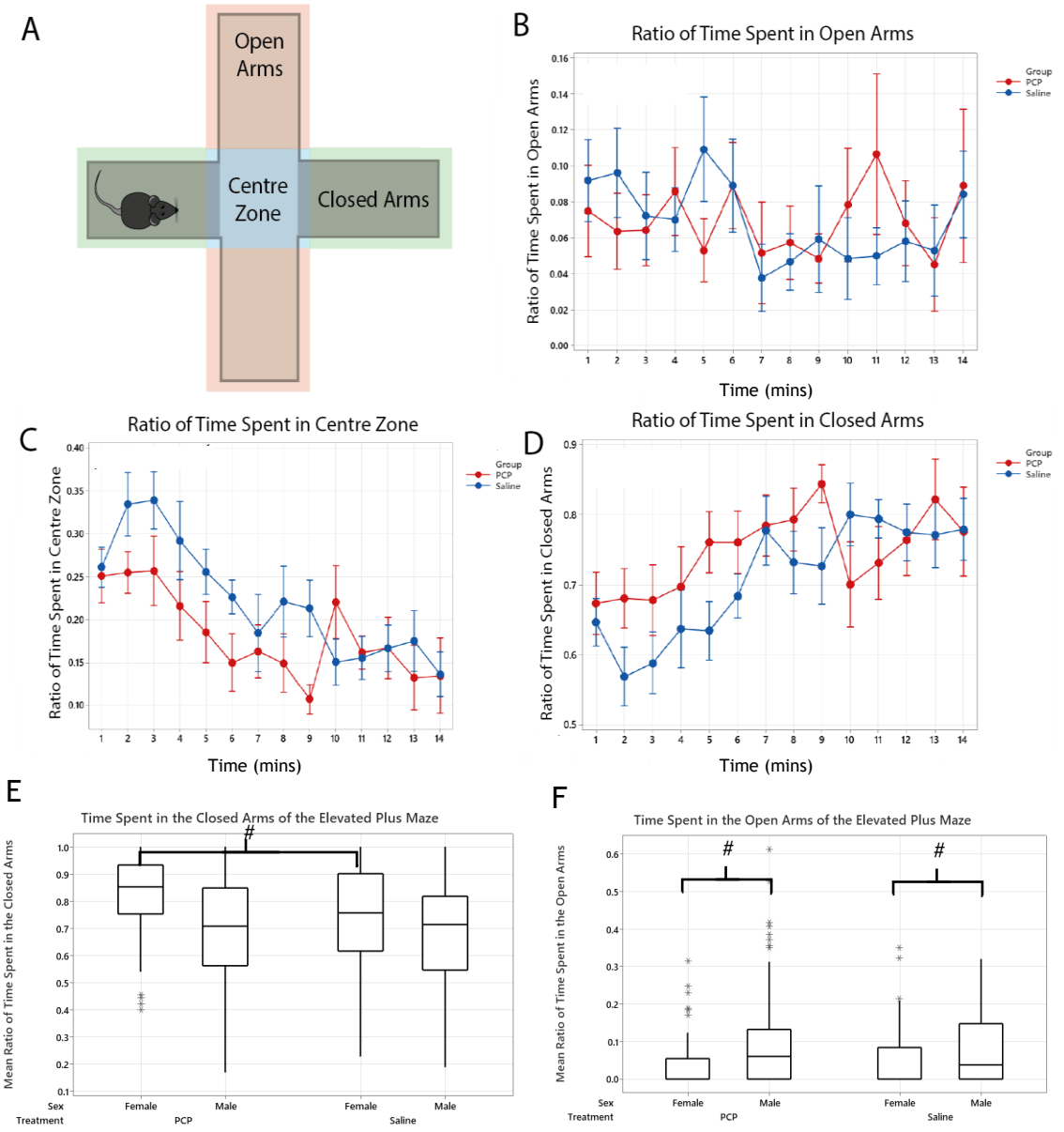

Figure 3.2: Acute, low-dose PCP increases anxiety related behaviours in females but not males with females spending more time in closed arms of the EPM following i.p PCP. A) diagram of the EPM. B) Ratio of time spent in the open-arms of the EPM over 15 minutes. C) Ratio of time spent in the centre zone of the EPM across 15 minutes. D) ratio of time spent in the closed-arms of the EPM over 15 minutes. E) Mean ratio of time spent in the EPM. F) Mean ratio of time spent in the open-arms of the EPM. *N = 9 females, 11 males. # indicates significant difference where p < 0.05.*

3.3.3 Home-cage behaviours

3.3.3.1 Locomotor Activity

Location of the mice were tracked every 15ms for 48 hours after either saline or PCP injection (2.5mg/kg) in a within-subjects design to check for changes in locomotor activity, as well as location in the cage to identify thigmotaxis behaviour (Figure 3.3a). Supplementary table 1 shows results of two-way nested ANOVA.

There were significant overall effects of sex on distance travelled in group housed mice in the home cage ($p < 0.01 \times 10^{-15}$). Although there was no overall effect of PCP on locomotion in the first 12 hours after injection ($p = 0.990$), there was an initial increase in activity in the first hour post-injection ($p = 1.86 \times 10^{-4}$) before dropping to a significantly decreased activity by the fourth hour post injection ($p = 0.009$). This was primarily driven by the female mice with male mice showing no increase in activity immediately after PCP (Figure 3.3c, 3.3d).

Thigmotaxis was also measured in the home-cage and showed an overall effect of PCP ($p = 0.004$). This was primarily driven by the males who showed a significant increase in thigmotaxis in the 12 hours after injection ($p = 8.32 \times 10^{-6}$) (Figure 3.3e), while females showed no overall effect of group on thigmotaxis ($p = 0.411$) (Figure 3.3f). The effect of PCP in the males was evident 1-2h and 7-9h after PCP administration (figure 3.3d).

Comparison	Locomotor Activity	
	F-Value	P-Value
Time	2.75	$<1 \times 10^{-16}$
Sex	380.7	$<1 \times 10^{-16}$
Treatment	2.07	0.149
Sex*Treatment	0.66	$<1 \times 10^{-16}$

Table 3-9: Results of Multi-factorial, nested ANOVA indicating relevant statistical results.

Mean Distance Travelled in the Home-cage (m)				
Group	N	Mean (Distance	Standard Error	Standard Deviation
PCP (Males)	9	251	10	417
PCP (Females)	9	554	20.3	844
Saline (Males)	9	269	11	471
Saline (Females)	9	593	23.6	981

Table 3-10: Summary data of the distance travelled (m) in the home-cage. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group	Tukey Method Grouping	
Female Saline	A	
Female PCP	A	
Male Saline		B
Male PCP		B

Table 3-11: The Tukey Method Grouping for differences in locomotor activity in the home cage. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

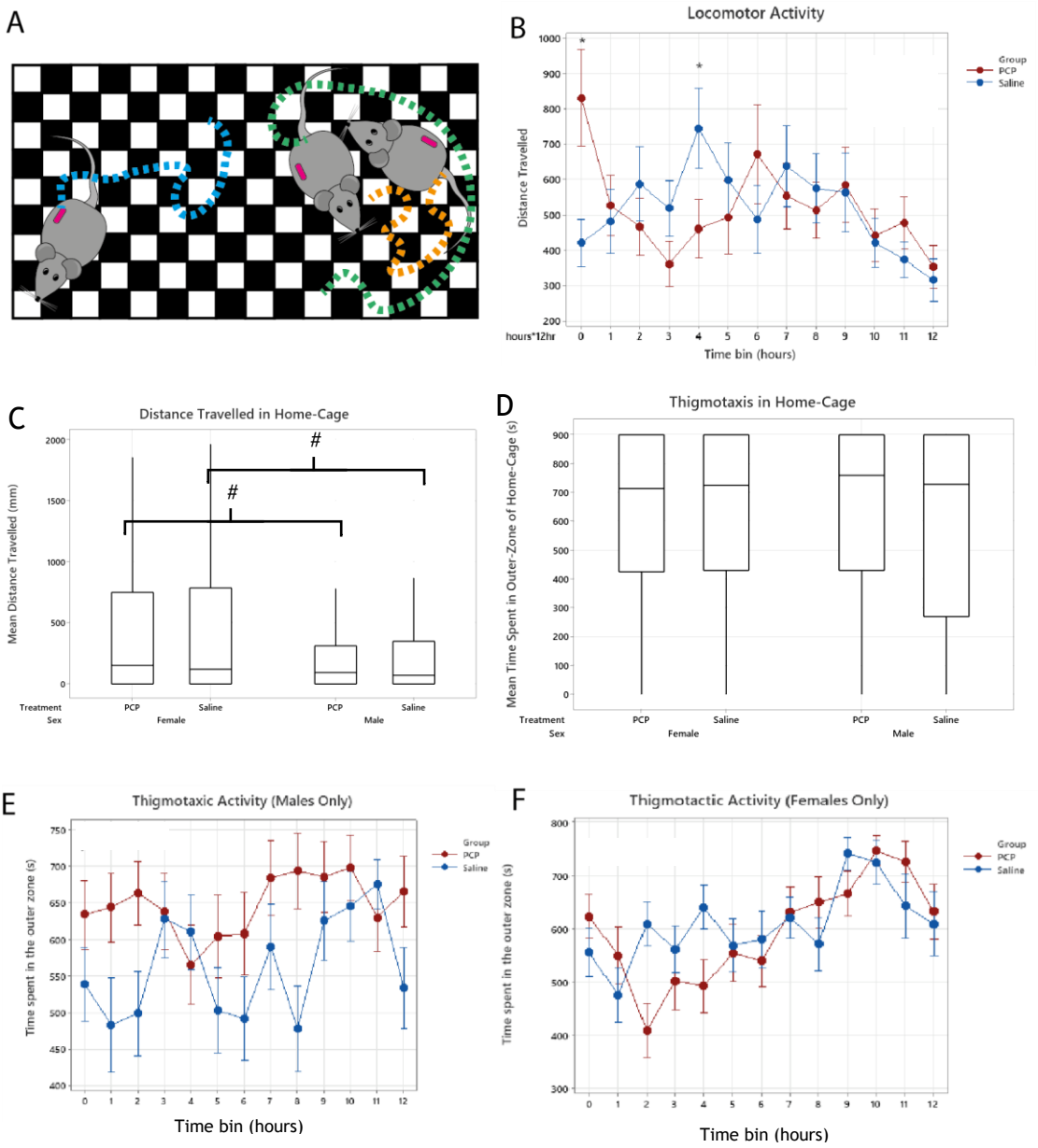

Figure 3.3: Acute low-dose PCP alters locomotor activity in the home-cage and increases thigmotaxis in male animals but not females. A) Diagram of locomotion tracking of micro-chipped animals on RFID tracking system in the home-cage. B). C) Mean locomotor activity in the home-cage over the first 12 hour period after injection D) Mean thigmotaxis behaviour in the home-cage over the first 12 hour period after injection. E) Thigmotaxis in the home-cage in male animals. F) Thigmotaxis in the home-cage in female animals. *N = 9 females, 8 males. P-values calculated by Two-way Nested ANOVA with Tukey's Pairwise multiple comparisons. # indicates significant difference where p < 0.05.*

3.3.3.2 Separation

Social behaviours were also measured by tracking location of one mouse in relation to its nearest cage-mate. Separation was defined as the distance to the nearest animal to determine levels of interactions between cage-mates (Figure 3.4a). Supplementary table 1 shows detailed results from two- way, nested ANOVA.

As with distance travelled, there was an effect of sex overall on separation behaviour ($p = 4.90 \times 10^{-7}$). Separation was generally greater in males than in females. Although there was no overall effect of acute PCP treatment in the 12 hours post injection ($p = 0.106$), there was an initial increase in separation in the first hour ($p = 0.002$) before an eventual decrease in separation 7 and 8 hours after injection (hour 7; $p = 0.037$, hour 8; $p = 1.67 \times 10^{-4}$). The early increase in separation was primarily driven by the male animals who show an overall increase in separation during the first 2 hours after injection ($p = 0.005$) (Figure 3.4d), while females have no overall difference in the first 2 hours ($p = 0.574$) (Figure 3.4c). In the third quarter of the 12-hour dark-phase following injection both males and females show a decrease in separation (females; $p = 0.001$, males; $p = 0.002$) (Figure 3.4e and f).

Comparisons	Distance to the nearest cage-mate.	
	F-Value	P-Value
Time	2.16	$<1x10^{-16}$
Sex	21.58	$3.45x10^{-6}$
Treatment	0.63	0.426
Sex*Treatment	1.56	0.212

Table 3-12: Results of Multi-factorial, nested ANOVA indicating relevant statistical results.

Distance to the nearest cage-mate (mm)				
Group	N	Mean	Standard Error	Standard Deviation
PCP (Males)	9	55	0.999	41.1
PCP (Females)	9	51	0.624	26
Saline (Males)	9	55	0.976	40.6
Saline (Females)	9	52	0.721	30

Table 3-13: Summary data of the distance to the nearest cage-mate in the home-cage. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group	Tukey Method Grouping		
Male PCP	A		
Male Saline	A	B	
Female Saline		B	C
Female PCP			C

Table 3-14: The Tukey Method Grouping for differences in distance to the nearest cage-mate in the home-cage. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another*.

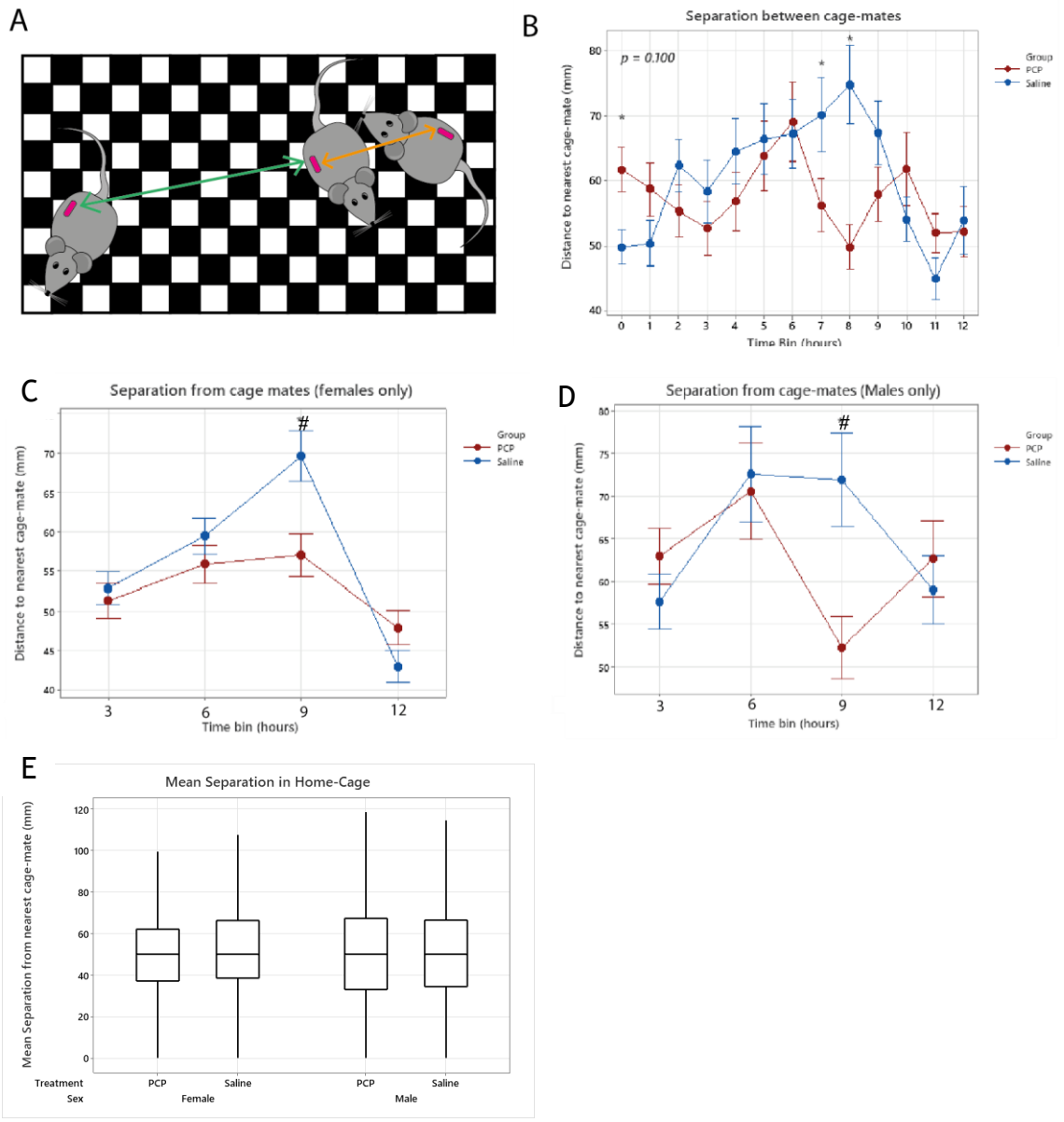

Figure 3.4: Acute, low-dose PCP alters sociability behaviours in the home-cage. A) Diagram of separation trackingon RFID tracking system. B) Separation from nearest cage-mate in the 12 hours after injection, beginning at the start of the dark phase. C) Separation from the nearest cage-mate in the 12-hours after injection in 3 hour time-bins for female animals. D) Separation from the nearest cage-mate in the 12-hours after injection in 3 hour time-bins for male animals. E) Mean separation from the nearest cage mate over the whole 12 hour period. *N = 9 females, 8 males. P-values calculated by Two-way Nested ANOVA with Tukey's Pairwise multiple comparisons. # indicates significant difference where p < 0.05.*

3.3.4 Novel object recognition

Novel object recognition ability was tested using the paradigm set out in Figure 14a, which included an object familiarisation phase before testing novel object recognition in animals injected with PCP or saline. See Methods section 3.2.5 for further details and objects used in the test. There was no overall effect of PCP in the novel object exploration index, defined as the time spent with the novel object ($p = 0.182$), subtracted from the time spent with the familiar object and divided by the total exploration time (Figure 3.5c). Additionally, there was no overall effect of sex on the novel object index (0.639) (Figure 3.5e).

However, there was a clear overall decrease in total exploration time following PCP compared to saline ($p = 0.003$) indicating that the innate drive for object exploration is decreased (Figure 3.5b). There was also a sex specific effect on total exploration time ($p = 0.009$) with a group and sex interaction ($p = 0.012$). This was primarily driven by the male animals showing a decrease in exploration time after administration of PCP compared to saline with no difference between drug group in the female animals (Figure 3.5d). In fact, male mice who had been injected with saline spent significantly more time exploring the objects than any other groups.

Comparison	Ratio of time spent exploring novel object	
	F-Value	P-Value
Sex	0.23	0.639
Treatment	1.9	0.182
Sex*Treatment	0.01	0.906

Table 3-15: **Results of Multi-factorial, nested ANOVA indicating relevant statistical results.**

Novel Object Index				
Group	N	Mean	Standard Error	Standard Deviation
PCP (Males)	8	0.250	0.109	0.326
PCP (Females)	6	0.202	0.214	0.479
Saline (Males)	8	0.081	0.091	0.186
Saline (Females)	6	0.0008	0.093	0.186

Table 3-16: **Summary data of the Novel Object Index.** *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group	Tukey Method Grouping	
Male PCP	A	
Female PCP	A	
Male Saline	A	
Female Saline	A	

Table 3-17: The Tukey Method Grouping for the Novel Object Index. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

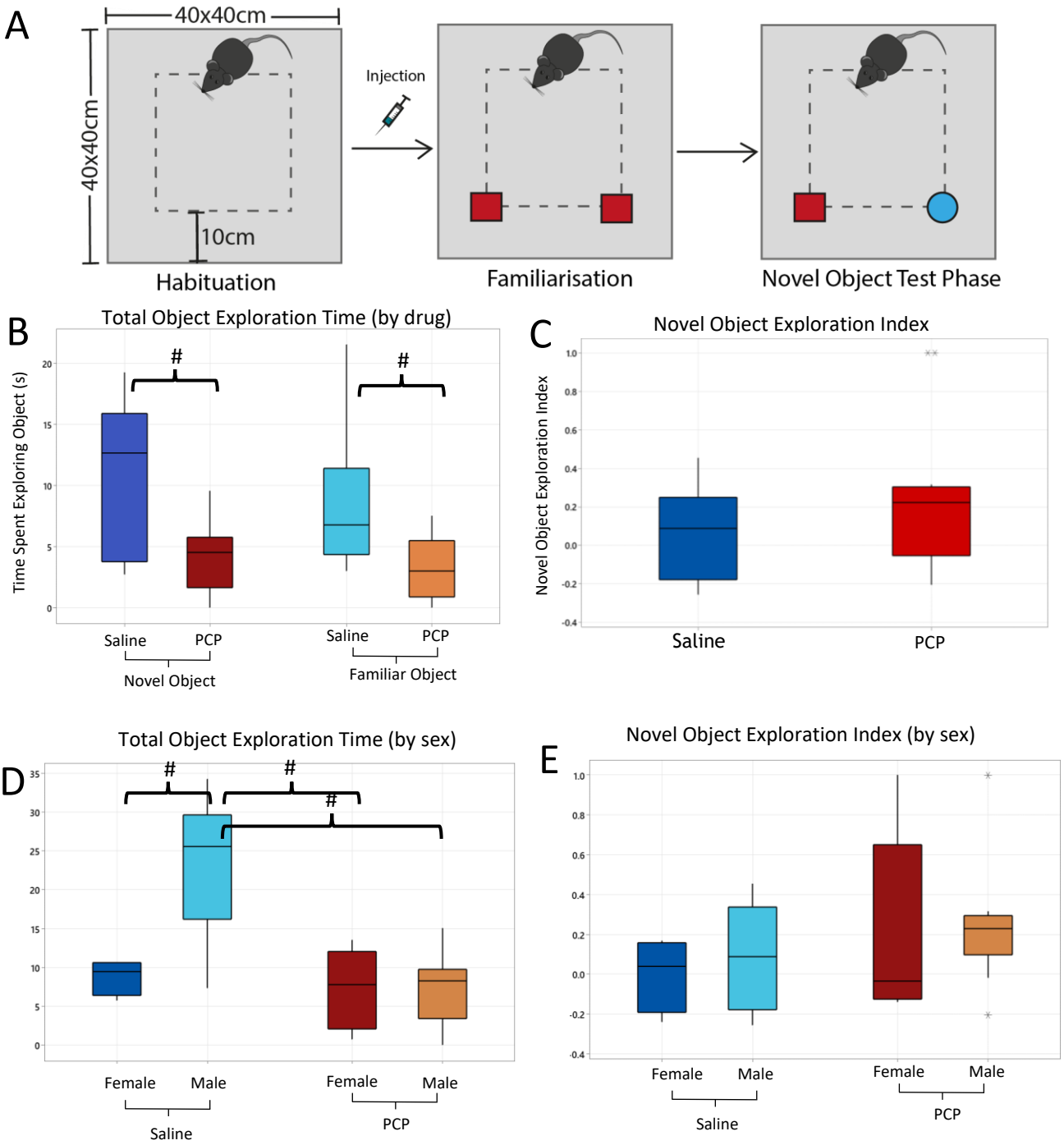

Figure 3.5: Novel Object Recognition. A) Phases of Novel Object Recognition tests. B) Total exploration time of novel and familiar objects within each group. C) Novel object exploration index (novel - familiar) / total exploration time. D) Total object exploration after injection of PCP or saline for male and female animals. E) Novel object exploration index after injection of PCP or saline for male or female animals. *N = 9 females, 11 males. P-values calculated by Two-way Nested ANOVA with Tukey's Pairwise multiple comparisons. # indicates significant difference where p < 0.05.*

3.3.5 Paired Discrimination and Reversal Learning

The stages in the paired discrimination task and reversal learning are indicated in Figure 2.8 and explained in detail in methods section 2.2.5. Mean accuracy for each session across all mice was calculated to estimate change in learning and memory over time. Full analysis of two-way nested ANOVA and Tukey's post hoc multiple comparisons are indicated in Supplementary table 2. Analysis of acquisition phases in paired discrimination showed a steady increase in accuracy over time as indicated by an overall effect of time ($p < 0.01x10^{-15}$) (Figure 3.6b). Visual inspection of the data suggests that acquisition of the task developed around session 10 of training. Since there was no effect of the sequence of reversal phases ($p = 0.823$) indicating that there was no difference between injection of PCP (2.5mg/kg) in the first reversal phase and receiving PCP in the second reversal phase, these data were combined to fulfil an adequate sample size for the group.

There was a significant effect of time during the reversal phases ($p < 0.01x10^{-15}$) suggesting a successful reversal learning period whereby accuracy dropped after reversal before steadily increasing over the course of the sessions. However, there was no effect of treatment group overall ($p = 0.819$) and there were no individual effects of PCP vs saline for sessions which were preceded by injection (Supplementary table 2) indicating no effect of PCP on learning. There was no analysis of sex in this experiment due to equipment breakdown which forcefully ended this experiment after only 7 female mice completed the task.

Comparisons	Accuracy	
	F-Value	P-Value
Time	21.37	$<1x10^{-16}$
Treatment	0.05	0.819
Reversal Learning Order	0.05	0.823

Table 3-18: Results of Multi-factorial, nested ANOVA indicating relevant statistical results.

Accuracy in Paired Discrimination (%)				
Group	N	Mean (Accuracy %)	Standard Error (Accuracy %)	Standard Deviation (Accuracy %)
PCP	9	61	2.3	20
Saline	9	61	2.5	22

Table 3-19: Summary data of Accuracy in the Paired Discrimination and reversal learning test. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group	Tukey Method Grouping	
PCP	A	
Saline	A	

Table 3-20: The Tukey Method Grouping for accuracy in the paired discrimination and reversal learning task. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

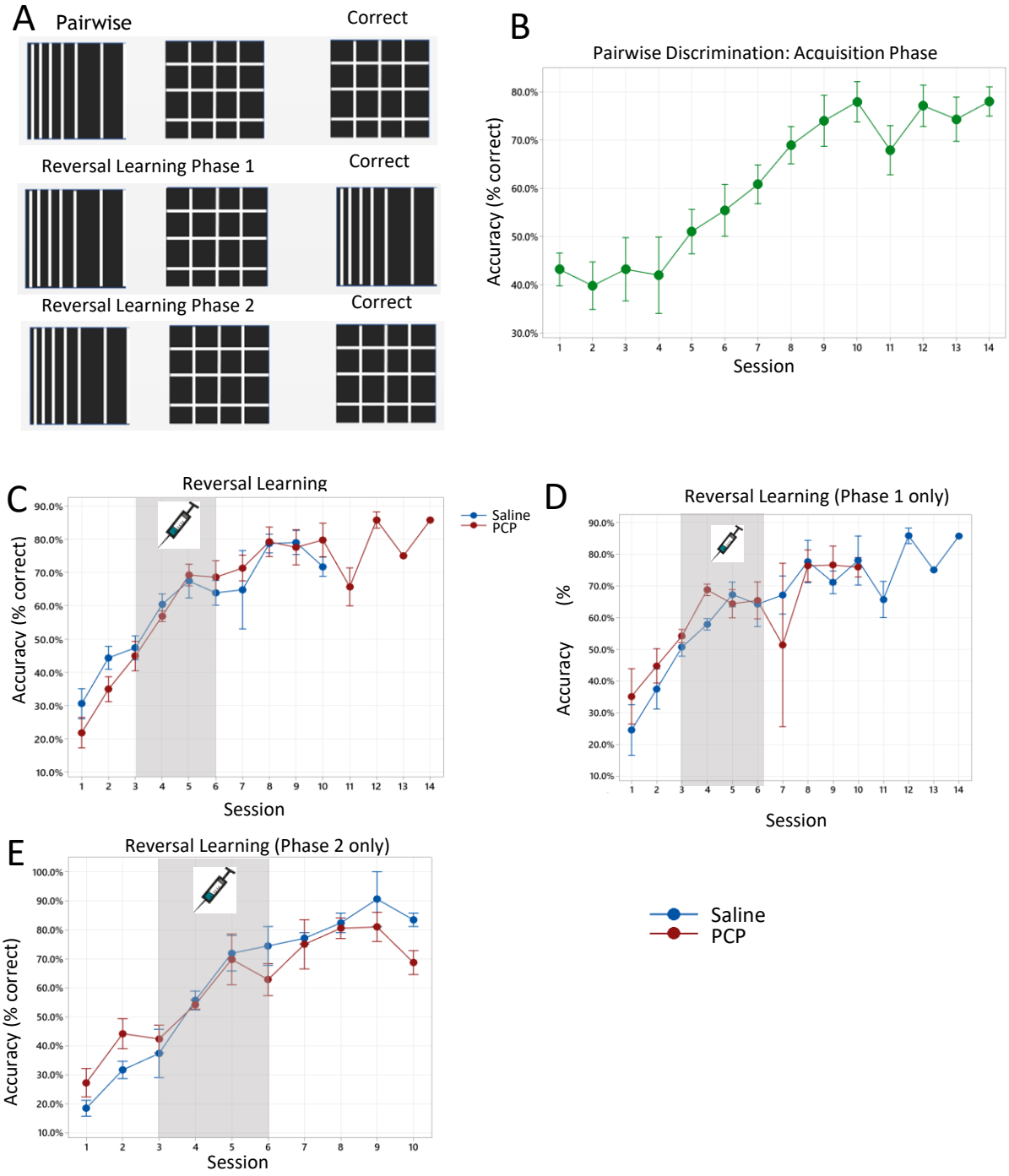

Figure 3.6: Paired Discrimination and Reversal Learning following PCP or Saline. A) Stages in behavioural paradigm. B) Accuracy of visual discrimination throughout the acquisition phase of the experiment. C) Accuracy of visual discrimination during the totalled reversal learning phase of the experiment with PCP or Saline injection 10 minutes prior to sessions 3-6. D) Accuracy of visual discrimination during the reversal learning phase of the experiment with PCP or Saline injection 10 minutes prior to sessions 3-6 of only reversal phase 1. E) Accuracy of visual discrimination during the reversal learning phase of the experiment with PCP or Saline injection 10 minutes prior to sessions 3-6 of only reversal phase 2. *N = 7 females. P-values calculated by Two-way Nested ANOVA with Tukey's Pairwise multiple comparisons. # indicates significant difference where p < 0.05.*

3.4 Discussion

Here, a series of behavioural tests were used to explore whether the sex differences that are apparent in schizophrenia (Leung and Chue, 2000) are also evident in the acute response to NMDA receptor antagonism in mice. Novel environmental contexts were used to measure sociability and locomotor activity over an extended period of time while more commonly used behavioural tests were used to measure anxiety behaviours. In addition, two cognitive tests were employed to assess the effects of PCP upon recognition memory using the widely used novel object recognition task and on learning and memory using a touchscreen task.

The main findings were firstly that, female, but not male mice exhibited increased anxiety related behaviours following PCP treatment in the EPM and an open field (thigmotaxis). By contrast PCP induced anxiety-relevant behaviours in a home-cage environment in male but not female mice. This double dissociation supports the view that environmental context is an important factor in determining drug-effects relevant to anxiety. Furthermore, PCP increased social withdrawal in male animals but not female animals replicating data seen in humans which indicate men with schizophrenia are more likely to exhibit social withdrawal.

3.4.1 Anxiety related behaviours

Anxiety is a core component of the negative symptoms of schizophrenia (Dickinson et al, 2018; Achim et al, 2011), contributing to the distress that those with schizophrenia experience. Interestingly, anxiety is also observed alongside the onset of psychosis in NMDA receptor encephalitis (S. Kayser and Dalmau, 2011; Ryan *et al.*, 2013; Vahter *et al.*, 2014; Espinola-Nadurille *et al.*, 2023), and long-term chronic PCP users show an increased incidence of anxiety (de Angelis and Goldstein, 1978), implying a close link between this symptom domain and NMDA receptor hypofunction. Various techniques were used to assess anxiety related behaviours in this study. The EPM has been used extensively in rodent models as a measure of anxiety since it exploits the innate

behaviour of rodents to remain within dark, enclosed areas (Walf and Frye, 2007). We found that female mice spent less time in the open arms than males, although there was no overt effect of PCP. However, across all animals the time spent in the open-arms was minimal. In fact, mice primarily stayed within the enclosed arms, or would venture to the centre zone and peer into the open-arm without fully exploring. Both males and females in general spent less time in the centre-zone following PCP administration which could indicate that PCP might increase anxiety behaviours across both sexes if this signified decreased exploratory behaviours. However, when looking only at time spent in the closed arms, males spent less time in these zones while females spent more time in the closed arms following PCP, suggesting that female mice were more anxious following PCP treatment than male mice. This indicates that although males also spent less time in the centre zone, they were likely exploring the open-arms instead suggesting reduced anxiety in the EPM following PCP. At the same time, females spent less time in the open arm and centre zone and more time in the closed arms following PCP contrasting to the results found in the males, suggesting an increase in anxiety related behaviour as a result of PCP.

These data are in apparent contrast to other studies (Turgeon, Anderson and O'Loughlin, 2010; Turgeon *et al.*, 2011), who reported a slight anxiogenic effect of PCP (15 mg/kg) in male rats, and a slight anxiolytic effect in females. However, this is clearly a much higher dose with much more overt locomotor effects. Nevertheless, since the EPM only records a short 15 minute period of time and the experimental environment likely affects the response, a second anxiety-related behaviour was analysed under two different conditions for an extended period of time. Thigmotaxis, the rodent preference of remaining close to the walls of area has been suggested to be another measure of anxiety (Treit and Fundytus, 1988; Simon, Dupuis and Costentin, 1994). Indeed, female rats show lower levels of anxiety than males (but higher levels of locomotor activity) in both open field thigmotaxis and in the various EPM measures (Knight *et al.*, 2022). After 2 hours in the open-field, females in general were more active than males although there was no effect of PCP on locomotor activity. However, there was a notable effect on thigmotaxis. Following injection of PCP,

thigmotaxis in males decreased while the opposite occurred in females. This reflects the results of the EPM, suggesting that PCP reduces anxiety in males with an increase in anxiety in females. With a longer period of time recorded where the animals are able to habituate to the open-field, this result strengthens the EPM finding. Additionally, the importance of analysing sex differences in behavioural experiments particularly since these results mimic the sex differences of schizophrenia in humans which show females with schizophrenia having higher incidence of anxiety co-morbidity.

Sex-dependent effects on thigmotaxic behaviour in mice are not well-characterised. A report using rats suggested that increased thigmotaxis in the open field following (low dose) MK801 administration was detected in males but not in females (Feinstein and Kritzer, 2013), although here the females showed slightly higher basal thigmotaxis. Furthermore, whilst animals were habituated to the environment in this case it would be pertinent to measure thigmotaxic behaviour during habituation as habituation to stress factor may operate differently to an animal previously habituated to the open-field. A low dose of ketamine reportedly produces equivalent thigmotaxic effects in male and female mice (Tarrés-Gatius *et al.*, 2020).

However, the open-field is still an artificially created environment which isolate animals from their natural setting. As a result, we also assessed thigmotaxis in the home-cage to determine whether a similar effect is seen in the natural environment in mice housed in groups of three. There were some clearly contrasting results to the open-field and EPM. In the home-cage, males showed an increase in thigmotaxis following PCP administration where females showed no overall difference in the 12 hours after injection. This is the opposite to what was found in the open-field and would imply increased anxiety in males and no change in females. However, it is worth considering what thigmotaxis behaviour could mean in the home-cage in the presence of other cage-mates. The element of stress which could be induced by artificial experimental environments and removal from cage-mates has been reduced. As a result, the potential for a fearful response which might result in searching for protection

near walls or under shelter is no longer present. This highlights the need to consider the experimental parameters when interpreting results from widely used behavioural tests such as the open-field and EPM. Do these tests truly assess anxiety or do they assess behavioural response to environmental stressors? The latter is still worth consideration for studying schizophrenia since humans with schizophrenia show impaired ability to adapt to stressful stimuli (Gispen-De Wied, 2000). However, these tests may not represent the baseline levels of anxiety that are seen in those with schizophrenia as has been suggested.

3.4.2 Sociability-related behaviours

Assessing behaviours in the home-cage with established cage-mates can allow us to assess changes in other behaviours under more natural circumstances, more accurately depicting effects of pharmacological interventions by excluding confounding effects of test environments. Peters and colleagues (2017) found that repeated PCP administration caused a reduction in social behaviours in (male) rats (Peters *et al.*, 2017). Here we looked at separation behaviours between cage mates as a measure of sociability in the animals. In mice containing the 16p11.2 duplication, a genetic mutation found to carry high risk for development of schizophrenia, separation was found to increase in mice containing this genetic variant indicating that decreased sociability can be identified as a phenotype of genetic variants carrying risk of schizophrenia (Bristow *et al.*, 2020). Mice in established colonies display different social proximity to cage-mates throughout the light cycle. When sleeping, animals tend to nest together while during the dark phase there is an increase in separation while animals explore, eat, drink or nest. However, here this pattern of behaviour was altered as a result of PCP injection. In the two hours post-injection, male separation from cage-mates increased while female separation did not. This suggests decreased sociability as a result of PCP only in males, an effect that mimics males with schizophrenia who display increased difficulties with social interaction, lower social functioning and lower improvement in sociability following treatment than in females. Additionally, it is possible this

indicates a secondary anxiety-like response which results in avoidance of cage-mates. Since male rodents have stronger hierarchical relationships in colonies than female mice it is possible changes in anxiety are more likely to affect sociability in males than females. When comparing this with the early increased thigmotaxis in the males in the home-cage this could be explained by this social avoidance with animals distancing themselves from each other more, resulting in their proximity to the edges of the cage. Mitchell et al also reported a loss of sociability immediately after PCP administration in male rats (Mitchell, Brett, *et al.*, 2020).

There is strong evidence that there are sex differences in pharmacokinetics of PCP in rats, with a substantially longer half-life in females (Nabeshima *et al.*, 1984; Shelnutt, Gunnell and Owens, 1999), and the pharmacokinetics in mice are very similar to rats (Slawson, Wilkins and Rollins, 1998). Hence a general trend towards larger effects of PCP in females is probably to be expected. That said, we observe more obvious effects of PCP on sociability in males, suggesting that males are particularly sensitive to NMDA receptor hypofunction in this behavioural domain.

On the other hand, 7-9 hours after injection there was a secondary effect of PCP in which both males and females decreased their separation behaviours showing an increased proximity to cage-mates. Since there was no effect of PCP on locomotor activity at this time point, we can assume this was not due to sleeping behaviours or altered sleeping patterns and thus is likely to be due to an increase in social interactions between waking animals. In fact, the main effects of acute PCP have previously been suggested to be within the first 4 hours after injection, but this highlights a potential after-effect of PCP which should be further investigated. It is not possible to determine if this increased sociability is related to altered anxiety behaviours as this could represent both increased and decreased anxiety levels. Either animals are seeking comfort in their cage-mates or anxiety levels have reduced and sociability has increased as a result. Overall, there is a clear extended effect of PCP on social interaction which continues well after other effects have disappeared. This is potentially

due to active metabolites which remain for an extended period of time, however the pharmacokinetic profile suggest this is unlikely. As a result, it is most likely to be due to a compensatory neuronal effect following the wash out of PCP.

3.4.3 Locomotor Activity

Furthermore, PCP has well-characterised acute stimulatory effects on locomotor activity, with agreement that doses above 5mg/kg induce hyperactivity a (Toth and Lajtha, 1986; Gleason and Shannon, 1997; Redmond *et al.*, 1999). This is important to consider when interpreting results of behavioural experiments such as the open-field which rely on locomotion to display behavioural traits such as anxiety. The low-dose of PCP (2.5 mg/kg) was chosen in an effort to avoid this effect, and in the open-field there was no effect on locomotor activity. This is consistent with previous findings that doses of PCP of <5mg/kg produce little if any detectable effects on locomotion (Daenen, Wolterink and Van Ree, 2003; Robinson and Riedel, 2014; Vitucci *et al.*, 2016). However, there were indications of hyperactivity in the first hour after injection in the home-cage which was primarily driven by the female mice. It is possible that in the open-field this behaviour was masked in the females, where they had overall higher activity than the males, likely as a result of increased stress-related behaviours which caused an increased in activity even on saline days. This once again highlights the importance of considering the experimental context as a factor in anxiety. It is interesting that there was also a suppression of locomotion 4-5h after PCP. A similar delayed suppression of locomotion after low-dose PCP has been observed in rats, also with group home-caged monitoring (Mitchell, Brett, *et al.*, 2020). The pharmacokinetics of PCP are complex, but there is a proportion of the dose present in the brain (in rodents) up to 15h after administration (Misra, Pontani and Bartolomeo, 1980). Such delayed effects may contribute to the long-lasting CNS changes that occur with repeated PCP administration (Cochran *et al.*, 2003; Morris, Cochran and Pratt, 2005).

