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Electrophysiological Investigation of Auditory Mismatch Negativity: A Brain-Based Biomarker of N-Methyl-D-Aspartate Signalling

Lisa-Marie Greenwood
University of Wollongong

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Electrophysiological Investigation of Auditory Mismatch Negativity:

A Brain-Based Biomarker of *N*-Methyl-D-Aspartate Signalling

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Lisa-Marie Greenwood

Supervisors:

Professor Rodney Croft

Professor Nadia Solowij

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School of Psychology, Faculty of Social Sciences

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To George and Uncle Junior.

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Declaration

I, *Lisa-Marie Greenwood*, declare that this thesis is submitted in partial fulfilment of the requirements for the conferral of the degree *Doctor of Philosophy*, from the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

Lisa-Marie Greenwood

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Publications

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Greenwood L-M, Leung S, Michie PT, Croft RJ. (2018). Dose-response relationship between glycine and mismatch negativity in healthy controls. *In Preparation.* (Chapter six).

Abstract

Inconsistent reports on the therapeutic efficacy of increasing synaptic glycine concentration have raised doubt as to the benefit of *N*-methyl-D-aspartate receptor (NMDAr) mediated treatments for schizophrenia. Categorising individuals based on broad diagnostic criteria does not appear to adequately identify individuals who will benefit from such treatments. Mismatch negativity (MMN) may be a suitable biomarker of NMDAr function, to help clarify the neurobiological relationship between pharmacological intervention and clinical treatment efficacy. MMN is an auditory event-related potential elicited following the presentation of a deviant stimulus, when it violates an established sequence stored in echoic memory. MMN is a robust deficit in schizophrenia and is categorised as a physiological element in the Cognitive Systems domain of the Research Domain Criteria framework. However, few studies have examined direct pharmacological modulation of MMN in schizophrenia patients. The aim of this thesis was to determine the nature of the relationship between MMN and NMDAr function, to inform the relative utility of MMN as a biomarker of NMDAr-mediated improvements in clinical symptoms in schizophrenia. To achieve this aim, three separate empirical studies were performed.

Study one aimed to determine the nature of the relationship between regular cannabis exposure and MMN in otherwise healthy subjects. A cross-sectional comparison between regular cannabis users and controls was used to infer the effects of regular cannabis exposure on endocannabinoid-mediated alterations in NMDAr excitability. Frequency MMN amplitude was smaller in the

overall sample of regular users and smaller duration MMN amplitude was linearly associated with more prolonged and heavier cannabis exposure. These findings suggest regular cannabis use alters cannabinoid receptor type-I (CB_1) mediated inhibition of NMDArs in auditory cortical networks important for MMN generation. Further, they suggest regular use alters neurobiological function in target pathways of NMDAr-mediated treatments. This is problematic when interpreting MMN deficits as pathophysiological correlates of core phenotypes and may confound NMDAr-mediated treatment efficacy in schizophrenia.

Study two aimed to determine whether acute glycine administration and adjunct glycine treatment increases MMN generation in chronic schizophrenia patients. In a randomised, double-blind, placebo-controlled, between-group trial, acute administration of low-dose glycine (0.2g/kg) increased MMN amplitude compared to placebo. Smaller duration MMN amplitude at baseline was linearly associated with greater severity of negative symptoms and predicted, at trend level, the degree of negative symptom improvement following 6-weeks of glycine treatment (incremented to 0.6g/kg/day). These findings support the view that NMDAr hypofunction contributes to robust MMN deficits observed in schizophrenia and demonstrates that MMN is a sensitive index of NMDAr hypofunction related to the pathophysiology of negative symptoms. Further, these findings support the utility of MMN to stratify neurobiological functioning of NMDArs and index change in neuronal function following target engagement of NMDAr-mediated treatments.

Study three aimed to determine the dose-response relationship between

glycine and MMN, in a randomised, double-blind, placebo-controlled, crossover trial. In an independent sample of healthy controls, this study observed an Inverted-U dose-response relationship between glycine dose and MMN amplitude. High-dose glycine (0.8g/kg) reduced MMN amplitude compared to low- and medium-doses (0.2g/kg and 0.4g/kg, respectively), but did not differ from placebo. Smaller baseline MMN amplitude was associated with greater increases in MMN following low-dose glycine, suggesting that increasing synaptic glycine concentration is more beneficial in the context of NMDAr remediation. These findings support MMN as a sensitive biomarker indexing change in NMDAr function and may help to inform mechanisms of clinical treatment efficacy following increased synaptic glycine concentrations.

Findings in this thesis support the utility of MMN to index NMDAr function and change in neuronal signalling following target engagement of NMDAr-mediated treatments. Alterations in MMN generation in regular cannabis users suggest MMN is sensitive to long-term plasticity changes in auditory-cortical networks. The efficacy of glycine to improve NMDAr neurotransmission in this thesis (indexed by MMN) appears to be mediated by NMDAr function prior to glycine administration and glycine dose amount. These findings support the potential for MMN to identify conditions for optimal treatment efficacy. Future studies confirming the presence of an Inverted-U dose-response relationship between MMN and other NMDAr agents, such as glycine reuptake inhibitors, may assist in tailoring effective treatments and better inform mechanisms of treatment heterogeneity in schizophrenia.

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Chapter Seven

Abbreviations

Δ^9-THC	Δ^9- tetrahydrocannabinol
AC	Auditory cortex
AEA	Arachidonoylethanolamide
AUDIT	Alcohol Use Disorder Identification Test
AI	Primary auditory cortex
AII	Secondary auditory cortex
BDI	Beck Depression Inventory
CATIE	Clinical Antipsychotic Trial of Intervention Effectiveness
Ca^{2+}	Calcium
CB_1	Cannabinoid receptor type-I
CBD	Cannabidiol
CDRS	Calgary Depression Rating Scale
CNS	Central nervous system
CAPE	Community Assessment of Psychic Experiences
CUtLASS	Cost Utility of the Latest Antipsychotic drugs in Schizophrenia Study
D_1	Dopamine receptor type-I
D_2	Dopamine receptor type-II
DLPFC	Dorsolateral prefrontal cortex
DSE	Depolarised induced suppression of excitation
DSM-V	Diagnostic and Statistical Manual – Fifth Edition (2013)
EEG	Electroencephalograph
ERP	Event-related potential

FGAs	First generation antipsychotics
fMRI	Functional magnetic resonance imaging
GABA	Gamma-aminobutyric acid
GDA	Glycyldodecylamide
GT1-RI	Glycine type-I reuptake inhibitor
HINT-1	Histidine triadnucleotide-binding protein 1
IC	Inferior colliculus
ICD-10	International Statistical Classification of Disease and Related Health Problems – 10th Revision (2016)
K10	Kessler Psychological Distress Scale – 10 item version
K^+	Potassium
Mg^{2+}	Magnesium
MGB	Medial geniculate body
MGBd	Medial geniculate body – dorsal division
MGBm	Medial geniculate body – medial division
MGBv	Medial geniculate body – ventral division
MINI	Mini-International Neuropsychiatric Interview
MMN	Mismatch negativity
MWC	Marijuana Withdrawal Checklist
NA	Nucleus accumbens
NAC	N-acetyl-cysteine
NA^+	Sodium
NMDAr	*N*-methyl-D-aspartate receptor

PANSS	Positive and Negative Syndrome Scale
PCP	Phencyclidine
PFC	Prefrontal cortex
SCZ-Placebo	Schizophrenia group under placebo treatment
SCZ-Glycine	Schizophrenia group under glycine treatment
SGAs	Second generation antipsychotics
SPQ	Schizotypal Personality Questionnaire
SSA	Stimulus-specific adaptation
STAI-I	State-Trait Anxiety Index – State measure
STAI-II	State-Trait Anxiety Index – Trait measure
STR	Striatum
VTA	Ventral tegmental area
WASI	Wechsler Abbreviated Scale of Intelligence
WSAS	Work and Social Adjustment Scale
WTAR	Wechsler Test of Adult Reading
2-AG	2-arachidonoylglycerol

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Chapter One

Schizophrenia

1.1 Chapter Introduction

Since the development of antipsychotics in the early 1970's, little progress has been made to improve drug efficacy and tolerability in schizophrenia, particularly in managing negative symptoms and cognitive deficits. Non-adherence to pharmacotherapy is approximately 50% in patients [1] and approximately one third do not respond to standard medications [2]. There is currently a lack of treatment available to increase motivation, emotional experience, attention, thought processes and ability to make judgements. Negative symptoms and cognitive deficits are evident in the prodromal phase and are associated with poor functional outcome and reduced quality of life in later stages of illness [3]. Greater understanding of the mechanisms underlying these core refractory symptoms may yield earlier diagnosis and improve symptom management for many patients.

The following chapter defines schizophrenia as a clinical disorder, before reviewing the underlying biological mechanisms of core phenotypes. The primary theoretical models of neurotransmitter dysfunction provide a framework to discuss the utility of novel *N*-methyl-D-aspartate receptor (NMDAr) mediated treatments, which aim to increase glutamatergic function. Given the role of the endocannabinoid system in regulating NMDArs and alterations in this regulatory mechanism following repeated cannabis use, the effects of cannabis in relation to the pathophysiology of clinical symptoms in schizophrenia are also discussed. The chapter concludes by identifying the need for biomarkers in schizophrenia to inform mechanisms of treatment heterogeneity following pharmacological invention with NMDAr-mediated treatments.

1.2 Schizophrenia Disorder

Schizophrenia is a chronic and debilitating mental disorder and is one of the most severe in terms of personal suffering and societal burden. The prevalence of schizophrenia is approximately 0.30-0.66 cases per 1,000 people, with an incidence of 10.2-22.0 new cases per 100,000 people, per year [4, 5]. Typical onset occurs in late adolescence or early adulthood, with an initial diagnosis at approximately 26 years in males and 30 years in females (for further review, see [6]). The behavioural phenotypes of schizophrenia cause great disruptions and suffering in the day-to-day life of patients, including reduced functional capacity, lower financial stability, increased health care needs, shorter life expectancy [7-9] and overall reduced quality of life [3]. The chronic nature of the syndrome also generates significant financial burden to the community [10], with an annual cost reported in Australia, for example, of approximately $2.6 billion in 2013 [5].

Two of the most widely used diagnostic criteria for validating the profile of schizophrenia are the Diagnostic and Statistical Manual (Version 10; DSM-V) [11], and the International Statistical Classification of Disease and Related Health Problems (Version 10; ICD-10) [12]. Characteristics of symptoms in the DSM-V are divided into two broad symptom domains: positive and negative symptoms. Although not included in the diagnostic criteria, neurocognitive decline is also considered a core feature of functional disability in schizophrenia [11, 13] and characterises the deteriorating nature of the disorder. Due to limited diagnostic stability, diverse treatment outcomes and discrete longitudinal course, the subtypes of schizophrenia have been removed from the DSM-V and replaced with

a dimensional structure, focusing on the stage and severity of presenting symptoms [14]. Classifying core phenotypes in a dimensional framework may offer greater predictive power for clinical outcomes [15], particularly when investigating clinical treatment efficacy of pharmacotherapies or behavioural interventions.

1.2.1 Positive Symptoms

The manifestation of schizophrenia is generally characterised by the onset of positive symptoms, which tend to be episodic over time and associated with increased risk of self-harm and hospitalisation [16]. Positive symptoms are an exacerbation of normal functioning, including delusions, hallucinations, disorganised thought and disorganised or catatonic behaviour [17]. Delusions are often conceptualised as misinterpretations of other people's intentions or beliefs and are regularly associated with an area of personal reference or significance. Patients may experience hallucinations or perceptual abnormalities in a range of uni- or multi-modal sensory systems, including olfactory, visual, gustational, and somatic [18]. Speech and thought patterns may often become incoherent or illogical, where the content of one topic does not contextually link to the next, or the original content of the thought is forgotten. The positive symptoms of schizophrenia often make it difficult for patients to identify components of their experience that are not part of reality.

1.2.2 Negative Symptoms

The manifestation of negative symptoms may precede the onset of the first psychotic episode and when pronounced during prodromal stages of illness, contribute to poorer clinical prognosis and long-term disability [19]. Negative symptoms are pervasive throughout the disorder, more stable over time and follow a longitudinally independent course when compared to positive symptoms [20]. The negative dimension of schizophrenia is characterised by absent or diminished emotional and behavioural responses, such as alogia (reduced quality or quantity of speech), avolition (reduced ability to initiate and follow through on plans), anhedonia (lack of pleasure), flattened affect (expressed as monotonous voice tone or immobile facial expressions) and social withdrawal (loss of interest in social engagement) [11]. The persistent nature of these symptoms impairs a patient's ability to maintain daily functioning and is associated with cognitive decline [21], an arrest in social development and attainment in usual social roles [22]. Despite a strong association, the shared variance between negative symptoms and cognitive deficits has been shown to be small, suggesting that each domain contributes independently to reduced functional outcomes [23].

1.2.3 Cognitive Deficits

Neurocognitive decline in schizophrenia represents a moderate-to-severe deviation below the norm [24] in areas important for daily functioning, including memory, learning, attention, visuo-spatial abilities, language and executive function [25-27]. Some patients present with reduced cognitive performance prior

to the onset of illness [28] and by the time of onset of the first psychotic episode, show stable impairment in several domains [29]. Cognitive deficits are associated with reduced ability to perform daily living tasks [30, 31] and reduced measures of global functioning and quality of life [3]. These debilitating performance outcomes remain consistent throughout chronic stages of illness in most patients [29]. Behavioural deficits may be indicative of abnormal neuronal development, aberrant neuroplasticity, structural and functional alterations [32], or unexpressed genetic components [33-36]. Conceptualising schizophrenia as a syndrome of cognitive dysfunction remains a core focus of clinical research, in an attempt to clarify the underlying mechanisms that give rise to and maintain these disabling features of the disorder.