3.4.4 Cognitive Behaviours

Finally, there was an initial attempt for comprehensive longer-term assessment of cognitive changes and sex differences using pairwise discrimination and reversal learning experiments in a touchscreen task, after acute low-dose PCP. However, due to equipment breakdown, this experiment was halted and only included female animals. As a result, there is no possibility to assess for sex differences following acute PCP in reversal learning ability during a crucial learning phase. However, PCP did not inhibit learning in reversal phases for the female mice which did complete the behavioural test.

However, other studies have shown that sub-chronic injection of PCP two-weeks prior to cognitive tasks results in altered reversal learning abilities in rats and mice (Brigman *et al.*, 2009; Fellini *et al.*, 2014; Talpos *et al.*, 2014; Savolainen *et al.*, 2021). It would be prudent therefore to continue to assess how acute, low-dose PCP may compare in relation to cognitive deficits as well as further research into sex differences under these conditions.

In order to continue assessment of cognitive changes, I instead used a commonly utilised measure of learning and memory through the novel object recognition task (Antunes and Biala, 2012). Despite seeing no effect on novel object discrimination, there was an interesting effect on total object exploration time whereby PCP resulted in decreased exploration time in total. There was no effect of locomotor activity in the open-field as a result of PCP even in the first ten minutes suggesting this unlikely due to any locomotor effects. However, the NOR requires more experimenter interference with requirement for increased handling while objects are placed. With open-field and EPM results showing altered anxiety response following PCP injection, it is important to assess how increased anxiety due to experimenter handling might affect cognitive behaviours under different conditions. In this case, males animals specifically showed a significant reduction in exploration time following PCP injection. Sex differences in the NOR behaviour also showed that male and female animals have different innate interests in exploration even with saline. Other studies have showed sex differences in NOR (Frick and Gresack, 2003;

Sutcliffe, Marshall and Neill, 2007) and so it is relevant to consider how this might mask effects of PCP if female animals are less likely to carry out the required behaviour. In this case, the question is raised of the meaning of exploratory behaviours in mice and how this might relate to humans. While this test is often used to describe changing cognitive abilities, exploration for a prey animal requires spatial awareness, reduced anxiety and desire to find shelter which may result in different prioritisation for mice over exploring new objects.

Furthermore, given the short period of time animals are given within the NOR behavioural paradigm it is difficult to approximate how this might relate to wider cognition. When giving further consideration to habituation effects seen here in the open-field beyond 30 minutes, it stands to reason that the increased stress response from handling, injection processes and habituation will have their own effects on results of NOR. Utilising a longer-term assessment of learning and memory such as the paired discrimination and reversal learning paradigm will provide a much wider view of overall cognitive abilities and can further provide nuance that will simply not be visible within a 15 minute experiment. Animals will additionally be more comprehensively acclimated to the test environment that is required for the paired discrimination limiting the potential effects of novel and stressful test environments. This further highlights that environmental and experimental context are an important but under-represented variable in behavioural neuroscience research.

3.4.5 Conclusions

To conclude, here we have shown that acute, low-dose PCP alters anxiety-related, social and cognitive behaviours in mice across a number of behavioural tests. Furthermore, these effects are sex specific and context dependent with behavioural experiments revealing variable results. Female mice in general displayed more anxiety related behaviours in response to PCP than males. However, females also showed altered activity patterns in home-cage environments and this must be further considered when interpreting anxiety-related behaviours. Males in general appeared to show reduced anxiety-related behaviours in some instances, whilst also displaying altered sociability in the

home-cage potentially indicating an increased anxiety response only in the home-cage. Males also depicted altered thigmotactic behaviours in the home-cage and whilst this may not directly indicate anxiety as in an open-field, combined with the altered social response this may depict either altered social functioning or be a secondary anxiety response. This pattern of affective output is relevant to the condition, in that females with schizophrenia have higher incidence of anxiety whilst males show reduced social functioning compared to females with schizophrenia. Cognitive changes were also assessed and although there was no evidence to suggest a reduction in cognitive ability, there were sex specific differences in desire to engage exploratory behaviours in males. It is important to therefore assess what this innate behaviour to explore new objects may represent in mice and how this might compare to human studies. Overall, these data highlight the importance of considering environmental context and sex in evaluating acute PCP induced changes in secondary and negative symptoms relevant to schizophrenia.

3.4.6 Future Directions

To further continue this research there are a number of additional experiments that would expand upon and give further insight into some of the main findings. Firstly, as discussed, a more comprehensive analysis of cognitive ability in learning and memory would provide valuable insight to any sex differences. Namely, continuing the paired discrimination and reversal learning paradigm by repeating the experiment in male animals would allow for this analysis. Furthermore, given that including this experiment alongside the other tests already carried out, there would be possibilities for comparison of how cognition, anxiety and locomotor activity can be affected in contrasting experimental environments. I would like to further explore this. While comparison of the home-cage to the open-field and EPM provides comparisons of motor activity and anxiety behaviours in familiar and novel experimental environments the home-cage also provides detailed analysis of social behaviour. I would like to compare this to more traditionally used social behaviour paradigms such as the three-chamber social behaviour experiment which

provides interesting insight into changes in sociability upon introduction to novel mice. With the home-cage providing social data on cage-mates it would be interesting to complete analysis on differences between these contexts.

Furthermore, a deeper analysis of social behaviours would be incredibly valuable. Whilst separation and time spent in isolation exposes interesting information around proximity to cage-mates, social behaviours are incredibly complex with hierarchical grouping, vocalisations, grooming behaviours and nesting patterns all revealing valuable insight into changing sociability. I would like to incorporate the use of DeepLabCut or other machine learning software to automatically track these more complex behaviour patterns providing more nuanced behavioural data. Considering how important social alterations are to those with schizophrenia, it is important the research in this area is developed further.

Finally, the use of PCP to mimic schizophrenia like behaviour in research primarily utilises two methodologies: acute administration or sub-chronic administration. Here, following research on the effect of acute injection replicating similar connectivity changes as found in humans with schizophrenia this method was implemented throughout. However, the sub-chronic administration of PCP has been shown in research to illicit clearer behavioural changes which more closely replicate the behavioural phenotype seen in humans with schizophrenia (Fellini *et al.*, 2014). Thus, to further continue this research it would be prudent to include the additional behavioural tests and then further repeat all experiments with a sub-chronic model for comparison. A further step would be to dissect the brains of animals on completion of behavioural experiments and assess for any neurobiological changes in transcription of distinct proteins with PCR, or assess cellular composition changes through histological analyses.

3.4.7 Limitations

Throughout these experiments there were a number of limitations which were imposed by different circumstances. Firstly, due to covid lockdowns, the time

between experiments on female animals and those on male animals spanned a number of months and it is important to consider seasonal change. While there is every effort to maintain rigorous routine for light cycle, cleaning of cages and staff there can be several changes over the course of a few months. Cage was included as a factor in the nested-ANOVA analysis in order to account for the possibility that external environmental changes may have affected results however the ideal process would be to carry out experiments at the same time. Other steps were taken to minimise environmental factors with regular handling in advance of behaviour and only one researcher (myself) involved in handling and experimental procedures, avoiding researcher induced behavioural changes.

Furthermore, due to equipment breakdown for paired discrimination and reversal learning tasks there was a small sample size of only 7 female mice for this behavioural paradigm. While this was due to circumstances of a world pandemic, it is still difficult to assess results under these conditions with only 3 or 4 animals in counter-balancing group. This was mitigated by doing analysis of reversal learning as a whole and including the phase as a factor rather than looking at each reversal learning phase separately however with a larger sample size it would be prudent to assess any differences between the reversal learning phases.

4 Results: Behavioural profile of dual-hit gene x environment model of schizophrenia

4.1 Introduction

Schizophrenia has a complex symptomology which encompasses a large number of behavioural variabilities that affect people who are diagnosed. These symptoms range from the commonly known positive symptoms such as delusions and hallucinations to more complex negative symptoms that involve anxiety and depression (Waltz and Gold, 2007; Betensky *et al.*, 2008; Regier, Kuhl and Kupfer, 2013). Recent research advances have focussed on developing methodology in rodents that can mimic aspects of the the schizophrenia phenotype in order to develop a more in depth understanding of underlying mechanisms that may contribute to development of schizophrenia. These include the use of NMDA receptor antagonists to imitate the NMDA receptor hypofunction seen in humans with schizophrenia (Morris, Cochran and Pratt, 2005; Javitt *et al.*, 2012; Grayson *et al.*, 2016; Moghadam *et al.*, 2021) or various genetic mouse lines which investigate high risk genes/CNVs for schizophrenia such as the 22q11 deletion or the 16p11.2 duplication (Benedetti *et al.*, 2022). Other studies have aimed to investigate how specific environmental factors, such as viral infections may alter brain development to result in schizophrenia (Schwartzer *et al.*, 2013; Babri, Doosti and Salari, 2014; Vitor-Vieira, Vilela and Giusti-Paiva, 2021; Woods *et al.*, 2023). However, whilst these studies have been very valuable in our understanding of how individual factors might influence the development of schizophrenia this does not reflect the natural progression of the disorder in people. In reality, no single factor has been identified as an absolute cause for schizophrenia development and so it is important that research begins to investigate how converging influences may interact to result in the neurobiological and behavioural changes that lead to schizophrenia.

4.1.1 Genetic Risk Factors

Investigations of genetic risk factors in schizophrenia has revealed a number of genes/CNVs of interest for the development of schizophrenia. These studies have identified the 16p11.2 locus as an area of interest in psychiatric and neurodevelopmental disorders (Weiss *et al.*, 2008; McCarthy *et al.*, 2009; Chang *et al.*, 2017). In fact, a deletion in this area has been highly associated with autism spectrum disorders (Weiss *et al.*, 2008; Kushima *et al.*, 2018; Niarchou *et al.*, 2019). On the other hand, a duplication of this region has shown a high risk of schizophrenia development in adulthood with an estimated 4.3 – 14% of those with the genetic variant having schizophrenia diagnosis (Kirov *et al.*, 2014; Marshall *et al.*, 2017). This suggests that the 16p11.2 duplication is a variant that carries a relatively high risk of diagnosis in comparison to other variants such as single nucleotide polymorphisms (SNPs) risk genes and as such must be a gene of interest in research going forward.

In animal models carrying this genotype, there are clear deficits which replicate some of the behavioural symptoms people with schizophrenia face. These include cognitive deficits in working memory (Yang *et al.*, 2015; Bristow *et al.*, 2020; Rein *et al.*, 2021), social interaction deficits (Bristow *et al.*, 2020; Rein *et al.*, 2021) and in anxiety related behaviours (Bristow *et al.*, 2020). It is clear therefore that not only does the 16p11.2 duplication carry high risk for schizophrenia development in humans, but behavioural deficits which mirror changes in humans with schizophrenia can be seen in animals carrying the variant. Utilising animals containing this genetic variant in research on schizophrenia therefore carries translational strength.

4.1.2 Environmental Risk Factors

There are a number of environmental stimuli which have been shown to increase the risk of schizophrenia development that can occur at multiple stages in key periods of neurodevelopment. Early childhood has been highlighted as one such key stage with people with schizophrenia more likely to have experienced early life stress or childhood trauma (Bebbington *et al.*, 2004;

Spauwen *et al.*, 2006; Morgan and Fisher, 2007; Carr *et al.*, 2013). Another key stage of neurobiological development which has been identified as particularly susceptible to environmental stimuli is during embryonic development. Events that occur during pregnancy such as maternal stress can increase likelihood of schizophrenia diagnosis (Khashan *et al.*, 2008). In fact, one environmental stimulus which has gained interest in schizophrenia research is that of maternal immune activation as a result of viral infection. Studies have shown that viral infection of a mother during pregnancy through viruses such as influenza, rubella, herpes simplex virus or syphilis have from 2-7 fold increased risk in diagnosis of schizophrenia in the offspring (Brown, 2006, 2011; Canetta and Brown, 2012; Janoutová *et al.*, 2016; Aguilar-Valles, Rodrigue and Matta-Camacho, 2020). Infection during the first and second trimesters has been shown to be particularly sensitive (Brown and Derkits, 2010; Brown, 2011).

With robust evidence that maternal immune activation can influence neurodevelopment in offspring, research aimed to determine if this effect could be artificially replicated in animals for further study. Several studies in rodents which utilise LPS or Poly IC have all shown long-term changes to social behaviours (Schwartzer *et al.*, 2013; Gzielo *et al.*, 2021; Vitor-Vieira, Vilela and Giusti-Paiva, 2021; Carbone *et al.*, 2023), cognitive ability (Ito *et al.*, 2010; Simões *et al.*, 2018; Sun *et al.*, 2022; Woods *et al.*, 2023) and anxiety-related behaviours (Babri, Doosti and Salari, 2014; Depino, 2015). With an ability to elicit clear behavioural deficits which mimic schizophrenia this is another model which can be therefore used to further study schizophrenia development.

4.1.3 A dual-hit model for schizophrenia

Whilst the existence of individual genetic and environmental risk factors has been well characterised in humans as well as in animal models (McDonald and Murray, 2000; Canetta and Brown, 2012; Janoutová *et al.*, 2016), there is still a question of how genes and environmental stimuli might interact. With no individual cause identified for schizophrenia, researchers hypothesise that genetic variants may predispose to diagnosis of schizophrenia whilst

environmental stimuli may interact with these variants to trigger development of neurobiological changes that result in schizophrenia diagnosis. These theories are based upon epidemiological studies of people with schizophrenia which highlight that increasing numbers of risk factors increase risk of diagnosis such as in the case of genetic vulnerability combined with maternal immune activation (Van Os, Rutten and Poulton, 2008; Prasad *et al.*, 2010). However, to truly assess this it is necessary to replicate this under experimental conditions in order to further identify how genes and environmental stimuli interact.

Here we utilise a genetic mouse line which carried the 16p11.2 duplication variant that carries high risk of schizophrenia development in humans (McCarthy *et al.*, 2009; Chang *et al.*, 2017) and has shown schizophrenia-like deficits in mice (Bristow *et al.*, 2020; Willis, Pratt and Morris, 2021). This was combined with an MIA protocol to replicate the environmental risk factor of maternal infection during embryonic development (Canetta and Brown, 2012; Janoutová *et al.*, 2016; Aguilar-Valles, Rodrigue and Matta-Camacho, 2020). Two different methods of MIA were utilised in order to also investigate mechanisms underlying multiple infection routes and their effects. These were Poly IC which induces an immune response through TLR3 activation and closely mimics double-stranded viral infection and, resiquimod, a TLR7 agonist which more accurately mimics single-stranded viral infections such as influenza and rubella (See Chapter 1.5.2).

Finally, adult mice that were born from maternally immune activated litters were investigated under a series of behavioural test in an effort to characterise a full behavioural profile of offspring. This included analysis of locomotor activity patterns, social interaction, anxiety-related behaviour and cognitive ability.

I hypothesised that mice containing the 16p11.2 duplication would exhibit hypoactivity, increased social deficits, cognitive dysfunctions and increased anxiety related behaviours. Additionally, mice from MIA groups would exhibit social deficits, cognitive dysfunction and heightened anxiety-like responses. I

further hypothesised that there would be a prevalent gene x environment interaction whereby 16p11.2 duplication mice that experienced MIA during embryonic development would show more severe behavioural effects compared to mice which only had one risk factor.

4.2 Methods

4.2.1 Animals

All animals were housed in social groups in a temperature-controlled room with light cycle reversed. Lights were switched off at 10am and switched on at 10pm and all experiments were done between the hours of 10am and 6pm. In total, 41 female and 32 males were used and were between 8 and 16 weeks old. There were 39 mice containing the 16p11.2 duplication and 34 wildtype mice resulting in a total of 73 mice. All animals were handled consistently 1 week prior to any behavioural experiments in order to habituate mice to the process of being picked up, weighed and placed into novel environments. All animals completed the open field test, elevated plus maze and the novel object recognition task. The home-cage monitoring has lower numbers of animals in each group due to the necessity for the home-cage monitoring apparatus to have a minimum of 3 animals per cage. Where possible female animals were combined at weaning to have a minimum or 3 or maximum of 4 animals per cage to maximise the numbers. It is not possible to introduce unfamiliar males to each other at this stage without risking aggression. Additionally, given the staggering of these experiments over the course of a year and the relatively low fecundity of the breeding it was not always possible to combine cages. This means that overall there was a fewer number of animals in cages of 3 to 4 which were available to complete home-cage monitoring analysis. Total numbers in each group are shown in the table below.

Group	**Number of animals: OF, EP, NOR**	**Number of animals: Home cage**
Female WT (Saline MIA)	7	3
Male WT (Saline MIA)	6	4
Female DUP (Saline MIA)	5	3
Male DUP (Saline MIA)	5	2
Female WT (Resiquimod MIA)	5	4

Male WT (Resiquimod MIA)	5	3
Female DUP (Resiquimod MIA)	11	10
Male DUP (Resiquimod MIA)	6	4
Female WT (Poly:IC MIA)	9	9
Male WT (Poly:IC MIA)	7	6
Female DUP (Poly:IC MIA)	4	4
Male DUP (Poly:IC MIA)	3	3
Total	**73**	**55**
Total Male	32	22
Total Female	41	33
Total WT	39	29
Total DUP	34	26

Table 4-1: Table of number of mice in each group for behavioural testing when assessing effects of a dual-hit model of schizophrenia. *DUP = 16p11.2 duplication. WT = wildtype.*

4.2.2 Home-cage behavioural monitoring

Mice were monitored in their home cage for a variety of behaviours such as movement, social interaction and time spent in isolation. To do this, mice were microchipped and placed into Actual Analytics Home-cage 24-hour monitoring equipment and microchips were tracked for movement across a 9x16 grid pattern of antennas as described in (methods section 2.2.1).

Mice were first placed into clear IVC cages in the Actual HCA™ system (Actual Analytics Ltd, UK) for 48 hours to habituate to the home-cage monitoring environment. Recording was then started at 10am at the beginning of a new dark-cycle and continued for a further 24 hours. At 10am the following day, recording was stopped and animals were returned to their previous home-cage environments.

4.2.3 Open-field Test

To monitor locomotor activity, mice were placed into a 40x40cm open-field and their movement tracked using an infra-red camera as described in (methods section 2.2.2). Animals were then placed into the open-field and recording commenced and continued for 2 hours. The centre-point of the animal was tracked using Noldus Ethovision Infrared tracking software which also identified placement of the animal in inner and outer zones of the open-field arena.

4.2.4 Elevated Plus Maze

To monitor anxiety behaviours, mice were placed into an EPM. Recording lasted 15 minutes and the centre point of the animal was tracked using Noldus Ethovision Infrared tracking software which assessed movement between open and closed arms as well as the centre zone of the EPM. Movement into the outer thirds of the open arms were also assessed. Further details can be found in section 2.2.3.

4.2.5 Novel Object Recognition

Cognitive abilities were measured by assessment of novel object recognition. Mice were first placed in the open-field to habituate 24 hours prior to test day for 15 minutes. 5 minutes of habituation was then recorded on the test day. Animals were briefly removed from the open-field while two identical objects were placed into the open field in locations indicated in methods section 2.2.4. Once animals had explored both objects for a total time of 15 seconds or had been in the open-field for 5 minutes they were briefly removed and one of the objects replaced with a novel object to provide a common base-line of familiarisation. Mice were placed back into test environment and recording continued for 5 minutes or until both objects had been explored for up to 30 seconds. Noldus Ethovision Infrared tracking software recorded the nose and tail points of each animal and their location in the open-field as well as their entrance into the 5x5cm zone in which the object was placed centrally.

4.2.6 Data Analyses

Data were analysed using Minitab, and all behavioural experiments were statistically tested using Two-way multi-factorial repeated-measures ANOVAs. Further details can be found in section 2.4. The independent variables in these experiments were treatment (three levels; PolyIC, Resiquimod and vehicle), genotype (two levels, 16p11.2 duplication and wildtype), sex (two levels; male and female) and time. Mouse ID and cage number were also included as nested factors within the independent variables of treatment, genotype and sex. The dependent variables which were analysed were distance travelled (for open field test, elevated plus maze and home-cage monitoring), time spent in the outer areas of the arena (for thigmotaxis measures in the open-field and home-cage), ratios of time spent in different zones of the EPM, mean distance to the nearest cage mate (during home-cage monitoring) ratio of time spent exploring the novel object (during novel object recognition) and accuracy during the paired discrimination and reversal learning tasks. Additionally, interactions between the independent variables of sex, genotype, treatment and time were also measured as part of the multi-factorial ANOVA. Tukey post-hoc tests were used to determine differences between individual means and significantly different groups are shown through Tukey's method grouping with full lists of pairwise comparisons provided in the supplementary materials. Tukey's method of grouping puts all groups which are not statistically significantly different from one another into a single group which is assigned a letter (e.g group A). Groups which are significantly different from each other are assigned a separate letter (e.g. group B). Some groups can be part of both group A and group B which means there is not significant difference in the means from both groups. However, groups which are only in Group A are significantly different to those in Group B.

4.3 Results

4.3.1 The Open-Field Test

Mice were placed in the open-field for a total of 2 hours and infra-red cameras tracked their location through Ethovision software. Location over time then provided information about overall distance travelled as a measure of locomotor activity or presence in different zones of the open-field as a measure of thigmotaxis. Results from multi-factorial, nested ANOVA are shown in Figure 4.1.

Comparison	Locomotor Activity		Thigmotaxis	
	F-value	p-value	F-value	p-value
Time	42.85	$< 1x10^{-16}$	4.82	$1.18x10^{-7}$
Genotype	15.18	$1.07x10^{-4}$	5.55	0.019
Sex	94.08	$< 1x10^{-16}$	41.39	$2.22x10^{-10}$
Treatment	138.67	$< 1x10^{-16}$	57.66	$< 1x10^{-16}$
Genotype*Sex	35.28	$4.38x10^{-9}$	0.59	0.442
Genotype*Treatment	162.08	$< 1x10^{-16}$	11.94	$7.86x10^{-6}$
Treatment*Sex	29.25	$5.88x10^{-14}$	47.42	$< 1x10^{-16}$
Genotype*Treatment*Sex	12.82	$3.35x10^{-6}$	0.40	0.673

Table 4-2: Results of Multi-factorial ANOVA statistics for open-field tests.

		Time spent in Outer Zone (s)			Total Distance Travelled (mm)		
Group	N	Mean	Standard Error	Standard Deviation	Mean	Standard Error	Standard Deviation
Saline, Males, WT	6	399	9.63	74.57	2498	114	885
Saline, Males, DUP	5	441	14.7	88.3	1826	165	993
Saline, Females, WT	7	309	12.7	98.5	2275	108	835
Saline, Females, DUP	5	334	13.6	124.8	2131	73	672
PolyIC, Males, WT	7	436	9.72	89.12	2240	87	799
PolyIC, Males, DUP	3	405	15.1	90.7	3244	262	1575
PolyIC, Females, WT	9	460	5.73	59.56	2633	82	854
PolyIC, Females, DUP	4	435	9.38	65.02	4901	615	421
Resiquimod, Males, WT	5	424	11.1	84.9	2800	115	880
Resiquimod, Males, DUP	6	442	11.3	94.9	2502	107	905
Resiquimod, Females, WT	5	402	11.1	85.2	3226	112	862
Resiquimod, Females, DUP	11	453	14.1	161.3	2933	86	987

Table 4-3: Summary data including the mean time spent in the outer zone of the open field (s) as well as the mean distance travelled (mm) for each group. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group	Tukey Method Grouping					
DUP PolyIC F	A					
DUP PolyIC M		B				
WT Resiquimod F		B				
DUP Resiquimod F		B				
WT Resiquimod M		B	C			
WT PolyIC F			C	D		
WT Saline M			C	D	E	
DUP Resiquimod M			C	D	E	
WT Saline F				D	E	F
WT PolyIC M					E	F
DUP Saline F					E	F
DUP Saline M						F

Table 4-4: The Tukey Method Grouping for distance travelled in the open-field. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

Group	Tukey Method Grouping			
WT PolyIC F	A			
DUP Resiquimod F	A			
DUP Resiquimod M	A	B		
DUP Saline M	A	B	C	
WT PolyIC M	A	B	C	

DUP PolyIC F	A	B	C	
WT Resiquimod M	A	B	C	
DUP PolyIC M		B	C	
WT Resiquimod F		B	C	
WT Saline M			C	
DUP Saline F				D
WT Saline F				D

Table 4-5: The Tukey Method Grouping for time spent in the outer zones of the open field. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

4.3.1.1 Locomotor Activity

In the open-field, distance travelled was measured to analyse any changes in locomotor activity. Multi-factorial, nested, repeated-measures ANOVA shows significant effects across a number of factors (Figure 4.1). First, time had a significant effect on locomotor activity ($p < 1x10^{-16}$) highlighting the habituation effect of mice to the experimental apparatus. There was an overall effect of genotype with 16p11.2 duplication containing mice showing increased locomotor activity ($p = 1.07x10^{-4}$) (Figure 4.2c) compared to wildtype animals. This is particularly evident in the last hour of the test (Figure 4.2d) and emphasises the importance of extended measurement. Female animals were also significantly more active than male animals ($p < 1x10^{-16}$) and there was as significant effect of treatment ($p < 1x10^{-16}$) with Tukey's post hoc analyses showing significantly lower activity in the saline group compared to Poly IC and Resiquimod groups ($p < 0.001$) as well as a significant difference between Poly IC and Resiquimod groups ($p < 0.001$) (Figure 4.2e). There were also significant effects of interactions between genotype, sex and treatment suggesting that each individual group had its own behavioural repertoire as a result of combining factors. In the case of genotype and treatment interactions, poly IC treated 16p11.2 duplication mice had the highest locomotor activity followed by resiquimod treated mice, followed by saline treated mice (figure, 4.2b).

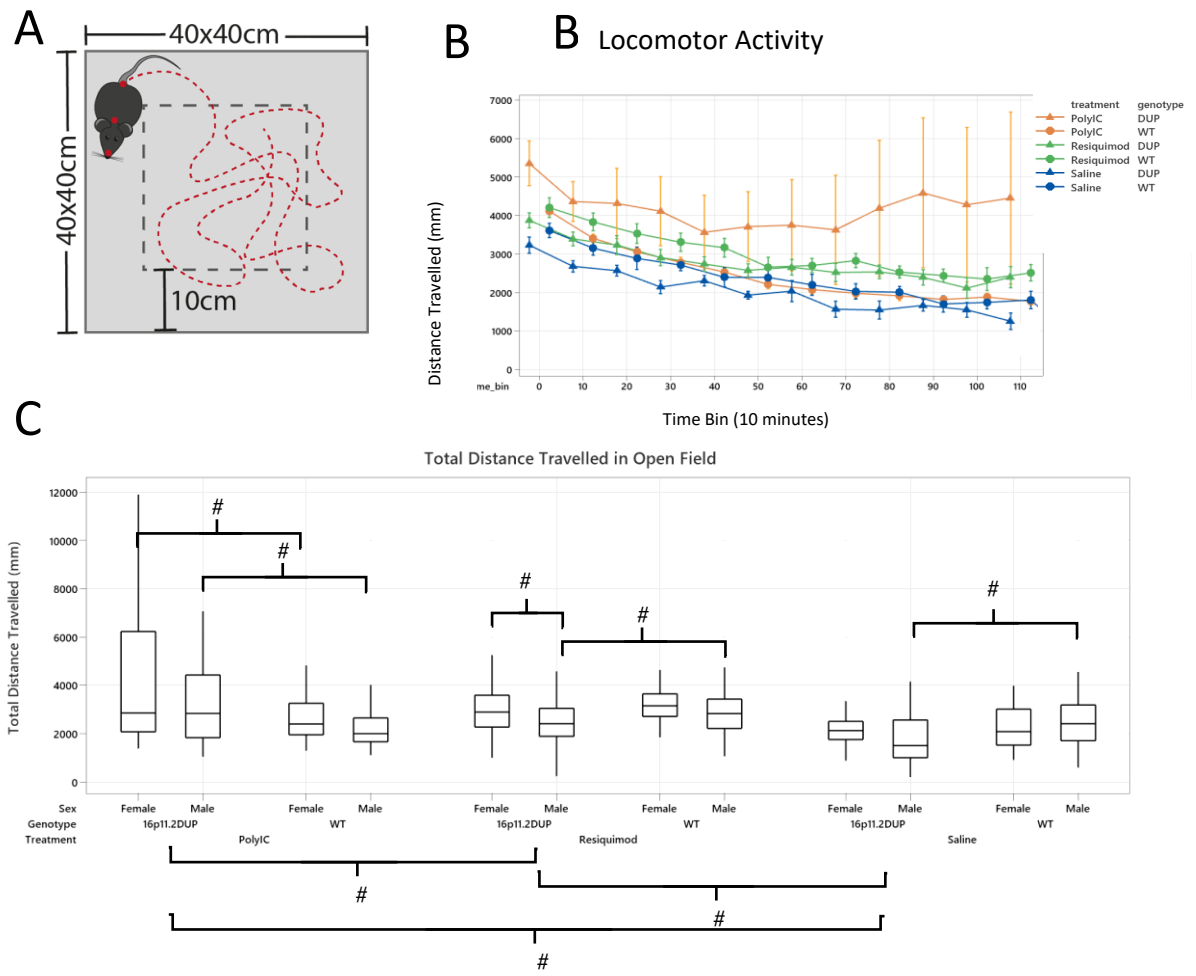

Figure 4.1: Locomotor Activity in the Open-Field Test in WT and DUP mice following MIA or saline. A) Diagram depicting the open-field apparatus. B) Locomotor activity over time of mice separated by genotype and MIA treatment groups. C) Total Locomotor activity of all groups over the whole experimental period. *N = 41 females, 32 males, 39 WT, 34 DUP, 23 saline, 27 resiquimod, 23 Poly IC. # indicates differences between means where p < 0.05.*

4.3.1.2 Thigmotaxis

Thigmotaxis was measured by recording the amount of time spent in the outer-zone of the open-field as a marker of increased anxiety (Figure 4.3a). There was an overall effect of genotype (p = 0.019) with wild type and 16p11.2 duplicating containing mice exhibiting variable changes in thigmotaxis over the experimental period (figure 4.3d). There was also an overall effect of sex, with female mice showing significantly lower levels of thigmotaxis compared to male animals ($p = 2.22 \times 10^{-10}$) (Figure 4.3c). Finally, there was a significant effect of treatment ($p < 1 \times 10^{-16}$) with saline groups showing a significantly lower amount of time spent in the outer zones compared to Poly IC ($p < 0.001$) and resiquimod ($p < 0.001$) but no significant difference between poly IC and resiquimod ($p = 0.323$) (Figure 4.3e). While there was no genotype and sex interaction, there was a significant interaction between genotype and treatment ($p = 7.86 \times 10^{-6}$) as well as treatment and sex ($p < 1 \times 10^{-16}$) with wildtype, saline treated groups showing the lowest thigmotaxis behaviour compared to resiquimod and Poly IC treated groups (figure 4.3b).

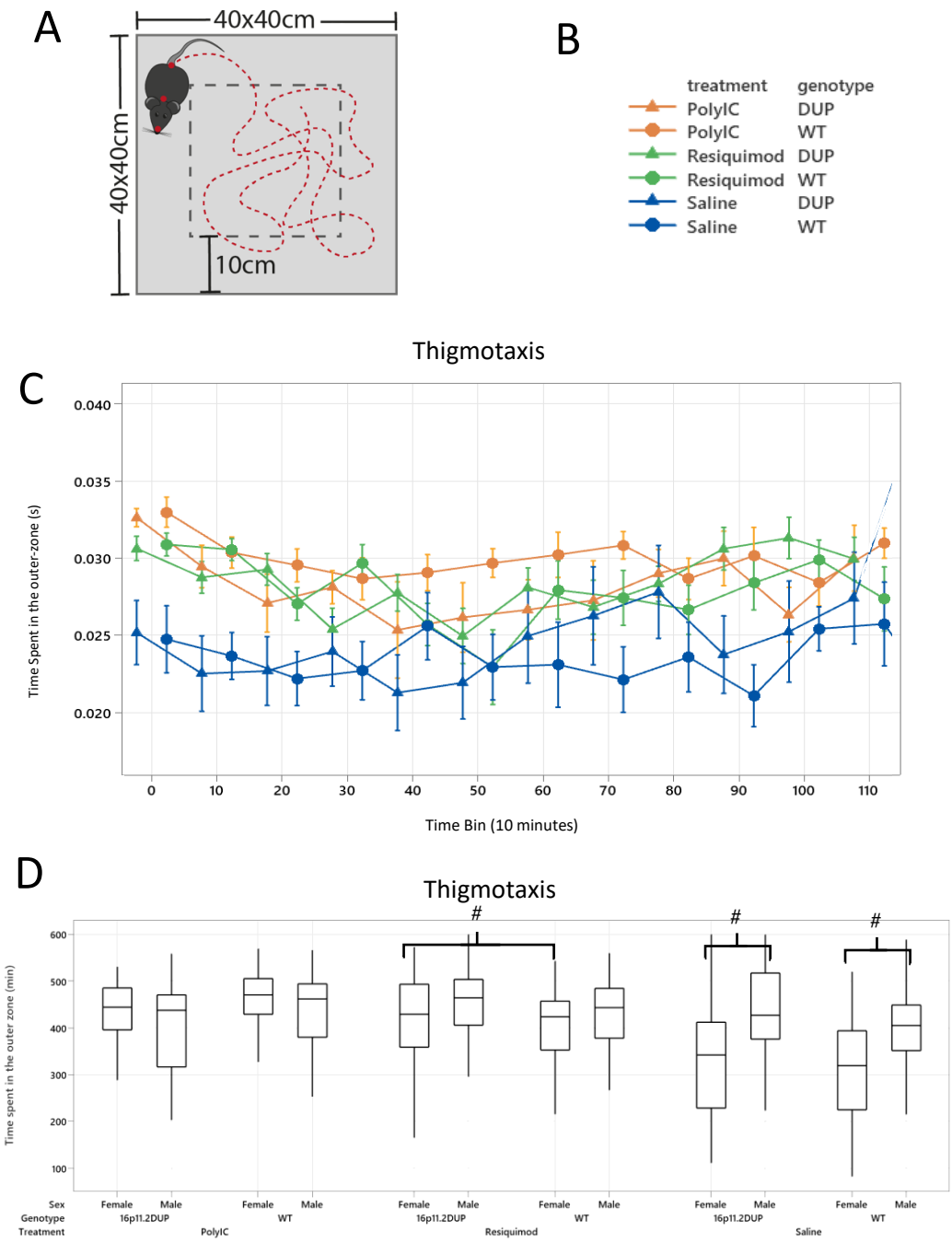

Figure 4.2: Thigmotaxis in the Open-Field Test in WT and DUP mice following MIA or saline. A) Diagram depicting the open-field apparatus. B) Key indicating different groups in line graphs. C) Total time spent in the outer zone of the open-field over time separated by genotype and MIA treatment groups. D) Total time spent in the outer zone of the open-field over the total experimental period of all groups, separated by genotype, sex and MIA treatment group. *N = 41 females, 32 males, 39 WT, 34 DUP, 23 saline, 27 resiquimod, 23 Poly IC. # indicates differences between means where p < 0.05.*

4.3.2 Elevated Plus Maze

Mice were placed into the elevated plus maze for a total of 15 minutes and their location in the maze over time was recorded using infrared cameras and Ethovision recording equipment. A ratio of time spent in the open arms of the maze in comparison to the other regions (centre-zone/closed arms) was calculated to determine differences between groups. Multi-factorial, nested ANOVA was used to determine overall effects of genotype, sex and treatment on this ratio. Results from multi-factorial nested ANOVA are shown in figure 4.4. There was an overall significant effect of treatment as well as genotype x treatment and treatment x sex interactions.

Genotype and sex showed no significant effects (figure 4.5) however there was a significant overall effect of treatment ($p = 2.86x10^{-7}$) (Figure 4.5b). This was primarily driven by saline animals showing significantly lower ratio of time spent in the open arm compared to poly IC ($p < 0.001$) and resiquimod ($p < 0.001$). There was no significant difference between Poly IC and resiquimod (p = 0.988). There were also significant interactions between treatment and sex and treatment and genotype although there was no genotype and sex interaction (Figure 4.4). This was primarily driven by the effects seen from an increased time spent in the open arms by 16p11.2 duplication mice in the Poly IC and resiquimod groups compared to their wildtype equivalents ($p < 0.001$).

Comparison	Statistic	
	F-value	p-value
Genotype	1.85	0.174
Sex	0.35	0.555
Treatment	15.32	$2.86x10^{-7}$
Genotype*Sex	0.30	0.584
Genotype*Treatment	28.17	$1.36x10^{-12}$
Treatment*Sex	11.88	$8.11x10^{-6}$
Genotype*Treatment*Sex	4.40	0.013

Table 4-6: Results of Multi-factorial ANOVA statistics for the elevated plus maze

Ratio of time spent in Open arms				
Group	N	Mean	Standard Error	Standard Deviation
Saline, Males, WT	6	0.15	0.02	0.14
Saline, Females, WT	7	0.08	0.09	0.09
Saline, Males, DUP	5	0.06	0.02	0.10
Saline, Females, DUP	5	0.06	0.008	0.09
PolyIC, Males, WT	7	0.11	0.01	0.13
PolyIC, Females, WT	9	0.15	0.01	0.15
PolyIC, Males, DUP	3	0.11	0.01	0.09
PolyIC, Females, DUP	4	0.16	0.02	0.12
Resiquimod, Males, WT	5	0.10	0.01	0.11
Resiquimod, Females, WT	5	0.09	0.01	0.11
Resiquimod, Males, DUP	6	0.21	0.02	0.18
Resiquimod, Females, DUP	11	0.09	0.01	0.12

Table 4-7: Summary data of the ratio of time spent in the open arms of the EPM. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group	**Tukey Method Grouping**				
Resiquimod, Male, DUP	A				
PolyIC, Female, DUP	A	B	C		
PolyIC, Female, WT	A	B			
Resiquimod, Female, DUP		B			
Saline, Male, WT	A	B	C	D	
PolyIC, Male, WT		B	C	D	E
PolyIC, Male, DUP		B	C	D	E
Resiquimod, Male, WT			C	D	E

Resiquimod, Female, WT				D	E
Saline, Female, WT				D	E
Saline, Female, DUP					E
Saline, Male, DUP					E

Table 4-8: The Tukey Method Grouping for ratio of time spent in the open arms of the EPM. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

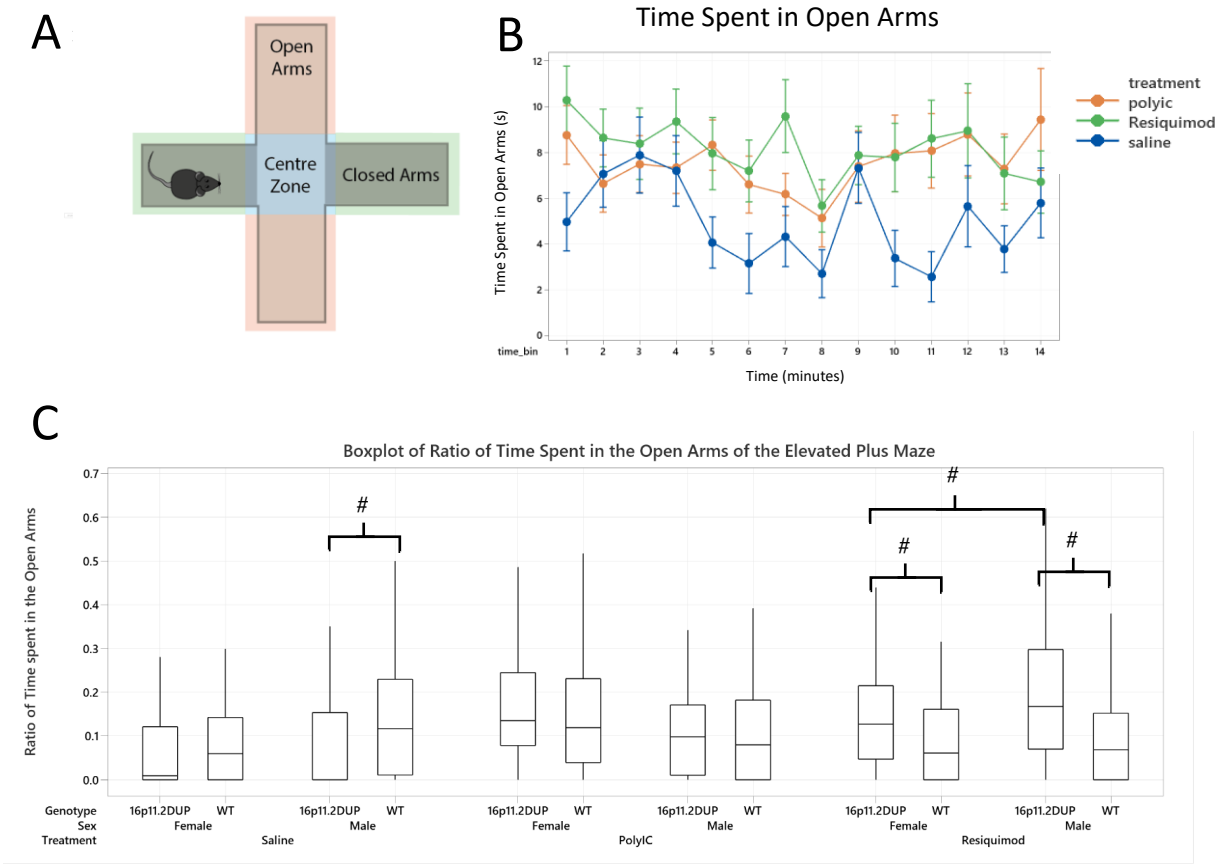

Figure 4.3: Behavioural Results of the Elevated Plus Maze in WT and DUP mice following MIA or saline. A) Diagram depicting the EPM apparatus. B) Total time spent the open arms of the EPM per minute, separated by MIA treatment groups over a 15-minute recording period. C) Diagram of time spent in the open arms of the EPM across the full experimental period, separated by genotype, sex and treatment. *N = 41 females, 32 males, 39 WT, 34 DUP, 23 saline, 27 resiquimod, 23 Poly IC. # indicates differences between means where p < 0.05. *indicates outlier data points.*

4.3.3 Home-cage continuous behavioural monitoring

Actual Analytics ™ home-cage monitoring equipment utilised RFID chip tracking across a grid pattern of antenna (as detailed in section 2.2.1). This allowed for measurement of distance travelled over time to assess locomotor activity as well as distance between mice. The latter measurement could indicate social separation by analysis of distance to the nearest cage mate. Multi-factorial, nested ANOVA was carried out to assess effects of MIA, genotype and sex on these parameters (Figure 4.6).