1.3 Pathophysiology of Schizophrenia

Advances in molecular biology, genetics and imaging techniques provide evidence of alterations in several neurotransmitter systems, including dopamine, glutamate, gamma-aminobutyric acid (GABA) and serotonin, which link abnormal neurochemistry to the phenotypic expressions of schizophrenia. These neurobiological frameworks aim to accommodate structural and functional abnormalities and disconnectivity between brain regions. The dopamine hypothesis of schizophrenia still remains the most relevant theory linking the pathophysiology of positive symptoms to the mechanism of current antipsychotic medications. However, the development of the glutamatergic hypothesis reconceptualised our understanding of the disorder and offers a new mechanism

of action and potential neurobiological target for treating core refractory symptoms. While these models are still in their infancy in explaining the aetiology of schizophrenia, their contribution to a neuropathophysiological framework of the brain usefully informs the manifestation and maintenance of core phenotypes. Further investigation and ongoing refinement of these models continues to advance the development of novel treatment interventions and their progression into clinical trials.

1.3.1 Dopamine Hypothesis

The initial hypothesis of excessive subcortical dopamine was derived from clinical benefits following administration of antipsychotics [37] and their potency for dopamine type-II (D_2) receptors [38]. Neuroimaging studies provide evidence of D_2 receptor hyperfunction in the mesolimbic pathway projecting from the ventral tegmental area to the nucleus accumbens [39] (Figure 1.1a). This increase in dopaminergic neurotransmission is associated with increased positive symptoms in schizophrenia and parallels fluctuations in psychotic episodes throughout the course of illness [40]. D_2 receptors are highly concentrated in the striatum, with lower concentrations in the prefrontal cortex and medial temporal regions. Dopamine dysfunction in the striatum, which receives inputs from both the ventral tegmental area and nucleus accumbens (Figure 1.1b), has been proposed as a final common pathway and mechanism of positive psychotics symptoms [41]. Overactive D_2 receptor expression in this region may also contribute to cortical-mediated cognitive deficits observed in schizophrenia [42].

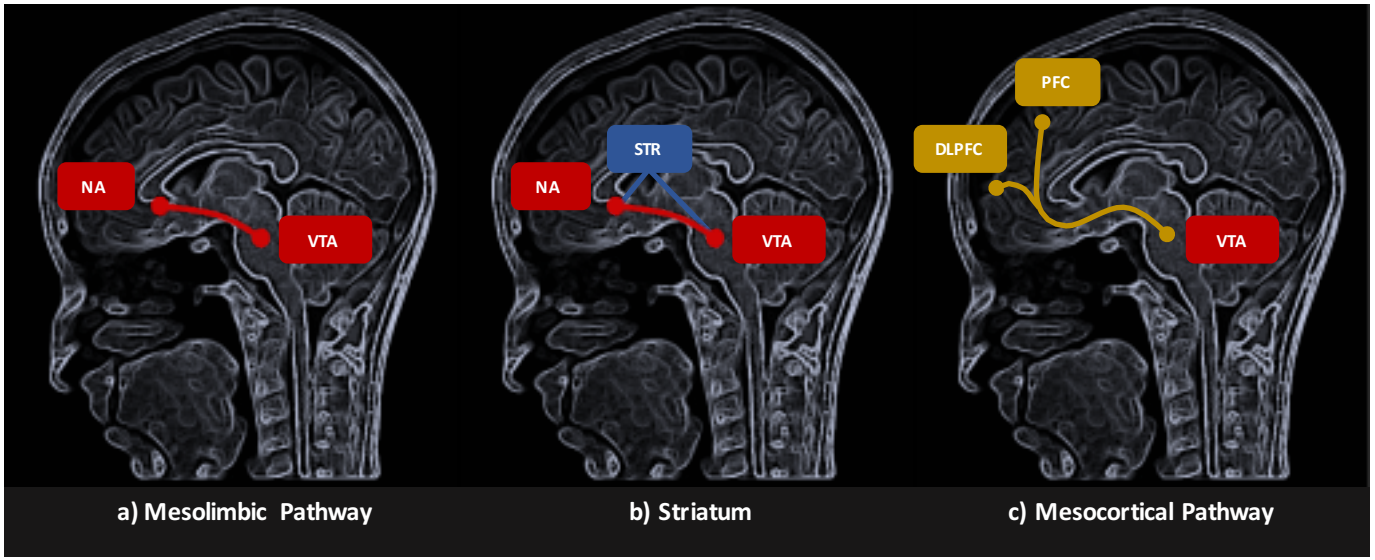

Figure 1.1. Mesolimbic and mesocortical pathways related to the dopamine hypothesis of schizophrenia: a) over-active dopaminergic function in the mesolimbic pathway, projecting from the ventral tegmental area to the nucleus accumbens, contributes to the manifestation of positive symptoms; b) the striatum is highly dense in dopamine D_2 receptors, receiving input from the ventral tegmental area and nucleus accumbens, and is the proposed final common pathway of positive symptoms in schizophrenia; c) under-active dopaminergic neurotransmission in the mesocortical pathway contributes to the manifestation of negative symptoms and cognitive deficits. DLPFC, dorsolateral prefrontal cortex; NA, nucleus accumbens; PFC, prefrontal cortex; STR, striatum; VTA, ventral tegmental area.

Hypoactivation of dopamine in the mesocortical pathway (Figure 1.1c) may play an important role in the generation of negative symptoms and cognitive impairment [43-46]. Activation of dopamine type-I (D_1) receptors located on glutamatergic neurons decreases presynaptic glutamate release, while those located on GABA interneurons promote inhibition of pyramidal neurons [47, 48]. Reduced D_1 receptor binding in the prefrontal cortex has been observed in drug-naïve schizophrenia patients and has shown to be associated with increased severity of negative symptoms and impaired cognitive performance [49]. Despite these associations, the dopamine hypothesis is limited to defining a pathophysiological understanding of psychosis. This neurobiological model is less able to define the aetiology of other neurotransmitter system dysfunction, such as glutamate, adenosine and serotonin, which accommodate broader phenotypic profiles in schizophrenia [50].

1.3.2 Glutamate Hypothesis

Decreased NMDAr function is thought to underlie neuronal atrophy and reduced excitatory networks in schizophrenia [51, 52]. The glutamatergic hypothesis proposes a preliminary dysregulation in prefrontal NMDAr function, which alters downstream dopaminergic neurotransmission [53, 54]. Hypofunctional NMDArs in the prefrontal cortex result in a weak GABA tone, attenuating the inhibition of secondary glutamate release (Figure 1.2). Increased secondary glutamate leads to excessive release of dopamine in the mesolimbic pathway (for further review, see [55]). This theory accommodates positive symptoms that are synonymous with

the dopamine hypothesis, as well as providing a neurobiological model inclusive of negative symptoms, cognitive deficits and additional structural and functional alterations reported in schizophrenia.

Support for glutamate dysfunction comes from acute models of dissociative anaesthetics that block NMDArs and decrease glutamate availability in the prefrontal cortex. NMDAr antagonists such as Phencyclidine (PCP) and Ketamine have been shown to give rise to schizophrenia-like symptoms in individuals without psychiatric history [51, 56] and worsen symptoms in schizophrenia patients [57, 58]. These acute pharmacological models induce positive and negative symptoms in a dose-response manner [59] and model cognitive impairments [53, 60], thought disorder [61] and eye tracking abnormalities [62, 63] that are reminiscent of schizophrenia. NMDAr co-agonists, such as glycine, have shown to inhibit PCP-induced hyperactivity [64], providing further evidence of altered NMDAr function underlying core schizophrenia phenotypes.

Patient studies report decreased glutamate levels in cerebrospinal fluid and increased NMDArs post-mortem [65]. This increase in NMDArs is likely a neuronal compensatory mechanism to manage the pervasive state of decreased glutamatergic function, which is evident throughout the chronicity of the disorder [66]. Individuals with complete dopamine D_2 receptor blockade persist with positive symptoms [67], suggesting that psychosis is mediated by additional neurotransmitter networks beyond hyperactive dopaminergic function in the mesolimbic pathway. Contrary to these conclusions, research utilising magnetic

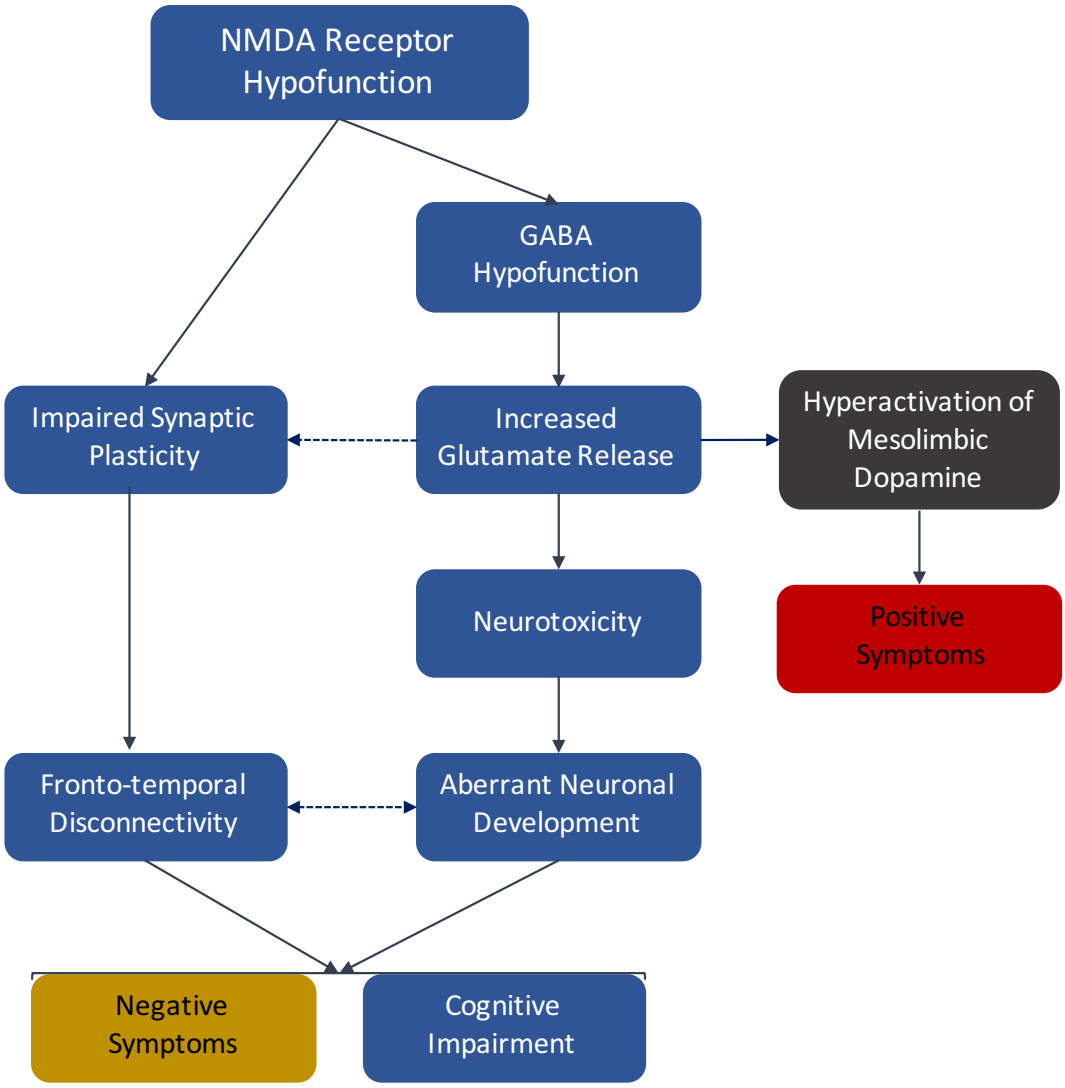

Figure 1.2. Schematic illustration of the glutamatergic hypothesis of schizophrenia. *N*-methyl-D-aspartate receptor-mediated positive symptoms, negative symptoms and cognitive deficits in the glutamatergic model of schizophrenia. NMDA, *N*-methyl-D-aspartate; GABA, Gamma-Aminobutyric Acid.

resonance spectroscopy techniques have failed to identify a consistent relationship between regional glutamate and glutamine levels across different stages of the disorder (for further review, see [68]). In order to inform the aetiology and maintenance of core refractory symptoms, there is need to clarify their relationship with the pathophysiology of altered NMDAr function in cortical and subcortical networks within the brain.

1.3.2.1 Glutamatergic Neurotransmitters

At resting potential, NMDArs are ligand-gated ion channels blocked by a magnesium (Mg^{2+}) gate. This block is relieved when the membrane potential is depolarised, allowing an influx of calcium (Ca^{2+}) to enter the neuron. For NMDArs to be activated, they require glycine (an NMDAr co-agonist) to bind to the NR1 subunit and glutamate to bind to the NR2 subunit of the receptor. When this occurs, Ca^{2+} activates a second messenger system that alters pre- and post-synaptic connections via long-term potentiation and long-term depression (for further review, see [69]). It is through this change in synaptic connection strength by which the brain learns and encodes new information. High levels of extracellular sodium (Na^{+}) and high intracellular potassium (k^{+}) concentrations allow Na^{+} pumps to reabsorb glutamate and amino acids back into the cell. Glutamate is either reabsorbed via this process or it is converted into glutamine by glial cells and transported to other neurons [70], before being converted back into glutamate. When high levels of glutamate accumulate outside of the cell and are not reabsorbed, NMDArs are re-activated, allowing further influx of Ca^{2+} ions

to enter the cell. This increased concentration of intracellular Ca^{2+} may lead to neuronal cell death or excitotoxicity and is likely a contributing factor of neuronal atrophy observed in schizophrenia [71].