Comparison	Locomotor Activity		Social Separation	
	F-value	p-value	F-value	p-value
Time	24.46	$< 1x10^{-16}$	11.07	$< 1x10^{-16}$
Genotype	8.77	$3.08x10^{-3}$	21.21	$4.23x10^{-6}$
Sex	30.34	$3.83x10^{-8}$	52.91	$4.089x10^{-14}$
Treatment	292.06	$< 1x10^{-16}$	69.73	$< 1x10^{-16}$
Genotype*Sex	11.6	$6.66x10^{-4}$	0.09	0.768
Genotype*Treatment	49.55	$< 1x10^{-16}$	15.86	$1.364x10^{-7}$
Treatment*Sex	26.64	$3.17x10^{-12}$	34.43	$1.4x10^{-15}$
Genotype*Treatment*Sex	6.83	0.001	6.56	$8.93x10^{-4}$

Table 4-9: Results of Multi-factorial ANOVA statistics for behaviours recorded in home-cage continuous behavioural monitoring.

		Time spent in Outer Zone (s)			Distance Travelled (mm)		
Group	N	Mean	Standard Error	Standard Deviation	Mean	Standard Error	Standard Deviation
Saline, Males, WT	4	46.8	0.784	29.7	332	11.5	435.2
Saline, Males, DUP	4	52.9	1.20	28.8	343	18.8	452.9
Saline, Females, WT	3	45.9	0.762	25.9	412	19.3	657.6
Saline, Females, DUP	4	46.9	0.793	26.9	435	19.4	658.4
PolyIC, Males, WT	6	59.1	0.862	31.0	257	8.29	371.43
PolyIC, Males, DUP	3	56.8	1.07	27.3	845	38.3	973.9
PolyIC, Females, WT	9	50.4	0.700	26.6	831	28.2	1071.8
PolyIC, Females, DUP	4	60.8	0.882	30	1214	41.3	1404
Resiquimod, Males, WT	3	43.5	1.11	32.2	394	19.7	571
Resiquimod, Males, DUP	4	59.9	1.71	49.8	169	9.31	271.4
Resiquimod, Females, WT	4	36.0	0.716	24.2	329	13.1	443.9
Resiquimod, Females, DUP	10	42.2	0.643	28.8	394	19.7	571

Table 4-10: Summary data of the distance travelled and thigmotaxis behaviour in the home-cage. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group (Distance travelled)	Tukey Method Grouping				
F DUP PolyIC	A				
M DUP PolyIC		B			
F WT PolyIC		B			
M WT PolyIC			C		
M WT Resiquimod				D	
F WT Saline				D	
M DUP Saline				D	
F DUP Saline				D	
M WT Saline				D	
F WT Resiquimod				D	E
F DUP Resiquimod				D	E
M DUP Resiquimod					E

Table 4-11: The Tukey Method Grouping for distance travelled in the home cage. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

Group (Separation)	Tukey Method Grouping				
M DUP Resiquimod	A				
F DUP PolyIC	A				

M WT PolyIC	A				
F WT PolyIC	A	B			
M DUP PolyIC	A	B	C		
M DUP Saline		B	C	D	
M WT Saline			C	D	
M WT Resiquimod			C	D	
F DUP Resiquimod				D	
F WT Saline				D	
F DUP Saline				D	
F WT Resiquimod					E

Table 4-12: The Tukey Method Grouping for distance to the nearest cage-mate in the home-cage. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

4.3.3.1 Locomotor Activity

Triangulation of RFID chips was taken ever 15ms and recorded for a period of 24 hours. Results showed a significant effect of time on locomotor activity with animals showing increased activity during the 12-hour dark period of the 24-hour light cycle ($p < 0.001$) *(Figure 4.7a-e)*. There was also an overall effect of genotype and sex with 16p11.2 duplication animals showing a significant increase in activity compared to wildtype animals *($p = 0.003$)* and females showing increased locomotor activity to male animals ($p < 0.001$) *(Figure 4.7c and 4.7d)*. Finally, there was a clear effect of treatment with Poly IC showing the highest activity levels over the 24-hour period. This was significantly increased compared to both resiquimod treated mice ($p < 0.001$) and saline treated animals ($p < 0.001$). Resiquimod showed a significant decrease in activity compared to saline treated animals ($p < 0.009$).

There were also significant interactions with genotype and sex, genotype and treatment, treatment and sex and with all three factors (figure 4.6). The genotype and treatment interaction is particularly evident with Poly IC treated 16p11.2 duplication mice showing significantly increased locomotor activity compared to poly IC treated wild type mice ($p < 0.001$) *(Figure 4.7a)*. Poly IC wild type animals also showed significant increases compared to wild type saline and resiquimod groups as well as saline 16p11.2 duplication mice ($p < 0.001$). Resiquimod treated 16p11.2 duplication mice showed a significantly decreased activity compared to all other groups ($p < 0.001$). The sex and genotype interaction was primarily driven by the Poly IC treated mice with females in this group showing a significantly higher locomotor activity than the males ($p < 0.001$). Finally, the genotype and sex interaction was due to female 16p11.2 duplication mice showed higher activity than all other groups ($p < 0.001$).

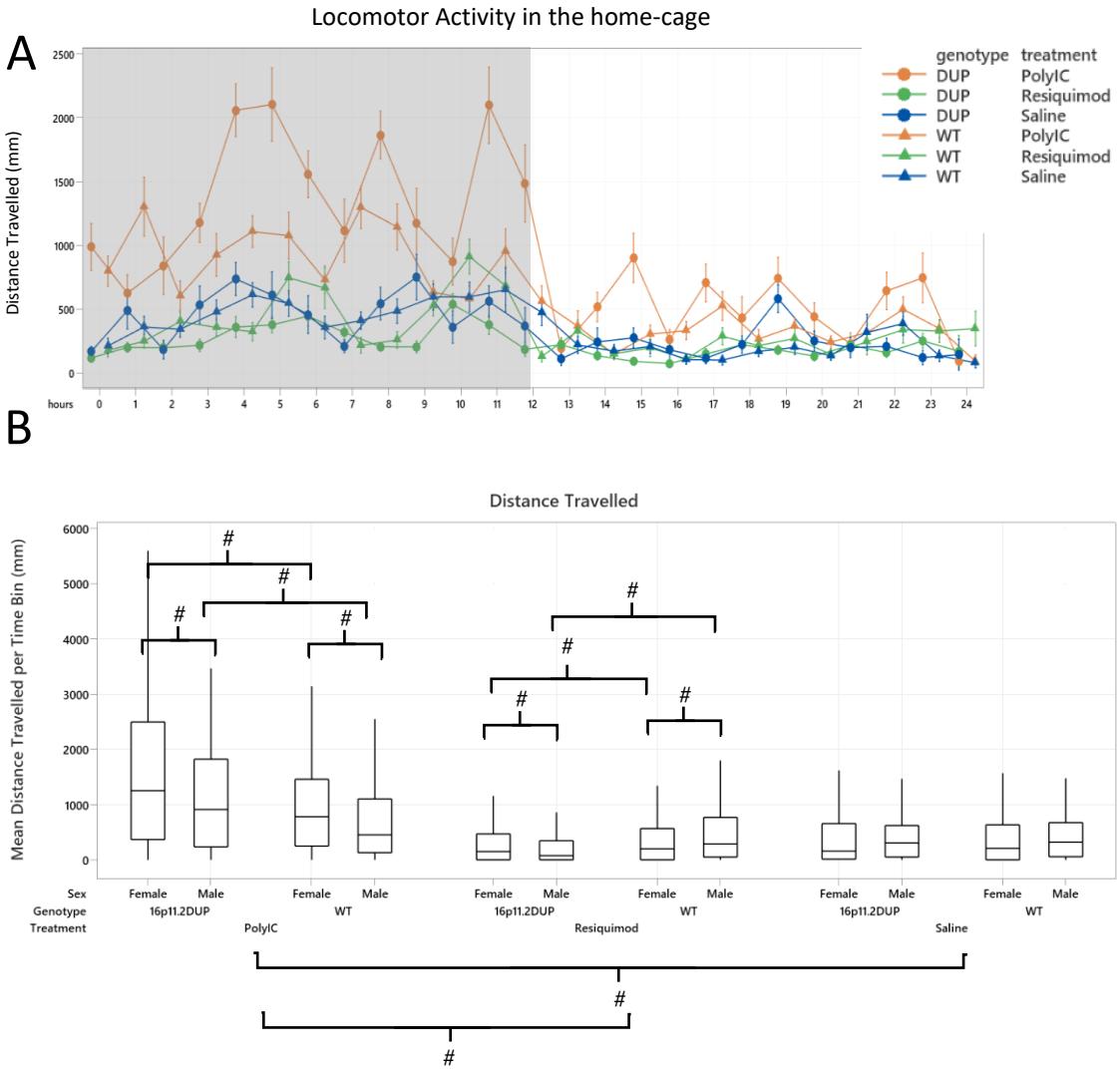

Figure 4.4: Locomotor Activity in the home cage in WT and DUP mice following MIA or saline. A) Diagram depicting the continuous home-cage behavioural monitoring equipment over a 24-hour period. B) Total distance travelled across all groups throughout the first 12-hour dark cycle. *Grey areas on each graph signify the dark, waking period of the 24-hour light cycle while white background signifies the light, sleeping period of the might cycle. # indicates differences between means where p < 0.05. *indicates outlier data points.*

4.3.3.2 Social Separation

Separation from cage mates was calculated as the mean distance to the nearest cage-mate per hour and provided information about proximity to other mice in the home-cage. This can be used as a basic measure of social interaction in the home environment. Multi-factorial, nested ANOVA was used to analyse effects of genotype, sex and treatment group on separation from cage mates over a 24-hour period. Tukey's post hoc analyses provided further information about significant difference between individual groups. There was an overall effect of time, with animals showing decreased separation during the light, sleeping period of the cycle *($p < 0.001$) (Figure 4.8)*. This demonstrates the nesting behaviours mice engage in during sleep and highlights how social behaviours can impact on separation as a measure. Genotype, sex and treatment all showed significant effects on separation (Figure 4.7). 16p11.2 duplication mice showed increased separation compared to their wildtype counterparts *($p < 0.001$) (Figure 4.8 c)* and male animals showed increased separation compared to their female counterparts *($p < 0.001$) (Figure 4.8d)*. Whilst poly IC treated animals showed higher levels of separation compared to both saline *($p < 0.001$)* and resiquimod treated animals *($p < 0.001$)*, there was no significant difference between saline and resiquimod groups *($p = 0.673$) (Figure 4.8b)*.

There were also significant interactions between genotype and treatment and treatment and sex (Figure 4.7 and 4.8a). The treatment and sex interaction was primarily driven by sex-specific responses to resiquimod mediated MIA. Male animals from resiquimod treated MIA litters showed a significant increase in separation behaviours compared to saline counter parts *($p < 0.001$)*. At the same time, female mice from resiquimod treated litters showed no significant difference from saline counterparts (*$p = 0.158$)*. Similarly, the genotype and treatment interaction was also driven by differing responses to resiquimod. Mice from resiquimod treated MIA letters with the 16p11.2 duplication showed increased separation compared to saline treated 16p11.2 duplication mice *($p < .001$)*. However, there was no difference in saline and resiquimod groups with the wildtype mice *($p =0.096$)*.

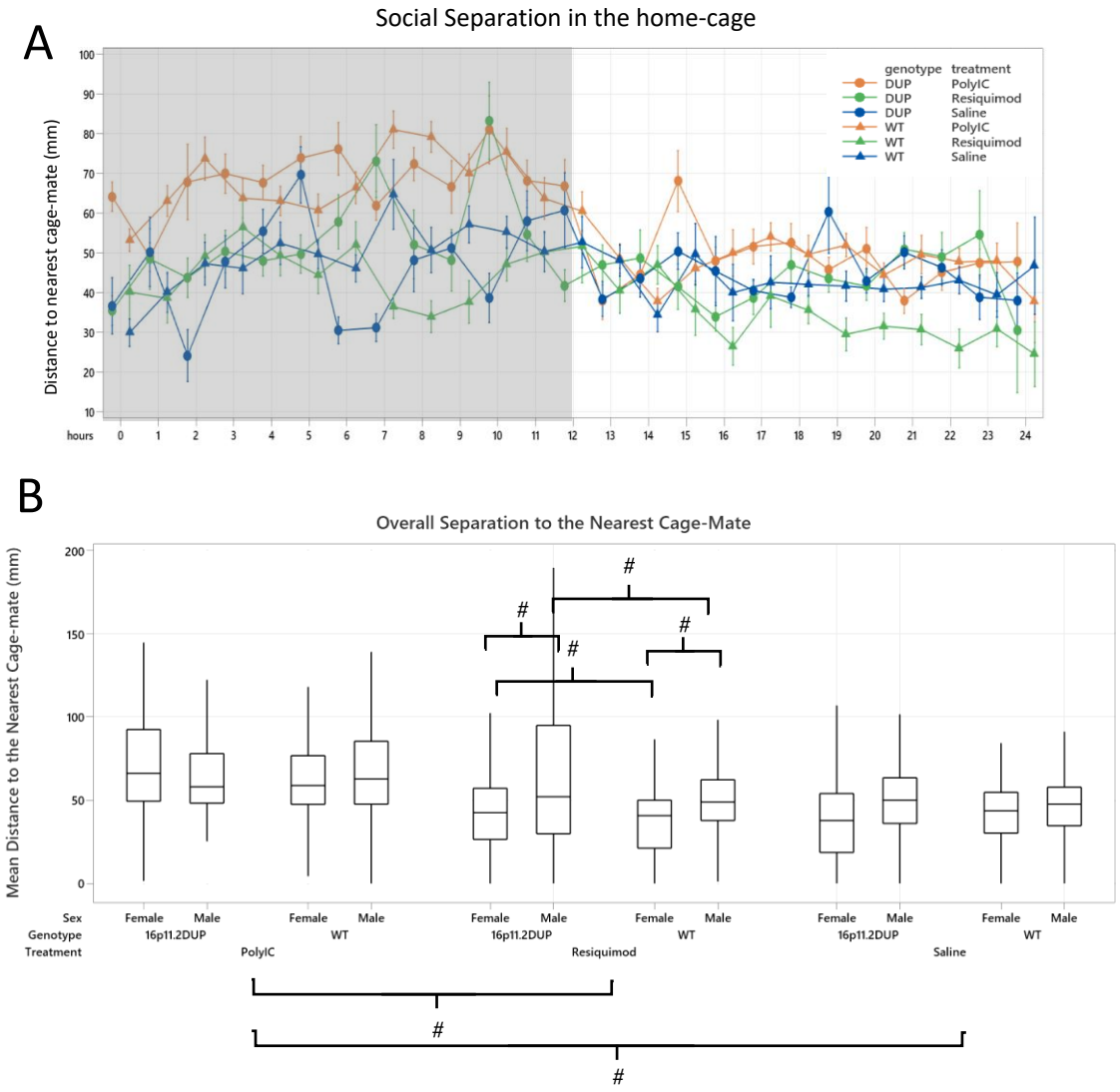

Figure 4.5: Separation from cage-mates in the home cage in WT and DUP mice following MIA or saline. A) Separation from nearest cage mate over a 24 time-period, separated by genotype and MIA treatment group. B) Mean separation from nearest cage mate in the first 12 hour dark-cycle, separated by genotype and MIA treatment group. *N = 54. Grey areas on each graph signify the dark, waking period of the 24 hour light cycle while white background signifies the light, sleeping period of the night cycle. . # indicates differences between means where p < 0.05. *indicates outlier data points.*

4.3.4 Novel Object Recognition

A novel object recognition paradigm was used (figure 4.10a) in order to assess cognitive abilities in the form of recognition of familiar vs novel items in an open field. Time spent with each object was measured before a ratio of exploration of the novel object compared to overall object exploration was calculated. Multi-factorial, nested ANOVA was used to determine overall effects of genotype, sex and treatment on this ratio. Mice failed to show a clear preference for the novel as opposed to the familiar object (ratio of novel/total exploration time not significantly different, $p > 0.5$). Furthermore, there were no significant effects of any factors on novel object recognition. There were also no significant interactions between these factors (Figure 4.9 and 4.10). Finally, there was an assessment of total exploration time, but no significant effects were found.

Comparison	Statistic	
	F-value	p-Value
Genotype	2.20	0.143
Sex	1.37	0.247
Treatment	1.82	0.171
Genotype*Sex	1.16	0.285
Genotype*Treatment	1.16	0.321
Treatment*Sex	0.89	0.414
Genotype*Treatment*Sex	1.72	0.188

Table 4-13: Results of Multi-factorial ANOVA statistics for Novel Object Recognition Tests.

Group	N	Mean	Standard Error	Standard Deviation
Saline, Males, WT	6	1.68	0.686	1.68
Saline, Males, DUP	7	1.74	0.940	2.10
Saline, Females, WT	5	4.29	1.57	3.51
Saline, Females, DUP	5	1.51	0.422	1.12
PolyIC, Males, WT	7	1.63	0.481	1.24
PolyIC, Males, DUP	9	1.30	0.315	0.55
PolyIC, Females, WT	3	1.91	0.434	1.30
PolyIC, Females, DUP	4	1.34	0.393	0.79
Resiquimod, Males, WT	5	1.59	0.518	1.04
Resiquimod, Males, DUP	5	1.39	0.469	1.05
Resiquimod, Females, WT	6	1.36	0.371	0.83
Resiquimod, Females, DUP	11	1.68	0.435	1.44

Table 4-14: Summary data of the Novel Object Index. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group	**Tukey Method Grouping**	
wt saline female	A	
wt polyic female	A	
dup saline male	A	
wt saline male	A	
dup resiquimod female	A	
wt polyic male	A	
wt resiquimod male	A	

dup saline female	A	
dup resiquimod male	A	
wt resiquimod female	A	
dup polyic female	A	
dup polyic male	A	

Table 4-15: The Tukey Method Grouping for the Novel Object Index. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

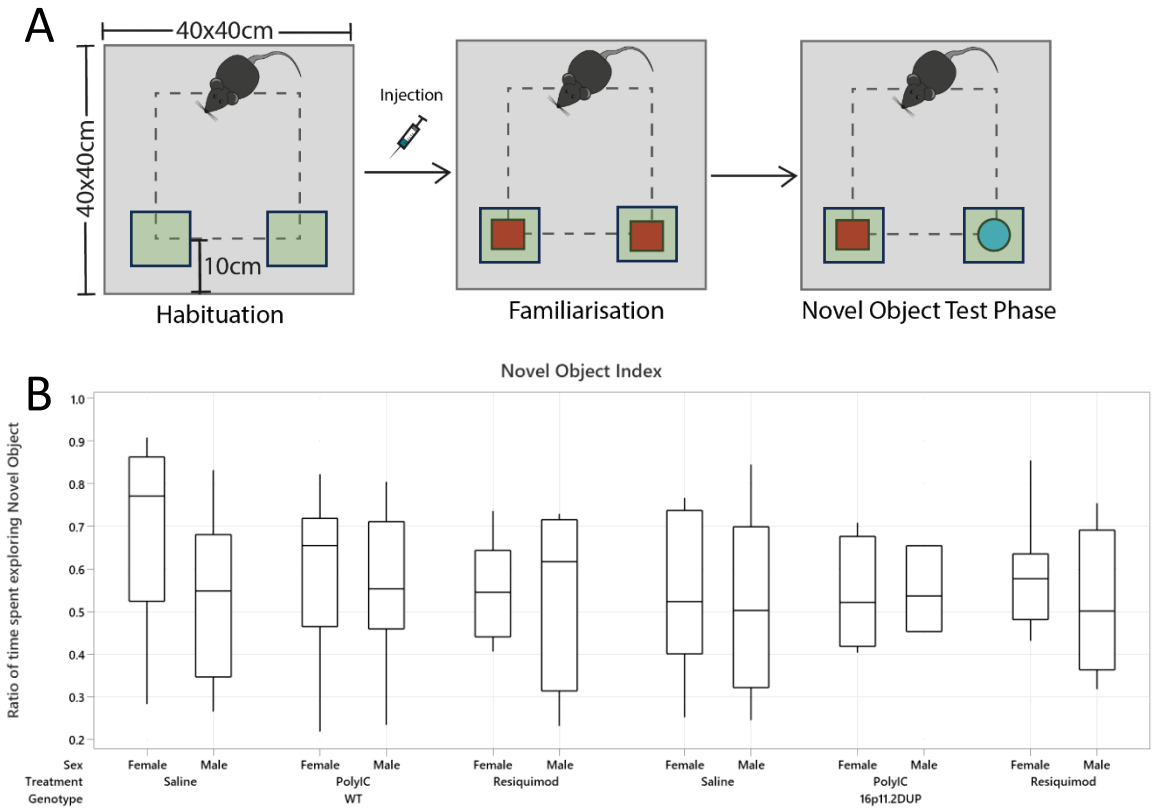

Figure 4.6: **Novel Object Recognition tests in WT and DUP mice following MIA or saline.** A) Diagram depicting the Novel-object recognition behavioural apparatus. B) Novel Object Index for each group. *N = 41 females, 32 males, 39 WT, 34 DUP, 23 saline, 27 resiquimod, 23 Poly IC. # indicates differences between means where p < 0.05. *indicates outlier data points.*

4.4 Discussion

People with schizophrenia show a number of behavioural changes that range from the well-established positive symptoms, such as delusions and hallucinations, to many more complex issues such as anxiety, depression, social withdrawal and cognitive dysfunction (Waltz and Gold, 2007; Betensky *et al.*, 2008; Regier, Kuhl and Kupfer, 2013). However, in animal research on schizophrenia the full repertoire of behavioural symptoms is rarely investigated. As a result, throughout this study, a series of behavioural tests were conducted in an effort to provide a detailed behavioural profile associated with the genetic and environmental risk factors.

The combination of genetic risk in the 16p11.2 duplication, alongside environmental influence through maternal immune activation was conducted as a dual-hit model of schizophrenia. Whilst several studies have identified the 16p11.2 duplication as a CNV which carries relatively high risk of schizophrenia diagnosis (McCarthy *et al.*, 2009; D'Angelo *et al.*, 2016; Niarchou *et al.*, 2019) there are currently no studies on how this risk variant may interact with environmental risk factors. As a result, here we utilise a methodology which aims to replicate the well-known environmental risk factor for schizophrenia development – maternal immune activation – in the 16p11.2 duplication genetic line. This provided valuable information on the effect of gene and environment interactions on overall behavioural outcomes. This has implications for future schizophrenia research as the use of dual-hit models can better replicate the development of schizophrenia in humans.

4.4.1 Anxiety-related behaviours

Anxiety is a well-established component of the negative symptoms of schizophrenia in humans and can result in great distress for those that have been diagnosed. There is evidence that women diagnosed with schizophrenia are more likely to experience anxiety symptoms compared to men (Castle and Murray, 1993; Leung and Chue, 2000; Sommer *et al.*, 2020). Here we aimed to assess changes in anxiety in mice through use of the open-field test, the

elevated plus maze and home-cage continuous behavioural monitoring. These tests provide data on overall locomotor activity as well as anxiety specific behaviours.

Interestingly, there was a marked effect on locomotor activity by several factors. Firstly, the 16p11.2 duplication caused a significant hyperactivity in the open field. However, this was only revealed in the second hour of the experiment. Bristow et al (2020) previously showed hypoactivity in the 16p11.2 dup males compared to their wildtype counterparts however this was in a 30-minute experiment. Given that locomotor activity changes over time as a result of habituation to the environment it is possible this is a habituation effect and the hyperactivity seen here can only be revealed after extended recording. Similarly, there was an effect of sex with females revealing an increase in activity compared to males. Finally, there was a strong effect of maternal immune activation with both Poly IC and resiquimod groups showing increased activity compared to saline groups. Furthermore, there was a clear gene and environment interaction with Poly IC treated 16p11.2 mice showing a marked hyperactivity compared to their wildtype counterparts. There were further interactions between genes, MIA groups and sex. Given that locomotor activity is often used as a measure to provide evidence that experimental interventions have not resulted in adverse effects on motor ability this was a surprising result. However, in this case hyperactivity has occurred rather than reduced motor function. Furthermore, these results are also replicated in the home cage, with locomotor activity increased in females, 16p11.2 duplication mice and poly IC groups. On the other hand, whilst resiquimod resulted in increased activity in the open-field, the opposite effect was found in the home-cage. This emphasises the importance of environmental context in assessing behaviour. While it is possible that the 16p11.2 duplication and female mice in general show increased activity, it is possible that the differences observed in locomotor activity in the open-field and the home cage in the resiquimod group is due to an increased anxiety response in the open-field test environment. In fact, a number of anxiety responses were recorded in the open-field and in the elevated plus maze.

The open-field also revealed interesting data around locomotor activity with a robust result which showed genotype and treatment effects. These effects were particularly evident in the later stages of the test indicating a potential habituation effect which masks potential variations in the early stages. The effect of Poly IC and Resiquimod is perhaps most interesting given that several studies have previously shown either no effect of MIA on locomotor activity, or have shown decreased activity (Smith *et al.*, 2007; Xuan and Hampson, 2014; Depino, 2015; Schaafsma *et al.*, 2017). Supplementary table 3 shows a review of MIA studies which assessed locomotor activity and thigmotaxis in the open-field. Furthermore, these studies also mostly showed decreased thigmotaxis (Smith *et al.*, 2007; Meyer, 2013; Depino, 2015; de Oliveira *et al.*, 2023; Haddad, De Oliveira and Schmid, 2023). This contrasts with the data seen here where MIA showed a clear increase intime spent in the outer edges of the open-field. It is likely that the increased locomotor activity and increased thigmotaxis are both effects of increased anxiety-related behaviours. It is possible that due to the mild stress of being placed in a test environment in the initial time period of the test masks this disparity in anxiety. This would further explain the contrasting results to other studies which record activity in the open-field for significantly shorter periods of time with some recording for only 5 minutes.

Overall, these results are indicative of increased anxiety related behaviours in the open-field with both thigmotaxis and locomotion increasing as a result of genotype, sex and MIA. In fact, these effects mirror data found in humans which show women having higher likelihood of anxiety than men. This suggests that the interactions between genotype, MIA group and sex are also of relevance to how risk factors may interplay in humans to increase severity of symptomology. In fact, there is some evidence in both rodents and humans that MIA can increase anxiety related disorders (Betts *et al.*, 2015; Quagliato, de Matos and Nardi, 2021).

Anxiety related behaviours were also measured in the elevated plus maze. The elevated plus maze has long been used as a measure of anxiety as it exploits the innate desire of rodents to hide in dark enclosed spaces, particularly in

higher stress situations. Mice carrying the 16p11.2 duplication variant have shown (anxiogenic) deficits in time spent in the EPM, particularly in male animals (Bristow *et al.*, 2020). However, another study found no difference between 16p11.2 duplication mice and wildtypes with no differences between sexes (Rein *et al.*, 2021). The EPM has also shown potential anxiety responses in MIA treated animals with MIA causing decreases in time spent in the open arms of the EPM indicating increased anxiety-related hiding in closed arms (Babri, Doosti and Salari, 2014; Depino, 2015; Hollins *et al.*, 2018). In this case, we found that the time spent in the outer arms of the maze increased following maternal immune activation, with both groups showing increased time spent in the open-arms of the maze compared to saline groups. This is the opposite to some other studies which have highlighted an increase in anxiolytic response in the EPM following MIA (Babri, Doosti and Salari, 2014; Depino, 2015; Hollins *et al.*, 2018), but consistent (Schaafsma *et al.*, 2017; de Oliveira *et al.*, 2023), albeit using LPS. There were no other effects with no sex differences or genotype differences identified. This is also perhaps in contrast to previous data which has highlighted a genotype and sex effect (Bristow *et al.*, 2020). With this contrasting data, it is necessary to further delve into potential anxiety responses, and how effectively and robustly they can be detected using this test.

Here we further assessed anxiety through an analysis of thigmotaxis behaviour in the open-field. Thigmotaxis is another innate rodent behaviour which results in proximity to walls rather than the central areas of an open-field. This has been suggested as another measure of anxiety in rodents with less anxious mice more likely to openly explore an arena than more anxious mice (Treit and Fundytus, 1988; Simon, Dupuis and Costentin, 1994). We found that there was no effect of genotype on thigmotaxis behaviour in the open-field. This suggests that despite the hyperactivity identified in the 16p11.2 duplication mice, this is unlikely due to an anxiety specific response. This is further supported by a lack of genotype effect in the EPM. Without further assessment of motor function, it is difficult to say what the hyperactivity may be attributed to. However, with genotype and treatment interactions, it is very likely that

alterations in locomotor activity may be driven further by MIA rather than genotype alone. In fact, MIA showed effects on the EPM and on thigmotaxis. However, the results from these behaviours were opposing with an increase in thigmotaxis in the open-field suggesting an increase in anxiety response while there was an increase in time spent in the outer arms of the EPM suggesting lower anxiety. It is clear therefore that environmental context may be a key factor in type of anxiety response.

Overall, experimental conditions each elicit differing responses that could be attributed to anxiety-related behaviour even within the same mouse groups. However, with effects on locomotor activity as a result of genotype, sex and MIA groups, it is possible this interferes with the recording of anxiety behaviours which rely on movement. Particularly in instances of activity in the open field. For example, female animals showing increased anxiety but decreased thigmotaxis suggests that they exhibit lower anxiety behaviour compared to males. However, if this hyperactivity is driven by a desire to escape the open field rather than to hide then it is possible thigmotaxis cannot measure the true anxiety effects. On the other hand this may indicate an increased desire to explore the arena as has been postulated in research (Treit and Fundytus, 1988; Simon, Dupuis and Costentin, 1994).

Despite differing responses in the two anxiety-related measures, there is a clear genotype and treatment interaction which is evident in locomotor activity, EPM results and in thigmotaxis behaviour. This is primarily driven by the 16p11.2 duplication-expressing mice which show stronger responses compared to the wild type counterparts within the poly IC and resiquimod MIA groups. In particular, the 16p11.2 mice in the Poly IC group often showed the strongest effects compared to other groups. This suggests a possible accumulative effect of genotype and environmental stimuli on the presentation of altered behavioural output and has implications for how we may assess schizophrenia risk factors in the future.

4.4.2 Social Interaction

Social withdrawal and social deficit is a common symptom of schizophrenia which can contribute to increasing isolation. This can be debilitating for those who rely on support networks to maintain treatment plans or for mediating other negative symptoms such as anxiety and depression. In humans, social withdrawal is more prevalent in men than in women (Usall *et al.*, 2002). In order to assess social dysfunction in mice, we utilised home-cage continuous behavioural monitoring to assess proximity to cage-mates over a 24-hour period. Distance to the nearest cage mate can then be used to measure social separation changes over time. This method has shown throughout multiple studies to be an effective measure of altered social behaviours (Bristow *et al.*, 2020; Mitchell, Thomson, *et al.*, 2020; Thomson *et al.*, 2021). There was a clear effect of dark and light periods in the light cycle on separation with mice showing decreased separation during the light period of the cycle. This provides evidence to support the theory that increased separation suggests a decreased social interaction given animals engage in huddling and nesting behaviours during the light, sleeping period of the light cycle and are more likely to engage in individual behaviours during the dark, waking period. As a result, this can be used as a basis for measuring social interaction in mice.

We found that there was a clear effect of genotype, with 16p11.2 duplication mice showing an overall increase in separation behaviour compared to wildtype animals. This concurs with other research which identified a social deficit in the home-cage as a result of the 16p11.2 duplication (Bristow *et al.*, 2020). Furthermore, another study which utilised the novel, social interaction test also identified deficits in the 16p11.2 duplication genotype (Rein *et al.*, 2021). With social withdrawal a major symptom of schizophrenia this is a very relevant result as we are able to replicate the social withdrawal seen in humans in mice.

Maternal immune activation was another factor in altered social withdrawal. Here we showed that maternal immune activation through the use of poly IC but not resiquimod increased separation from cage mates highlighting a social deficit. While resiquimod had no effect in this case, the effect of MIA on social

withdrawal has been established previously. Several studies in rats and in mice have shown social deficits in the novel social interaction test as well as in social play behaviours as a result of maternal immune activation with LPS and Poly IC (Schwartzer *et al.*, 2013; Gzielo *et al.*, 2021; Vitor-Vieira, Vilela and Giusti-Paiva, 2021; Carbone *et al.*, 2023). Combined with results in this study, it is clear that maternal immune activation can have long lasting effects on social ability. It is worth considering how this may be related to the anxiety related effects. If MIA results in increased risk of anxiety related disorders then it is likely this may impact on social interactions. However, social withdrawal is a behaviour more often seen in males both in rodents and humans while females who show increased anxiety, are less likely to exhibit social withdrawal. As a result, it remains plausible that these are two independent behaviours which are impacted separately.

Most importantly, we were able to identify a number of interactions between genetic and environmental factors. There was a clear interaction between genotype and MIA treatment group driven primarily by results from litters who received MIA trough resiquimod. Whilst Poly IC groups showed the highest level of separation from cage-mates there was a significant increase in separation from 16p11.2 mice in the resiquimod group compared to wildtype counterparts and all saline animals. This suggests that the interaction between genotype and MIA is required to elicit behavioural responses as a result of resiquimod. This contrasts with the Poly IC data which shows no difference between 16p11.2 duplication and wildtype mice and suggests the behavioural effects are down to poly IC alone. Showing that resiquimod elicits a genotype specific response to social deficits is of importance to schizophrenia research for two reasons. Firstly, resiquimod more directly mimics the immune response associated with viral infections that have been shown to increase likelihood of schizophrenia diagnosis. As a result, it is possible that this mechanistically replicates events which occur to result in schizophrenia more accurately. Secondly, this provides evidence to support the theory that specific genes may combine with environmental stimuli to predispose to development of psychiatric disorders.

Resiquimod also elicited a sex-specific response with male animals from resiquimod litters showing significantly increased separation compared to female counterparts and saline groups. This concurs with research on schizophrenia in humans in which men are more likely to experience social deficits compared to women. This is another case in which resiquimod MIA more accurately represents the natural variations of symptomology than Poly IC in which there was no sex dependent effects.

4.4.3 Cognitive abilities

Another key behavioural phenotype of people with schizophrenia is a cognitive deficit which can be traced to earlier life prior to diagnosis and into childhood (Waltz and Gold, 2007; Nuechterlein et al, 2004). These cognitive deficits are seen in both men and women with schizophrenia and can result in difficulties with working memory, spatial memory and visual or verbal learning processes as well as dysfunction in more basic cognitive processes such as reasoning and attention (Nuechterlein et al, 2004). Studies in animal models relevant to schizophrenia have also identified cognitive deficits. Several investigations which utilise NMDA receptor antagonists to imitate the NMDA receptor hypofunction found in schizophrenia have identified cognitive deficits in different behavioural paradigms such as cognitive flexibility, visual learning and memory (Brigman *et al.*, 2009; Fellini *et al.*, 2014; Hauser, Isbrandt and Roeper, 2017; Savolainen *et al.*, 2021). Maternal immune activation with LPS or Poly IC have also resulted in cognitive impairments in both mice and rats ranging from spatial learning and memory to attention(Ito *et al.*, 2010; Simões *et al.*, 2018; Sun *et al.*, 2022; Woods *et al.*, 2023). Finally, cognitive deficits have also been identified in mice with the 16p11.2 duplication including working memory and spatial or visual learning and memory (Yang *et al.*, 2015; Bristow *et al.*, 2020; Rein *et al.*, 2021).

In order to assess cognitive ability in mice, we utilised a commonly used measure of visual working memory; the novel object recognition test (Antunes and Biala, 2012; Lueptow, 2017). By exploiting the innate desire of a mouse to explore new objects in an environment it is possible to take a measure of visual

and spatial memory with mice more likely to explore a novel object compared to a familiar object (Antunes and Biala, 2012). However, in this case we did not identify any specific preferences between groups for the novel object. In fact, in this case mice explored the novel object for only marginally longer than the familiar object. This suggests that even the control group of wildtype mice from saline MIA groups did not show a novel object preference. It is highly likely that this is due to the experimental design of the NOR test. Mice are given a short 5-minute habituation period to the open field before removal from the test arena whilst two identical objects are placed into the open-field for the familiarisation phase. Mice are then placed back into the open-field and given another short period of 5 minutes in the open-field or a maximum of 15 seconds of exploration time. The fast nature of this is experiment is often cited as a valuable reason for its use (Antunes and Biala, 2012; Lueptow, 2017). However, given that the open-field experiments here have shown that habituation effects can last for up to an hour in the open-field and combined with the added handling involved in the NOR, it is highly likely that animals are showing heightened stress responses at this early period. Increased anxiety has been shown to impair working and spatial memory in mouse anxiety models (Santin *et al.*, 2009; Darcet *et al.*, 2014). In this case, if the test environment is increasing stress response it is potentially masking complex cognitive function.

Furthermore, the total exploration time in both the familiarisation phase and the novel object phase is very short. It is possible the familiarisation phase of the experiment is not long enough for animals to become truly familiar with the object. Particularly in instances where higher stress scenarios might influence desire for exploratory behaviour. With these factors in mind, it would be prudent to more extensively assess cognitive behaviour in these animals utilising a longitudinal experiment such as paired discrimination and reversal learning protocols. This would allow for adequate habituation to a test environment over a number of days with a longitudinal study of learning across multiple test sessions. Utilising this behavioural paradigm would therefore provide a better characterisation of cognitive abilities in these mice.