1.3.2.2 GABAergic Neurotransmitters

Attention to GABAergic function in schizophrenia was rejuvenated following developments in dopaminergic and glutamatergic hypotheses. GABAergic neurons contribute to a holistic framework of both deficits [72] and potential therapeutic interventions [73] in schizophrenia. This is particularly relevant to understanding alterations in neuronal plasticity within the disorder, as both glutamate and GABA play an important role in filtering information transmitted to cortical pyramidal neurons. When information is transferred down the neuronal dendritic spine, GABAergic synapses moderate neuronal plasticity by filtering glutamatergic signals, before they propagate to the cell soma to generate an action potential (for further review, see [74]; Figure 1.3). Glutamatergic neurons provide the excitatory drive for GABAergic interneurons, whereby reduced glutamatergic function leads to a loss of inhibitory filtering and subsequent hyper-activation of pyramidal neurons (Figure 1.2). Therefore, reduced cortical function of glutamate may initiate GABA-mediated cognitive deficits in schizophrenia.

1.4 Neurobiological Alterations

A structural model of schizophrenia is supported by findings of smaller whole brain volume [75, 76], reduced hippocampal volume [77], decreased grey matter

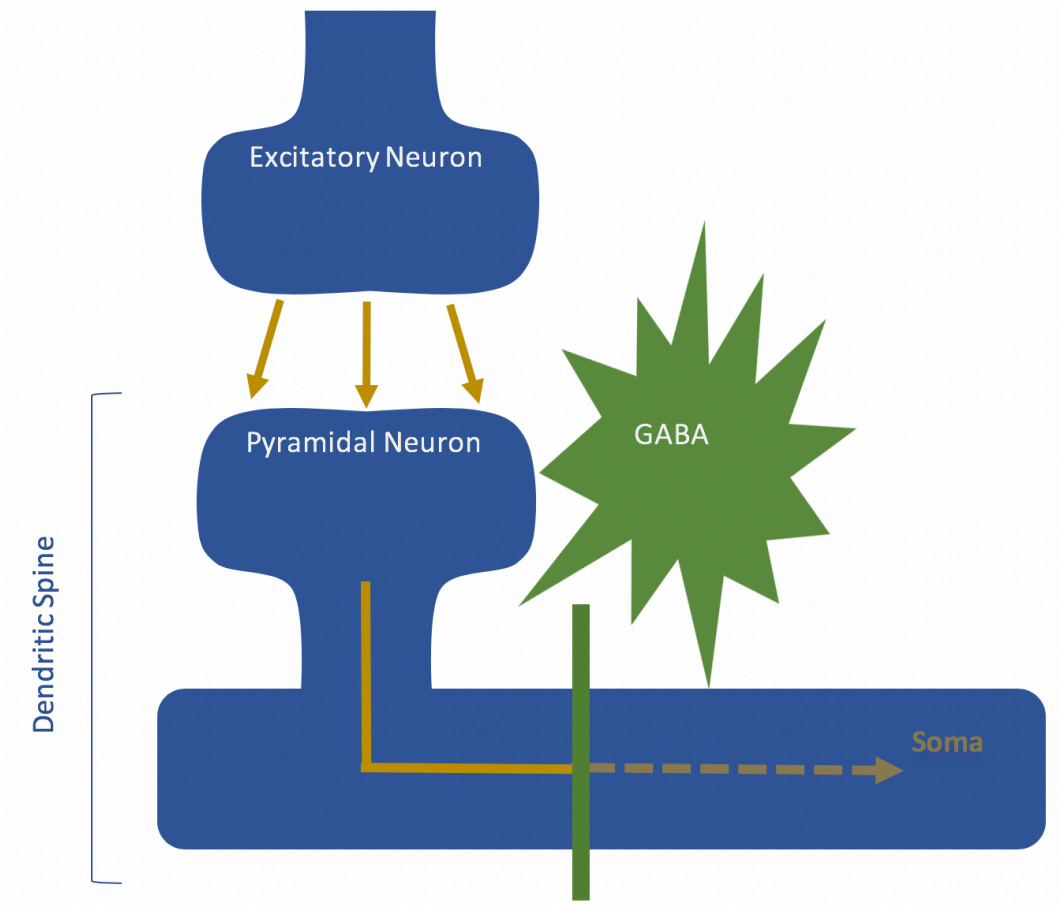

Figure 1.3. Gamma-aminobutyric acid filtering of pyramidal neurons. Gamma-Aminobutyric Acid (GABA) interneurons filter (green vertical bar) excitatory inputs on the dendritic spines of cortical pyramidal neurons, before reaching the cell soma. When glutamatergic regulation of GABA interneurons is decreased, the GABA-mediated inhibitory filtering is reduced, resulting in hyper-activation of cortical pyramidal neurons. GABA, Gamma-Aminobutyric Acid.

[75, 78, 79], enlarged ventricles [80], focal alterations of white matter tracts and brain atrophy in regions such as the prefrontal cortex [16]. Alterations in structural and functional connectivity appear evident across different stages of the disorder [81, 82] , while structural alterations in family members provide support for a genetic contribution (for further review, see [83]). Findings of neurobiological alterations in schizophrenia has facilitated the characterisation of different stages of the disorder, including the development of premorbid risk factors (for further review, see [84]). Individuals at high risk for developing psychosis are reported to have pronounced grey matter deficits [85], reduced whole brain volume, and left and right prefrontal and temporal lobe volume [76]. Diffusion tensor imaging techniques report that abnormal white matter development in temporal regions in schizophrenia predicts functional outcomes in later stages of illness [86].

Neuroscience has directed much attention to understanding phenotypes arising from aberrant neuronal networks and integration between brain regions, suggesting that many phenotypes can only be explained by considering the relationship between a range of cognitive processes. Instead of attributing *structural* plasticity, symptoms are postulated to result from *synaptic* plasticity - the activity dependent modelling of the pattern and strength of synaptic connections (for further review, see [87]). A series of post-mortem studies report reduced excitatory feed-forward circuits extending from the auditory cortex (AC) in chronic patients [88, 89], which may result in poor adaptation to perceptual changes in the environment. A disconnectivity framework of schizophrenia proposes that the brain may still show regionally specific structural abnormalities,

but that these abnormalities are secondary to the more pervasive problem of deficient integration and communication of information [87].

1.5 Antipsychotic Treatments

In the acute psychotic state, schizophrenia patients exhibit an increase in dopamine synthesis and synaptic dopamine concentration [90], providing a clear and logical link to first-generation antipsychotics (FGAs) targeting dopamine D_2 receptor function [91]. A limitation of FGAs, which includes agents such as haloperidol and chlorpromazine, is the manifestation of extrapyramidal side effects following acute and chronic D_2 receptor blockade (Figure 1.4). Following administration of haloperidol, dopamine D_2 receptor occupancy rates above 65% have demonstrated therapeutic efficacy, while occupancy rates above 78% elicited extrapyramidal side-effects and no further symptom improvement [91]. High doses of FGAs can also block activation in the mesocortical pathway, contributing to secondary negative symptoms and cognitive deficits [92].

The profound side effects of FGAs led to the development of second-generation antipsychotics (SGAs). An advantage of SGAs is reduced specificity for dopaminergic receptors and indirect modulation of dopamine via other neurotransmitter systems. For example, risperidone and ziprasidone have a high affinity ratio for 5HT2A-to-D_2 receptors, where 5HT2A has an additional regulatory effect on dopaminergic function (for example, see [93, 94]). These properties allow the drugs to maintain their therapeutic benefit while lowering the risk of extrapyramidal and secondary negative symptoms. Contrary to the proposed

benefits of SGAs, these drugs also incur increased cardio and metabolic side-effects, such as weight gain and glucose dysregulation (for further review, see [95]). Overall, these treatments have not met expectations with regards to reduced side-effect profiles or increased tolerability when directly compared to FGAs (for further review, see [96]).

Clozapine is almost considered a third class of antipsychotic, due to its ability to treat up to 50-60% of treatment-refractory patients (i.e. patients who have not previously responded to antipsychotic medication) [94, 97]. However, this drug demonstrates limited efficacy when administered to treat first episode psychosis [98]. Clozapine has lower-affinity and short-term high occupancy at D_2 receptors, which is sufficient to maintain antipsychotic properties without over-occupying the receptor [99, 100]. In addition, clozapine has high affinity for 5HT2A, Muscarinic M1 and α2-adrenoceptors (for further review, see [101]), supporting significant involvement of neurotransmitter systems beyond direct dopamine activation that contribute to its effectiveness. There are additional adverse side effects involved in treatment with clozapine, most notably, haematological reactions, dose-related reduction in seizure threshold, myocarditis and cardio-myopathy [102]. These risk factors require close monitoring, limiting the practical utility of administering clozapine in treatment-resistant or chronically ill patients.

While antipsychotics show some efficacy in reducing psychotic symptoms and preventing relapse in schizophrenia [91, 103], they have modest effects in treating negative symptoms and cognitive deficits [104]. Recent large-scale clinical trials also raise concern over the naturalistic efficacy of SGAs (compared to FGAs)

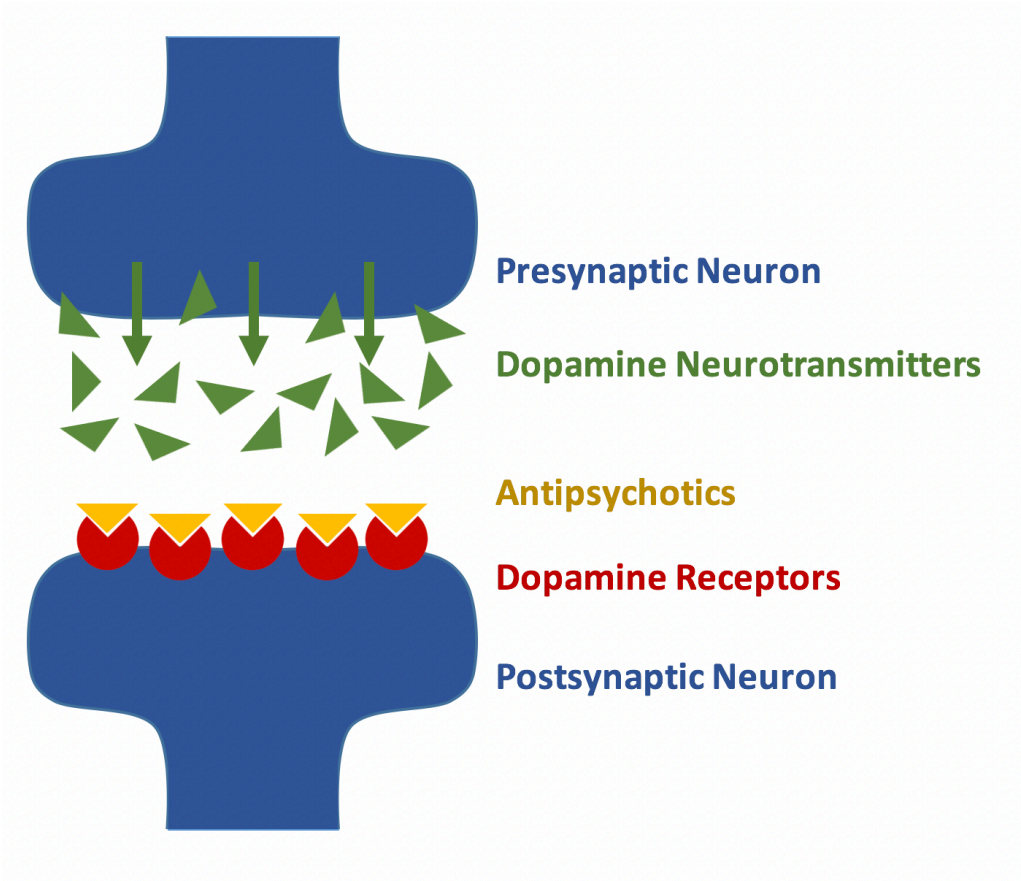

Figure 1.4. Antipsychotic blockade of dopamine D_2 receptors. First generation antipsychotics have high affinity for dopamine D_2 receptors, while second generation antipsychotics have high potency but reduced specificity for dopamine. Both acute and chronic blockade of D_2 receptors contribute to the unwanted side-effect profile of antipsychotics in the treatment of schizophrenia.

when assessing real world outcomes. The Clinical Antipsychotic Trial of Intervention Effectiveness (CATIE) [105] and the Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study (CUtLASS) [106] both failed to demonstrate superior efficacy for either FGAs or SGAs on measures of treatment discontinuation, improved psychotic symptoms, or increased quality of life. In a meta-analysis examining the efficacy of fifteen different antipsychotics, only small effects sizes were observed for amisulpride, olanzapine, and risperidone, all of which were developed in the first series of SGAs [107].