4.4.4 Conclusions

To conclude, in order to assess how genes and environmental stimuli might interact to increase risk of schizophrenia diagnosis a dual-hit model of schizophrenia risk was used. Using mice with the 16p11.2 duplication genotype which carries high risk for schizophrenia development, this was combined with a maternal immune activation protocol that compared saline, poly IC and resiquimod. There was some differences in anxiety-related behaviours that were dependent on behavioural tests used and potential effects of locomotor activity. In the EPM, males and 16p11.2 duplication mice appeared to show decreased anxiety related responses but thigmotaxis behaviours in the open-field demonstrated the opposite. MIA group also appeared to show increased thigmotaxis in the open field but the opposite in the EPM. This highlights how environmental context can elicit contrasting results and as a result produce opposing effects. These data highlight the importance of considering the environmental context of experimental tests as a factor in future research. Whilst there were no significant effects on cognitive ability in the NOR for any factors, the acuity of the test to detect cognitive processes is unclear, and the influence of environment is likely at play. Longitudinal assessments of cognitive ability where animals are able to more effectively habituate and acclimate to experimental environments would likely provide richer data on cognitive functions in this case.

On the other hand, there were a number of clear social deficits which concur with the literature on different models related to schizophrenia. There was an increase in social withdrawal in the 16p11.2 duplication mice compared to wildtype counterparts as well as an overall effect of poly IC. However, there were also interesting interactions within the resiquimod group which perhaps more accurately replicate the accumulative effects of risk factors for schizophrenia development in humans. Namely, resiquimod groups showed a sex-dependent and genotype dependent social withdrawal. Males and 16p11.2 duplication containing mice in this group showed increased social withdrawal compared to female and wildtype counterparts. Indeed, people carrying this

duplication are particularly impaired in social cognitive processes (Taylor et al., 2023; Gur et al., 2024; Verbesselt et al., 2024). With evidence in humans to show males diagnosed with schizophrenia more often experiencing social withdrawal and theories that genes and environmental stimuli may interact to result in schizophrenia these results have important implications for future schizophrenia research.

In summary examining social behaviours in a home-cage environment has revealed important differences between MIA treatments as well as demonstrating an important gene-environment interaction in 16p11.2 duplication mice receiving resiquimod *in utero* through MIA. This has implications for neuropsychiatric research with potential for the creation of more translational research which more closely mimics intrinsic development of complex neurobiological disorders.

4.4.5 Future Directions

There are a number of further areas of research which have arisen from these results that given more time would be useful to continue. Firstly, expanding the behavioural repertoire in order to do a full assessment of environmental contexts and how they may influence behaviour would be prudent given the results here. This would include carrying out another measure of sociability by using the 3-chamber novel social interaction test. This test is carried out over a shorter recording period and relies on placing mice in a novel environment with a mouse it has not met before to determine how often it decides to interact with the novel mouse. This can be very stressful for both animals involved considering the territorial nature of rodents and compounded with the novel test environment it would be interesting to investigate how this might elicit differing responses to social behaviours seen in the home cage. Furthermore, assessing cognitive abilities utilising a more complex, longitudinal cognitive experiment such as the paired discrimination and reversal learning paradigm could provide rich data on learning and memory capabilities. Comparing this to the results in the NOR experiments here would further

highlight whether there is a strong effect of test environment on behavioural output.

Additionally, given the contrasting results seen here on anxiety-related behaviours it would be prudent to do further analysis. With the rich data that can be analysed within the home-cage developing the technology further to assess more complex behaviours could provide valuable information. This could include vocalisations, grooming behaviours, nesting behaviours and individual social interactions. Ability to assess these in more detail would allow a clear picture of baseline anxiety levels. Comparing this further to current assessments could clarify the true effects of each risk factor on anxiety.

4.4.6 Limitations

The primary limitation of this study is in the group sizes which are variable when split into each factor. This is due to difficulty in producing balanced numbers for sex and genotype during breeding. To minimise waste of animals in line with ARRIVE guidelines and the 3Rs, all mice went through behavioural analysis and breeding continued until a minimum of three animals per group existed. In the end, there were group sizes of 3-11 mice resulting in a relatively large variability in group size.

There are also a number of limitations within behavioural testing which can influence results including time of day for behavioural tests, time of year and staffing. Given these experiments occurred over the period of COVID lockdowns and there were variable number of people within the lab environments at different times of year it is possible some variability exists. To mitigate this, mice from each group were assessed at different times of the day to ensure there was no circumstance in which mice from one group were always tested in the morning and others in the afternoon.

5 Results: Neurobiological profile of dual-hit gene x environment model of schizophrenia

5.1 Introduction

Research into schizophrenia has identified a number of factors which individually carry risk for the development of schizophrenia including genetic and environmental factors (McDonald and Murray, 2000; Canetta and Brown, 2012; Janoutová *et al.*, 2016). The 16p11.2 duplication carries a relatively high risk for the development of schizophrenia in adulthood and has become a copy number variant of interest in research (McCarthy *et al.*, 2009; Chang *et al.*, 2017). However, it is clear that while individual genetic variants may increase likelihood of schizophrenia there are other factors at play. Epidemiology studies have identified that maternal immune activation as a result of viral infection during pregnancy is another risk factor for the development of a number of psychiatric disorders (Canetta and Brown, 2012; Janoutová *et al.*, 2016; Aguilar-Valles, Rodrigue and Matta-Camacho, 2020). Researchers have therefore hypothesised that there is an accumulative effect of genetic and environmental risk factors which combine to result in eventual diagnosis. There is a growing literature in investigating the neurobiological impact of gene-environment interactions in rodent models relevant to schizophrenia (reviewed in Kannan, Sawa and Pletnikov, 2013). However, to date no studies have explored the impact of maternal immune activation combined with CNVs that confer a high penetrance for developing schizophrenia Here we utilise a dual-hit model, combining the 16p11.2 duplication risk variant with a maternal immune activation protocol to determine if there are lasting biological changes to the brain which may replicate changes that are found in humans with schizophrenia.

Post-mortem studies of patients with schizophrenia have shown significant changes in neuronal markers compared to the brains of those with no diagnosed psychiatric disorders. The identification of NMDA receptor hypofunction as a potential mechanism for symptoms of schizophrenia was highlighted when researchers showed that PCP could result in a schizophrenia-like psychosis. This lead to further research into the NMDA receptor theory of schizophrenia before post-mortem studies confirmed that NMDA receptor expression was indeed decreased in patients with schizophrenia (Weickert *et al.*, 2013). Dysfunctional prefrontal cortex (PFC) interconnectivity with the thalamus has also highlighted functional deficits in schizophrenia (Dawson, Morris and Pratt, 2015) as well as dorsal PFC and connectivity with the hippocampus (Meyer-Lindenberg *et al.*, 2005). Finally, post-mortem studies of human brains have indicated that altered inflammatory markers are also found in patients with (Trépanier *et al.*, 2016) while peripheral inflammation was also associated with decreased cortical and hippocampal volume as well as more severe clinical outcomes in patients with schizophrenia (Upthegrove and Khandaker, 2020; Lizano *et al.*, 2021). Overall, there are a number of neuronal markers of interest in schizophrenia which have been identified through post-mortem human brain studies and animal research.

5.1.1 Neuronal Signalling Markers

The NMDA receptor hypofunction theory of schizophrenia suggests the basis of altered brain activity and effects on behaviour could be due to neuronal signalling dysfunction. As a result, research has looked into GABAergic interneuron function, expression and activity in people with schizophrenia. Parvalbumin positive (PV) interneurons are important in fast-spiking GABAergic activity and have shown deficits in schizophrenia. Post-mortem studies identified not only a reduction in density of PV expressing neurons (Kaar *et al.*, 2019) but further deficits indicated decreased density of excitatory neurons in contact with PV neurons (Chung, Fish and Lewis, 2016). This has also been replicated in mouse models of schizophrenia which have identified altered PV expression. In a 16p11.2 duplication mouse model of schizophrenia PV neurons had reduced expression (Willis, Pratt and Morris, 2022). This has been evident

in a number of other studies in animal models which also identified reduced expression of PV neurons (Belforte *et al.*, 2010; delPino *et al.*, 2013).

These studies support the potential role of GABAergic signalling dysfunction in schizophrenia and as such, studies investigate further how this may be affected in other cell types. Somatostatin positive interneurons (SST) also have a fundamental role in GABAergic signalling but contrastingly to PV expressing neurons exhibit slow-spiking neuronal activity. Similarly to PV, post-mortem studies have shown a decreased expression of SST expressing interneurons (Morris, Hashimoto and Lewis, 2008; Konradi *et al.*, 2011) in humans with schizophrenia. In mice, a study which investigated the effects of knock-down of SST and PV demonstrated behavioural deficits related to schizophrenia as well as similar structural changes in PFC and hippocampal interconnectivity that has been seen in patients with schizophrenia (Perez, Boley and Lodge, 2019). Decreased SST expression has also been identified in other studies on schizophrenia (Van Derveer *et al.*, 2021; Dienel, Fish and Lewis, 2023).

Overall, it is clear that PV and SST interneurons are of relevance to the biological phenotype of schizophrenia and that any mouse model would more robustly replicate schizophrenia if these neuronal signalling markers were reduced.

5.1.2 Inflammatory Signalling Markers

The role of inflammation in schizophrenia has become a prevalent area of interest due to a clear correlation between schizophrenia and increased inflammatory cytokine production. In particular, the cytokine IL6 which has been shown in the literature to be increased in schizophrenia (Fillman *et al.*, 2013; Trépanier *et al.*, 2016; Miller and Goldsmith, 2019; Lizano *et al.*, 2021). Other inflammatory markers have also been shown to be increased in schizophrenia. A number of studies have identified TNFa as a relevant marker of inflammation in patients with schizophrenia (Paterson *et al.*, 2006; Dimitrov *et al.*, 2014; Hoseth *et al.*, 2017; Lizano *et al.*, 2021). Additionally, a microglial cell activation marker – CD68 – has also shown an increase in expression in

schizophrenia (De Picker *et al.*, 2021; Snijders *et al.*, 2021; Jenkins, Lewis and Volk, 2023).

Since maternal immune activation has been identified as a risk factor for the development of schizophrenia, it is clear that immune involvement is an important factor from early in neurodevelopment. These studies corroborate this. However, the exact mechanism by which this may occur is unclear. Microglial activation during embryonic brain development resulting in increased synaptic pruning has been hypothesised as a potential mechanism of action (Boksa, 2012; Sellgren *et al.*, 2019). It seems logical therefore that inflammatory cytokines such as TNFa and IL6, or markers of microglial activation such as CD68 would be raised. However, these studies have all highlighted that increases in these markers are evident in adult humans. As a result, it appears that prolonged inflammation beyond embryonic development is a key factor in schizophrenia and assessing sustained inflammation should be an area of focus in schizophrenia research.

5.1.3 Dock Proteins

Recent CNV and exome studies have identified a family of proteins which may be of future interest in understanding schizophrenia development with individual genetic variants identified in people with schizophrenia (Shi, 2013; Samani *et al.*, 2022). These dedicators of cytokines (DOCKs) are a family of 11 Rho GTPase regulators which function as guanine nucleotide exchange factors (GEFs) to mediate a variety of cellular pathways. DOCKs primarily activate two forms from the Rho GTPase family: Rac1 and CDC42, which each have varied roles in intracellular signalling. A number of these DOCKS have been implicated in schizophrenia and other psychiatric disorders (Namekata *et al.*, 2012, 2014, 2019, 2020; Guo *et al.*, 2021; Umehara *et al.*, 2021; Samani *et al.*, 2022).

DOCK8 is highly expressed in the microglial cells (Mortazavi *et al.*, 2008) and has been shown to have a genetic variation associated with schizophrenia (Glessner *et al.*, 2017). Furthermore, DOCK8 is hypothesised to be important for microglial activation and regulates microglial functions during

neuroinflammatory conditions (Namekata et al., 2019, 2020; Sellgren et al., 2019). Therefore, it stands to reason that DOCK8 may also be involved in the excessive synaptic pruning which is hypothesised to be a result of microglial overactivation (Sellgren *et al.*, 2019) during embryonic development, increasing risk of schizophrenia development.

DOCK11 is also found in the microglial cells of the CNS (Mortazavi *et al.*, 2008). Two different GEO data sets showed a strong link between DOCK11 and maternal immune activation with MIA through LPS or Poly IC both increasing expression of DOCK 11 immediately after injection as well as once pups were born. Despite the lack of direct studies into DOCK11, a search into a GEO data set of maternal immune activation studies yielded a strong link between MIA and DOCK11 (Namekata et al, 2012; GEO Accession number: GSE34058; Oskvig et al, 2012; GEO Accession number: GSE117327). With the relevance of microglial cell activity in synaptic pruning (Sellgren *et al.*, 2019) and microglial activation markers such as CD68 showing upregulation (De Picker *et al.*, 2021; Snijders *et al.*, 2021; Jenkins, Lewis and Volk, 2023) it may be prudent to investigate further the mechanisms by which DOCK 11 may be involved.

5.1.4 Biological markers in a two-hit model for schizophrenia

Here I utilised a two-hit model for schizophrenia which exhibited a number of behavioural effects (see MIA results chapter 4). This model utilised the 16p11.2 duplication genetic mouse line which has been shown to replicate similar changes in regional brain connectivity to that in patients with schizophrenia (Bristow *et al.*, 2020) as well as similar alterations in neuronal markers such as PV and SST (Willis, Pratt and Morris, 2021, 2022). Additionally, in both wild-type and 16p11.2 dup mice a maternal immune activation protocol was induced using saline, Poly IC or resiquimod. Whilst Poly IC is a commonly used immunogenic for maternal immune activation due to the robust evidence of an altered immune response in mice (Hameete *et al.*, 2021) it was decided to include a second immunogenic which more accurately mimics TLR7 receptor activation. TLR7 receptor activation occurs during infection of single-stranded viruses such as influenza and rubella which have been shown to increase risk of

schizophrenia (Hemmi *et al.*, 2002; Isobe *et al.*, 2006; Kwon *et al.*, 2021). Whilst Poly IC induces an immune response this occurs through TLR3 activation, more closely mimicking double-stranded viral infection. As a result, resiquimod, a TLR7 agonist was used here for comparison.

I hypothesise that in this two-hit model there will be a number of effects. Firstly, 16p11.2 dup genotype mice will have altered neuronal signalling marker changes in PV and SST expression. Furthermore, there will be a treatment and genotype interaction for changes in PV and SST expression. I hypothesise that MIA will increase expression of IL6, TNFa and CD68 compared to saline groups. Finally, DOCK 8 and DOCK 11 expression will be increased following MIA treatments compared to saline groups with a genotype and treatment interaction.

5.2 Methods

5.2.1 Animals

Mice used throughout these experiments are as indicated in figure (Figure 2.2). All animals were housed in social groups in a temperature-controlled room with light cycle reversed. In total, 41 female and 32 males were used and were between 8 and 16 weeks old. There were 39 wild type and 34 16p11.2 duplication mice. Animals were taken from litters which had undergone a maternal immune activation protocol as described in section (methods chapter subsection 2.1.3) and as such had been treated during embryonic development with subcutaneous saline, resiquimod (2 mg/kg) or Poly I:C. (20mg/kg). For each immunogenic treatment there were male and female animals of each genetic line resulting in groups of varying sizes (n = 3-11; see Figure 2.2 in methods section).

Once mice had reached adulthood they underwent behavioural experiments as detailed in (Section 2.2 and Chapter 3) before brains were collected and dissected to isolate PFC and hippocampus as well as spleen. Samples were stored at -80°C until used for biochemical analysis. Total number of animals in each group are shown below.

Group	**Number of animals**
Female WT (Saline MIA)	7
Male WT (Saline MIA)	6
Female DUP (Saline MIA)	5
Male DUP (Saline MIA)	5
Female WT (Resiquimod MIA)	5
Male WT (Resiquimod MIA)	5
Female DUP (Resiquimod MIA)	11
Male DUP (Resiquimod MIA)	6
Female WT (Poly:IC MIA)	9

Male WT (Poly:IC MIA)	7
Female DUP (Poly:IC MIA)	4
Male DUP (Poly:IC MIA)	3
Total	**73**
Total Male	32
Total Female	41
Total WT	39
Total DUP	34

Table 5-1: Table depicting number of animals in each sub group, separated by genotype, sex and MIA treatment

5.2.2 cDNA Extraction

5.2.2.1 RNA Isolation

Samples were removed from the -80°C freezer and placed on ice. 10µl of ß-mercaptoethanol was added to each 1ml of Buffer RLT with enough solution made for the number of samples. Samples which weighed less than 20mg were placed into Lysis Matrix tubes containing 1.4mm Zirconium Silicate Spheres with 350µl ß-mercaptoethanol and Buffer RLT solution. If the sample weighed between 20mg and 30mg, 600µl ß-mercaptoethanol and Buffer RLT solution was added instead. If the sample weighed more than 30g it was sliced to a smaller size and excess sample stored at -80°C.

Tubes were then placed into a Hybaid Ribolyser for 60 seconds in 20 second increments before being centrifuged. Supernatant was collected and centrifuged again before being added to a 1:1 equivalent volume of 70% ethanol. This was then loaded onto RNeasy minicolumns with a maximum of 700µl loaded at a time. Minicolumns were centrifuged and flowthrough discarded before loading any remaining sample.

Once all samples were loaded onto RNeasy minicolumns, 350µl of buffer RW1 was added and centrifuges with flowthrough discarded. Then 80µl of DNase

incubation mix was added and left at room temperature for 15 minutes. A second load of 350µl of buffer RW1 was added and centrifuged with flowthrough discarded. 2 cycles of loading 500µl Buffer RPE and centrifuging before discarding flowthrough were completed. Finally, 50µl of RNase free water were added to the column and left at room temperature for 5 minutes. After centrifuging the sample of isolated RNA was stored at -80°C or if possible immediately used to synthesize cDNA.

5.2.2.2 cDNA Synthetics from isolated RNA

First the quantity of RNA in each sample was measured using a nanodrop. The volume of sample which contained 0.2µg of RNA was calculated and combined with RNAase free water to bring the total volume to 9µl. This was mixed with 10µl of RT Buffer and 1µl of 20X high capacity enzyme mix. Samples were then placed into a thermocycler for 60 minute at 37°C then 5 minutes at 95°C to deactivate enzymes before being cooled to 4°C until collected. 80µl of nuclease free water was added to dilute the samples before storage at -20°C.

5.2.3 Reverse Transcription Quantitative Polymerase Chain Reaction (RT qPCR)

A master-mix containing; 5µl 2X Fast SYBR Green Master Mix (Agilent), 0.3µl of the relevant forward and reverse primer, 25µl of NFW and 0.15µl of SYBR Dye was made for the appropriate number of samples. 9µl of this master-mix and 1µl of cDNA was then pipetted into 10µl wells of a 48-well plate before the plate was sealed. The plate was first shaken for 30 seconds before being centrifuged to ensure no bubbles were present in the wells. The RT qPCR was then started with 1 cycle of 2 minutes at 50°C followed by 1 cycle at 95°C for 2 minutes. There were then 40 cycles of 10 seconds at 95°C followed by 30 seconds at 60°C. Once completed the cycle number at which the sample reached the fluorescence threshold of SYBR Green (Ct) is collected and can be directly related to the expression of cDNA.

5.2.4 Data Analyses

After collection of RT-qPCR data the 2^-ΔΔCt method was used for analysis to normalise the data to GAPDH which was used as the housekeeping gene with WT saline animals used as the control group. Data were then analysed using Minitab, and all experiments were statistically tested using multi-factorial, nested ANOVAs. The independent variables in these experiments were treatment (three levels; PolyIC, Resiquimod and vehicle), genotype (two levels, 16p11.2 duplication and wildtype) and sex (two levels; male and female). Mouse ID and PCR were also included as nested factors within the independent variables of treatment, genotype and sex to account for individual differences for these factors. The dependent variables which were analysed was the expression of PV, SST, IL6, TNFa, CD68, DOCK8 and DOCK11. Additionally, interactions between the independent variables of sex, genotype, treatment and time were also measured as part of the multi-factorial ANOVA. Tukey post-hoc tests were used to determine differences between individual means and significantly different groups are shown through Tukey's method grouping with full lists of pairwise comparisons provided in the supplementary materials. Tukey's method of grouping puts all groups which are not statistically significantly different from one another into a single group which is assigned a letter (e.g group A). Groups which are significantly different from each other are assigned a separate letter (e.g. group B). Some groups can be part of both group A and group B which means there is not significant difference in the means from both groups. However, groups which are only in Group A are significantly different to those in Group B.

5.3 Results

5.3.1 Neuronal Signalling Markers

Following dissection of PFC from brains of mice derived from a dual-hit model of schizophrenia whereby, RT-qPCR tests were carried out to identify changes in neuronal signalling markers PV and SST. This would identify in effects of MIA and 16p11.2 duplication on these markers as well as interactions between genotype, sex and MIA group. A summary of multi-factorial, nested ANOVA for these markers are shown in Figure 5.1. These results showed that there was no significant effect of genotype, sex or treatment on paravalbumin expression however there was a significant interaction of treatment and sex (table, p = 0.022) driven primarily by males in the resiquimod treatment group who showed a significant decrease in PV expression compared to females with saline (p = 0.042). For somatostatin there were no significant effects of genotype, sex or treatment and there were no significant interactions.

Comparison	PV		SST	
	F-value	p-value	F-value	p-value
Genotype	0.84	0.364	1.13	0.293
Sex	2.27	0.137	1.38	0.246
Treatment	0.93	0.401	0.69	0.504
Genotype*Sex	0.41	0.523	0.15	0.704
Genotype*Treatment	1.32	0.274	0.63	0.535
Treatment*Sex	4.09	*0.022	0.36	0.698
Genotype*Treatment*Sex	2.11	0.131	0.83	0.441

Table 5-2: Statistical tests for neuronal signalling markers in mouse PFC. *N = 73. *p < 0.05.*

		PV			SST		
Group	N	Mean	Standard Error	Standard Deviation	Mean	Standard Error	Standard Deviation
Saline, Males, WT	6	-1.481	0.361	1.022	-1.139	0.257	0.727
Saline, Males, DUP	5	-1.027	0.224	0.388	-0.684	0.217	0.376
Saline, Females, WT	7	-0.765	0.088	0.216	-1.749	0.368	0.902
Saline, Females, DUP	5	-1.066	0.162	0.429	-1.151	0.381	1.009
PolyIC, Males, WT	7	-0.850	0.097	0.257	-1.169	0.328	0.868
PolyIC, Males, DUP	3	-1.061	0.027	0.047	-0.624	0.144	0.249
PolyIC, Females, WT	9	-1.934	0.478	1.264	-1.010	0.266	0.703
PolyIC, Females, DUP	4	-0.867	0.089	0.179	-1.049	0.270	0.540
Resiquimod, Males, WT	5	-1.700	0.231	0.517	-1.060	0.270	0.604
Resiquimod, Males, DUP	6	-2.030	0.616	1.509	-1.222	0.219	0.536
Resiquimod, Females, WT	5	-1.256	0.449	1.004	-1.363	0.498	1.113

Resiquimod, Females, DUP	11	-1.121	0.285	0.854	-1.601	0.725	2.176

Table 5-3: Summary data for expression of PV and SST as measured by RT-qPCR. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group (PV)	**Tukey Method Grouping**	
WT F PolyIC	A	
WT M Resiquimod	A	
DUP M Resiquimod	A	
WT M Saline	A	
DUP M Saline	A	
DUP F Saline	A	
WT F Resiquimod	A	
DUP M PolyIC	A	
WT M PolyIC	A	
DUP F Resiquimod	A	
DUP F PolyIC	A	
WT F Saline	A	

Table 5-4: The Tukey Method Grouping for the expression of PV as measured by RT-qPCR. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

Group (SST)	Tukey Method Grouping	
WT F Saline	A	
DUP F Resiquimod	A	
DUP M Resiquimod	A	
WT M PolyIC	A	
WT F Resiquimod	A	
WT M Resiquimod	A	
DUP F Saline	A	
DUP F PolyIC	A	
WT F PolyIC	A	
WT M Saline	A	
DUP M Saline	A	
DUP M PolyIC	A	

Table 5-5: The Tukey Method Grouping for the expression of SST as measured by RT-qPCR. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

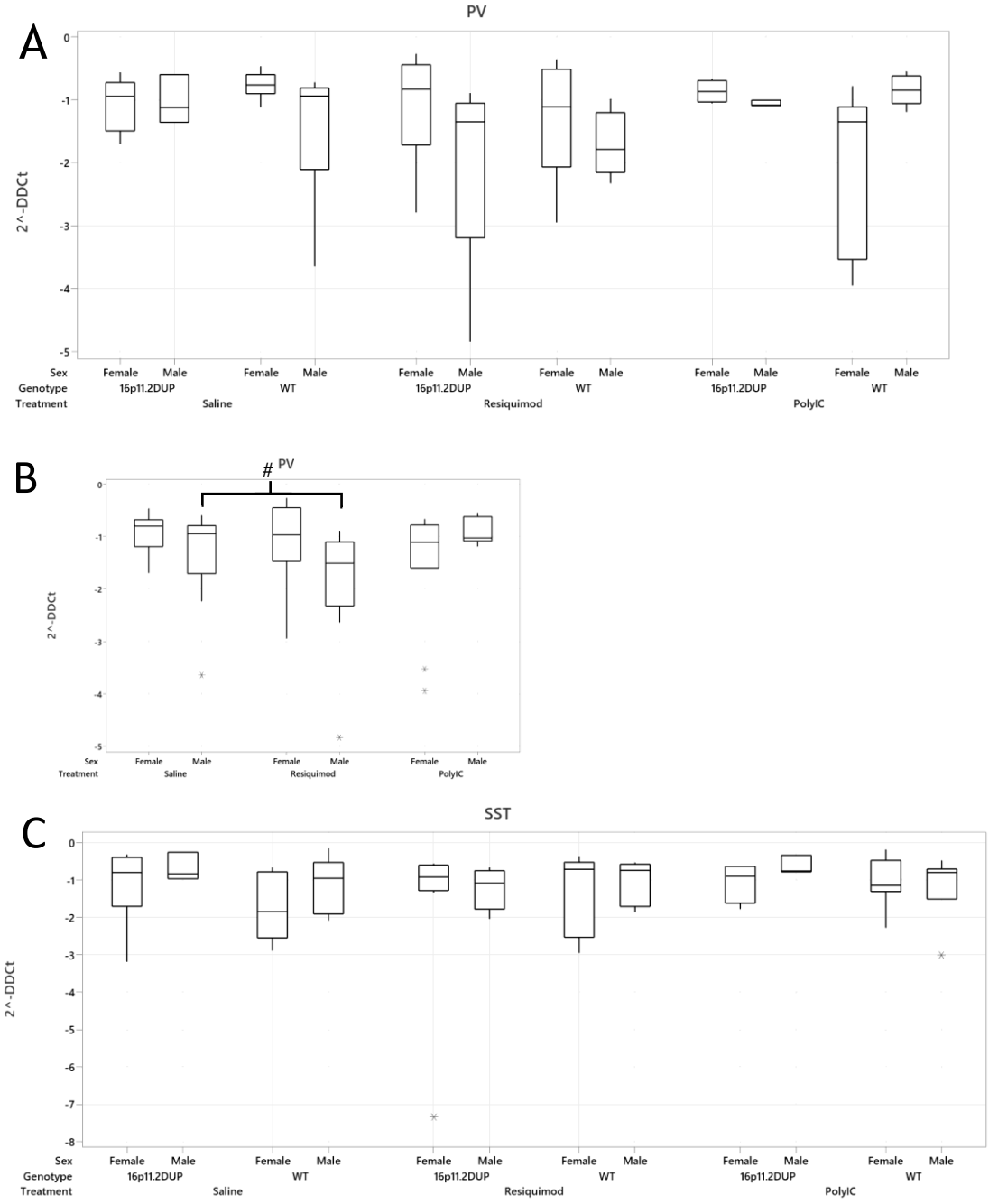

Figure 5.1: Neuronal signalling markers in the PFC of adult wt and 16p11.2 duplication mice following MIA protocols with saline, resiquimod or poly IC. A) PV expression in PFC of mice separated by MIA treatment, genotype and sex. B) PV expression in PFC of mice separated by MIA treatment and sex C) SST expression in PFC of mice separated by MIA treatment, genotype and sex *N = 41 females, 32 males, 39 WT, 34 DUP, 23 saline, 27 resiquimod, 23 Poly IC. P# indicates differences between means where p < 0.05. *signify outlier data points.*

5.3.2 Inflammatory Markers

Following dissection of PFC from brains of mice derived from a dual-hit model of schizophrenia whereby, RT-qPCR tests were carried out to identify changes in inflammatory markers TNFa, CD68 and IL 6. The impact of MIA in 16p11.2 duplication carrying mice on inflammatory markers is summarised in Figure 5.3.

It is first important to note that during RT-qPCR experiments for TNFa and CD68, a fluorescence was recorded in the RT-qPCR wells which did not contain enzymes necessary for the formation of cDNA from the extracted mRNA. This suggest that a small amount of cellular DNA was extracted alongside mRNA.

Multi-factorial ANOVA showed there weas no significant effect of genotype, sex or treatment on TNFa. However, there was a significant interaction with treatment and sex ($p = 0.036$) and this was driven by a difference in male and female responses to resiquimod ($p = 0.038$). Treatment did have a significant effect on the expression of IL6 ($p = 0.001$) and on CD68 ($p = 0.004$). Post-hoc analysis showed that there was a significant increase in expression of IL6 following Poly IC MIA compared to Saline ($p = 0.001$) and Resiquimod ($p = 0.006$) groups. Whilst in CD68, there was a significant increase in expression following Poly IC compared to Resiquimod ($p= 0.004$) but not saline ($p = 0.442$).

In summary, whilst there were no gene-environment interactions, there were differences between PolyIC and resiquimod in their ability to alter IL6 and CD68 expression.

Comparison	TNFa		IL6		CD68	
	F-value	p-value	F-value	p-value	F-value	p-value
Genotype	0.52	0.473	1.71	0.197	0.00	0.993
Sex	2.16	0.147	0.54	0.467	0.35	0.559
Treatment	1.52	0.228	8.15	0.001*	6.07	0.004*
Genotype*Sex	0.57	0.454	0.01	0.921	1.13	0.292
Genotype*Treatment	0.48	0.624	1.30	0.281	0.65	0.526
Treatment*Sex	3.53	0.036*	1.78	0.179	0.11	0.897
Genotype*Treatment*Sex	0.54	0.587	0.04	0.962	1.31	0.279

Table 5-6: Statistical tests for inflammatory markers in mouse PFC. N = 73. *p < 0.05.

		TNFa		
Group	N	Mean	Standard Error	Standard Deviation
Saline, Males, WT	6	-1.051	0.153	0.433
Saline, Males, DUP	5	-0.811	0.053	0.092
Saline, Females, WT	7	-1.103	0.178	0.436
Saline, Females, DUP	5	-1.104	0.206	0.545
PolyIC, Males, WT	7	-0.908	0.179	0.474
PolyIC, Males, DUP	3	-0.683	0.138	0.239
PolyIC, Females, WT	9	-0.878	0.195	0.516
PolyIC, Females, DUP	4	-0.568	0.208	0.417
Resiquimod, Males, WT	5	-0.438	0.053	0.118
Resiquimod, Males, DUP	6	-1.018	0.360	0.882
Resiquimod, Females, WT	5	-1.487	0.377	0.843
Resiquimod, Females, DUP	11	-1.357	0.337	1.010

Table 5-7: Summary data for expression of TNFa as measured by RT-qPCR. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

		IL6		
Group	N	Mean	Standard Error	Standard Deviation
Saline, Males, WT	6	-1.451	0.351	0.993
Saline, Males, DUP	5	-1.562	0.364	0.631
Saline, Females, WT	7	-0.999	0.212	0.520
Saline, Females, DUP	5	-1.067	0.162	0.429
PolyIC, Males, WT	7	-1.012	0.279	0.739
PolyIC, Males, DUP	3	-0.398	0.041	0.071
PolyIC, Females, WT	9	-0.776	0.274	0.725
PolyIC, Females, DUP	4	-0.269	0.084	0.167
Resiquimod, Males, WT	5	-1.126	0.324	0.725
Resiquimod, Males, DUP	6	-1.063	0.409	1.001
Resiquimod, Females, WT	5	-1.488	0.362	0.809
Resiquimod, Females, DUP	11	-1.055	0.207	0.621

Table 5-8: Summary data for expression of IL6 as measured by RT-qPCR. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

		CD68		
Group	N	Mean	Standard Error	Standard Deviation
Saline, Males, WT	6	-1.373	0.402	1.137
Saline, Males, DUP	5	-2.556	0.569	0.985
Saline, Females, WT	7	-1.760	0.791	1.937
Saline, Females, DUP	5	-2.502	0.638	1.689
PolyIC, Males, WT	7	-2.342	0.680	1.800
PolyIC, Males, DUP	3	-0.796	0.315	0.546
PolyIC, Females, WT	9	-2.138	0.696	1.842
PolyIC, Females, DUP	4	-1.048	0.510	1.021
Resiquimod, Males, WT	5	-2.137	0.651	1.457
Resiquimod, Males, DUP	6	-5.470	2.93	7.17
Resiquimod, Females, WT	5	-2.916	0.873	1.952
Resiquimod, Females, DUP	11	-1.328	0.449	1.357

Table 5-9: Summary data for expression of CD68 as measured by RT-qPCR. *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group (TNFa)	Tukey Method Grouping	
WT F Resiquimod	A	
DUP F Resiquimod	A	
WT F Saline	A	
WT M Saline	A	
DUP F Saline	A	
WT M PolyIC	A	
DUP M Saline	A	
WT F PolyIC	A	
DUP M PolyIC	A	
DUP M Resiquimod	A	
DUP F PolyIC	A	
WT M Resiquimod	A	

Table 5-10: The Tukey Method Grouping for the expression of TNFa as measured by RT-qPCR. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

Group (IL6)	Tukey Method Grouping	
DUP M Saline	A	
WT F Resiquimod	A	
WT M Saline	A	
DUP F Saline	A	
DUP F Resiquimod	A	

WT M Resiquimod	A	
WT F Saline	A	
WT M PolyIC	A	
DUP M Resiquimod	A	
WT F PolyIC	A	
DUP M PolyIC	A	
DUP F PolyIC	A	

Table 5-11: The Tukey Method Grouping for the expression of TNFa as measured by RT-qPCR. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

Group (CD68)	Tukey Method Grouping	
DUP M Resiquimod	A	
WT F Resiquimod	A	
DUP F Resiquimod	A	
DUP F Saline	A	
WT F Saline	A	
WT M Resiquimod	A	
WT M Saline	A	
WT F PolyIC	A	
DUP M Saline	A	
WT M PolyIC	A	
DUP M PolyIC	A	
DUP F PolyIC	A	

Table 5-12: The Tukey Method Grouping for the expression of TNFa as measured by RT-qPCR. *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

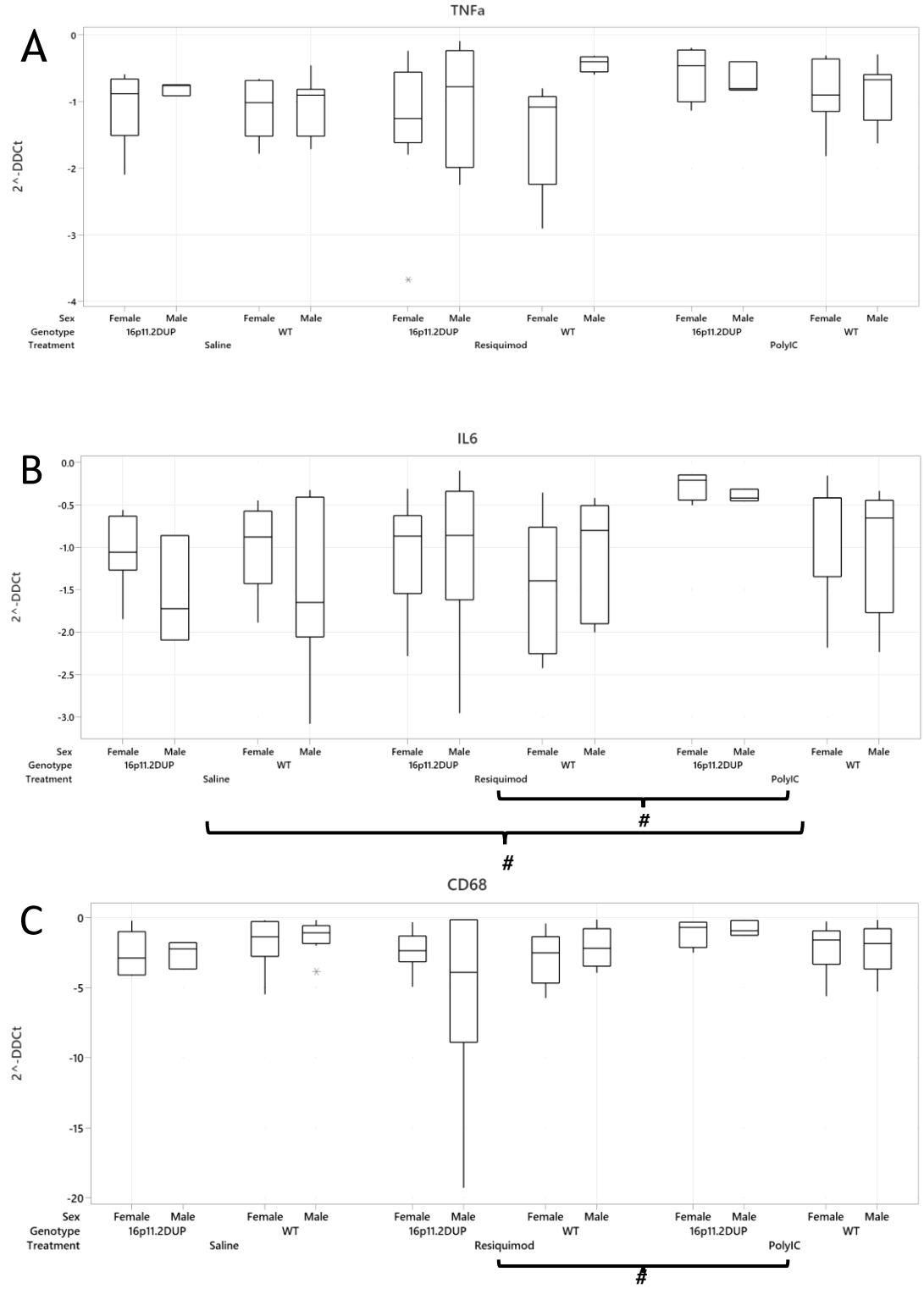

Figure 5.2: **Inflammatory markers in the PFC of adult wt and 16p11.2 duplication mice following MIA protocols with saline, resiquimod or poly IC.** A) TNFa expression in PFC of mice separated by group. B) IL6 expression in PFC of mice separated by group. C) CD68 expression in PFC of mice separated by group. *N = 41 females, 32 males, 39 WT, 34 DUP, 23 saline, 27 resiquimod, 23 Poly IC. # indicates differences between means where p < 0.05. *signify outlier data points.*

5.3.3 DOCK Proteins

After dissection of PFC from brains of mice derived from the dual hit MIA and 16p11.2 duplication model of schizophrenia, RT-qPCR were carried out to assess changes in expression of key microglial activation proteins; DOCK 8 and DOCK 11. Multifactorial, nested ANOVA results are summarised in Figure 5.5. Results showed no effect of genotype or sex on DOCK proteins however there was a significant effect of treatment group on expression of both DOCK 8 (table, $p = 0.022$) and DOCK 11 (table, $p = 0.001$). In Dock 8 this was primarily driven by a significant difference in expression between resiquimod and Poly IC ($p = 0.017$) while in DOCK 11 this was driven by a significant increase in expression following Poly IC compared to Saline ($p = 0.001$). Furthermore, there was a significant interaction between genotype and treatment in Dock 8 expression with DUP Poly IC animals showing a significant increase in expression compared to WT Resiquimod animals ($p = 0.011$).

Overall, Poly IC and resiquimod produced differential effects on DOCK8 and DOCK11, in mouse prefrontal cortex.

Comparison	DOCK 8		DOCK 11	
	F-value	p-value	F-value	p-value
Genotype	2.59	0.113	2.01	0.163
Sex	0.30	0.586	0.12	0.732
Treatment	4.10	0.022*	7.78	0.001*
Genotype*Sex	0.06	0.803	0.02	0.882
Genotype*Treatment	3.18	0.049*	0.19	0.828
Treatment*Sex	1.27	0.288	1.98	0.151
Genotype*Treatment*Sex	2.15	0.127	0.81	0.450

Table 5-13: Statistical tests for inflammatory markers in mouse PFC. N = 73. *p < 0.05.