1.6 Glutamatergic Treatments

The challenge for current pharmacological research is to address the under-recognised and treatment refractoriness of core negative symptoms and cognitive deficits, and to optimise conditions for pharmaceutical benefit. A glutamatergic model postulates hypofunction of prefrontal NMDArs that lead to reduced excitatory networks and alterations in brain structure, function and downstream neurotransmitter pathways [55, 108-110]. Direct activation of glutamatergic receptors leads to neuronal cell death and is not a feasible option to manage the hypofunctional NMDAr state of schizophrenia. Alternatively, activation of the glycine modulatory site is one proposed mechanism of increasing glutamatergic neurotransmission (Figure 1.5). In animal models of schizophrenia, glycine reduced PCP-mediated psychotic symptoms [111, 112]. The same authors report glycine increased NMDAr-mediated inhibition of dopamine release in the striatum [113, 114], while glycyldodecylamide (GDA), a glycine type-1 reuptake inhibitor

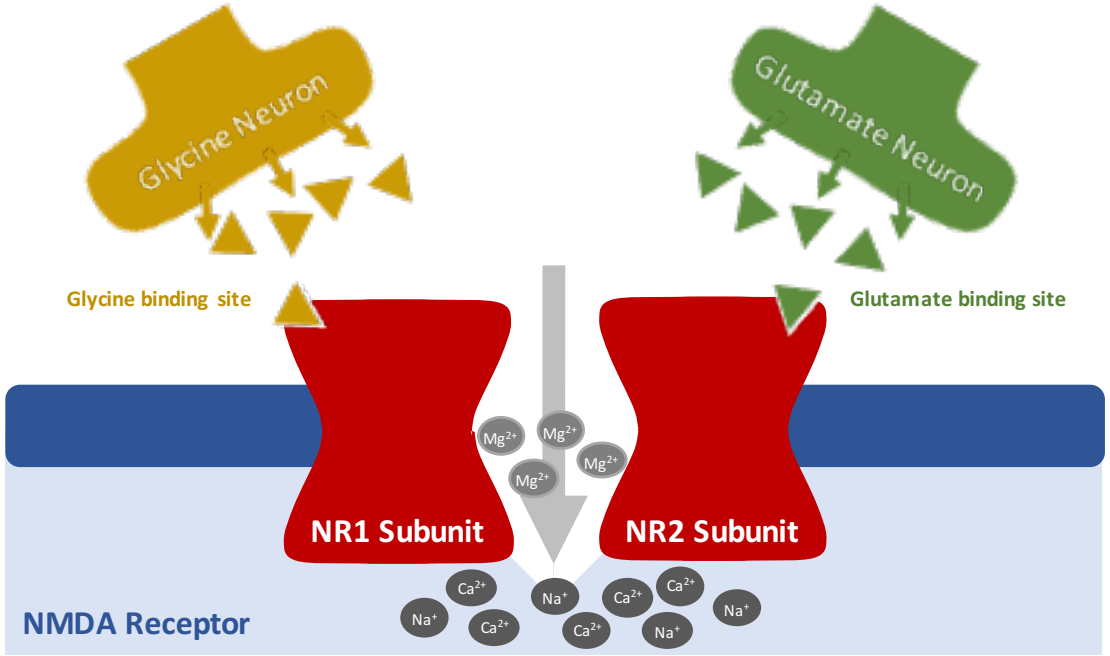

Figure 1.5. Glycine binding on *N*-methyl-D-aspartate receptors. Directly activating the glutamate binding site on *N*-methyl-D-aspartate receptors (NMDArs) may lead to neuronal cell death or excitotoxicity. One alternative way to increase NMDAr function in schizophrenia is to activate the glycine modulatory site. Novel therapeutic targets aim to increase synaptic glycine concentrations to rectify the hypofunctional NMDAr state in schizophrenia. NMDA, *N*-methyl-D-aspartate ; CA^{2+}, Calcium ions; Na^{+}, Sodium ions; MG^{2+}, Magnesium ions.

(GT1-RI) stimulated NMDAr-mediated GABA release in the same region [114].

NMDAr agonists such as glycine [115-117] and D-serine [118, 119], as well as the glutathione precursor N-acetyl-cysteine (NAC) [120], have demonstrated improved clinical symptoms in patients on stable antipsychotic medication, although some studies have failed to replicate these findings (for further review, see [121]). While high-dose glycine has shown to improve negative symptoms [117, 122, 123] and cognitive deficits [124] in treatment-resistant patients, increasing synaptic glycine concentration under clozapine may saturate the glycine modulatory site and initiate increased negative symptoms; glycine may downregulate NMDAr activity [125] and D-cycloserine may displace fully occupied sites [126].

Proof of concept studies administering GT1-RIs have shown promising results for improving positive and negative symptoms [127]. However, a recent phase-III clinical trial failed to support any benefit of the GT1-RI bitopertin when compared to placebo [128], raising doubts as to the benefit of increasing synaptic glycine concentration in schizophrenia. An editorial by Beck and colleagues [2] raises concern of secondary negative symptoms inflating a placebo effect, particularly in chronic patients. The authors further suggest the need for stratifying biological dysfunction in clinical trials and the need for biological markers to further inform mechanisms of treatment efficacy. Such markers may help clarify inconsistent reports on therapeutic outcomes following increased synaptic glycine concentration in schizophrenia.

1.7 Cannabis Use in Schizophrenia

Substance use disorder is highly prevalent in schizophrenia, with approximately 50% of substance use in patients compared to 16% in the general population [129]. Notably, cannabis use is significantly higher in schizophrenia [130, 131] and in individuals with a psychotic illness more generally [132]. A meta-analysis estimated the prevalence of a cannabis use disorder in schizophrenia, indicating clinically significant distress or impairment [11], for current use at 16% and lifetime use at 27.1% [133]. In the Australian and New Zealand Clinical Practice Guidelines cannabis is identified as the most serious comorbidity in schizophrenia due to its widespread use [134]. Cannabis has been reported to worsen outcomes in schizophrenia patients by enhancing cognitive deficits and psychotic symptoms and increases the risk of relapse [135-137]. Chronic cannabis users, without psychiatric history, also exhibit many cognitive phenotypes that are proposed vulnerability markers of schizophrenia [138].

1.7.1 Structural and Functional Alterations

Disruption of normal endocannabinoid functioning may lead to alterations in brain networks important for neuronal and cognitive development [139]. Cognitive deficits in heavy and long-term cannabis users are thought to be mediated by alterations in the hippocampus, prefrontal cortex and cerebellum [140]. These regions are critically involved in memory and higher order cognitive processing and are dense with cannabinoid receptors [141, 142]. The most commonly reduced functions following acute and chronic cannabis exposure are attention

and verbal learning and memory, with some evidence of ongoing impairment after prolonged cessation of use (for further review, see [143]). These findings are supported by animal models reporting learning and memory impairment after acute and chronic cannabinoid administration [144, 145].

Similarities in structural and functional deficits between cannabis users and schizophrenia patients suggest a common underlying pathology [138]. Reduced hippocampal volume in cannabis users has been associated with cumulative exposure to cannabis and increased development of subclinical psychotic symptoms, where hippocampal reductions were of similar magnitude to that observed in schizophrenia [146]. Molecular and electrophysiological techniques have been used to demonstrate cannabinoid type-I (CB_1) receptor mediation of Δ^9-Tetrahydrocannabinol (Δ^9THC) induced reductions in long-term potentiation [147]. In this study, Δ^9THC was shown to down-regulate glutamatergic receptor subunits in mice and induce their endocytosis via CB_1 receptors. Repeated exposure to cannabis has also been shown to suppress long-term potentiation in the CA1 region of the hippocampus [148, 149]. These findings suggest a complex interaction between endocannabinoid and glutamatergic neurotransmitter function that is adversely affected by repeated Δ^9THC exposure.