		DOCK 8 (2^-DDCt)			DOCK 11 (2^-DDCt)		
Group	N	Mean	Standard Error	Standard Deviation	Mean	Standard Error	Standard Deviation
Saline, Males, WT	6	-1.371	0.293	0.828	-1.202	0.135	0.404
Saline, Males, DUP	5	-1.081	0.153	0.266	-0.834	0.172	0.345
Saline, Females, WT	7	-0.853	0.131	0.321	-0.912	0.063	0.141
Saline, Females, DUP	5	-1.322	0.246	0.651	-0.846	0.151	0.399
PolyIC, Males, WT	7	-1.203	0.113	0.299	-0.462	0.060	0.158
PolyIC, Males, DUP	3	-0.679	0.152	0.264	-0.627	0.334	0.579
PolyIC, Females, WT	9	-1.096	0.128	0.339	-0.557	0.061	0.160
PolyIC, Females, DUP	4	-0.868	0.304	0.608	-0.362	0.059	0.118
Resiquimod, Males, WT	5	-1.156	0.173	0.387	-1.294	0.287	0.642
Resiquimod, Males, DUP	6	-1.713	0.695	1.702	-1.530	0.526	1.288
Resiquimod, Females, WT	5	-2.310	0.279	0.623	-0.521	0.088	0.196
Resiquimod, Females, DUP	11	-1.198	0.262	0.786	-0.788	0.254	0.763

Table 5-14: **Summary data for expression of DOCK8 and DOCK11 as measured by RT-qPCR.** *Number of animals in each group as well as standard error from the mean and the standard deviation are also indicated.*

Group (Dock 8)	Tukey Method Grouping	
WT F Resiquimod	A	
DUP F Saline	A	B
WT M PolyIC	A	B
DUP M Saline	A	B
DUP M Resiquimod	A	B
WT M Resiquimod	A	B
DUP F Resiquimod	A	B
WT M Saline	A	B
WT F PolyIC	A	B
WT F Saline		B
DUP F PolyIC		B
DUP M PolyIC		B

Table 5-15: **The Tukey Method Grouping for the expression of DOCK8 as measured by RT-qPCR.** *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

Group (Dock11)	Tukey Method Grouping	
WT M Saline	A	
WT F Saline	A	
dup M Resiquimod	A	
dup M Saline	A	

dup F Saline	A	
WT F PolyIC	A	
dup F Resiquimod	A	
WT M Resiquimod	A	
WT M PolyIC	A	
WT F Resiquimod	A	
dup F PolyIC	A	
dup M PolyIC	A	

Table 5-16: **The Tukey Method Grouping for the expression of DOCK11 as measured by RT-qPCR.** *Groups which share a letter grouping do not have significantly different means. If groups do not share a common letter in the Tukey's method grouping they are statistically different from one another.*

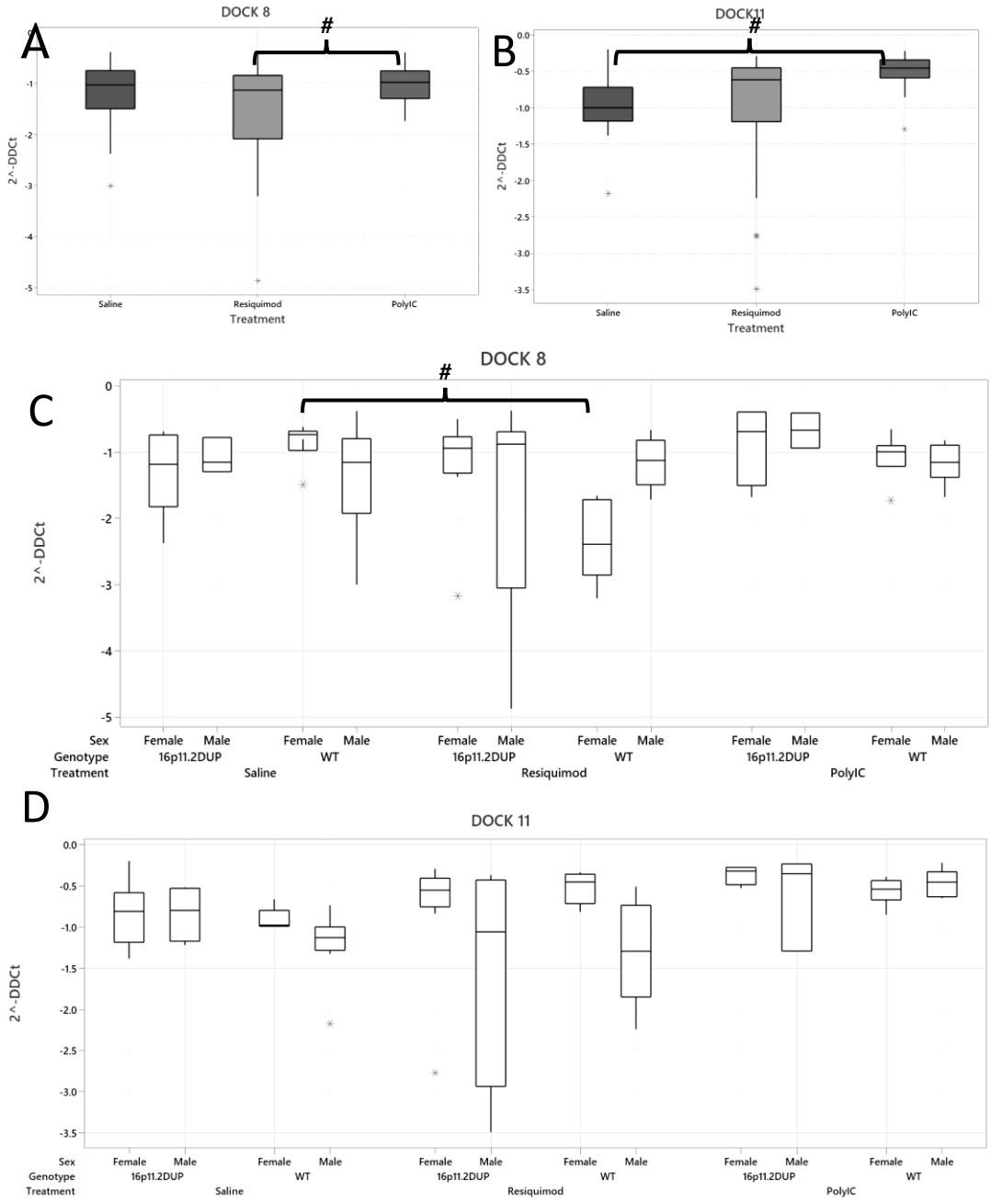

Figure 5.3: DOCK protein expression in the PFC of adult WT and 16p11.2 duplication mice following MIA protocols with saline, resiquimod or poly IC. A) DOCK8 expression in PFC of mice separated by MIA group. B) DOCK11 expression in PFC of mice separated by MIA group. C) DOCK8 expression in PFC of mice separated by sex, genotype and MIA treatment group. D) DOCK 11 expression in PFC of mice separated by sex, genotype and MIA treatment group. *N = 41 females, 32 males, 39 WT, 34 DUP, 23 saline, 27 resiquimod, 23 Poly IC. # indicates differences between means where p < 0.05. *signify outlier data points.*

5.4 Discussion

With the aim of assessing the long-term biological impact of a two-hit model of schizophrenia, a genetic mouse line which carries a variant that conveys a high-risk for schizophrenia – the 16p11.2 duplication – and maternal immune activation through Poly IC or Resiquimod were used. This experimental design more realistically replicates real-life environmental and genetic interaction which studies hypothesise accumulate to result in schizophrenia in humans. Therefore, a dual-hit model may be a more accurate translational research method for understanding complex psychiatric disorders with no singular cause being thus far, identified.

It is clear that in this two-hit model there are a number of behavioural effects upheld through to adulthood as a result of specific genotype and maternal immune activation (Chapter 4). However, it is also important to determine if this type of experimental design can also replicate the neurobiological changes that can be seen in people with schizophrenia. With the PFC a key region of biological variability in people with schizophrenia, a number of neuronal and inflammatory markers were assessed in the PFC of the adult mice from the litters of 16p11.2 duplication mouse lines and immune activated dams. Quantifying transcription of key genes provided interesting information about the long-term effects of maternal immune activation. I found Poly IC and resiquimod produced differential effects on neuronal signalling, neuroinflammation and microglial activation. However, there were no overall effects of genotype on any of the markers assessed nor evidence for any biologically significant gene-MIA interactions. These findings are discussed further below.

5.4.1 Neuronal Signalling Markers

In humans, there is robust evidence that neuronal signalling is dysfunctional in people with schizophrenia particularly in the PFC. Specifically, several post-mortem studies have identified deficits in parvalbumin expressing interneurons in the PFC (Hashimoto *et al.*, 2003, 2008; Fung *et al.*, 2014; Glausier, Fish and

Lewis, 2014). Chung et al, 2016 showed that this may be due to the deficit in formation of excitatory synapses by PV interneurons. Research in mouse models has further shown evidence of PV interneuron disruption in schizophrenia. In a study investigating a gene of interest in schizophrenia – Erbb4 – a reduction in excitatory synapses on PV interneurons was identified (delPino *et al.*, 2013). Another study which investigated the NMDA receptor hypofunction theory of schizophrenia through use of a genetic mouse line containing dysfunction in a NMDA receptor subunit also highlighted dysfunction in PV interneuron expression (Belforte *et al.*, 2010). Willis, Pratt and Morris (2021) and Bristow et al (2020) also showed that in mice containing the 16p11.2 duplication risk gene for schizophrenia that PV interneuron expression was reduced. There was further evidence to suggest this could have been due to changes in the development of perineuronal nets, a key structure for PV neuron functionality and synapse formation.

Here, there was no significant difference in PV expression between wild type and 16p11.2 duplication containing mice in this instance. This is in contrast to Willis, Pratt and Morris (2021) and Bristow et al (2020). However, research has shown that PV dysfunction may go beyond deficits in density of PV neurons themselves and could be due to presentation of excitatory synapses on the interneurons (Belforte *et al.*, 2010; delPino *et al.*, 2013; Chung, Fish and Lewis, 2016) or more complex structures in perineuronal nets (Willis, Pratt and Morris, 2021). As a result, further in-depth analysis of PV interneuron structure and function would provide more detailed analysis of any changes that could be due to the 16p11.2 duplication beyond simple expression of PV. This would be especially prudent considering the 16p11.2 duplication alone showed marked changes in anxiety-related behaviours and in sociability indicated the genotype does have effects relevant to neuropsychiatric disorders.

There was however a significant interaction between maternal immune activation group and sex with male mice from litters who were immune activated with resiquimod showing a decrease in expression in PV compared to female, saline treated animals. Tukey's post hoc analysis did not identify any

further significant differences between the other groups. Resiquimod resulting in decreased PV expression in male animals could suggest that resiquimod has a sex-specific response. Interestingly this may reflect data found in humans. Resiquimod replicates infection of single-stranded viruses (Hemmi *et al.*, 2002; Isobe *et al.*, 2006; Kwon *et al.*, 2021) which increases risk of schizophrenia when occurring during embryonic development. Additionally, men show a higher incidence of schizophrenia diagnosis (McDonald and Murray, 2000; Canetta and Brown, 2012; Janoutová *et al.*, 2016) and here we have found that resiquimod may affect neuronal signalling in only male animals. This is a potentially important finding that should elicit further investigation.

GABAergic interneuron dysfunction in schizophrenia is also seen in post-mortem studies of SST expressing interneurons (Hashimoto *et al.*, 2003; Fung *et al.*, 2014; Dienel, Fish and Lewis, 2023). Similarly to PV expression, animal studies which investigate different aspects of schizophrenia have also identified reduced SST expression (Belforte *et al.*, 2010). The 16p11.2 duplication has been shown to result in reduced SST expression in the PFC (Bristow *et al.*, 2020; Willis, Pratt and Morris, 2021). However, this was not evident in this study. With no significant effects of genotype, MIA group or sex there is no evidence to suggest dysfunction in SST neurons. Given the robust literature which indicates otherwise, further investigation is required to determine if functionality of these neurons is altered despite no difference in expression of SST.

Overall, there is little evidence in the current study to suggest widespread dysfunction in GABAergic signalling interneurons in this dual-hit model of schizophrenia. However, these results differ from previous findings in 16p11.2 duplication mice (Willis, Pratt and Morris, 2021). Further investigation into why this may be the case are warranted. Potentially environmental factors relating to differences in mouse housing could be a factor. However, this is more likely due to differing methodology in assessing expression. Here a relatively crude dissection followed by RT qPCR of the general region was carried out. Willis et al (2021) utilised in situ hybridisation which highlights more subtle changes. It

is possible in this case that assessing expression of GABAergic signalling in a more specific methodology would reveal more detailed results.

5.4.2 Inflammatory Signalling Markers

Inflammation has recently become an area of interest in schizophrenia research with evidence suggesting that increased inflammatory markers are linked with psychiatric disorders (Trépanier *et al.*, 2016; Kose *et al.*, 2021; Lizano *et al.*, 2021). At the same time, maternal immune activation has been linked with increased risk of schizophrenia development (Canetta and Brown, 2012; Aguilar-Valles, Rodrigue and Matta-Camacho, 2020; Choudhury and Lennox, 2021). However, whether maternal immune activation can result in sustained inflammatory response through to adulthood is still unclear. Furthermore, there is little research into dual-hit models which might determine whether risk-genes for schizophrenia can pre-dispose to inflammatory response.

In this case, analysing key neuroinflammatory markers provided detail into sustained immune response in the brain through to adulthood following maternal immune activation during embryonic development. Here, we showed increased transcription of IL6 is sustained into adulthood following maternal immune activation with Poly IC. This replicates results found in other animal models of schizophrenia risk factors as well as in data from humans which highlight IL6 as an increased marker of inflammation in the peripheral immune system of people with schizophrenia. In fact, IL6 is consistently highlighted as an inflammatory marker of interest in schizophrenia research (Fillman *et al.*, 2013; Trépanier *et al.*, 2016; Miller and Goldsmith, 2019; Lizano *et al.*, 2021). Replicating this effect in an animal model therefore highlights how maternal immune activation is a translational experimental approach. Furthermore, it is clear that maternal immune activation during embryonic development can have long lasting effects on inflammation in the brain through to adulthood.

There was also a sex specific effect for resiquimod with female mice showing increased TNFa expression compared to males. Similarly to IL6, TNFa has shown evidence of sustained elevation in schizophrenia (Miller *et al.*, 2011; Hoseth *et*

al., 2017). Additionally, while there was an overall effect of treatment on CD68 expression, this was due to a difference in Poly IC and Resiquimod groups rather than an effect of immune activation compared to no immune activation. However, it is relevant to consider how discrepancies between immunogenic treatments might be relevant to humans. With research utilising Poly IC as an immune activation agent despite the mechanism of action being different the viral infection it is relevant to compare to immunogenic compounds such as resiquimod which do replicate viral infection (Hemmi *et al.*, 2002; Isobe *et al.*, 2006). Nevertheless, it appears that Poly IC creates a more robust immune response compared to resiquimod and in this case, the effects on long term immune markers are relevant to data found in humans.

5.4.3 Dock Proteins

DOCK proteins are a family of RhoGTPases which have been linked to a number of conditions such as autism and schizophrenia (Namekata *et al.*, 2012, 2014; Guo *et al.*, 2021; Umehara *et al.*, 2021; Samani *et al.*, 2022). However, there is still relatively little research into their function. Whilst DOCK 8 and DOCK 11 have been linked with microglial function their mechanisms are still to be determined. Regardless, studies have shown that these two DOCK proteins have shown altered expression in the brains of mice following maternal immune activation with LPS and Poly (Namekata *et al.*, 2012, 2014, 2019, 2020; Oskvig *et al.*, 2012). Given the importance of microglial cells in neurodevelopment during pregnancy and the clear link between maternal immune activation and schizophrenia, it stands to reason that these proteins may be of interest in schizophrenia research.

Here, we identified a clear increase in expression of DOCK 11 in the PFC of adult mice after maternal immune activation with Poly IC. This is in line with other research on MIA and DOCK 11 expression immediately following MIA and in early adulthood (Namekata *et al.*, 2012; Oskvig *et al.*, 2012). Thus far, research suggests that DOCK 11 functions to regulate cell cycle, in activation or in migration of immune cells (Sinha and Yang, 2008). This could be of

importance in research which aims to identify how immune regulation might increase risk of schizophrenia.

While DOCK 8 showed a treatment effect this was due to a significant difference in expression following resiquimod compared to Poly IC. Again, this highlights the need to investigate more closely the mechanism of action of maternal immune activation in humans although this does not necessarily provide relevant information about the specific effect of immune activation compared to no immune activation. Furthermore, while there was a gene and treatment interaction identified this was again a discrepancy in responses to Poly IC and resiquimod. There is no evidence in this study to suggest that DOCK8 shows prolonged increase in expression following MIA in embryonic development.

5.4.4 Conclusion

In this case there were no effects of genotype on any inflammatory or signalling markers. Given there is little evidence that the 16p11.2 duplication has direct immune involvement this is perhaps not surprising. However, with the robust data which shows altered expression of GABAergic interneuron expression in the 16p11.2 duplication this is somewhat surprising (Bristow *et al.*, 2020; Willis, Pratt and Morris, 2021). This necessitates further research into the underlying interneuron cell function and activation beyond only expression of PV or SST. Furthermore, it also highlights that with minimal interaction of genetic risk and immune activation there is unlikely to be an accumulation effect of risk factors on these biological markers. However, there was a clear effect of maternal immune activation on sustained immune changes in the PFC including in IL6 and in DOCK 11, a protein important in microglial cell function. This suggests that while it is not possible to say there is an accumulation effect of risk factors with no specific genotype and MIA interactions, there may be individual effects of immune response that cause long lasting neuroinflammatory changes. Importantly, with further investigation into other markers of neuronal signalling, function and structural changes it is possible that additional genotype effects will be identified.

5.4.5 Future Directions

There are a number of future directions for this research that given more time I would have performed. Firstly, analysing other brain regions, particularly the hippocampus, would be an important next step to determine if there are any long lasting effects beyond the PFC. This would be particularly important when looking at GABAergic interneuron function given that the PFC and hippocampal axis is a key structural component that shows dysfunction in schizophrenia (Bristow *et al.*, 2020). Furthermore, the spleens from these animals were also dissected and could provide valuable information about systemic inflammation so it would be prudent to further assess inflammatory marker expression here.

Secondly, further research into the function of GABAergic signalling beyond expression of PV and SST would provide richer data into neuronal signalling capabilities. Analysis of excitatory synapse formation and PNNs would provide more detailed information about PV neuron activity patterns and would more closely relate to data found in humans which has shown deficits in these areas.

Finally, analysis of other markers which are relevant to schizophrenia would be paramount. For example, GAD 67, BDNF and JNK have been shown to be involved in dysfunction related to neuronal signalling (Willis, Pratt and Morris, 2021); CRP is an inflammatory marker that has = been shown to be increased in humans with schizophrenia (Lizano *et al.*, 2021) and studies have identified links with DOCK 4 to schizophrenia and other psychiatric disorders (Alkelai *et al.*, 2012; Guo *et al.*, 2021).

5.4.6 Limitations

There were a number of limitations that occurred during these experiments that must be considered alongside the results. During RT-qPCR, a signal was detected in control samples which did not contain enzyme that converted mRNA to cDNA. This is most likely due to primers which may overlap with cellular DNA regions causing amplification of potentially un-transcribed cellular DNA. However, whilst this means it may be difficult to differentiate between un-transcribed DNA and transcribed mRNA presence it is still relevant to consider

the results from these experiments. Future steps will ensure that the primers for these targets will be redesigned in a way that excludes the potential for amplification of cellular DNA and instead, isolates extracted mRNA. Additionally, given the number of animals in this research, it was necessary to carry out these experiments across several PCR plates. This can introduce some variability in fluorescence readings and in experimental error. However, to mitigate this, PCR plate was included in statistical analyses to ensure that this variability was included in calculations of significant effects.

Finally, there were uneven group sizes throughout this experiment when groups were split into sex, genotype and MIA treatment groups. This is primarily due to the complexity of the breeding processes which meant it was not possible to predict which genotype and sexes would arise from any given litter. As a result, groups consisted of 3 -11 mice to ensure that no animal that had entered the experimental process was unused in line with recommendations from the 3R guidelines.

6 General Discussion

Throughout this thesis there has been an exploration of how neuropharmacological intervention, genetics and environmental stimuli can result in schizophrenia-like behavioural and biological phenotypes in mice. The aims were to:

1. Assess detectable behavioural changes that can occur with neuropharmacological intervention which mimic the neural activation pattern seen in people with schizophrenia.
2. Determine whether these behavioural changes can be seen in a novel dual-hit model for schizophrenia that combined genetic and environmental risk factors.
3. Begin to classify the neurobiological changes that occur after combined genetic and environmental risk factors.

The purpose of these experiments is primarily to address one of the fundamental questions of mental health disorders such as schizophrenia: if there are no specific genes which carry 100% risk for development of schizophrenia then how do risk genes interact with environmental risk factors to increase likelihood of development? Furthermore, is it possible to recreate the accumulation of risk factors in animals to increase severity of schizophrenia-like behavioural and biological phenotypes?

To address these aims, first, PCP – a commonly used neuropharmacological intervention which mimics the neural activation patterns seen in brains of people with schizophrenia (Morris, Cochran and Pratt, 2005; Javitt *et al.*, 2012; Morris and Pratt, 2014; Grayson *et al.*, 2016; Moghadam *et al.*, 2021) – was used to assess the types of schizophrenia related behavioural changes which could be detected in mice. PCP was administered prior to or during a series of behavioural tests which assess cognitive ability, social behaviours and anxiety-related behaviours (Treit and Fundytus, 1988; Simon, Dupuis and Costentin, 1994; Walf and Frye, 2007; Antunes and Biala, 2012; Mitchell, Thomson, *et al.*, 2020). Since people with schizophrenia often exhibit anxiety, social withdrawal

and cognitive deficits (Waltz and Gold, 2007; Betensky *et al.*, 2008) these were suitable tests to determine the presence of a schizophrenia like phenotype. The hypotheses were that PCP would induce social deficits, increase anxiety and induce cognitive deficits as can be seen in humans with schizophrenia.

Next, a dual-hit model of schizophrenia was used to determine how environmental stimuli and genetics may interact with behaviour. Since pre-natal infection of the mother can increase risk of schizophrenia (Brown, 2006, 2011; Canetta and Brown, 2012; Janoutová *et al.*, 2016; Aguilar-Valles, Rodrigue and Matta-Camacho, 2020), maternal immune activation was induced using resiquimod or Poly I:C, two immunogenic agents that mimic infections (Hameete *et al.*, 2021; Kwon *et al.*, 2021). This was carried out in dams with the 16p11.2 duplication, a high-risk genetic variant for the development of schizophrenia in humans (Kirov *et al.*, 2014; Marshall *et al.*, 2017). This yielded WT and 16p11.2 duplication carrying mice who were either exposed to saline or immune activation during embryonic development. Once these animals had grown to adulthood, a number of behavioural assessments were carried out. The behavioural tests used were those that had already shown behavioural changes following neuropharmacological intervention with PCP (Chapter 4) and so would reliably identify schizophrenia-like phenotypes in cognitive ability, social behaviours and anxiety-related behaviours. In this case, the hypothesis was that the gene alone would induce anxiety and show deficits in social behaviour and cognition. Furthermore, MIA would show the same effects. Finally, it was hypothesised that the combination of MIA and 16p11.2 duplication would increase the severity of these behavioural changes.

Last, we aimed to determine if there were sustained neurobiological changes in the same WT and 16p11.2 duplication carrying animals who underwent MIA protocols. Once behavioural tests were completed on these animals, the prefrontal cortex was dissected, and a series of RT-qPCR tests were carried out to determine if there were sustained changes in neuronal signalling, inflammatory markers and markers of microglial activity. This would indicate whether events such as MIA could have sustained effects which last permanently

into adulthood that could influence neurodevelopmental processes and result in adulthood disorders like schizophrenia. It was hypothesised that 16p11.2 duplication would result in reduced neuronal signalling markers but have no effects on inflammation or microglial activity whilst MIA would have effects on all markers. Furthermore, the combination of genetic variant and MIA would show interactions that also show reduced neuronal signalling markers and increased inflammatory markers imitating data found in post-mortem brains of humans with schizophrenia.

Together these experiments assess behavioural changes that can be seen using well-established models of schizophrenia, assess whether these same effects can be seen in the use of a dual-hit model of schizophrenia and whether there are sustained biological changes in the prefrontal cortex with this dual hit model.

6.1 Summary of Main Findings

6.1.1 Behavioural changes following neuropharmacological intervention

There were a number of behavioural changes that occurred after administration of acute, low-dose PCP to WT mice. These included alterations in anxiety-related, social and cognitive behaviours that mirror changes seen in humans. Moreover, there were also sex-specific effects that directly mimic the sex-specific symptom repertoire of humans with schizophrenia (Castle and Murray, 1993; Leung and Chue, 2000; Usall *et al.*, 2002; Sommer *et al.*, 2020). Specifically, female mice showed an increased anxiety-related response in both open-field tests and elevated plus maze tests, whilst males showed decreased anxiety-related responses. Furthermore, female mice showed no specific changes in social behaviours following PCP however PCP in male mice resulted in increased social withdrawal with separation between cage-mates increasing in the period immediately following administration (chapter 3). Although there were no indications of reduced cognitive ability in the novel object recognition test, the sex-specific alterations in anxiety and social behaviour can also be

seen in people with schizophrenia. Whilst there is little difference in presentation of positive symptoms of schizophrenia, women are more likely to experience affective disorders such as anxiety and depression (Castle and Murray, 1993; Leung and Chue, 2000; Sommer *et al.*, 2020). At the same time, men are far more likely to experience social withdrawal or difficulties with social interaction (Leung and Chue, 2000; Usall *et al.*, 2002). It is clear therefore that the administration of acute, low-dose PCP has induced a similar behavioural output as can be seen in humans.

On the other hand, there were a number of context-driven responses which call into question the methodology that is used as standard within research to assess behavioural effects. This is particularly true, when these are used to draw conclusions about complex neuropsychiatric disorders like schizophrenia. For example, here male mice showed decreased thigmotaxis in the open-field but increased thigmotaxis in the home-cage. Thigmotaxis is suggested as a measure of anxiety in mice as the behaviour exploits the natural inclination of rodents to find safer areas in an open-field, particularly when in fear (Treit and Fundytus, 1988; Simon, Dupuis and Costentin, 1994). However, there is little information around what the same behaviour could indicate in the home-cage. Theoretically, this could indicate a repetitive behaviour of walking in circles at the edge of the cage, particularly if there are changes in locomotor activity. However, this could also indicate anxiety or be a side-effect of the increase in social withdrawal seen in these animals considering the limited space available to retreat from cage-mates in the monitoring set-up. If this does indicate anxiety, it calls into question how different environmental contexts could elicit different behavioural phenotypes and it is important to take this into consideration for future behavioural neuroscience research.

6.1.2 Behavioural changes following combined genetic and environmental risk factors

Combining genetic risk through the use of mice carrying the high risk genetic variant of the 16p11.2 duplication (Kirov *et al.*, 2014; Rees *et al.*, 2014;

Marshall *et al.*, 2017) with the environmental risk of MIA (Brown, 2006, 2011; Canetta and Brown, 2012; Janoutová *et al.*, 2016; Aguilar-Valles, Rodrigue and Matta-Camacho, 2020) provided a dual-hit model for schizophrenia which more closely replicates the combination of risk factors which theoretically accumulate in humans to result in eventual diagnosis of schizophrenia in adulthood. There were a number of interesting behavioural changes that occurred within the different groups. On a wide scale, the 16p11.2 duplication showed an overall increase in anxiety-related behaviours in the elevated plus maze, but decreased thigmotaxis in the open-field. MIA groups on the other hand showed the opposite effect with increased thigmotaxis in the open-field but reduced anxiety behaviour in the elevated plus maze. 16p11.2 duplication carrying mice also showed a clear social deficit with increased separation from cage mates compared to WT animals. These results are not unexpected given that they mimic the behavioural deficits in the 16p11.2 duplication or in MIA that have been previously characterised in our lab and by others (Ito *et al.*, 2010; Babri, Doosti and Salari, 2014; Depino, 2015; Simões *et al.*, 2018; Bristow *et al.*, 2020; Gzielo *et al.*, 2021; Carbone *et al.*, 2023).

However, what is particularly interesting in these results is the more specific effects found in the subgroups. A clear gene x environment interaction can be seen, with resiquimod and 16p11.2 duplication carrying mice showing increased social withdrawal compared to saline and wild-type counterparts or compared to Poly IC groups. There was also a sex x gene interaction, with male 16p11.2 duplication animals showing a significant increase in social withdrawal compared to female or wild-type counterparts. This is a novel finding which has implications for how complex disorders which are developed as a result of combining risk factors are studied. If it is evident that genetics and environmental stimuli interact, this provides a basis for further research into the genes in this area and how expression or activity may be influenced by the environment to increase severity or indeed presence of behavioural deficits. This is further supported by the fact that the sex-specific effects are also mimicking data found in the symptomology of humans, with men experiencing

more instances of social withdrawal than women (Leung and Chue, 2000; Usall *et al.*, 2002).

On the other hand, the data here have also further highlighted the importance of experimental context given the discrepancies in indicators of anxiety in different experiments. For example, comparing the EPM to the open field highlights a number of instances which could affect results of anxiety measures. In the EPM, the test lasted a total of 15 minutes whilst the open-field test continued for two hours. Despite identical levels of handling, with the same handler and similar habituation times, it is clear in the locomotor activity in the open-field that there is a habituation effect over time (Chapter 3 and 4). In the EPM, there are no indications of a habituation effect and indeed, the time period is too short to accurately assess this. If this habituation effect in the open-field is due to increased initial anxiety due to being placed into an experimental environment, away from the home-cage, food, water and cage-mates it stands to reason that there could be confounding effects seen in the EPM on anxiety behaviour given the short testing period. This should also be considered when assessing results of NOR. The test itself involves multiple instances of lifting animals in and out of the test field and lasts for a maximum of 5 minutes. It is highly improbable that animals are not experiencing mild stress during this experiment and as such it is important to consider how that might affect exploratory behaviours in mice. Finally, there were changes to locomotor activity of female mice that were seen in the home-cage but not in the open-field. Again, it is important to consider whether increased anxiety responses, such as can be seen in thigmotaxis in the open-field, can mask behavioural changes that could be seen in familiar and safe spaces such as the home-cage.

Overall, it is clear from these results that experimental context does have effects on behavioural measurements. This has implications for research since this is often not taken into consideration when using standard experimental practice with tests used here such as the EPM, NOR or open-field as well as others like the 3-chamber novel social interaction test, forced-swim tests and

other experiments which aim to assess complex behavioural characteristics in simplified, short-term paradigms.

6.1.3 Biological changes following combined genetic and environmental risk factors

Whilst there were no identified effects of genotype in this case, there were a number of sustained effects as a result of maternal immune activation in inflammatory markers as well as markers of microglial cell function in the prefrontal cortex (Chapter 6). With evidence that humans with schizophrenia show similar increases in inflammation (Trépanier *et al.*, 2016; Lizano *et al.*, 2021), to replicate this after one instance of MIA suggests that immune activity during key stages of neuronal development can have lasting effects not only on neuronal development but also on the immune system. Whilst there were no changes here on neuronal signalling, contrary to similar studies it is important to highlight that these studies utilise different methods for expression measurement, namely in-situ hybridisation (Openshaw *et al.*, 2023). Here, a relatively crude method of dissection following by mRNA extraction was used which can elicit data on whole regions. However, more detailed experiments using in-situ hybridisation would more accurately identify subtle, region-specific changes in expression. Furthermore, a pertinent assessment of neural circuitry and connectivity would be required given humans with schizophrenia often show decreased hippocampal-prefrontal cortex connectivity perhaps through the use of in-vivo calcium imaging which can provide live data about neural activity in live animals (Russell, 2011).

Nevertheless, the finding that MIA can elicit sustained alterations in neuroinflammatory markers and microglial cell activity has implications for further research. The role of the immune system in neuropsychiatric disorders has recently become an area of interest with an increasing number of studies in MIA, childhood immune activation and immune markers in adolescence (Brown and Derkits, 2010; Brown, 2011; Fillman *et al.*, 2013; Janoutová *et al.*, 2016; Trépanier *et al.*, 2016; Miller and Goldsmith, 2019; Aguilar-Valles,

Rodrigue and Matta-Camacho, 2020; Lizano *et al.*, 2021). To confirm that a single event of MIA can show altered immunological markers through to adulthood as is indicated here (chapter 5) supports the theory that the immune system must have a role alongside other risk factors such as genetics. This further identifies mechanisms by which neuropsychiatric conditions such as schizophrenia can develop and therefore provides potential for the identification of treatment targets, interventions and potentially diagnostic support.

6.2 Overall Discussion

6.2.1 Modelling Schizophrenia

Research on mental health conditions requires the use of translational studies using mice so that we can better understand complex neurobiology, neuropharmacology and underlying mechanisms which contribute to these disorders. However, to do this it is important that the experimental model that is used is relevant to the translational research. In this case, schizophrenia is an extremely complex disorder with no one-cause being identified. In fact, epidemiology studies on humans theorise that numerous individual risk factors may combine to increase likelihood of development (Schwartzer *et al.*, 2013; Vitor-Vieira, Vilela and Giusti-Paiva, 2021; Benedetti *et al.*, 2022). Despite this, research has continued to look at manipulation of individual factors in mice when researching the disorder, such as through the use of genetic lines (Yang *et al.*, 2015; Bristow *et al.*, 2020; Rein *et al.*, 2021; Openshaw *et al.*, 2023), MIA (Schwartzer *et al.*, 2013; Gzielo *et al.*, 2021; Vitor-Vieira, Vilela and Giusti-Paiva, 2021; Carbone *et al.*, 2023), early-life stress paradigms (Bebbington *et al.*, 2004; Spauwen *et al.*, 2006; Morgan and Fisher, 2007) or through the use of pharmacological interventions (Javitt *et al.*, 2012; Grayson *et al.*, 2016; Moghadam *et al.*, 2021). Whilst these studies have provided valuable data on underlying mechanisms, it is important that research which aims to create more translational paradigms are incorporated to provide a wider understanding of risk interaction, and mechanistic changes which can affect one another. As a

result, here a full analysis of a traditional model of schizophrenia (Chapter 3) was used alongside a longer-term dual-hit paradigm (Chapter 4 and 5). Behavioural repertoire and biological changes were assessed in order to elicit novel data on gene x environment interaction (Chapter 4 and 5).

First, the full behavioural repertoire of mice in response to PCP, an NMDA receptor antagonist which mimics the NMDA receptor hypofunction seen in people with schizophrenia, was established. The use of PCP is commonly used in schizophrenia research and as expected behavioural tests identified clear effects on anxiety and social withdrawal (Chapter 3).

Next, a dual-hit model was used to mimic the accumulation of risk factors that occur in humans who are diagnosed with schizophrenia. It was then possible to determine if behavioural changes found in these mice replicate the data found in the PCP animal model as well as their relevance to data on humans. Indeed, altered social behaviours and anxiety were seen in different groups showing effects of MIA, genotype and sex replicating the symptomology seen in humans (Chapter 4). However, whilst the PCP experiment did identify sex differences, there were complex changes between groups with gene x environment interactions identified as well as gene x sex interactions which cannot be seen in a more simplified model like PCP. This data provides the basis for future research into mechanistic involvement of MIA with 16p11.2 duplications or other genetic risk factors as well as the relevance of hormonal or chromosomal differences associated with different sexes and underlying mechanisms of these sex differences.

Finally, the expression of key neuronal signalling and inflammatory markers found in the prefrontal cortex of these mice were investigated. Whilst there were clear behavioural effects as a result of both genotype and MIA (Chapter 4), these mice showed no effects of genotype on neurobiological markers (Chapter 5). This is somewhat surprising given previous studies (Bristow *et al.*, 2020; Openshaw *et al.*, 2023) however; it is likely these results are due to experimental method and more refined neurobiological experimentation could reveal further relevant data. Particularly since the behavioural changes appear

to be significantly robust throughout this study it is likely that there are neurobiological changes in these mice still to be identified.

Nevertheless, a key finding was that MIA did show long-term lasting effects in neuroinflammatory markers. Combined with the behavioural data which also showed overall effects of MIA this supports research which is investigating the role of inflammation in development of schizophrenia. Whilst there were no gene x environment interactions in these biological markers, it is possible that there are individual effects which are not seen. For example, the 16p11.2 duplication could have effects which are not directly influenced by immune activation and so would not necessarily interact with MIA. Overall, it is not possible to conclude here that there are no interactions between gene and environment given that the behavioural data indicates the opposite, and so further research could interrogate a greater range of neurobiological markers to provide greater insight into a potential gene x environment interaction.

It is also important to note at this point the discrepancies between the two modalities of MIA. As described, Poly IC is commonly used in MIA research, however the underlying mechanism of action is via TLR3. This more accurately replicated infection by bacteria or double-stranded RNA viruses (Kwon *et al.*, 2021). However, since epidemiology studies have identified that single-stranded viruses such as influenza or rubella are those that increase risk of schizophrenia (Brown, 2006, 2012) I also assessed response to MIA by resiquimod, a TLR7 agonist. This more accurately replicated viral infection by single-stranded viruses and therefore represents MIA through more translational methods. However, results here indicated that both behaviourally and neurobiologically, Poly IC showed more robust changes than resiquimod. On the other hand, both Poly IC and Resiquimod showed gene x environment interactions in different experiments. This likely suggests that mechanism of infection is less likely to be involved in actively increasing risk for schizophrenia and is instead due to immune response maternally and in the developing brain. This further highlights areas of focus for future research in neuroinflammatory causes for psychiatric disorders.

6.2.2 Assessing animal behaviour

A major consideration throughout this research has been in the interpretation of behavioural data. Behavioural tests were chosen in an effort to assess behaviour using paradigms that are commonly used in research such as the EPM alongside longitudinal experiments that are less common such as the home-cage continuous behavioural monitoring. The purpose of this was primarily to determine if differences between these experiments are evident. It is clear that experimental context does have an effect on the behavioural output of animals (Chapter 3 and 4). However, studies rarely address this when drawing conclusions from behavioural research.

One such discrepancy can be seen when monitoring locomotor activity in the home-cage compared to the open-field (Chapter 3 and 4). The activity patterns of females in particular highlighted that these two environmental contexts can present contrasting behavioural output despite researchers often ignoring "context" and considering these measurements to be the same. Furthermore, comparison between the extended open-field test used here compared to the short EPM tests for anxiety further highlights how experimental context can produce variable results despite the inference that each test measures the same behaviour (Chapter 4). These variables are most likely to be due to the mild stress that is induced as a result of handling of a prey animal, which develops natural fear responses to novel environments, lighting changes, smells and movements. In fact, despite handling and habituation to handlers, mice who are more predisposed to anxiety behaviours such as the 16p11.2 duplication (Chapter 4; Bristow et al., 2020; Openshaw et al., 2023) carrying mice are unlikely to be in a calm state during these experiments.

This begins to further call into question other tests that it was not possible to provide comparison for here. The NOR is commonly used to examine cognitive ability as a measure of working and spatial memory. However, taking into consideration the potentially mild stress that is likely to be affecting the mice in this paradigm, it is important to reassess why and how mice may explore objects. In fact, it was identified here that male animals spent more time

engaging in exploratory behaviours in the PCP behavioural tests compared to females (Chapter 3). Given females exhibited increased anxiety-related behaviours, the decreased desire to explore any objects (novel or familiar) could be a secondary effect of stress. If this is the case, it is then not possible to determine if any differences are actually due to cognitive ability or are instead confounded by the anxiety state of the animals. In future, I suggest the use of longitudinal assessments for cognitive ability such as the paired discrimination and reversal learning paradigm that is detailed in Chapter 2 and 3. Whilst animals are still likely to be mildly stressed by the novel environment, the regular habituation as part of this paradigm for extended periods, every day over the course of several weeks increases the likelihood that animals can familiarise with the surroundings and become more accustomed to the equipment. Furthermore, the more complex cognitive requirements over a number of days are likely to give a clearer picture of overall ability than a short 5-minute assessment.

Furthermore, the use of continuous home-cage behavioural monitoring provides a wide area of measures for analysis that can provide valuable information on sociability, anxiety behaviours and locomotor activity with minimal interference to normal mouse activity. In fact, social behaviours from this experiment are likely to provide a far more translational assessment than a 3-chamber novel social interaction test, as the sociability of mice in their home environment and in their natural hierarchy more closely relates to the social withdrawal from friends and family members seen in humans with schizophrenia. Furthermore, there is potential for continuous monitoring to develop further through the use of machine learning and AI to provide data on mouse vocalisation, individual behaviours such as grooming, nesting, eating and repetitive actions as well as individual cage-mate relationships. With these developments, studies can identify changes in complex behaviours with minimal interference of human interaction, providing richer data for research in neuropsychiatric and behavioural disorders.

6.3 Future directions

Each of these experiments has provided data that address the aims stated at the beginning of this thesis. Moreover, each experiment has also identified a number of directions for future research. To further investigate PCP as a model for schizophrenia behavioural research, it would be pertinent to utilise the sub-chronic model and compare behavioural output to acute use. Additionally, including additional behavioural tests to the dual-hit model as well as the PCP model, such as the 3-chamber social interaction test, and the paired discrimination and reversal learning test, would allow for a direct comparison between acute experiments and longitudinal experiments, and therefore the full impact of environmental context. Finally, further assessment of neurobiological markers related to neuronal signalling or inflammatory signalling such as BDNF, JNK or IL 10, would elicit more detail into long-term effects of gene and environmental risk factors. Additionally, investigation of new regions in the brain such as the hippocampus which has also shown dysfunction in humans with schizophrenia would be a useful next stage. Further investigations using more region-specific and detailed measures for expression of neuronal signalling markers such as in-situ hybridisation, would generate a clear picture of genotype specific changes.

Whilst each of these experiments individually can be expanded, the overall aims of assessing how dual-hit models can identify gene x environment interactions is also an area of research which can be grown exponentially. Incorporating other environmental stimuli such as prolonged maternal stress or early life stress (Bebbington *et al.*, 2004; Spauwen *et al.*, 2006; Khashan *et al.*, 2008) alongside investigation of other high-risk genetic variants such as the 22q11 deletion (Benedetti *et al.*, 2022) could provide new contexts for investigation. Additionally, it would also be important to continue investigation of neuronal changes at multiple developmental stages, such as immediately after MIA in the embryonic brain, at P5 in early development or during early adolescence as well as in adulthood, in order to determine the progression of altered neuronal signalling and inflammatory markers. Finally, assessing behavioural changes

during adolescence as well as in adulthood would also provide rich data for comparison and could elicit information around the long-term development of behavioural change. Overall, there are many avenues for potential research to continue or new research to begin based on the findings demonstrated throughout this thesis.

6.4 Final conclusions

To conclude, I aimed to determine the behavioural repertoire that could be detected using a commonly used model for schizophrenia. Next, I aimed to assess whether a similar behavioural repertoire would be seen in a dual-hit model for schizophrenia combining genetic and environmental risk factors. Finally, I aimed to determine whether this dual-hit model would also result in long-term neurobiological changes. Here, we found that PCP has sex-dependent and context dependent effects on social behaviours and anxiety as can be seen in humans. These effects were also seen as a result of combining genetic and environmental risk factors in a dual-hit model of schizophrenia using high-risk genes and MIA. Furthermore, gene x environment interactions were seen to affect behavioural outputs as well as gene x sex interactions that resulted in complex behavioural changes which mimic data found in humans. These studies also highlighted the importance of considering experimental context and its effect on behavioural output when drawing conclusions about complex behavioural phenotypes. Finally, MIA induced long term neurobiological changes to neuroinflammatory markers which highlight the relevance of the immune system in neurodevelopment and the role of inflammation in mental health conditions such as schizophrenia. These data provide the basis for several continuing future directions that could advance the area of research to a more translational approach which minimises experimental interference but provides rich data for analysis.

7 References

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8 Supplementary Materials

8.1 Tukey's Simultaneous Pairwise Comparisons for behavioural tests in PCP experiments

8.1.1 Open field Test

Difference of treat*sex Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(pcp m) - (pcp f)	-3470	379	(-4442, -2498)	-9.16	0.000
(saline f) - (pcp f)	-2737	381	(-3714, -1761)	-7.19	0.000
(saline m) - (pcp f)	-1581	363	(-2512, -649)	-4.36	0.000
(saline f) - (pcp m)	733	379	(-240, 1705)	1.93	0.214
(saline m) - (pcp m)	1889	361	(963, 2816)	5.23	0.000
(saline m) - (saline f)	1157	363	(225, 2088)	3.19	0.008

Table 8-1: Tukey's Simultaneous Tests for Differences between means in time spent in outer zones of open-field test.

Difference of sex*treat Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(f saline) - (f pcp)	65.2	82.2	(-145.7, 276.1)	0.79	0.858

(m pcp) - (f pcp)	-1379.7	80.1	(-1585.2, -1174.1)	-17.23	0.000
(m saline) - (f pcp)	-1547.7	78.3	(-1748.8, -1346.7)	-19.76	0.000
(m pcp) - (f saline)	-1444.8	80.1	(-1650.4, -1239.3)	-18.04	0.000
(m saline) - (f saline)	-1612.9	78.3	(-1814.0, -1411.8)	-20.59	0.000
(m saline) - (m pcp)	-168.1	76.2	(-363.5, 27.4)	-2.21	0.121

Table 8-2: Tukey's Simultaneous Tests for Differences between means in time spent in distance travelled in open-field test.

8.1.2 Elevated Plus Maze

Difference of sex*treat Levels	**Difference of Means**	**SE of Difference**	**Simultaneous 95% CI**	**T-Value**	**Adjusted P-Value**
(f saline) - (f pcp)	0.0113	0.0133	(-0.0228, 0.0454)	0.85	0.829
(m pcp) - (f pcp)	0.0555	0.0121	(0.0244, 0.0866)	4.58	0.000
(m saline) - (f pcp)	0.0454	0.0119	(0.0150, 0.0759)	3.83	0.001
(m pcp) - (f saline)	0.0442	0.0121	(0.0131, 0.0753)	3.65	0.001

(m saline) - (f saline)	0.0341	0.0119	(0.0037, 0.0646)	2.88	0.021
(m saline) - (m pcp)	-0.0101	0.0106	(-0.0372, 0.0170)	-0.96	0.774

Table 8-3: Tukey's Simultaneous Tests for Differences between means in time spent in open arms of EPM.

8.1.3 Home Cage Monitoring

Difference of sex*treat Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(f saline) - (f PCP)	36.0	22.5	(-21.7, 93.6)	1.60	0.379
(m PCP) - (f PCP)	-298.0	22.6	(-356.1, -239.9)	-13.17	0.000
(m saline) - (f PCP)	-288.0	22.5	(-345.8, -230.3)	-12.81	0.000
(m PCP) - (f saline)	-334.0	22.6	(-392.0, -276.0)	-14.78	0.000
(m saline) - (f saline)	-324.0	22.5	(-381.7, -266.3)	-14.42	0.000
(m saline) - (m PCP)	10.0	22.6	(-48.1, 68.0)	0.44	0.971

Table 8-4: Tukey's Simultaneous Tests for Differences between means in distance travelled in the home-cage.

Difference of sex*treat Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(f saline) - (f PCP)	1.67	1.15	(-1.28, 4.62)	1.45	0.468
(m PCP) - (f PCP)	4.81	1.16	(1.84, 7.78)	4.15	0.000
(m saline) - (f PCP)	4.44	1.15	(1.48, 7.39)	3.86	0.001
(m PCP) - (f saline)	3.14	1.16	(0.17, 6.11)	2.72	0.033
(m saline) - (f saline)	2.77	1.15	(-0.18, 5.72)	2.41	0.075
(m saline) - (m PCP)	-0.37	1.16	(-3.34, 2.60)	-0.32	0.989

Table 8-5: Tukey's Simultaneous Tests for Differences between means in separation from nearest cage-mate in the home cage.

8.1.4 Novel Object Recognition

Difference of group*sex Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(saline m) - (saline f)	0.080	0.197	(-0.465, 0.625)	0.41	0.977
(pcp f) - (saline f)	0.201	0.220	(-0.407, 0.809)	0.91	0.797
(pcp m) - (saline f)	0.249	0.197	(-0.296, 0.794)	1.26	0.594

(pcp f) - (saline m)	0.121	0.183	(-0.384, 0.627)	0.66	0.910
(pcp m) - (saline m)	0.169	0.155	(-0.258, 0.597)	1.09	0.696
(pcp m) - (pcp f)	0.048	0.183	(-0.458, 0.554)	0.26	0.994

Table 8-6: Tukey's Simultaneous Tests for Differences between means in Novel Object Index.

8.1.5 Paired Discrimination and Reversal Learning

Difference of Treat_rev Levels	**Difference of Means**	**SE of Difference**	**Simultaneous 95% CI**	**T-Value**	**Adjusted P-Value**
pcp - sal	-0.0049	0.0212	(-0.0468, 0.0371)	-0.23	0.819

Table 8-7: Tukey's Simultaneous Tests for Differences between means in accuracy of paired discrimination.

8.2 Tukey's Simultaneous Pairwise Comparisons for behavioural tests in PCP experiments

8.2.1 Open-Field Test

Difference of genotype*treatment*sex Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(DUP PolyIC M) - (DUP PolyIC F)	-1657	153	(-2158, -1157)	-10.82	0.000
(DUP Resiquimod F) - (DUP PolyIC F)	-1968	117	(-2351, -1585)	-16.78	0.000
(DUP Resiquimod M) - (DUP PolyIC F)	-2406	130	(-2831, -1982)	-18.53	0.000
(DUP Saline F) - (DUP PolyIC F)	-2770	126	(-3181, -2360)	-22.03	0.000
(DUP Saline M) - (DUP PolyIC F)	-3075	153	(-3576, -2575)	-20.07	0.000
(WT PolyIC F) - (DUP PolyIC F)	-2268	121	(-2662, -1874)	-18.81	0.000
(WT PolyIC M) - (DUP PolyIC F)	-2660	126	(-3071, -2250)	-21.16	0.000
(WT Resiquimod F) - (DUP PolyIC F)	-1698	135	(-2140, -1257)	-12.57	0.000
(WT Resiquimod M) - (DUP PolyIC F)	-2105	135	(-2547, -1664)	-15.58	0.000

(WT Saline F) - (DUP PolyIC F)	-2626	135	(-3066, -2187)	-19.52	0.000
(WT Saline M) - (DUP PolyIC F)	-2404	135	(-2843, -1964)	-17.86	0.000
(DUP Resiquimod F) - (DUP PolyIC M)	-310	131	(-738, 117)	-2.37	0.424
(DUP Resiquimod M) - (DUP PolyIC M)	-749	142	(-1214, -284)	-5.27	0.000
(DUP Saline F) - (DUP PolyIC M)	-1113	138	(-1565, -661)	-8.04	0.000
(DUP Saline M) - (DUP PolyIC M)	-1418	164	(-1953, -883)	-8.66	0.000
(WT PolyIC F) - (DUP PolyIC M)	-610	134	(-1047, -174)	-4.56	0.000
(WT PolyIC M) - (DUP PolyIC M)	-1003	138	(-1455, -551)	-7.25	0.000
(WT Resiquimod F) - (DUP PolyIC M)	-41	147	(-521, 440)	-0.28	1.000
(WT Resiquimod M) - (DUP PolyIC M)	-448	147	(-928, 32)	-3.05	0.096
(WT Saline F) - (DUP PolyIC M)	-969	146	(-1447, -490)	-6.61	0.000
(WT Saline M) - (DUP PolyIC M)	-746	146	(-1225, -268)	-5.09	0.000
(DUP Resiquimod M) - (DUP Resiquimod F)	-439	102	(-773, -104)	-4.28	0.001

(DUP Saline F) - (DUP Resiquimod F)	-802.4	97.1	(-1119.8, -485.1)	-8.26	0.000
(DUP Saline M) - (DUP Resiquimod F)	-1107	131	(-1535, -680)	-8.47	0.000
(WT PolyIC F) - (DUP Resiquimod F)	-300.0	90.3	(-595.1, -4.9)	-3.32	0.042
(WT PolyIC M) - (DUP Resiquimod F)	-692.5	97.1	(-1009.9, -375.2)	-7.13	0.000
(WT Resiquimod F) - (DUP Resiquimod F)	270	109	(-86, 626)	2.47	0.356
(WT Resiquimod M) - (DUP Resiquimod F)	-137	109	(-493, 219)	-1.26	0.984
(WT Saline F) - (DUP Resiquimod F)	-658	108	(-1012, -304)	-6.08	0.000
(WT Saline M) - (DUP Resiquimod F)	-436	108	(-790, -82)	-4.02	0.003
(DUP Saline F) - (DUP Resiquimod M)	-364	112	(-730, 2)	-3.25	0.053
(DUP Saline M) - (DUP Resiquimod M)	-669	142	(-1133, -204)	-4.70	0.000
(WT PolyIC F) - (DUP Resiquimod M)	139	106	(-208, 486)	1.30	0.979
(WT PolyIC M) - (DUP Resiquimod M)	-254	112	(-620, 112)	-2.27	0.501
(WT Resiquimod F) - (DUP Resiquimod M)	708	122	(308, 1108)	5.78	0.000

(WT Resiquimod M) - (DUP Resiquimod M)	301	122	(-99, 701)	2.46	0.366
(WT Saline F) - (DUP Resiquimod M)	-220	122	(-618, 178)	-1.80	0.816
(WT Saline M) - (DUP Resiquimod M)	3	122	(-395, 401)	0.02	1.000
(DUP Saline M) - (DUP Saline F)	-305	138	(-757, 147)	-2.20	0.548
(WT PolyIC F) - (DUP Saline F)	502	101	(172, 833)	4.97	0.000
(WT PolyIC M) - (DUP Saline F)	110	107	(-240, 460)	1.02	0.997
(WT Resiquimod F) - (DUP Saline F)	1072	118	(686, 1458)	9.08	0.000
(WT Resiquimod M) - (DUP Saline F)	665	118	(279, 1051)	5.63	0.000
(WT Saline F) - (DUP Saline F)	144	117	(-240, 528)	1.23	0.987
(WT Saline M) - (DUP Saline F)	367	117	(-17, 750)	3.12	0.077
(WT PolyIC F) - (DUP Saline M)	807	134	(370, 1244)	6.04	0.000
(WT PolyIC M) - (DUP Saline M)	415	138	(-37, 867)	3.00	0.109
(WT Resiquimod F) - (DUP Saline M)	1377	147	(897, 1857)	9.37	0.000

(WT Resiquimod M) - (DUP Saline M)	970	147	(490, 1450)	6.60	0.000
(WT Saline F) - (DUP Saline M)	449	146	(-30, 928)	3.06	0.091
(WT Saline M) - (DUP Saline M)	672	146	(193, 1150)	4.58	0.000
(WT PolyIC M) - (WT PolyIC F)	-393	101	(-723, -62)	-3.88	0.006
(WT Resiquimod F) - (WT PolyIC F)	570	113	(202, 937)	5.06	0.000
(WT Resiquimod M) - (WT PolyIC F)	163	113	(-205, 530)	1.44	0.955
(WT Saline F) - (WT PolyIC F)	-358	112	(-724, 7)	-3.20	0.061
(WT Saline M) - (WT PolyIC F)	-136	112	(-501, 230)	-1.21	0.988
(WT Resiquimod F) - (WT PolyIC M)	962	118	(577, 1348)	8.15	0.000
(WT Resiquimod M) - (WT PolyIC M)	555	118	(169, 941)	4.70	0.000
(WT Saline F) - (WT PolyIC M)	34	117	(-350, 418)	0.29	1.000
(WT Saline M) - (WT PolyIC M)	257	117	(-127, 641)	2.19	0.560
(WT Resiquimod M) - (WT Resiquimod F)	-407	128	(-825, 11)	-3.18	0.065

(WT Saline F) - (WT Resiquimod F)	-928	127	(-1345, -512)	-7.28	0.000
(WT Saline M) - (WT Resiquimod F)	-706	127	(-1122, -289)	-5.54	0.000
(WT Saline F) - (WT Resiquimod M)	-521	127	(-937, -105)	-4.09	0.003
(WT Saline M) - (WT Resiquimod M)	-298	127	(-715, 118)	-2.34	0.447
(WT Saline M) - (WT Saline F)	223	127	(-192, 637)	1.76	0.842

Table 8-8: Tukey's Simultaneous Tests for Differences between means in distance travelled in the open field.

Difference of genotype*treatment*sex Levels	**Difference of Means**	**SE of Difference**	**Simultaneous 95% CI**	**T-Value**	**Adjusted P-Value**
(DUP PolyIC M) - (DUP PolyIC F)	-29.6	16.0	(-81.8, 22.5)	-1.86	0.787
(DUP Resiquimod F) - (DUP PolyIC F)	17.8	12.2	(-22.1, 57.7)	1.46	0.952
(DUP Resiquimod M) - (DUP PolyIC F)	7.7	13.5	(-36.5, 51.9)	0.57	1.000
(DUP Saline F) - (DUP PolyIC F)	-101.2	13.1	(-144.0, -58.4)	-7.73	0.000
(DUP Saline M) - (DUP PolyIC F)	5.6	16.0	(-46.5, 57.8)	0.35	1.000

(WT PolyIC F) - (DUP PolyIC F)	25.0	12.6	(-16.0, 66.0)	1.99	0.700
(WT PolyIC M) - (DUP PolyIC F)	0.3	13.1	(-42.5, 43.1)	0.02	1.000
(WT Resiquimod F) - (DUP PolyIC F)	-33.2	14.1	(-79.2, 12.8)	-2.36	0.434
(WT Resiquimod M) - (DUP PolyIC F)	-11.2	14.1	(-57.2, 34.8)	-0.79	1.000
(WT Saline F) - (DUP PolyIC F)	-126.7	14.0	(-172.5, -80.9)	-9.03	0.000
(WT Saline M) - (DUP PolyIC F)	-36.8	14.0	(-82.6, 9.0)	-2.62	0.267
(DUP Resiquimod F) - (DUP PolyIC M)	47.4	13.6	(2.9, 91.9)	3.48	0.025
(DUP Resiquimod M) - (DUP PolyIC M)	37.3	14.8	(-11.1, 85.7)	2.52	0.328
(DUP Saline F) - (DUP PolyIC M)	-71.6	14.4	(-118.7, -24.5)	-4.97	0.000
(DUP Saline M) - (DUP PolyIC M)	35.2	17.1	(-20.5, 91.0)	2.07	0.648
(WT PolyIC F) - (DUP PolyIC M)	54.6	13.9	(9.1, 100.1)	3.92	0.005
(WT PolyIC M) - (DUP PolyIC M)	29.9	14.4	(-17.2, 77.0)	2.07	0.643
(WT Resiquimod F) - (DUP PolyIC M)	-3.6	15.3	(-53.6, 46.4)	-0.24	1.000

(WT Resiquimod M) - (DUP PolyIC M)	18.4	15.3	(-31.6, 68.5)	1.20	0.989
(WT Saline F) - (DUP PolyIC M)	-97.0	15.3	(-146.9, -47.2)	-6.36	0.000
(WT Saline M) - (DUP PolyIC M)	-7.1	15.3	(-57.0, 42.7)	-0.47	1.000
(DUP Resiquimod M) - (DUP Resiquimod F)	-10.1	10.7	(-45.0, 24.8)	-0.95	0.999
(DUP Saline F) - (DUP Resiquimod F)	-119.0	10.1	(-152.1, -86.0)	-11.76	0.000
(DUP Saline M) - (DUP Resiquimod F)	-12.2	13.6	(-56.7, 32.3)	-0.89	0.999
(WT PolyIC F) - (DUP Resiquimod F)	7.20	9.41	(-23.54, 37.95)	0.77	1.000
(WT PolyIC M) - (DUP Resiquimod F)	-17.5	10.1	(-50.6, 15.5)	-1.73	0.853
(WT Resiquimod F) - (DUP Resiquimod F)	-51.0	11.4	(-88.1, -13.9)	-4.49	0.000
(WT Resiquimod M) - (DUP Resiquimod F)	-29.0	11.4	(-66.1, 8.1)	-2.55	0.308
(WT Saline F) - (DUP Resiquimod F)	-144.5	11.3	(-181.3, -107.6)	-12.80	0.000
(WT Saline M) - (DUP Resiquimod F)	-54.6	11.3	(-91.4, -17.7)	-4.83	0.000
(DUP Saline F) - (DUP Resiquimod M)	-108.9	11.7	(-147.1, -70.8)	-9.33	0.000

(DUP Saline M) - (DUP Resiquimod M)	-2.1	14.8	(-50.5, 46.3)	-0.14	1.000
(WT PolyIC F) - (DUP Resiquimod M)	17.3	11.1	(-18.9, 53.4)	1.56	0.922
(WT PolyIC M) - (DUP Resiquimod M)	-7.4	11.7	(-45.6, 30.7)	-0.64	1.000
(WT Resiquimod F) - (DUP Resiquimod M)	-40.9	12.8	(-82.6, 0.8)	-3.21	0.060
(WT Resiquimod M) - (DUP Resiquimod M)	-18.9	12.8	(-60.6, 22.8)	-1.48	0.946
(WT Saline F) - (DUP Resiquimod M)	-134.4	12.7	(-175.8, -92.9)	-10.58	0.000
(WT Saline M) - (DUP Resiquimod M)	-44.5	12.7	(-86.0, -3.0)	-3.50	0.023
(DUP Saline M) - (DUP Saline F)	106.9	14.4	(59.7, 154.0)	7.41	0.000
(WT PolyIC F) - (DUP Saline F)	126.2	10.5	(91.8, 160.6)	11.99	0.000
(WT PolyIC M) - (DUP Saline F)	101.5	11.2	(65.0, 138.0)	9.09	0.000
(WT Resiquimod F) - (DUP Saline F)	68.0	12.3	(27.8, 108.2)	5.53	0.000
(WT Resiquimod M) - (DUP Saline F)	90.0	12.3	(49.9, 130.2)	7.32	0.000
(WT Saline F) - (DUP Saline F)	-25.4	12.2	(-65.4, 14.5)	-2.08	0.639

(WT Saline M) - (DUP Saline F)	64.5	12.2	(24.5, 104.4)	5.27	0.000
(WT PolyIC F) - (DUP Saline M)	19.4	13.9	(-26.1, 64.9)	1.39	0.966
(WT PolyIC M) - (DUP Saline M)	-5.4	14.4	(-52.5, 41.7)	-0.37	1.000
(WT Resiquimod F) - (DUP Saline M)	-38.9	15.3	(-88.9, 11.2)	-2.54	0.317
(WT Resiquimod M) - (DUP Saline M)	-16.8	15.3	(-66.8, 33.2)	-1.10	0.995
(WT Saline F) - (DUP Saline M)	-132.3	15.3	(-182.1, -82.4)	-8.67	0.000
(WT Saline M) - (DUP Saline M)	-42.4	15.3	(-92.2, 7.5)	-2.78	0.190
(WT PolyIC M) - (WT PolyIC F)	-24.7	10.5	(-59.1, 9.7)	-2.35	0.441
(WT Resiquimod F) - (WT PolyIC F)	-58.2	11.7	(-96.5, -19.9)	-4.97	0.000
(WT Resiquimod M) - (WT PolyIC F)	-36.2	11.7	(-74.5, 2.1)	-3.09	0.086
(WT Saline F) - (WT PolyIC F)	-151.7	11.7	(-189.7, -113.6)	-13.01	0.000
(WT Saline M) - (WT PolyIC F)	-61.8	11.7	(-99.8, -23.7)	-5.30	0.000
(WT Resiquimod F) - (WT PolyIC M)	-33.5	12.3	(-73.7, 6.7)	-2.72	0.215

(WT Resiquimod M) - (WT PolyIC M)	-11.4	12.3	(-51.6, 28.7)	-0.93	0.999
(WT Saline F) - (WT PolyIC M)	-126.9	12.2	(-166.9, -86.9)	-10.37	0.000
(WT Saline M) - (WT PolyIC M)	-37.0	12.2	(-77.0, 3.0)	-3.03	0.101
(WT Resiquimod M) - (WT Resiquimod F)	22.0	13.3	(-21.5, 65.6)	1.65	0.889
(WT Saline F) - (WT Resiquimod F)	-93.4	13.3	(-136.8, -50.1)	-7.04	0.000
(WT Saline M) - (WT Resiquimod F)	-3.5	13.3	(-46.9, 39.8)	-0.27	1.000
(WT Saline F) - (WT Resiquimod M)	-115.5	13.3	(-158.8, -72.1)	-8.70	0.000
(WT Saline M) - (WT Resiquimod M)	-25.6	13.3	(-69.0, 17.8)	-1.93	0.743
(WT Saline M) - (WT Saline F)	89.9	13.2	(46.7, 133.1)	6.80	0.000

Table 8-9: Tukey's Simultaneous Tests for Differences between means in time spent in the outer zones of the open field.

8.2.2 Elevated Plus Maze

Difference of treatment*sex*genotype Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(saline female wt) - (saline female dup)	0.0216	0.0185	(-0.0388, 0.0821)	1.17	0.991
(saline male dup) - (saline female dup)	-0.0020	0.0205	(-0.0688, 0.0649)	-0.10	1.000
(saline male wt) - (saline female dup)	0.0852	0.0172	(0.0289, 0.1415)	4.94	0.000
(polyic female dup) - (saline female dup)	0.0989	0.0185	(0.0384, 0.1594)	5.34	0.000
(polyic female wt) - (saline female dup)	0.0945	0.0147	(0.0465, 0.1424)	6.43	0.000
(polyic male dup) - (saline female dup)	0.0474	0.0185	(-0.0130, 0.1079)	2.56	0.302
(polyic male wt) - (saline female dup)	0.0493	0.0163	(-0.0040, 0.1027)	3.02	0.102
(Resiquimod female dup) - (saline female dup)	0.0889	0.0140	(0.0430, 0.1348)	6.33	0.000
(Resiquimod female wt) - (saline female dup)	0.0276	0.0172	(-0.0287, 0.0839)	1.60	0.909
(Resiquimod male dup) - (saline female dup)	0.1434	0.0163	(0.0900, 0.1967)	8.78	0.000

(Resiquimod male wt) - (saline female dup)	0.0355	0.0172	(-0.0208, 0.0918)	2.06	0.651
(saline male dup) - (saline female wt)	-0.0236	0.0231	(-0.0990, 0.0518)	-1.02	0.997
(saline male wt) - (saline female wt)	0.0635	0.0203	(-0.0027, 0.1298)	3.13	0.075
(polyic female dup) - (saline female wt)	0.0773	0.0214	(0.0074, 0.1471)	3.61	0.016
(polyic female wt) - (saline female wt)	0.0728	0.0182	(0.0135, 0.1322)	4.01	0.004
(polyic male dup) - (saline female wt)	0.0258	0.0214	(-0.0441, 0.0956)	1.21	0.989
(polyic male wt) - (saline female wt)	0.0277	0.0195	(-0.0361, 0.0914)	1.42	0.960
(Resiquimod female dup) - (saline female wt)	0.0673	0.0177	(0.0096, 0.1249)	3.81	0.008
(Resiquimod female wt) - (saline female wt)	0.0059	0.0203	(-0.0603, 0.0722)	0.29	1.000
(Resiquimod male dup) - (saline female wt)	0.1217	0.0195	(0.0580, 0.1855)	6.24	0.000
(Resiquimod male wt) - (saline female wt)	0.0139	0.0203	(-0.0524, 0.0801)	0.68	1.000
(saline male wt) - (saline male dup)	0.0871	0.0221	(0.0150, 0.1593)	3.95	0.005

(polyic female dup) - (saline male dup)	0.1009	0.0231	(0.0254, 0.1763)	4.37	0.001
(polyic female wt) - (saline male dup)	0.0964	0.0202	(0.0306, 0.1623)	4.78	0.000
(polyic male dup) - (saline male dup)	0.0494	0.0231	(-0.0260, 0.1248)	2.14	0.594
(polyic male wt) - (saline male dup)	0.0513	0.0214	(-0.0185, 0.1211)	2.40	0.406
(Resiquimod female dup) - (saline male dup)	0.0909	0.0197	(0.0266, 0.1552)	4.62	0.000
(Resiquimod female wt) - (saline male dup)	0.0296	0.0221	(-0.0426, 0.1017)	1.34	0.974
(Resiquimod male dup) - (saline male dup)	0.1453	0.0214	(0.0755, 0.2152)	6.80	0.000
(Resiquimod male wt) - (saline male dup)	0.0375	0.0221	(-0.0346, 0.1096)	1.70	0.870
(polyic female dup) - (saline male wt)	0.0137	0.0203	(-0.0525, 0.0800)	0.68	1.000
(polyic female wt) - (saline male wt)	0.0093	0.0169	(-0.0458, 0.0644)	0.55	1.000
(polyic male dup) - (saline male wt)	-0.0377	0.0203	(-0.1040, 0.0285)	-1.86	0.784
(polyic male wt) - (saline male wt)	-0.0358	0.0183	(-0.0956, 0.0240)	-1.96	0.722
(Resiquimod female dup) - (saline male wt)	0.0038	0.0163	(-0.0495, 0.0570)	0.23	1.000

(Resiquimod female wt) - (saline male wt)	-0.0576	0.0191	(-0.1200, 0.0049)	-3.01	0.105
(Resiquimod male dup) - (saline male wt)	0.0582	0.0183	(-0.0016, 0.1180)	3.18	0.065
(Resiquimod male wt) - (saline male wt)	-0.0496	0.0191	(-0.1121, 0.0128)	-2.60	0.281
(polyic female wt) - (polyic female dup)	-0.0045	0.0182	(-0.0638, 0.0549)	-0.25	1.000
(polyic male dup) - (polyic female dup)	-0.0515	0.0214	(-0.1213, 0.0184)	-2.41	0.400
(polyic male wt) - (polyic female dup)	-0.0496	0.0195	(-0.1133, 0.0142)	-2.54	0.314
(Resiquimod female dup) - (polyic female dup)	-0.0100	0.0177	(-0.0676, 0.0477)	-0.57	1.000
(Resiquimod female wt) - (polyic female dup)	-0.0713	0.0203	(-0.1376, -0.0051)	-3.52	0.022
(Resiquimod male dup) - (polyic female dup)	0.0445	0.0195	(-0.0193, 0.1082)	2.28	0.492
(Resiquimod male wt) - (polyic female dup)	-0.0634	0.0203	(-0.1296, 0.0029)	-3.13	0.076
(polyic male dup) - (polyic female wt)	-0.0470	0.0182	(-0.1064, 0.0123)	-2.59	0.286
(polyic male wt) - (polyic female wt)	-0.0451	0.0159	(-0.0972, 0.0069)	-2.83	0.166

(Resiquimod female dup) - (polyic female wt)	-0.0055	0.0136	(-0.0499, 0.0389)	-0.41	1.000
(Resiquimod female wt) - (polyic female wt)	-0.0669	0.0169	(-0.1220, -0.0118)	-3.97	0.004
(Resiquimod male dup) - (polyic female wt)	0.0489	0.0159	(-0.0031, 0.1010)	3.07	0.090
(Resiquimod male wt) - (polyic female wt)	-0.0589	0.0169	(-0.1140, -0.0039)	-3.50	0.024
(polyic male wt) - (polyic male dup)	0.0019	0.0195	(-0.0619, 0.0656)	0.10	1.000
(Resiquimod female dup) - (polyic male dup)	0.0415	0.0177	(-0.0162, 0.0992)	2.35	0.440
(Resiquimod female wt) - (polyic male dup)	-0.0198	0.0203	(-0.0861, 0.0464)	-0.98	0.998
(Resiquimod male dup) - (polyic male dup)	0.0959	0.0195	(0.0322, 0.1597)	4.92	0.000
(Resiquimod male wt) - (polyic male dup)	-0.0119	0.0203	(-0.0782, 0.0543)	-0.59	1.000
(Resiquimod female dup) - (polyic male wt)	0.0396	0.0153	(-0.0105, 0.0897)	2.58	0.290
(Resiquimod female wt) - (polyic male wt)	-0.0217	0.0183	(-0.0815, 0.0381)	-1.19	0.990
(Resiquimod male dup) - (polyic male wt)	0.0940	0.0175	(0.0370, 0.1511)	5.39	0.000

(Resiquimod male wt) - (polyic male wt)	-0.0138	0.0183	(-0.0736, 0.0460)	-0.75	1.000
(Resiquimod female wt) - (Resiquimod female dup)	-0.0613	0.0163	(-0.1146, -0.0081)	-3.76	0.009
(Resiquimod male dup) - (Resiquimod female dup)	0.0544	0.0153	(0.0043, 0.1046)	3.55	0.020
(Resiquimod male wt) - (Resiquimod female dup)	-0.0534	0.0163	(-0.1067, -0.0001)	-3.28	0.049
(Resiquimod male dup) - (Resiquimod female wt)	0.1158	0.0183	(0.0560, 0.1756)	6.32	0.000
(Resiquimod male wt) - (Resiquimod female wt)	0.0079	0.0191	(-0.0545, 0.0704)	0.41	1.000
(Resiquimod male wt) - (Resiquimod male dup)	-0.1079	0.0183	(-0.1677, -0.0480)	-5.89	0.000

Table 8-10: Tukey's Simultaneous Tests for Differences between means in time spent in the open arms of the EPM.

8.2.3 Home-cage Monitoring

Difference of sex*genotype*treatment Levels	**Difference of Means**	**SE of Difference**	**Simultaneous 95% CI**	**T-Value**	**Adjusted P-Value**
(F DUP Resiquimod) - (F DUP PolyIC)	-908.4	39.8	(-1038.5, -778.2)	-22.80	0.000
(F DUP Saline) - (F DUP PolyIC)	-822.7	48.4	(-980.7, -664.6)	-17.00	0.000

(F WT PolyIC) - (F DUP PolyIC)	-452.0	42.5	(-590.8, -313.2)	-10.64	0.000
(F WT Resiquimod) - (F DUP PolyIC)	-857.3	44.8	(-1003.7, -711.0)	-19.14	0.000
(F WT Saline) - (F DUP PolyIC)	-796.5	48.4	(-954.5, -638.5)	-16.46	0.000
(M DUP PolyIC) - (F DUP PolyIC)	-448.9	49.0	(-609.1, -288.7)	-9.15	0.000
(M DUP Resiquimod) - (F DUP PolyIC)	-1006.0	48.7	(-1165.1, -847.0)	-20.66	0.000
(M DUP Saline) - (F DUP PolyIC)	-801.2	55.7	(-983.0, -619.3)	-14.39	0.000
(M WT PolyIC) - (F DUP PolyIC)	-607.9	41.3	(-742.8, -473.0)	-14.72	0.000
(M WT Resiquimod) - (F DUP PolyIC)	-781.6	49.3	(-942.7, -620.4)	-15.84	0.000
(M WT Saline) - (F DUP PolyIC)	-840.4	42.6	(-979.5, -701.4)	-19.74	0.000
(F DUP Saline) - (F DUP Resiquimod)	85.7	43.8	(-57.5, 228.9)	1.95	0.724
(F WT PolyIC) - (F DUP Resiquimod)	456.4	37.2	(334.7, 578.0)	12.25	0.000
(F WT Resiquimod) - (F DUP Resiquimod)	51.0	39.8	(-79.1, 181.2)	1.28	0.982
(F WT Saline) - (F DUP Resiquimod)	111.9	43.8	(-31.4, 255.1)	2.55	0.308

(M DUP PolyIC) - (F DUP Resiquimod)	459.5	44.6	(313.9, 605.1)	10.31	0.000
(M DUP Resiquimod) - (F DUP Resiquimod)	-97.7	44.2	(-242.0, 46.6)	-2.21	0.541
(M DUP Saline) - (F DUP Resiquimod)	107.2	51.7	(-61.8, 276.2)	2.07	0.643
(M WT PolyIC) - (F DUP Resiquimod)	300.5	35.9	(183.3, 417.6)	8.38	0.000
(M WT Resiquimod) - (F DUP Resiquimod)	126.8	44.9	(-19.7, 273.3)	2.83	0.169
(M WT Saline) - (F DUP Resiquimod)	67.9	37.3	(-54.0, 189.9)	1.82	0.808
(F WT PolyIC) - (F DUP Saline)	370.7	46.3	(219.5, 521.8)	8.01	0.000
(F WT Resiquimod) - (F DUP Saline)	-34.7	48.4	(-192.7, 123.4)	-0.72	1.000
(F WT Saline) - (F DUP Saline)	26.2	51.7	(-142.8, 195.1)	0.51	1.000
(M DUP PolyIC) - (F DUP Saline)	373.8	52.3	(202.8, 544.8)	7.14	0.000
(M DUP Resiquimod) - (F DUP Saline)	-183.4	52.0	(-353.3, -13.5)	-3.53	0.022
(M DUP Saline) - (F DUP Saline)	21.5	58.6	(-169.9, 212.9)	0.37	1.000
(M WT PolyIC) - (F DUP Saline)	214.8	45.2	(67.3, 362.3)	4.76	0.000

(M WT Resiquimod) - (F DUP Saline)	41.1	52.6	(-130.8, 213.0)	0.78	1.000
(M WT Saline) - (F DUP Saline)	-17.8	46.3	(-169.1, 133.6)	-0.38	1.000
(F WT Resiquimod) - (F WT PolyIC)	-405.3	42.5	(-544.1, -266.5)	-9.54	0.000
(F WT Saline) - (F WT PolyIC)	-344.5	46.3	(-495.6, -193.4)	-7.45	0.000
(M DUP PolyIC) - (F WT PolyIC)	3.1	46.9	(-150.2, 156.5)	0.07	1.000
(M DUP Resiquimod) - (F WT PolyIC)	-554.0	46.6	(-706.2, -401.9)	-11.89	0.000
(M DUP Saline) - (F WT PolyIC)	-349.2	53.8	(-525.0, -173.3)	-6.49	0.000
(M WT PolyIC) - (F WT PolyIC)	-155.9	38.8	(-282.6, -29.2)	-4.02	0.003
(M WT Resiquimod) - (F WT PolyIC)	-329.6	47.3	(-484.0, -175.2)	-6.97	0.000
(M WT Saline) - (F WT PolyIC)	-388.4	40.1	(-519.6, -257.3)	-9.68	0.000
(F WT Saline) - (F WT Resiquimod)	60.8	48.4	(-97.2, 218.9)	1.26	0.984
(M DUP PolyIC) - (F WT Resiquimod)	408.5	49.0	(248.3, 568.7)	8.33	0.000
(M DUP Resiquimod) - (F WT Resiquimod)	-148.7	48.7	(-307.8, 10.3)	-3.05	0.093

(M DUP Saline) - (F WT Resiquimod)	56.2	55.7	(-125.7, 238.0)	1.01	0.998
(M WT PolyIC) - (F WT Resiquimod)	249.5	41.3	(114.6, 384.3)	6.04	0.000
(M WT Resiquimod) - (F WT Resiquimod)	75.8	49.3	(-85.4, 237.0)	1.54	0.931
(M WT Saline) - (F WT Resiquimod)	16.9	42.6	(-122.2, 156.0)	0.40	1.000
(M DUP PolyIC) - (F WT Saline)	347.6	52.3	(176.7, 518.6)	6.64	0.000
(M DUP Resiquimod) - (F WT Saline)	-209.5	52.0	(-379.4, -39.6)	-4.03	0.003
(M DUP Saline) - (F WT Saline)	-4.7	58.6	(-196.1, 186.7)	-0.08	1.000
(M WT PolyIC) - (F WT Saline)	188.6	45.2	(41.1, 336.1)	4.18	0.002
(M WT Resiquimod) - (F WT Saline)	14.9	52.6	(-157.0, 186.8)	0.28	1.000
(M WT Saline) - (F WT Saline)	-43.9	46.3	(-195.3, 107.4)	-0.95	0.999
(M DUP Resiquimod) - (M DUP PolyIC)	-557.2	52.6	(-729.1, -385.3)	-10.59	0.000
(M DUP Saline) - (M DUP PolyIC)	-352.3	59.1	(-545.5, -159.1)	-5.96	0.000
(M WT PolyIC) - (M DUP PolyIC)	-159.0	45.8	(-308.7, -9.3)	-3.47	0.026

(M WT Resiquimod) - (M DUP PolyIC)	-332.7	53.2	(-506.6, -158.8)	-6.25	0.000
(M WT Saline) - (M DUP PolyIC)	-391.6	47.0	(-545.2, -238.0)	-8.33	0.000
(M DUP Saline) - (M DUP Resiquimod)	204.9	58.8	(12.7, 397.0)	3.48	0.025
(M WT PolyIC) - (M DUP Resiquimod)	398.2	45.5	(249.6, 546.8)	8.75	0.000
(M WT Resiquimod) - (M DUP Resiquimod)	224.5	52.9	(51.8, 397.2)	4.25	0.001
(M WT Saline) - (M DUP Resiquimod)	165.6	46.7	(13.2, 318.0)	3.55	0.020
(M WT PolyIC) - (M DUP Saline)	193.3	52.9	(20.5, 366.1)	3.65	0.014
(M WT Resiquimod) - (M DUP Saline)	19.6	59.3	(-174.2, 213.4)	0.33	1.000
(M WT Saline) - (M DUP Saline)	-39.3	53.9	(-215.3, 136.8)	-0.73	1.000
(M WT Resiquimod) - (M WT PolyIC)	-173.7	46.2	(-324.6, -22.8)	-3.76	0.009
(M WT Saline) - (M WT PolyIC)	-232.6	38.9	(-359.6, -105.6)	-5.98	0.000
(M WT Saline) - (M WT Resiquimod)	-58.9	47.3	(-213.5, 95.7)	-1.24	0.985

Table 8-11: Tukey's Simultaneous Tests for Differences between means in distance travelled in the home-cage.

Difference of sex*genotype*treatment Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(F DUP Resiquimod) - (F DUP PolyIC)	-18.26	1.93	(-24.56, -11.96)	-9.47	0.000
(F DUP Saline) - (F DUP PolyIC)	-18.91	2.34	(-26.56, -11.26)	-8.08	0.000
(F WT PolyIC) - (F DUP PolyIC)	-5.19	2.06	(-11.91, 1.53)	-2.52	0.325
(F WT Resiquimod) - (F DUP PolyIC)	-27.08	2.17	(-34.16, -19.99)	-12.49	0.000
(F WT Saline) - (F DUP PolyIC)	-18.86	2.34	(-26.51, -11.22)	-8.06	0.000
(M DUP PolyIC) - (F DUP PolyIC)	-7.03	2.37	(-14.78, 0.73)	-2.96	0.120
(M DUP Resiquimod) - (F DUP PolyIC)	0.86	2.36	(-6.83, 8.56)	0.37	1.000
(M DUP Saline) - (F DUP PolyIC)	-11.11	2.69	(-19.91, -2.31)	-4.12	0.002
(M WT PolyIC) - (F DUP PolyIC)	-2.32	2.00	(-8.85, 4.21)	-1.16	0.992
(M WT Resiquimod) - (F DUP PolyIC)	-13.77	2.39	(-21.57, -5.97)	-5.77	0.000
(M WT Saline) - (F DUP PolyIC)	-13.46	2.06	(-20.19, -6.73)	-6.53	0.000

(F DUP Saline) - (F DUP Resiquimod)	-0.65	2.12	(-7.58, 6.28)	-0.31	1.000
(F WT PolyIC) - (F DUP Resiquimod)	13.07	1.80	(7.18, 18.96)	7.25	0.000
(F WT Resiquimod) - (F DUP Resiquimod)	-8.82	1.93	(-15.11, -2.52)	-4.57	0.000
(F WT Saline) - (F DUP Resiquimod)	-0.61	2.12	(-7.54, 6.32)	-0.29	1.000
(M DUP PolyIC) - (F DUP Resiquimod)	11.23	2.16	(4.19, 18.28)	5.21	0.000
(M DUP Resiquimod) - (F DUP Resiquimod)	19.12	2.14	(12.14, 26.10)	8.95	0.000
(M DUP Saline) - (F DUP Resiquimod)	7.15	2.50	(-1.03, 15.33)	2.86	0.157
(M WT PolyIC) - (F DUP Resiquimod)	15.94	1.74	(10.27, 21.61)	9.19	0.000
(M WT Resiquimod) - (F DUP Resiquimod)	4.49	2.17	(-2.61, 11.58)	2.07	0.647
(M WT Saline) - (F DUP Resiquimod)	4.80	1.81	(-1.10, 10.70)	2.66	0.247
(F WT PolyIC) - (F DUP Saline)	13.72	2.24	(6.41, 21.03)	6.13	0.000
(F WT Resiquimod) - (F DUP Saline)	-8.17	2.34	(-15.81, -0.52)	-3.49	0.025
(F WT Saline) - (F DUP Saline)	0.04	2.50	(-8.13, 8.22)	0.02	1.000

(M DUP PolyIC) - (F DUP Saline)	11.88	2.53	(3.61, 20.16)	4.69	0.000
(M DUP Resiquimod) - (F DUP Saline)	19.77	2.52	(11.55, 27.99)	7.86	0.000
(M DUP Saline) - (F DUP Saline)	7.80	2.84	(-1.46, 17.06)	2.75	0.201
(M WT PolyIC) - (F DUP Saline)	16.59	2.19	(9.45, 23.73)	7.59	0.000
(M WT Resiquimod) - (F DUP Saline)	5.13	2.55	(-3.18, 13.45)	2.02	0.682
(M WT Saline) - (F DUP Saline)	5.45	2.24	(-1.87, 12.78)	2.43	0.384
(F WT Resiquimod) - (F WT PolyIC)	-21.89	2.06	(-28.60, -15.17)	-10.64	0.000
(F WT Saline) - (F WT PolyIC)	-13.68	2.24	(-20.99, -6.36)	-6.11	0.000
(M DUP PolyIC) - (F WT PolyIC)	-1.84	2.27	(-9.26, 5.59)	-0.81	1.000
(M DUP Resiquimod) - (F WT PolyIC)	6.05	2.25	(-1.31, 13.42)	2.69	0.233
(M DUP Saline) - (F WT PolyIC)	-5.92	2.61	(-14.43, 2.59)	-2.27	0.497
(M WT PolyIC) - (F WT PolyIC)	2.87	1.88	(-3.26, 9.00)	1.53	0.933
(M WT Resiquimod) - (F WT PolyIC)	-8.59	2.29	(-16.06, -1.11)	-3.75	0.010

(M WT Saline) - (F WT PolyIC)	-8.27	1.94	(-14.61, -1.92)	-4.26	0.001
(F WT Saline) - (F WT Resiquimod)	8.21	2.34	(0.56, 15.86)	3.51	0.023
(M DUP PolyIC) - (F WT Resiquimod)	20.05	2.37	(12.30, 27.80)	8.45	0.000
(M DUP Resiquimod) - (F WT Resiquimod)	27.94	2.36	(20.24, 35.64)	11.86	0.000
(M DUP Saline) - (F WT Resiquimod)	15.97	2.69	(7.17, 24.77)	5.93	0.000
(M WT PolyIC) - (F WT Resiquimod)	24.76	2.00	(18.23, 31.28)	12.39	0.000
(M WT Resiquimod) - (F WT Resiquimod)	13.30	2.39	(5.50, 21.10)	5.57	0.000
(M WT Saline) - (F WT Resiquimod)	13.62	2.06	(6.89, 20.35)	6.61	0.000
(M DUP PolyIC) - (F WT Saline)	11.84	2.53	(3.57, 20.11)	4.67	0.000
(M DUP Resiquimod) - (F WT Saline)	19.73	2.52	(11.51, 27.95)	7.84	0.000
(M DUP Saline) - (F WT Saline)	7.76	2.84	(-1.51, 17.02)	2.74	0.209
(M WT PolyIC) - (F WT Saline)	16.55	2.19	(9.41, 23.68)	7.57	0.000
(M WT Resiquimod) - (F WT Saline)	5.09	2.55	(-3.23, 13.41)	2.00	0.694

(M WT Saline) - (F WT Saline)	5.41	2.24	(-1.92, 12.73)	2.41	0.398
(M DUP Resiquimod) - (M DUP PolyIC)	7.89	2.55	(-0.43, 16.21)	3.10	0.083
(M DUP Saline) - (M DUP PolyIC)	-4.08	2.86	(-13.43, 5.27)	-1.43	0.959
(M WT PolyIC) - (M DUP PolyIC)	4.71	2.22	(-2.54, 11.95)	2.12	0.607
(M WT Resiquimod) - (M DUP PolyIC)	-6.75	2.58	(-15.16, 1.67)	-2.62	0.268
(M WT Saline) - (M DUP PolyIC)	-6.43	2.28	(-13.86, 1.00)	-2.83	0.169
(M DUP Saline) - (M DUP Resiquimod)	-11.97	2.85	(-21.27, -2.67)	-4.21	0.002
(M WT PolyIC) - (M DUP Resiquimod)	-3.18	2.20	(-10.37, 4.01)	-1.45	0.954
(M WT Resiquimod) - (M DUP Resiquimod)	-14.64	2.56	(-23.00, -6.28)	-5.72	0.000
(M WT Saline) - (M DUP Resiquimod)	-14.32	2.26	(-21.69, -6.95)	-6.34	0.000
(M WT PolyIC) - (M DUP Saline)	8.79	2.56	(0.43, 17.15)	3.43	0.029
(M WT Resiquimod) - (M DUP Saline)	-2.67	2.87	(-12.04, 6.71)	-0.93	0.999
(M WT Saline) - (M DUP Saline)	-2.35	2.61	(-10.87, 6.17)	-0.90	0.999

(M WT Resiquimod) - (M WT PolyIC)	-11.46	2.24	(-18.76, -4.15)	-5.13	0.000
(M WT Saline) - (M WT PolyIC)	-11.14	1.88	(-17.28, -4.99)	-5.92	0.000
(M WT Saline) - (M WT Resiquimod)	0.32	2.29	(-7.16, 7.80)	0.14	1.000

Table 8-12: Tukey's Simultaneous Tests for Differences between means in separation from nearest cage-mate in the home cage.

8.2.4 Novel Object Recognition

Difference of genotype*treatment*sex Levels	**Difference of Means**	**SE of Difference**	**Simultaneous 95% CI**	**T-Value**	**Adjusted P-Value**
(dup polyic male) - (dup polyic female)	-0.04	1.20	(-4.12, 4.03)	-0.04	1.000
(dup resiquimod female) - (dup polyic female)	0.336	0.916	(-2.781, 3.453)	0.37	1.000
(dup resiquimod male) - (dup polyic female)	0.05	1.05	(-3.53, 3.63)	0.04	1.000
(dup saline female) - (dup polyic female)	0.174	0.984	(-3.172, 3.520)	0.18	1.000
(dup saline male) - (dup polyic female)	0.40	1.05	(-3.18, 3.98)	0.38	1.000
(wt polyic female) - (dup polyic female)	0.576	0.943	(-2.632, 3.784)	0.61	1.000
(wt polyic male) - (dup polyic female)	0.290	0.984	(-3.056, 3.636)	0.29	1.000

(wt resiquimod female) - (dup polyic female)	0.02	1.05	(-3.56, 3.60)	0.02	1.000
(wt resiquimod male) - (dup polyic female)	0.25	1.11	(-3.53, 4.02)	0.22	1.000
(wt saline female) - (dup polyic female)	2.95	1.05	(-0.63, 6.53)	2.80	0.205
(wt saline male) - (dup polyic female)	0.35	1.01	(-3.10, 3.79)	0.34	1.000
(dup resiquimod female) - (dup polyic male)	0.38	1.02	(-3.10, 3.86)	0.37	1.000
(dup resiquimod male) - (dup polyic male)	0.09	1.15	(-3.81, 3.99)	0.08	1.000
(dup saline female) - (dup polyic male)	0.22	1.08	(-3.47, 3.90)	0.20	1.000
(dup saline male) - (dup polyic male)	0.44	1.15	(-3.46, 4.34)	0.39	1.000
(wt polyic female) - (dup polyic male)	0.62	1.05	(-2.94, 4.18)	0.59	1.000
(wt polyic male) - (dup polyic male)	0.33	1.08	(-3.35, 4.02)	0.31	1.000
(wt resiquimod female) - (dup polyic male)	0.07	1.15	(-3.83, 3.97)	0.06	1.000
(wt resiquimod male) - (dup polyic male)	0.29	1.20	(-3.79, 4.37)	0.24	1.000
(wt saline female) - (dup polyic male)	3.00	1.15	(-0.90, 6.89)	2.61	0.296

(wt saline male) - (dup polyic male)	0.39	1.11	(-3.39, 4.16)	0.35	1.000
(dup resiquimod male) - (dup resiquimod female)	-0.289	0.847	(-3.168, 2.590)	-0.34	1.000
(dup saline female) - (dup resiquimod female)	-0.162	0.759	(-2.743, 2.419)	-0.21	1.000
(dup saline male) - (dup resiquimod female)	0.063	0.847	(-2.816, 2.942)	0.07	1.000
(wt polyic female) - (dup resiquimod female)	0.240	0.705	(-2.160, 2.639)	0.34	1.000
(wt polyic male) - (dup resiquimod female)	-0.046	0.759	(-2.627, 2.535)	-0.06	1.000
(wt resiquimod female) - (dup resiquimod female)	-0.312	0.847	(-3.191, 2.567)	-0.37	1.000
(wt resiquimod male) - (dup resiquimod female)	-0.088	0.916	(-3.205, 3.029)	-0.10	1.000
(wt saline female) - (dup resiquimod female)	2.617	0.847	(-0.262, 5.496)	3.09	0.109
(wt saline male) - (dup resiquimod female)	0.011	0.797	(-2.699, 2.720)	0.01	1.000
(dup saline female) - (dup resiquimod male)	0.127	0.919	(-2.999, 3.253)	0.14	1.000
(dup saline male) - (dup resiquimod male)	0.352	0.993	(-3.025, 3.728)	0.35	1.000

(wt polyic female) - (dup resiquimod male)	0.529	0.875	(-2.449, 3.506)	0.60	1.000
(wt polyic male) - (dup resiquimod male)	0.243	0.919	(-2.883, 3.368)	0.26	1.000
(wt resiquimod female) - (dup resiquimod male)	-0.023	0.993	(-3.400, 3.353)	-0.02	1.000
(wt resiquimod male) - (dup resiquimod male)	0.20	1.05	(-3.38, 3.78)	0.19	1.000
(wt saline female) - (dup resiquimod male)	2.906	0.993	(-0.470, 6.282)	2.93	0.158
(wt saline male) - (dup resiquimod male)	0.300	0.950	(-2.933, 3.532)	0.32	1.000
(dup saline male) - (dup saline female)	0.225	0.919	(-2.901, 3.350)	0.24	1.000
(wt polyic female) - (dup saline female)	0.402	0.791	(-2.289, 3.092)	0.51	1.000
(wt polyic male) - (dup saline female)	0.115	0.839	(-2.738, 2.969)	0.14	1.000
(wt resiquimod female) - (dup saline female)	-0.150	0.919	(-3.276, 2.975)	-0.16	1.000
(wt resiquimod male) - (dup saline female)	0.074	0.984	(-3.272, 3.420)	0.07	1.000
(wt saline female) - (dup saline female)	2.779	0.919	(-0.347, 5.904)	3.02	0.128
(wt saline male) - (dup saline female)	0.172	0.873	(-2.798, 3.142)	0.20	1.000

(wt polyic female) - (dup saline male)	0.177	0.875	(-2.801, 3.155)	0.20	1.000
(wt polyic male) - (dup saline male)	-0.109	0.919	(-3.235, 3.017)	-0.12	1.000
(wt resiquimod female) - (dup saline male)	-0.375	0.993	(-3.751, 3.001)	-0.38	1.000
(wt resiquimod male) - (dup saline male)	-0.15	1.05	(-3.73, 3.43)	-0.14	1.000
(wt saline female) - (dup saline male)	2.554	0.993	(-0.822, 5.930)	2.57	0.318
(wt saline male) - (dup saline male)	-0.052	0.950	(-3.285, 3.180)	-0.05	1.000
(wt polyic male) - (wt polyic female)	-0.286	0.791	(-2.976, 2.404)	-0.36	1.000
(wt resiquimod female) - (wt polyic female)	-0.552	0.875	(-3.530, 2.426)	-0.63	1.000
(wt resiquimod male) - (wt polyic female)	-0.328	0.943	(-3.536, 2.880)	-0.35	1.000
(wt saline female) - (wt polyic female)	2.377	0.875	(-0.601, 5.355)	2.72	0.245
(wt saline male) - (wt polyic female)	-0.229	0.827	(-3.043, 2.584)	-0.28	1.000
(wt resiquimod female) - (wt polyic male)	-0.266	0.919	(-3.392, 2.860)	-0.29	1.000
(wt resiquimod male) - (wt polyic male)	-0.042	0.984	(-3.388, 3.304)	-0.04	1.000

(wt saline female) - (wt polyic male)	2.663	0.919	(-0.463, 5.789)	2.90	0.169
(wt saline male) - (wt polyic male)	0.057	0.873	(-2.913, 3.027)	0.07	1.000
(wt resiquimod male) - (wt resiquimod female)	0.22	1.05	(-3.36, 3.81)	0.21	1.000
(wt saline female) - (wt resiquimod female)	2.929	0.993	(-0.447, 6.305)	2.95	0.150
(wt saline male) - (wt resiquimod female)	0.323	0.950	(-2.910, 3.555)	0.34	1.000
(wt saline female) - (wt resiquimod male)	2.70	1.05	(-0.88, 6.29)	2.57	0.320
(wt saline male) - (wt resiquimod male)	0.10	1.01	(-3.35, 3.54)	0.10	1.000
(wt saline male) - (wt saline female)	-2.606	0.950	(-5.839, 0.626)	-2.74	0.232

Table 8-13: Tukey's Simultaneous Tests for Differences between means in the Novel Object Index.

8.3 Tukey's Simultaneous Pairwise Comparisons for expression of biological markers in the prefrontal cortex.

8.3.1 Parvalbumin

Difference of Genotype*Sex*Treatment Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(DUP F Resiquimod) - (DUP F Saline)	-0.122	0.112	(-0.506, 0.262)	-1.09	0.994
(DUP F PolyIC) - (DUP F Saline)	-0.127	0.137	(-0.596, 0.341)	-0.93	0.998
(DUP M Saline) - (DUP F Saline)	0.006	0.151	(-0.511, 0.523)	0.04	1.000
(DUP M Resiquimod) - (DUP F Saline)	0.180	0.122	(-0.238, 0.597)	1.47	0.943
(DUP M PolyIC) - (DUP F Saline)	-0.079	0.154	(-0.603, 0.446)	-0.51	1.000
(WT F Saline) - (DUP F Saline)	-0.178	0.122	(-0.595, 0.240)	-1.45	0.947
(WT F Resiquimod) - (DUP F Saline)	-0.032	0.128	(-0.469, 0.405)	-0.25	1.000
(WT F PolyIC) - (DUP F Saline)	0.220	0.117	(-0.178, 0.619)	1.89	0.761
(WT M Saline) - (DUP F Saline)	0.051	0.115	(-0.341, 0.443)	0.44	1.000

(WT M Resiquimod) - (DUP F Saline)	0.185	0.128	(-0.252, 0.622)	1.45	0.948
(WT M PolyIC) - (DUP F Saline)	-0.083	0.117	(-0.481, 0.316)	-0.71	1.000
(DUP F PolyIC) - (DUP F Resiquimod)	-0.005	0.132	(-0.455, 0.444)	-0.04	1.000
(DUP M Saline) - (DUP F Resiquimod)	0.128	0.147	(-0.374, 0.630)	0.87	0.999
(DUP M Resiquimod) - (DUP F Resiquimod)	0.302	0.116	(-0.096, 0.699)	2.59	0.310
(DUP M PolyIC) - (DUP F Resiquimod)	0.043	0.146	(-0.455, 0.542)	0.30	1.000
(WT F Saline) - (DUP F Resiquimod)	-0.055	0.116	(-0.453, 0.342)	-0.48	1.000
(WT F Resiquimod) - (DUP F Resiquimod)	0.090	0.122	(-0.328, 0.507)	0.73	1.000
(WT F PolyIC) - (DUP F Resiquimod)	0.342	0.112	(-0.040, 0.725)	3.06	0.120
(WT M Saline) - (DUP F Resiquimod)	0.173	0.108	(-0.195, 0.541)	1.60	0.901
(WT M Resiquimod) - (DUP F Resiquimod)	0.307	0.122	(-0.110, 0.725)	2.51	0.355
(WT M PolyIC) - (DUP F Resiquimod)	0.039	0.112	(-0.343, 0.422)	0.35	1.000
(DUP M Saline) - (DUP F PolyIC)	0.134	0.169	(-0.443, 0.710)	0.79	1.000

(DUP M Resiquimod) - (DUP F PolyIC)	0.307	0.141	(-0.174, 0.788)	2.18	0.570
(DUP M PolyIC) - (DUP F PolyIC)	0.049	0.167	(-0.521, 0.618)	0.29	1.000
(WT F Saline) - (DUP F PolyIC)	-0.050	0.141	(-0.531, 0.431)	-0.36	1.000
(WT F Resiquimod) - (DUP F PolyIC)	0.095	0.146	(-0.405, 0.595)	0.65	1.000
(WT F PolyIC) - (DUP F PolyIC)	0.348	0.138	(-0.123, 0.818)	2.52	0.348
(WT M Saline) - (DUP F PolyIC)	0.178	0.134	(-0.279, 0.635)	1.33	0.971
(WT M Resiquimod) - (DUP F PolyIC)	0.313	0.146	(-0.187, 0.812)	2.14	0.600
(WT M PolyIC) - (DUP F PolyIC)	0.045	0.138	(-0.426, 0.515)	0.33	1.000
(DUP M Resiquimod) - (DUP M Saline)	0.174	0.156	(-0.360, 0.707)	1.11	0.993
(DUP M PolyIC) - (DUP M Saline)	-0.085	0.182	(-0.707, 0.537)	-0.47	1.000
(WT F Saline) - (DUP M Saline)	-0.184	0.156	(-0.717, 0.350)	-1.18	0.989
(WT F Resiquimod) - (DUP M Saline)	-0.039	0.160	(-0.585, 0.508)	-0.24	1.000
(WT F PolyIC) - (DUP M Saline)	0.214	0.151	(-0.301, 0.729)	1.42	0.954

(WT M Saline) - (DUP M Saline)	0.045	0.151	(-0.472, 0.561)	0.29	1.000
(WT M Resiquimod) - (DUP M Saline)	0.179	0.160	(-0.368, 0.726)	1.12	0.992
(WT M PolyIC) - (DUP M Saline)	-0.089	0.151	(-0.603, 0.426)	-0.59	1.000
(DUP M PolyIC) - (DUP M Resiquimod)	-0.258	0.155	(-0.787, 0.270)	-1.67	0.874
(WT F Saline) - (DUP M Resiquimod)	-0.357	0.125	(-0.785, 0.071)	-2.85	0.189
(WT F Resiquimod) - (DUP M Resiquimod)	-0.212	0.132	(-0.662, 0.238)	-1.61	0.898
(WT F PolyIC) - (DUP M Resiquimod)	0.041	0.122	(-0.375, 0.457)	0.33	1.000
(WT M Saline) - (DUP M Resiquimod)	-0.129	0.118	(-0.532, 0.274)	-1.09	0.994
(WT M Resiquimod) - (DUP M Resiquimod)	0.006	0.132	(-0.444, 0.455)	0.04	1.000
(WT M PolyIC) - (DUP M Resiquimod)	-0.262	0.122	(-0.678, 0.154)	-2.15	0.589
(WT F Saline) - (DUP M PolyIC)	-0.099	0.155	(-0.628, 0.430)	-0.64	1.000
(WT F Resiquimod) - (DUP M PolyIC)	0.046	0.160	(-0.501, 0.593)	0.29	1.000
(WT F PolyIC) - (DUP M PolyIC)	0.299	0.153	(-0.224, 0.823)	1.95	0.722

(WT M Saline) - (DUP M PolyIC)	0.129	0.148	(-0.376, 0.635)	0.87	0.999
(WT M Resiquimod) - (DUP M PolyIC)	0.264	0.160	(-0.283, 0.811)	1.65	0.883
(WT M PolyIC) - (DUP M PolyIC)	-0.004	0.153	(-0.527, 0.520)	-0.02	1.000
(WT F Resiquimod) - (WT F Saline)	0.145	0.132	(-0.305, 0.595)	1.10	0.993
(WT F PolyIC) - (WT F Saline)	0.398	0.122	(-0.018, 0.814)	3.27	0.073
(WT M Saline) - (WT F Saline)	0.228	0.118	(-0.175, 0.631)	1.93	0.734
(WT M Resiquimod) - (WT F Saline)	0.363	0.132	(-0.087, 0.813)	2.75	0.230
(WT M PolyIC) - (WT F Saline)	0.095	0.122	(-0.321, 0.511)	0.78	1.000
(WT F PolyIC) - (WT F Resiquimod)	0.253	0.127	(-0.183, 0.688)	1.98	0.702
(WT M Saline) - (WT F Resiquimod)	0.083	0.124	(-0.342, 0.508)	0.67	1.000
(WT M Resiquimod) - (WT F Resiquimod)	0.218	0.137	(-0.251, 0.686)	1.58	0.907
(WT M PolyIC) - (WT F Resiquimod)	-0.050	0.127	(-0.485, 0.385)	-0.39	1.000
(WT M Saline) - (WT F PolyIC)	-0.170	0.114	(-0.560, 0.221)	-1.48	0.939

(WT M Resiquimod) - (WT F PolyIC)	-0.035	0.127	(-0.470, 0.400)	-0.28	1.000
(WT M PolyIC) - (WT F PolyIC)	-0.303	0.116	(-0.699, 0.093)	-2.61	0.300
(WT M Resiquimod) - (WT M Saline)	0.134	0.124	(-0.291, 0.560)	1.08	0.994
(WT M PolyIC) - (WT M Saline)	-0.133	0.114	(-0.524, 0.257)	-1.17	0.990
(WT M PolyIC) - (WT M Resiquimod)	-0.268	0.127	(-0.703, 0.168)	-2.10	0.625

Table 8-14: Tukey's Simultaneous Tests for Differences between means in the expression of PV in the PFC as measured by RT-qPCR.

8.3.2 Somatostatin

Difference of Genotype*Sex*Treatment Levels	**Difference of Means**	**SE of Difference**	**Simultaneous 95% CI**	**T-Value**	**Adjusted P-Value**
(DUP F Resiquimod) - (DUP F Saline)	0.071	0.157	(-0.466, 0.608)	0.45	1.000
(DUP F PolyIC) - (DUP F Saline)	-0.012	0.192	(-0.667, 0.643)	-0.06	1.000
(DUP M Saline) - (DUP F Saline)	-0.110	0.211	(-0.832, 0.612)	-0.52	1.000
(DUP M Resiquimod) - (DUP F Saline)	0.068	0.171	(-0.516, 0.652)	0.40	1.000

(DUP M PolyIC) - (DUP F Saline)	-0.259	0.215	(-0.992, 0.474)	-1.21	0.986
(WT F Saline) - (DUP F Saline)	0.198	0.171	(-0.385, 0.782)	1.16	0.990
(WT F Resiquimod) - (DUP F Saline)	0.053	0.179	(-0.557, 0.664)	0.30	1.000
(WT F PolyIC) - (DUP F Saline)	-0.032	0.163	(-0.589, 0.524)	-0.20	1.000
(WT M Saline) - (DUP F Saline)	-0.045	0.160	(-0.593, 0.503)	-0.28	1.000
(WT M Resiquimod) - (DUP F Saline)	0.015	0.179	(-0.596, 0.625)	0.08	1.000
(WT M PolyIC) - (DUP F Saline)	0.066	0.163	(-0.490, 0.623)	0.41	1.000
(DUP F PolyIC) - (DUP F Resiquimod)	-0.083	0.184	(-0.711, 0.545)	-0.45	1.000
(DUP M Saline) - (DUP F Resiquimod)	-0.181	0.205	(-0.883, 0.521)	-0.88	0.999
(DUP M Resiquimod) - (DUP F Resiquimod)	-0.003	0.163	(-0.559, 0.552)	-0.02	1.000
(DUP M PolyIC) - (DUP F Resiquimod)	-0.330	0.204	(-1.027, 0.367)	-1.62	0.895
(WT F Saline) - (DUP F Resiquimod)	0.127	0.163	(-0.429, 0.683)	0.78	1.000
(WT F Resiquimod) - (DUP F Resiquimod)	-0.018	0.171	(-0.602, 0.566)	-0.11	1.000

(WT F PolyIC) - (DUP F Resiquimod)	-0.104	0.157	(-0.638, 0.431)	-0.66	1.000
(WT M Saline) - (DUP F Resiquimod)	-0.116	0.151	(-0.630, 0.399)	-0.77	1.000
(WT M Resiquimod) - (DUP F Resiquimod)	-0.057	0.171	(-0.640, 0.527)	-0.33	1.000
(WT M PolyIC) - (DUP F Resiquimod)	-0.005	0.157	(-0.539, 0.530)	-0.03	1.000
(DUP M Saline) - (DUP F PolyIC)	-0.098	0.236	(-0.903, 0.707)	-0.42	1.000
(DUP M Resiquimod) - (DUP F PolyIC)	0.080	0.197	(-0.593, 0.752)	0.40	1.000
(DUP M PolyIC) - (DUP F PolyIC)	-0.247	0.233	(-1.043, 0.548)	-1.06	0.995
(WT F Saline) - (DUP F PolyIC)	0.210	0.197	(-0.462, 0.882)	1.07	0.995
(WT F Resiquimod) - (DUP F PolyIC)	0.065	0.204	(-0.633, 0.763)	0.32	1.000
(WT F PolyIC) - (DUP F PolyIC)	-0.021	0.193	(-0.678, 0.637)	-0.11	1.000
(WT M Saline) - (DUP F PolyIC)	-0.033	0.187	(-0.671, 0.605)	-0.18	1.000
(WT M Resiquimod) - (DUP F PolyIC)	0.026	0.204	(-0.672, 0.724)	0.13	1.000
(WT M PolyIC) - (DUP F PolyIC)	0.078	0.193	(-0.580, 0.736)	0.41	1.000

(DUP M Resiquimod) - (DUP M Saline)	0.178	0.218	(-0.568, 0.923)	0.81	1.000
(DUP M PolyIC) - (DUP M Saline)	-0.149	0.254	(-1.018, 0.720)	-0.59	1.000
(WT F Saline) - (DUP M Saline)	0.308	0.218	(-0.437, 1.053)	1.41	0.956
(WT F Resiquimod) - (DUP M Saline)	0.163	0.224	(-0.601, 0.927)	0.73	1.000
(WT F PolyIC) - (DUP M Saline)	0.077	0.211	(-0.642, 0.797)	0.37	1.000
(WT M Saline) - (DUP M Saline)	0.065	0.211	(-0.656, 0.786)	0.31	1.000
(WT M Resiquimod) - (DUP M Saline)	0.124	0.224	(-0.640, 0.888)	0.56	1.000
(WT M PolyIC) - (DUP M Saline)	0.176	0.211	(-0.543, 0.895)	0.84	0.999
(DUP M PolyIC) - (DUP M Resiquimod)	-0.327	0.216	(-1.066, 0.412)	-1.51	0.931
(WT F Saline) - (DUP M Resiquimod)	0.130	0.175	(-0.468, 0.728)	0.74	1.000
(WT F Resiquimod) - (DUP M Resiquimod)	-0.015	0.184	(-0.644, 0.614)	-0.08	1.000
(WT F PolyIC) - (DUP M Resiquimod)	-0.100	0.170	(-0.682, 0.481)	-0.59	1.000
(WT M Saline) - (DUP M Resiquimod)	-0.113	0.165	(-0.676, 0.451)	-0.68	1.000

(WT M Resiquimod) - (DUP M Resiquimod)	-0.054	0.184	(-0.682, 0.575)	-0.29	1.000
(WT M PolyIC) - (DUP M Resiquimod)	-0.002	0.170	(-0.583, 0.580)	-0.01	1.000
(WT F Saline) - (DUP M PolyIC)	0.457	0.216	(-0.282, 1.196)	2.11	0.617
(WT F Resiquimod) - (DUP M PolyIC)	0.312	0.224	(-0.452, 1.076)	1.39	0.960
(WT F PolyIC) - (DUP M PolyIC)	0.226	0.214	(-0.505, 0.958)	1.06	0.995
(WT M Saline) - (DUP M PolyIC)	0.214	0.207	(-0.492, 0.920)	1.04	0.996
(WT M Resiquimod) - (DUP M PolyIC)	0.273	0.224	(-0.491, 1.038)	1.22	0.985
(WT M PolyIC) - (DUP M PolyIC)	0.325	0.214	(-0.406, 1.057)	1.52	0.929
(WT F Resiquimod) - (WT F Saline)	-0.145	0.184	(-0.774, 0.484)	-0.79	1.000
(WT F PolyIC) - (WT F Saline)	-0.230	0.170	(-0.812, 0.351)	-1.35	0.967
(WT M Saline) - (WT F Saline)	-0.243	0.165	(-0.806, 0.321)	-1.47	0.942
(WT M Resiquimod) - (WT F Saline)	-0.184	0.184	(-0.812, 0.445)	-1.00	0.997
(WT M PolyIC) - (WT F Saline)	-0.132	0.170	(-0.713, 0.449)	-0.77	1.000

(WT F PolyIC) - (WT F Resiquimod)	-0.086	0.178	(-0.694, 0.523)	-0.48	1.000
(WT M Saline) - (WT F Resiquimod)	-0.098	0.174	(-0.692, 0.496)	-0.56	1.000
(WT M Resiquimod) - (WT F Resiquimod)	-0.039	0.192	(-0.694, 0.616)	-0.20	1.000
(WT M PolyIC) - (WT F Resiquimod)	0.013	0.178	(-0.595, 0.621)	0.07	1.000
(WT M Saline) - (WT F PolyIC)	-0.012	0.160	(-0.558, 0.533)	-0.08	1.000
(WT M Resiquimod) - (WT F PolyIC)	0.047	0.178	(-0.562, 0.655)	0.26	1.000
(WT M PolyIC) - (WT F PolyIC)	0.099	0.162	(-0.455, 0.652)	0.61	1.000
(WT M Resiquimod) - (WT M Saline)	0.059	0.174	(-0.535, 0.653)	0.34	1.000
(WT M PolyIC) - (WT M Saline)	0.111	0.160	(-0.435, 0.657)	0.69	1.000
(WT M PolyIC) - (WT M Resiquimod)	0.052	0.178	(-0.556, 0.660)	0.29	1.000

Table 8-15: Tukey's Simultaneous Tests for Differences between means in the expression of SST in the PFC as measured by RT-qPCR.

8.3.3 Tumour Necrosis Factor

Difference of Genotype*Sex*Treatment Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(DUP F Resiquimod) - (DUP F Saline)	0.043	0.147	(-0.460, 0.545)	0.29	1.000
(DUP F PolyIC) - (DUP F Saline)	-0.321	0.180	(-0.934, 0.293)	-1.79	0.819
(DUP M Saline) - (DUP F Saline)	-0.097	0.198	(-0.773, 0.579)	-0.49	1.000
(DUP M Resiquimod) - (DUP F Saline)	-0.183	0.160	(-0.729, 0.364)	-1.14	0.991
(DUP M PolyIC) - (DUP F Saline)	-0.153	0.201	(-0.839, 0.533)	-0.76	1.000
(WT F Saline) - (DUP F Saline)	0.027	0.160	(-0.520, 0.573)	0.17	1.000
(WT F Resiquimod) - (DUP F Saline)	0.138	0.167	(-0.433, 0.710)	0.83	0.999
(WT F PolyIC) - (DUP F Saline)	-0.121	0.153	(-0.642, 0.400)	-0.79	1.000
(WT M Saline) - (DUP F Saline)	0.015	0.150	(-0.498, 0.528)	0.10	1.000
(WT M Resiquimod) - (DUP F Saline)	-0.358	0.167	(-0.929, 0.214)	-2.14	0.600
(WT M PolyIC) - (DUP F Saline)	-0.096	0.153	(-0.617, 0.425)	-0.63	1.000

(DUP F PolyIC) - (DUP F Resiquimod)	-0.363	0.172	(-0.951, 0.225)	-2.11	0.618
(DUP M Saline) - (DUP F Resiquimod)	-0.140	0.192	(-0.796, 0.517)	-0.73	1.000
(DUP M Resiquimod) - (DUP F Resiquimod)	-0.225	0.152	(-0.746, 0.295)	-1.48	0.940
(DUP M PolyIC) - (DUP F Resiquimod)	-0.196	0.191	(-0.848, 0.456)	-1.03	0.996
(WT F Saline) - (DUP F Resiquimod)	-0.016	0.152	(-0.536, 0.504)	-0.11	1.000
(WT F Resiquimod) - (DUP F Resiquimod)	0.096	0.160	(-0.451, 0.642)	0.60	1.000
(WT F PolyIC) - (DUP F Resiquimod)	-0.164	0.147	(-0.664, 0.336)	-1.12	0.992
(WT M Saline) - (DUP F Resiquimod)	-0.027	0.141	(-0.509, 0.454)	-0.19	1.000
(WT M Resiquimod) - (DUP F Resiquimod)	-0.400	0.160	(-0.947, 0.146)	-2.50	0.360
(WT M PolyIC) - (DUP F Resiquimod)	-0.139	0.147	(-0.639, 0.362)	-0.95	0.998
(DUP M Saline) - (DUP F PolyIC)	0.224	0.221	(-0.530, 0.978)	1.01	0.997
(DUP M Resiquimod) - (DUP F PolyIC)	0.138	0.184	(-0.491, 0.767)	0.75	1.000
(DUP M PolyIC) - (DUP F PolyIC)	0.167	0.218	(-0.577, 0.912)	0.77	1.000

(WT F Saline) - (DUP F PolyIC)	0.347	0.184	(-0.282, 0.976)	1.88	0.763
(WT F Resiquimod) - (DUP F PolyIC)	0.459	0.191	(-0.195, 1.113)	2.40	0.425
(WT F PolyIC) - (DUP F PolyIC)	0.199	0.180	(-0.416, 0.815)	1.11	0.993
(WT M Saline) - (DUP F PolyIC)	0.336	0.175	(-0.262, 0.934)	1.92	0.741
(WT M Resiquimod) - (DUP F PolyIC)	-0.037	0.191	(-0.691, 0.617)	-0.19	1.000
(WT M PolyIC) - (DUP F PolyIC)	0.225	0.180	(-0.391, 0.840)	1.25	0.982
(DUP M Resiquimod) - (DUP M Saline)	-0.086	0.204	(-0.784, 0.612)	-0.42	1.000
(DUP M PolyIC) - (DUP M Saline)	-0.056	0.238	(-0.870, 0.757)	-0.24	1.000
(WT F Saline) - (DUP M Saline)	0.123	0.204	(-0.574, 0.821)	0.60	1.000
(WT F Resiquimod) - (DUP M Saline)	0.235	0.209	(-0.480, 0.950)	1.12	0.992
(WT F PolyIC) - (DUP M Saline)	-0.025	0.197	(-0.698, 0.649)	-0.12	1.000
(WT M Saline) - (DUP M Saline)	0.112	0.198	(-0.563, 0.787)	0.57	1.000
(WT M Resiquimod) - (DUP M Saline)	-0.261	0.209	(-0.976, 0.454)	-1.25	0.982

(WT M PolyIC) - (DUP M Saline)	0.001	0.197	(-0.673, 0.674)	0.00	1.000
(DUP M PolyIC) - (DUP M Resiquimod)	0.029	0.203	(-0.662, 0.721)	0.15	1.000
(WT F Saline) - (DUP M Resiquimod)	0.209	0.164	(-0.351, 0.769)	1.28	0.979
(WT F Resiquimod) - (DUP M Resiquimod)	0.321	0.172	(-0.268, 0.910)	1.86	0.776
(WT F PolyIC) - (DUP M Resiquimod)	0.061	0.159	(-0.483, 0.605)	0.39	1.000
(WT M Saline) - (DUP M Resiquimod)	0.198	0.154	(-0.329, 0.725)	1.28	0.978
(WT M Resiquimod) - (DUP M Resiquimod)	-0.175	0.172	(-0.764, 0.414)	-1.02	0.997
(WT M PolyIC) - (DUP M Resiquimod)	0.087	0.159	(-0.458, 0.631)	0.54	1.000
(WT F Saline) - (DUP M PolyIC)	0.180	0.203	(-0.512, 0.872)	0.89	0.999
(WT F Resiquimod) - (DUP M PolyIC)	0.292	0.209	(-0.424, 1.007)	1.39	0.960
(WT F PolyIC) - (DUP M PolyIC)	0.032	0.201	(-0.653, 0.717)	0.16	1.000
(WT M Saline) - (DUP M PolyIC)	0.169	0.194	(-0.493, 0.830)	0.87	0.999
(WT M Resiquimod) - (DUP M PolyIC)	-0.205	0.209	(-0.920, 0.511)	-0.98	0.998

(WT M PolyIC) - (DUP M PolyIC)	0.057	0.201	(-0.628, 0.742)	0.28	1.000
(WT F Resiquimod) - (WT F Saline)	0.112	0.172	(-0.477, 0.700)	0.65	1.000
(WT F PolyIC) - (WT F Saline)	-0.148	0.159	(-0.692, 0.396)	-0.93	0.998
(WT M Saline) - (WT F Saline)	-0.011	0.154	(-0.539, 0.516)	-0.07	1.000
(WT M Resiquimod) - (WT F Saline)	-0.384	0.172	(-0.973, 0.204)	-2.23	0.537
(WT M PolyIC) - (WT F Saline)	-0.123	0.159	(-0.667, 0.422)	-0.77	1.000
(WT F PolyIC) - (WT F Resiquimod)	-0.260	0.167	(-0.829, 0.310)	-1.56	0.917
(WT M Saline) - (WT F Resiquimod)	-0.123	0.163	(-0.679, 0.433)	-0.76	1.000
(WT M Resiquimod) - (WT F Resiquimod)	-0.496	0.180	(-1.109, 0.117)	-2.76	0.225
(WT M PolyIC) - (WT F Resiquimod)	-0.234	0.167	(-0.804, 0.335)	-1.41	0.957
(WT M Saline) - (WT F PolyIC)	0.137	0.150	(-0.374, 0.647)	0.91	0.999
(WT M Resiquimod) - (WT F PolyIC)	-0.236	0.167	(-0.806, 0.333)	-1.42	0.955
(WT M PolyIC) - (WT F PolyIC)	0.025	0.152	(-0.493, 0.544)	0.17	1.000

(WT M Resiquimod) - (WT M Saline)	-0.373	0.163	(-0.929, 0.183)	-2.29	0.495
(WT M PolyIC) - (WT M Saline)	-0.111	0.150	(-0.622, 0.399)	-0.75	1.000
(WT M PolyIC) - (WT M Resiquimod)	0.262	0.167	(-0.308, 0.831)	1.57	0.913

Table 8-16: Tukey's Simultaneous Tests for Differences between means in the expression of TNFa in the PFC as measured by RT-qPCR.

8.3.4 Interleukin 6

Difference of Genotype*Sex*Treatment Levels	**Difference of Means**	**SE of Difference**	**Simultaneous 95% CI**	**T-Value**	**Adjusted P-Value**
(DUP F Resiquimod) - (DUP F Saline)	-0.012	0.157	(-0.548, 0.523)	-0.08	1.000
(DUP F PolyIC) - (DUP F Saline)	-0.581	0.191	(-1.234, 0.073)	-3.04	0.127
(DUP M Saline) - (DUP F Saline)	0.127	0.211	(-0.593, 0.847)	0.60	1.000
(DUP M Resiquimod) - (DUP F Saline)	-0.173	0.170	(-0.755, 0.410)	-1.01	0.997
(DUP M PolyIC) - (DUP F Saline)	-0.355	0.214	(-1.086, 0.376)	-1.66	0.878
(WT F Saline) - (DUP F Saline)	-0.060	0.170	(-0.642, 0.522)	-0.35	1.000
(WT F Resiquimod) - (DUP F Saline)	0.084	0.178	(-0.525, 0.693)	0.47	1.000

(WT F PolyIC) - (DUP F Saline)	-0.290	0.163	(-0.845, 0.266)	-1.78	0.821
(WT M Saline) - (DUP F Saline)	0.048	0.160	(-0.498, 0.595)	0.30	1.000
(WT M Resiquimod) - (DUP F Saline)	-0.037	0.178	(-0.646, 0.572)	-0.21	1.000
(WT M PolyIC) - (DUP F Saline)	-0.130	0.163	(-0.686, 0.425)	-0.80	1.000
(DUP F PolyIC) - (DUP F Resiquimod)	-0.568	0.183	(-1.195, 0.058)	-3.10	0.109
(DUP M Saline) - (DUP F Resiquimod)	0.139	0.205	(-0.561, 0.839)	0.68	1.000
(DUP M Resiquimod) - (DUP F Resiquimod)	-0.160	0.162	(-0.715, 0.394)	-0.99	0.997
(DUP M PolyIC) - (DUP F Resiquimod)	-0.343	0.203	(-1.038, 0.352)	-1.69	0.867
(WT F Saline) - (DUP F Resiquimod)	-0.048	0.162	(-0.602, 0.507)	-0.29	1.000
(WT F Resiquimod) - (DUP F Resiquimod)	0.096	0.170	(-0.486, 0.679)	0.57	1.000
(WT F PolyIC) - (DUP F Resiquimod)	-0.277	0.156	(-0.811, 0.256)	-1.78	0.823
(WT M Saline) - (DUP F Resiquimod)	0.060	0.150	(-0.453, 0.574)	0.40	1.000
(WT M Resiquimod) - (DUP F Resiquimod)	-0.025	0.170	(-0.607, 0.557)	-0.15	1.000

(WT M PolyIC) - (DUP F Resiquimod)	-0.118	0.156	(-0.651, 0.415)	-0.76	1.000
(DUP M Saline) - (DUP F PolyIC)	0.707	0.235	(-0.096, 1.511)	3.01	0.135
(DUP M Resiquimod) - (DUP F PolyIC)	0.408	0.196	(-0.262, 1.079)	2.08	0.639
(DUP M PolyIC) - (DUP F PolyIC)	0.226	0.232	(-0.568, 1.019)	0.97	0.998
(WT F Saline) - (DUP F PolyIC)	0.521	0.196	(-0.150, 1.191)	2.65	0.277
(WT F Resiquimod) - (DUP F PolyIC)	0.665	0.204	(-0.031, 1.361)	3.26	0.074
(WT F PolyIC) - (DUP F PolyIC)	0.291	0.192	(-0.365, 0.947)	1.52	0.930
(WT M Saline) - (DUP F PolyIC)	0.629	0.186	(-0.008, 1.266)	3.37	0.056
(WT M Resiquimod) - (DUP F PolyIC)	0.543	0.204	(-0.153, 1.240)	2.67	0.271
(WT M PolyIC) - (DUP F PolyIC)	0.450	0.192	(-0.206, 1.106)	2.34	0.460
(DUP M Resiquimod) - (DUP M Saline)	-0.299	0.218	(-1.043, 0.444)	-1.38	0.963
(DUP M PolyIC) - (DUP M Saline)	-0.482	0.254	(-1.348, 0.385)	-1.90	0.754
(WT F Saline) - (DUP M Saline)	-0.187	0.218	(-0.930, 0.557)	-0.86	0.999

(WT F Resiquimod) - (DUP M Saline)	-0.043	0.223	(-0.805, 0.720)	-0.19	1.000
(WT F PolyIC) - (DUP M Saline)	-0.416	0.210	(-1.134, 0.301)	-1.98	0.703
(WT M Saline) - (DUP M Saline)	-0.079	0.211	(-0.798, 0.641)	-0.37	1.000
(WT M Resiquimod) - (DUP M Saline)	-0.164	0.223	(-0.926, 0.598)	-0.74	1.000
(WT M PolyIC) - (DUP M Saline)	-0.257	0.210	(-0.975, 0.460)	-1.22	0.985
(DUP M PolyIC) - (DUP M Resiquimod)	-0.183	0.216	(-0.920, 0.555)	-0.85	0.999
(WT F Saline) - (DUP M Resiquimod)	0.113	0.175	(-0.484, 0.709)	0.65	1.000
(WT F Resiquimod) - (DUP M Resiquimod)	0.257	0.184	(-0.370, 0.884)	1.40	0.959
(WT F PolyIC) - (DUP M Resiquimod)	-0.117	0.170	(-0.697, 0.463)	-0.69	1.000
(WT M Saline) - (DUP M Resiquimod)	0.221	0.165	(-0.341, 0.783)	1.34	0.969
(WT M Resiquimod) - (DUP M Resiquimod)	0.135	0.184	(-0.492, 0.762)	0.74	1.000
(WT M PolyIC) - (DUP M Resiquimod)	0.042	0.170	(-0.538, 0.622)	0.25	1.000
(WT F Saline) - (DUP M PolyIC)	0.295	0.216	(-0.442, 1.032)	1.37	0.965

(WT F Resiquimod) - (DUP M PolyIC)	0.439	0.223	(-0.323, 1.202)	1.97	0.712
(WT F PolyIC) - (DUP M PolyIC)	0.065	0.214	(-0.664, 0.795)	0.31	1.000
(WT M Saline) - (DUP M PolyIC)	0.403	0.206	(-0.301, 1.108)	1.96	0.720
(WT M Resiquimod) - (DUP M PolyIC)	0.318	0.223	(-0.444, 1.080)	1.42	0.953
(WT M PolyIC) - (DUP M PolyIC)	0.225	0.214	(-0.505, 0.954)	1.05	0.996
(WT F Resiquimod) - (WT F Saline)	0.144	0.184	(-0.483, 0.771)	0.78	1.000
(WT F PolyIC) - (WT F Saline)	-0.230	0.170	(-0.810, 0.350)	-1.35	0.967
(WT M Saline) - (WT F Saline)	0.108	0.165	(-0.454, 0.670)	0.66	1.000
(WT M Resiquimod) - (WT F Saline)	0.023	0.184	(-0.605, 0.650)	0.12	1.000
(WT M PolyIC) - (WT F Saline)	-0.071	0.170	(-0.650, 0.509)	-0.42	1.000
(WT F PolyIC) - (WT F Resiquimod)	-0.374	0.178	(-0.981, 0.233)	-2.10	0.622
(WT M Saline) - (WT F Resiquimod)	-0.036	0.174	(-0.629, 0.557)	-0.21	1.000
(WT M Resiquimod) - (WT F Resiquimod)	-0.122	0.191	(-0.775, 0.532)	-0.64	1.000

(WT M PolyIC) - (WT F Resiquimod)	-0.215	0.178	(-0.822, 0.392)	-1.21	0.986
(WT M Saline) - (WT F PolyIC)	0.338	0.159	(-0.206, 0.882)	2.12	0.611
(WT M Resiquimod) - (WT F PolyIC)	0.252	0.178	(-0.354, 0.859)	1.42	0.954
(WT M PolyIC) - (WT F PolyIC)	0.159	0.162	(-0.393, 0.712)	0.98	0.997
(WT M Resiquimod) - (WT M Saline)	-0.085	0.174	(-0.678, 0.507)	-0.49	1.000
(WT M PolyIC) - (WT M Saline)	-0.179	0.159	(-0.723, 0.366)	-1.12	0.992
(WT M PolyIC) - (WT M Resiquimod)	-0.093	0.178	(-0.700, 0.514)	-0.52	1.000

Table 8-17: Tukey's Simultaneous Tests for Differences between means in the expression of IL6 in the PFC as measured by RT-qPCR.

8.3.5 Cluster of Differentiation 68

Difference of Genotype*Sex*Treatment Levels	**Difference of Means**	**SE of Difference**	**Simultaneous 95% CI**	**T-Value**	**Adjusted P-Value**
(DUP F Resiquimod) - (DUP F Saline)	0.049	0.105	(-0.311, 0.409)	0.46	1.000
(DUP F PolyIC) - (DUP F Saline)	-0.191	0.129	(-0.631, 0.249)	-1.48	0.939
(DUP M Saline) - (DUP F Saline)	-0.088	0.142	(-0.572, 0.397)	-0.62	1.000

(DUP M Resiquimod) - (DUP F Saline)	0.178	0.115	(-0.213, 0.570)	1.55	0.918
(DUP M PolyIC) - (DUP F Saline)	-0.159	0.144	(-0.650, 0.333)	-1.10	0.993
(WT F Saline) - (DUP F Saline)	-0.048	0.115	(-0.440, 0.343)	-0.42	1.000
(WT F Resiquimod) - (DUP F Saline)	0.151	0.120	(-0.258, 0.561)	1.26	0.981
(WT F PolyIC) - (DUP F Saline)	-0.078	0.109	(-0.452, 0.295)	-0.72	1.000
(WT M Saline) - (DUP F Saline)	-0.077	0.108	(-0.445, 0.291)	-0.72	1.000
(WT M Resiquimod) - (DUP F Saline)	-0.049	0.120	(-0.459, 0.361)	-0.41	1.000
(WT M PolyIC) - (DUP F Saline)	-0.106	0.109	(-0.480, 0.268)	-0.97	0.998
(DUP F PolyIC) - (DUP F Resiquimod)	-0.240	0.123	(-0.661, 0.182)	-1.94	0.728
(DUP M Saline) - (DUP F Resiquimod)	-0.136	0.138	(-0.607, 0.335)	-0.99	0.997
(DUP M Resiquimod) - (DUP F Resiquimod)	0.129	0.109	(-0.244, 0.502)	1.18	0.988
(DUP M PolyIC) - (DUP F Resiquimod)	-0.207	0.137	(-0.675, 0.260)	-1.52	0.930
(WT F Saline) - (DUP F Resiquimod)	-0.097	0.109	(-0.470, 0.276)	-0.89	0.999

(WT F Resiquimod) - (DUP F Resiquimod)	0.103	0.115	(-0.289, 0.494)	0.90	0.999
(WT F PolyIC) - (DUP F Resiquimod)	-0.127	0.105	(-0.486, 0.232)	-1.21	0.986
(WT M Saline) - (DUP F Resiquimod)	-0.126	0.101	(-0.471, 0.219)	-1.24	0.982
(WT M Resiquimod) - (DUP F Resiquimod)	-0.098	0.115	(-0.490, 0.294)	-0.85	0.999
(WT M PolyIC) - (DUP F Resiquimod)	-0.155	0.105	(-0.513, 0.204)	-1.47	0.942
(DUP M Saline) - (DUP F PolyIC)	0.103	0.158	(-0.437, 0.644)	0.65	1.000
(DUP M Resiquimod) - (DUP F PolyIC)	0.369	0.132	(-0.082, 0.820)	2.79	0.211
(DUP M PolyIC) - (DUP F PolyIC)	0.032	0.156	(-0.501, 0.566)	0.21	1.000
(WT F Saline) - (DUP F PolyIC)	0.143	0.132	(-0.309, 0.594)	1.08	0.994
(WT F Resiquimod) - (DUP F PolyIC)	0.342	0.137	(-0.126, 0.811)	2.50	0.364
(WT F PolyIC) - (DUP F PolyIC)	0.113	0.129	(-0.329, 0.554)	0.87	0.999
(WT M Saline) - (DUP F PolyIC)	0.114	0.125	(-0.314, 0.542)	0.91	0.999
(WT M Resiquimod) - (DUP F PolyIC)	0.142	0.137	(-0.327, 0.610)	1.03	0.996

(WT M PolyIC) - (DUP F PolyIC)	0.085	0.129	(-0.356, 0.526)	0.66	1.000
(DUP M Resiquimod) - (DUP M Saline)	0.266	0.146	(-0.234, 0.766)	1.81	0.803
(DUP M PolyIC) - (DUP M Saline)	-0.071	0.171	(-0.654, 0.512)	-0.42	1.000
(WT F Saline) - (DUP M Saline)	0.039	0.146	(-0.461, 0.539)	0.27	1.000
(WT F Resiquimod) - (DUP M Saline)	0.239	0.150	(-0.274, 0.752)	1.59	0.905
(WT F PolyIC) - (DUP M Saline)	0.009	0.141	(-0.474, 0.492)	0.06	1.000
(WT M Saline) - (DUP M Saline)	0.011	0.142	(-0.473, 0.494)	0.07	1.000
(WT M Resiquimod) - (DUP M Saline)	0.038	0.150	(-0.474, 0.551)	0.26	1.000
(WT M PolyIC) - (DUP M Saline)	-0.018	0.141	(-0.501, 0.464)	-0.13	1.000
(DUP M PolyIC) - (DUP M Resiquimod)	-0.337	0.145	(-0.833, 0.159)	-2.32	0.476
(WT F Saline) - (DUP M Resiquimod)	-0.227	0.118	(-0.628, 0.175)	-1.93	0.737
(WT F Resiquimod) - (DUP M Resiquimod)	-0.027	0.124	(-0.449, 0.395)	-0.22	1.000
(WT F PolyIC) - (DUP M Resiquimod)	-0.257	0.114	(-0.647, 0.133)	-2.25	0.525

(WT M Saline) - (DUP M Resiquimod)	-0.255	0.111	(-0.633, 0.123)	-2.30	0.486
(WT M Resiquimod) - (DUP M Resiquimod)	-0.227	0.124	(-0.649, 0.195)	-1.84	0.789
(WT M PolyIC) - (DUP M Resiquimod)	-0.284	0.114	(-0.674, 0.106)	-2.49	0.369
(WT F Saline) - (DUP M PolyIC)	0.110	0.145	(-0.386, 0.606)	0.76	1.000
(WT F Resiquimod) - (DUP M PolyIC)	0.310	0.150	(-0.203, 0.823)	2.07	0.648
(WT F PolyIC) - (DUP M PolyIC)	0.080	0.144	(-0.411, 0.571)	0.56	1.000
(WT M Saline) - (DUP M PolyIC)	0.082	0.139	(-0.392, 0.556)	0.59	1.000
(WT M Resiquimod) - (DUP M PolyIC)	0.110	0.150	(-0.403, 0.622)	0.73	1.000
(WT M PolyIC) - (DUP M PolyIC)	0.053	0.144	(-0.438, 0.544)	0.37	1.000
(WT F Resiquimod) - (WT F Saline)	0.200	0.124	(-0.222, 0.622)	1.62	0.895
(WT F PolyIC) - (WT F Saline)	-0.030	0.114	(-0.420, 0.360)	-0.26	1.000
(WT M Saline) - (WT F Saline)	-0.029	0.111	(-0.407, 0.349)	-0.26	1.000
(WT M Resiquimod) - (WT F Saline)	-0.001	0.124	(-0.423, 0.421)	-0.01	1.000

(WT M PolyIC) - (WT F Saline)	-0.058	0.114	(-0.448, 0.332)	-0.50	1.000
(WT F PolyIC) - (WT F Resiquimod)	-0.230	0.120	(-0.638, 0.178)	-1.92	0.740
(WT M Saline) - (WT F Resiquimod)	-0.228	0.117	(-0.627, 0.170)	-1.96	0.719
(WT M Resiquimod) - (WT F Resiquimod)	-0.201	0.129	(-0.640, 0.239)	-1.56	0.916
(WT M PolyIC) - (WT F Resiquimod)	-0.257	0.120	(-0.666, 0.151)	-2.15	0.589
(WT M Saline) - (WT F PolyIC)	0.001	0.107	(-0.365, 0.368)	0.01	1.000
(WT M Resiquimod) - (WT F PolyIC)	0.029	0.120	(-0.379, 0.437)	0.24	1.000
(WT M PolyIC) - (WT F PolyIC)	-0.028	0.109	(-0.399, 0.344)	-0.25	1.000
(WT M Resiquimod) - (WT M Saline)	0.028	0.117	(-0.371, 0.426)	0.24	1.000
(WT M PolyIC) - (WT M Saline)	-0.029	0.107	(-0.395, 0.337)	-0.27	1.000
(WT M PolyIC) - (WT M Resiquimod)	-0.057	0.120	(-0.465, 0.351)	-0.47	1.000

Table 8-18: Tukey's Simultaneous Tests for Differences between means in the expression of CD68 in the PFC as measured by RT-qPCR.

8.3.6 Dedicator of Cytokines 11

Difference of Genotype*Sex*Treatment Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
(DUP F Resiquimod) - (DUP F Saline)	-0.044	0.110	(-0.420, 0.332)	-0.40	1.000
(DUP F PolyIC) - (DUP F Saline)	-0.232	0.134	(-0.691, 0.226)	-1.73	0.846
(DUP M Saline) - (DUP F Saline)	-0.011	0.148	(-0.517, 0.494)	-0.08	1.000
(DUP M Resiquimod) - (DUP F Saline)	-0.018	0.120	(-0.427, 0.391)	-0.15	1.000
(DUP M PolyIC) - (DUP F Saline)	-0.295	0.150	(-0.809, 0.218)	-1.97	0.713
(WT F Saline) - (DUP F Saline)	-0.183	0.120	(-0.592, 0.226)	-1.53	0.926
(WT F Resiquimod) - (DUP F Saline)	0.266	0.125	(-0.162, 0.693)	2.12	0.611
(WT F PolyIC) - (DUP F Saline)	-0.055	0.114	(-0.445, 0.335)	-0.48	1.000
(WT M Saline) - (DUP F Saline)	-0.050	0.112	(-0.434, 0.334)	-0.44	1.000
(WT M Resiquimod) - (DUP F Saline)	-0.043	0.125	(-0.471, 0.385)	-0.34	1.000
(WT M PolyIC) - (DUP F Saline)	-0.009	0.114	(-0.399, 0.381)	-0.08	1.000

(DUP F PolyIC) - (DUP F Resiquimod)	-0.189	0.129	(-0.628, 0.251)	-1.46	0.944
(DUP M Saline) - (DUP F Resiquimod)	0.033	0.144	(-0.459, 0.524)	0.23	1.000
(DUP M Resiquimod) - (DUP F Resiquimod)	0.026	0.114	(-0.363, 0.415)	0.23	1.000
(DUP M PolyIC) - (DUP F Resiquimod)	-0.252	0.143	(-0.739, 0.236)	-1.76	0.831
(WT F Saline) - (DUP F Resiquimod)	-0.139	0.114	(-0.528, 0.250)	-1.22	0.985
(WT F Resiquimod) - (DUP F Resiquimod)	0.309	0.120	(-0.099, 0.718)	2.59	0.313
(WT F PolyIC) - (DUP F Resiquimod)	-0.011	0.110	(-0.385, 0.363)	-0.10	1.000
(WT M Saline) - (DUP F Resiquimod)	-0.006	0.105	(-0.366, 0.354)	-0.06	1.000
(WT M Resiquimod) - (DUP F Resiquimod)	0.001	0.120	(-0.408, 0.410)	0.01	1.000
(WT M PolyIC) - (DUP F Resiquimod)	0.035	0.110	(-0.339, 0.409)	0.32	1.000
(DUP M Saline) - (DUP F PolyIC)	0.221	0.165	(-0.343, 0.785)	1.34	0.970
(DUP M Resiquimod) - (DUP F PolyIC)	0.215	0.138	(-0.256, 0.685)	1.56	0.917
(DUP M PolyIC) - (DUP F PolyIC)	-0.063	0.163	(-0.620, 0.494)	-0.39	1.000

(WT F Saline) - (DUP F PolyIC)	0.050	0.138	(-0.421, 0.520)	0.36	1.000
(WT F Resiquimod) - (DUP F PolyIC)	0.498	0.143	(0.009, 0.987)	3.48	0.042
(WT F PolyIC) - (DUP F PolyIC)	0.177	0.135	(-0.283, 0.638)	1.32	0.973
(WT M Saline) - (DUP F PolyIC)	0.182	0.131	(-0.265, 0.629)	1.39	0.960
(WT M Resiquimod) - (DUP F PolyIC)	0.190	0.143	(-0.299, 0.679)	1.32	0.972
(WT M PolyIC) - (DUP F PolyIC)	0.223	0.135	(-0.237, 0.684)	1.66	0.879
(DUP M Resiquimod) - (DUP M Saline)	-0.007	0.153	(-0.529, 0.515)	-0.04	1.000
(DUP M PolyIC) - (DUP M Saline)	-0.284	0.178	(-0.893, 0.324)	-1.60	0.903
(WT F Saline) - (DUP M Saline)	-0.172	0.153	(-0.694, 0.350)	-1.12	0.992
(WT F Resiquimod) - (DUP M Saline)	0.277	0.157	(-0.258, 0.812)	1.77	0.828
(WT F PolyIC) - (DUP M Saline)	-0.044	0.148	(-0.548, 0.460)	-0.30	1.000
(WT M Saline) - (DUP M Saline)	-0.039	0.148	(-0.544, 0.466)	-0.26	1.000
(WT M Resiquimod) - (DUP M Saline)	-0.032	0.157	(-0.567, 0.503)	-0.20	1.000

(WT M PolyIC) - (DUP M Saline)	0.002	0.148	(-0.502, 0.506)	0.01	1.000
(DUP M PolyIC) - (DUP M Resiquimod)	-0.278	0.152	(-0.795, 0.240)	-1.83	0.793
(WT F Saline) - (DUP M Resiquimod)	-0.165	0.123	(-0.584, 0.254)	-1.35	0.969
(WT F Resiquimod) - (DUP M Resiquimod)	0.283	0.129	(-0.157, 0.724)	2.20	0.558
(WT F PolyIC) - (DUP M Resiquimod)	-0.037	0.119	(-0.444, 0.370)	-0.31	1.000
(WT M Saline) - (DUP M Resiquimod)	-0.032	0.116	(-0.427, 0.362)	-0.28	1.000
(WT M Resiquimod) - (DUP M Resiquimod)	-0.025	0.129	(-0.465, 0.415)	-0.19	1.000
(WT M PolyIC) - (DUP M Resiquimod)	0.009	0.119	(-0.398, 0.416)	0.07	1.000
(WT F Saline) - (DUP M PolyIC)	0.113	0.152	(-0.405, 0.630)	0.74	1.000
(WT F Resiquimod) - (DUP M PolyIC)	0.561	0.157	(0.026, 1.096)	3.58	0.032
(WT F PolyIC) - (DUP M PolyIC)	0.241	0.150	(-0.272, 0.753)	1.60	0.900
(WT M Saline) - (DUP M PolyIC)	0.245	0.145	(-0.249, 0.740)	1.70	0.862
(WT M Resiquimod) - (DUP M PolyIC)	0.253	0.157	(-0.283, 0.788)	1.61	0.897

(WT M PolyIC) - (DUP M PolyIC)	0.286	0.150	(-0.226, 0.799)	1.91	0.748
(WT F Resiquimod) - (WT F Saline)	0.448	0.129	(0.008, 0.889)	3.48	0.042
(WT F PolyIC) - (WT F Saline)	0.128	0.119	(-0.279, 0.535)	1.07	0.995
(WT M Saline) - (WT F Saline)	0.133	0.116	(-0.262, 0.527)	1.15	0.991
(WT M Resiquimod) - (WT F Saline)	0.140	0.129	(-0.300, 0.580)	1.09	0.994
(WT M PolyIC) - (WT F Saline)	0.174	0.119	(-0.233, 0.581)	1.46	0.945
(WT F PolyIC) - (WT F Resiquimod)	-0.321	0.125	(-0.747, 0.105)	-2.57	0.322
(WT M Saline) - (WT F Resiquimod)	-0.316	0.122	(-0.732, 0.100)	-2.59	0.310
(WT M Resiquimod) - (WT F Resiquimod)	-0.308	0.134	(-0.767, 0.150)	-2.30	0.492
(WT M PolyIC) - (WT F Resiquimod)	-0.275	0.125	(-0.701, 0.151)	-2.20	0.556
(WT M Saline) - (WT F PolyIC)	0.005	0.112	(-0.377, 0.387)	0.04	1.000
(WT M Resiquimod) - (WT F PolyIC)	0.012	0.125	(-0.414, 0.438)	0.10	1.000
(WT M PolyIC) - (WT F PolyIC)	0.046	0.114	(-0.342, 0.434)	0.40	1.000

(WT M Resiquimod) - (WT M Saline)	0.007	0.122	(-0.409, 0.423)	0.06	1.000
(WT M PolyIC) - (WT M Saline)	0.041	0.112	(-0.341, 0.423)	0.37	1.000
(WT M PolyIC) - (WT M Resiquimod)	0.034	0.125	(-0.392, 0.460)	0.27	1.000

Table 8-19: Tukey's Simultaneous Tests for Differences between means in the expression of DOCK11 in the PFC as measured by RT-qPCR.

8.3.7 Dedicator of Cytokines 8

Difference of Genotype*Sex*Treatment Levels	**Difference of Means**	**SE of Difference**	**Simultaneous 95% CI**	**T-Value**	**Adjusted P-Value**
(dup F Resiquimod) - (dup F Saline)	-0.132	0.117	(-0.533, 0.270)	-1.12	0.992
(dup F PolyIC) - (dup F Saline)	-0.210	0.153	(-0.734, 0.314)	-1.37	0.964
(dup M Saline) - (dup F Saline)	0.011	0.197	(-0.663, 0.686)	0.06	1.000
(dup M Resiquimod) - (dup F Saline)	0.011	0.156	(-0.522, 0.545)	0.07	1.000
(dup M PolyIC) - (dup F Saline)	-0.329	0.206	(-1.034, 0.375)	-1.60	0.901
(WT F Saline) - (dup F Saline)	0.097	0.122	(-0.319, 0.512)	0.79	1.000
(WT F Resiquimod) - (dup F Saline)	-0.194	0.136	(-0.659, 0.272)	-1.42	0.953

(WT F PolyIC) - (dup F Saline)	-0.025	0.148	(-0.531, 0.481)	-0.17	1.000
(WT M Saline) - (dup F Saline)	0.099	0.172	(-0.489, 0.686)	0.58	1.000
(WT M Resiquimod) - (dup F Saline)	-0.132	0.194	(-0.797, 0.534)	-0.68	1.000
(WT M PolyIC) - (dup F Saline)	-0.175	0.189	(-0.822, 0.472)	-0.93	0.998
(dup F PolyIC) - (dup F Resiquimod)	-0.078	0.161	(-0.627, 0.471)	-0.49	1.000
(dup M Saline) - (dup F Resiquimod)	0.143	0.210	(-0.577, 0.863)	0.68	1.000
(dup M Resiquimod) - (dup F Resiquimod)	0.143	0.173	(-0.447, 0.734)	0.83	0.999
(dup M PolyIC) - (dup F Resiquimod)	-0.197	0.219	(-0.946, 0.551)	-0.90	0.999
(WT F Saline) - (dup F Resiquimod)	0.228	0.120	(-0.182, 0.639)	1.90	0.752
(WT F Resiquimod) - (dup F Resiquimod)	-0.062	0.116	(-0.457, 0.333)	-0.54	1.000
(WT F PolyIC) - (dup F Resiquimod)	0.107	0.155	(-0.423, 0.637)	0.69	1.000
(WT M Saline) - (dup F Resiquimod)	0.230	0.187	(-0.409, 0.870)	1.23	0.983
(WT M Resiquimod) - (dup F Resiquimod)	-0.000	0.208	(-0.712, 0.712)	-0.00	1.000

(WT M PolyIC) - (dup F Resiquimod)	-0.043	0.203	(-0.738, 0.651)	-0.21	1.000
(dup M Saline) - (dup F PolyIC)	0.221	0.238	(-0.594, 1.036)	0.93	0.998
(dup M Resiquimod) - (dup F PolyIC)	0.221	0.206	(-0.482, 0.925)	1.08	0.994
(dup M PolyIC) - (dup F PolyIC)	-0.119	0.246	(-0.960, 0.721)	-0.49	1.000
(WT F Saline) - (dup F PolyIC)	0.306	0.149	(-0.203, 0.816)	2.06	0.654
(WT F Resiquimod) - (dup F PolyIC)	0.016	0.179	(-0.598, 0.630)	0.09	1.000
(WT F PolyIC) - (dup F PolyIC)	0.185	0.126	(-0.248, 0.618)	1.46	0.944
(WT M Saline) - (dup F PolyIC)	0.309	0.218	(-0.436, 1.054)	1.42	0.955
(WT M Resiquimod) - (dup F PolyIC)	0.078	0.236	(-0.730, 0.886)	0.33	1.000
(WT M PolyIC) - (dup F PolyIC)	0.035	0.232	(-0.758, 0.827)	0.15	1.000
(dup M Resiquimod) - (dup M Saline)	0.000	0.148	(-0.507, 0.508)	0.00	1.000
(dup M PolyIC) - (dup M Saline)	-0.340	0.167	(-0.911, 0.230)	-2.04	0.664
(WT F Saline) - (dup M Saline)	0.085	0.212	(-0.640, 0.810)	0.40	1.000

(WT F Resiquimod) - (dup M Saline)	-0.205	0.225	(-0.976, 0.566)	-0.91	0.999
(WT F PolyIC) - (dup M Saline)	-0.036	0.234	(-0.838, 0.766)	-0.15	1.000
(WT M Saline) - (dup M Saline)	0.087	0.122	(-0.329, 0.504)	0.72	1.000
(WT M Resiquimod) - (dup M Saline)	-0.143	0.163	(-0.701, 0.415)	-0.88	0.999
(WT M PolyIC) - (dup M Saline)	-0.186	0.137	(-0.656, 0.283)	-1.36	0.966
(dup M PolyIC) - (dup M Resiquimod)	-0.341	0.152	(-0.861, 0.180)	-2.24	0.530
(WT F Saline) - (dup M Resiquimod)	0.085	0.174	(-0.511, 0.681)	0.49	1.000
(WT F Resiquimod) - (dup M Resiquimod)	-0.205	0.190	(-0.857, 0.447)	-1.08	0.994
(WT F PolyIC) - (dup M Resiquimod)	-0.036	0.201	(-0.725, 0.652)	-0.18	1.000
(WT M Saline) - (dup M Resiquimod)	0.087	0.124	(-0.337, 0.511)	0.70	1.000
(WT M Resiquimod) - (dup M Resiquimod)	-0.143	0.134	(-0.601, 0.314)	-1.07	0.995
(WT M PolyIC) - (dup M Resiquimod)	-0.187	0.130	(-0.632, 0.259)	-1.43	0.951
(WT F Saline) - (dup M PolyIC)	0.426	0.220	(-0.327, 1.179)	1.93	0.732

(WT F Resiquimod) - (dup M PolyIC)	0.135	0.233	(-0.662, 0.933)	0.58	1.000
(WT F PolyIC) - (dup M PolyIC)	0.304	0.242	(-0.523, 1.132)	1.26	0.981
(WT M Saline) - (dup M PolyIC)	0.428	0.152	(-0.094, 0.949)	2.81	0.207
(WT M Resiquimod) - (dup M PolyIC)	0.197	0.160	(-0.351, 0.746)	1.23	0.984
(WT M PolyIC) - (dup M PolyIC)	0.154	0.144	(-0.339, 0.647)	1.07	0.995
(WT F Resiquimod) - (WT F Saline)	-0.290	0.139	(-0.767, 0.187)	-2.08	0.637
(WT F PolyIC) - (WT F Saline)	-0.121	0.140	(-0.601, 0.358)	-0.87	0.999
(WT M Saline) - (WT F Saline)	0.002	0.188	(-0.642, 0.647)	0.01	1.000
(WT M Resiquimod) - (WT F Saline)	-0.228	0.209	(-0.945, 0.488)	-1.09	0.994
(WT M PolyIC) - (WT F Saline)	-0.272	0.204	(-0.971, 0.428)	-1.33	0.971
(WT F PolyIC) - (WT F Resiquimod)	0.169	0.174	(-0.427, 0.765)	0.97	0.998
(WT M Saline) - (WT F Resiquimod)	0.292	0.203	(-0.404, 0.989)	1.44	0.950
(WT M Resiquimod) - (WT F Resiquimod)	0.062	0.223	(-0.702, 0.825)	0.28	1.000

(WT M PolyIC) - (WT F Resiquimod)	0.019	0.218	(-0.729, 0.766)	0.08	1.000
(WT M Saline) - (WT F PolyIC)	0.124	0.213	(-0.607, 0.854)	0.58	1.000
(WT M Resiquimod) - (WT F PolyIC)	-0.107	0.232	(-0.902, 0.688)	-0.46	1.000
(WT M PolyIC) - (WT F PolyIC)	-0.150	0.228	(-0.930, 0.629)	-0.66	1.000
(WT M Resiquimod) - (WT M Saline)	-0.230	0.141	(-0.715, 0.254)	-1.63	0.890
(WT M PolyIC) - (WT M Saline)	-0.274	0.125	(-0.702, 0.154)	-2.19	0.562
(WT M PolyIC) - (WT M Resiquimod)	-0.043	0.147	(-0.546, 0.460)	-0.30	1.000

Table 8-20: Tukey's Simultaneous Tests for Differences between means in the expression of DOCK8 in the PFC as measured by RT-qPCR.

8.4 Other Supplementary Tables

Experiment	Time		Treatment		Sex		Treatment X Sex		Time X Treatment		Time x Treatment x Sex	
	F-value	p-value	F-value	p-value	F-value	p-value	F-value	p-value	F-value	P-value	F-value	p-value
Open field (distance travelled)	3.1	$5.09x10^{-4}$	0.06	0.807	329.3	$<0.01x10^{-15}$	1.04	0.309	0.73	0.711	0.15	0.999
Open field (thigmotaxis)	1.12	0.344	2.61	0.107	19.44	$1.3x10^{-5}$	77.79	$<0.01x10^{-15}$	1.14	0.329	0.63	0.801
Elevated Plus Maze (closed arm ratio)	4.31	$9.73x10^{-7}$	8.8	0.003	38.89	$1.28x10^{-9}$	6.16	0.014	1.47	0.125	0.59	0.853
Home-cage (distance travelled)	0.83	0.805	$1.5x10^{-4}$	0.990	133.7	$<0.01x10^{-15}$	3.65	0.056	0.953	0.672	0.9013	0.672
Home-cage (separation)	1.07	0.395	2.62	0.106	25.5	$4.9x10^{-7}$	0.04	0.837	1.23	0.134	1.04	0.395
Novel Object Recognition (total time exploring)	N/A	N/A	11.15	0.003	8.04	0.009	7.4	0.012	N/A	N/A	N/A	N/A
Novel Object Recognition Index	N/A	N/A	1.90	0.182	0.23	0.639	0.01	0.906	N/A	N/A	N/A	N/A

Table 8-21: *Results from Two-way nested ANOVA for behavioural experiments following PCP injection.*

Experiment	Time		Treatment		Time X Treatment	
	F-value	p-value	F-value/T-value	p-value	F-value	P-value
Overall Acquisition Phase	10.33	$<0.01x10^{-15}$	N/A	N/A	N/A	N/A
Overall Reversal Learning	28.07	$<0.01x10^{-15}$	0.05	0.827	0.78	0.632
Reversal Learning Phase 1	8.75	$<0.01x10^{-15}$	0.26	0.615	0.57	0.814
Reversal Learning Phase 2	26.00	$<0.01x10^{-15}$	0.12	0.727	1.20	0.324
Reversal Learning Session 3	N/A	N/A	0.37	1.000	N/A	N/A
Reversal Learning Session 4	N/A	N/A	0.54	1.000	N/A	N/A
Reversal Learning Session 5	N/A	N/A	-0.27	1.000	N/A	N/A
Reversal Learning Session 6	N/A	N/A	-0.71	1.000	N/A	N/A
Reversal Learning Session 7	N/A	N/A	-0.99	1.000	N/A	N/A

Table 8-22: Results from Two-way nested ANOVA for paired discrimination and reversal learning cognitive test following PCP injection.

Citation	Rodent	Year	Agent	OF time	LMA	Centre time	+ maze open time
Depino	mice	2015	LPS	5’ only	↓	↓	↓
Xuan	mice	2014	LPS	-	-	-	
Schaafsma	mice	2017	LPS	home cage	↓ (dark only)		↑
De Oliveira	mice	2023	LPS	10’ only	(↓) (ns)	↓	↑
Meyer	mice	2005	polyIC		-	↓ (entries)	
Meyer	mice	2006	polyIC		-	↓ (entries)	
Smith	mice	2007	polyIC	10’ only	↓	↓ (entries)	
Hsiao	mice	2012	polyIC		-	↓	
Abazyan	mice	2010	polyIC		-		-
Gumusoglu	mice	2010	polyIC				↓
Hollins	rats	2018	polyIC				↓
Li	rats	2021	polyIC		-	↓	↓
Khan	mice	2014	polyIC		-		
Xuan	mice	2014	polyIC	60’	↓	-	
Guerrin	rats	2022	polyIC		-	↓	
Guerrin	rats	2023	polyIC	5’	↑	-	-
Raymann	mice	2023	polyIC		-	-	
Haddad	mice	2023	polyIC		-	↓	
Missig	mice	2023	imiquimod	?	- (↑24h dark)	↑	-
Sheng	mice	2024	resiquimod	10’ only	-	-	

Table 8-23: Review of behavioural outcomes following MIA.