1.7.2 Endogenous Cannabinoids

Endocannabinoids and their receptors modulate physiological functioning in a range of neuronal networking systems within the brain [150] and play an important role in behavioural processes such as locomotion, anxiety, learning and

memory [151]. The two main cannabinoid receptors, CB_1 and cannabinoid type-II (CB_2), belong to the family of G-protein coupled receptors. CB_1 receptors are expressed in the central nervous system (CNS), with highest concentrations in the basal ganglia, hippocampus, and prefrontal and anterior cingulate cortex, while CB_2 receptors are mainly found in the immune cells and peripheral tissues [152]. Endocannabinoids are lipid transmitters that serve as natural ligands for cannabinoid receptors, with the main endocannabinoids being arachidonoylethanolamide (anandamide or AEA) and 2-arachidonoylglycerol (2-AG). Endocannabinoid synthesis is located on membrane phospholipids in response to postsynaptic intracellular CA^{2+}, a process that may be aided by post-synaptic G-protein couple receptor activation.

Cannabinoid receptors mediate the inhibition of neurotransmitter release throughout the central nervous system, including glutamate, dopamine and GABA [153-155]. Depolarised induced suppression of excitation (DSE), the process of inhibiting neurotransmitter release from glutamatergic neurons, occurs when CB_1 receptors inhibit voltage-gated Ca^{2+} channels and K^+ conductance [156]. DSE occurs when 2-AG is released from CA1 pyramidal neurons during depolarisation and act in a retrograde manner to activate CB_1 receptors on Schaffer collateral axon terminals [156]. CB_1 receptors regulate activation of NMDArs, via coupling of histidine triadnucleotide-binding protein 1 (HINT-1) [157], to prevent further CA^{2+} influx and therefore protect against neuronal excitotoxicity. As NMDArs become highly activated, cannabinoids are recruited on demand to co-internalise the NR1 subunit of the receptor, negatively controlling NMDAr function via retrograde

synaptic messaging [158].

1.7.3 Exogenous Cannabinoids

The endocannabinoid system is the binding site of exogenous cannabinoids, such as Δ^9-THC, which disrupt normal endocannabinoid regulation of neuronal excitability within the brain [159]. Δ^9THC is the main psychotropic constituent in cannabis [160] and is a partial agonist at CB_1 and CB_2 receptors. CB_1 receptors located on glutamatergic neurons appear to be activated at lower concentrations of Δ^9THC compared to those on GABAergic neurons, suggesting a bell-shaped dose-response excitatory curve [161]. Cannabidiol (CBD), also an exogenous cannabinoid found in cannabis plant matter and partial agonist at CB_1 and CB_2 receptors, has purported anxiolytic and antipsychotic properties (for further review, see [162]). Although CBD has low affinity at CB_1 receptors, it has negative allosteric modulator properties [163, 164] that reduce the ability of CB_1 agonists, such as Δ^9THC, to bind to the receptor.

Increased activation of CB_1 receptors has been shown to induce psychotic states in vulnerable individuals [165, 166] and worsen symptoms in schizophrenia patients [135]. Alterations in endocannabinoid regulation of glutamatergic function, whereby CB_1 receptors restrict NMDAr activation, may lead to prolonged states of NMDAr hypofunction or downregulation of NMDArs, conditions that are synonymous with the pathophysiology of schizophrenia (see section 1.3). Over-activation of pre-synaptic CB_1 receptors may inhibit glutamate release in the synaptic cleft, while post-synaptic CB_1 receptors may alter NMDAr signalling

pathways. Prolonged states of reduced NMDAr signalling may lead to alterations in downstream neurotransmitter functioning, such as dopamine [167], providing a mechanism for which repeated exposure to Δ^9THC may precipitate psychotic symptoms in vulnerable individuals.

It is unclear whether smoked cannabis alters CB_1-NMDAr associations to differentially affect the neuronal response to NMDAr-mediated treatments in schizophrenia. The efficacy of increasing synaptic glycine concentration may be reduced under conditions of elevated CB_1-mediated inhibition of NMDAr activation. While most of the pharmacological action of exogenous cannabinoids is reported for CB_1 receptors, Δ^9THC and CBD may also alter glutamatergic and GABAergic function via CB_1-independent mechanisms (for review of additional molecular targets not discussed here, see [168]). In neurons located in the ventral tegmental area in mice, Δ^9THC potentiates glycine receptor-mediated currents via allosteric mechanisms in a dose-response manner [161]. These findings suggest exogenous cannabinoids alter neural activation and downstream neurotransmitter release in brain networks implicated in the pathophysiology of core schizophrenia phenotypes (see section 1.3.1). Of particular relevance to the current thesis is that different combinations of Δ^9THC and CBD potency found in cannabis plant matter [169], as well as evidence for their dose-dependent outcomes (for further review, see [170]), may lead to differential effects of cannabis on NMDArs and associated signalling pathways. There is need to further clarify the effects of repeated cannabis use on vulnerability markers indexing hypofunctional NMDAr activity in schizophrenia.

1.8 Chapter Summary

While neuroscience has advanced our understanding of the biological underpinnings of schizophrenia, there remains vast heterogeneity in response to pharmacological treatments. A dopaminergic hypothesis provides a clear link to antipsychotics targeting dopamine D_2 receptor function. However, a glutamatergic hypothesis proposes preliminary dysregulation that accommodates core refractory symptoms and cognitive deficits, specifying NMDArs as a logical neuronal target for pharmacological intervention. The shift from a narrowly defined dopamine hypothesis, to the refinement of broader neurobiological models such as glutamate, has guided the development of alternative treatments which are progressing through preclinical and clinical phases of testing. A challenge for current neuropsychopharmacological research is to clarify mechanisms of improved clinical outcomes and to increase the specificity and sensitivity of diagnostic and treatment tools [171].

Increasing synaptic glycine concentration is one potential method to rectify the hypofunctional NMDAr state in schizophrenia. Phase-II clinical trials have found some evidence that this method improves positive and negative symptoms [119, 127, 172, 173], while other studies have failed to replicate these findings [128]; this raises some doubt as to the benefit of increasing post-synaptic glycine concentrations in schizophrenia. The endocannabinoid system plays a key role in regulating NMDAr activation and disruption of this regulatory mechanism, such as that following regular cannabis use, may alter neurotransmitter functioning in target pathways of NMDAr-mediated treatments. Clarifying the role

of the endocannabinoid system (both related and unrelated to regular cannabis use) on cortical and subcortical networks deficient in schizophrenia, may help to inform mechanisms of core refractory phenotypes. The application of biomarkers in glutamatergic-mediated pharmacotherapy trials may be a useful means of informing the relationship between functional target engagement and improved clinical outcomes. Further, they may help clarify inconsistent reports on the benefits of increasing synaptic glycine concentration to improve clinical symptoms in schizophrenia.

Chapter Two

Auditory Mismatch Negativity

2.1 Chapter Introduction

In pharmacotherapy trials, biomarkers aim to clarify the relationship between neuronal occupancy and expected therapeutic benefits. Absence of such measures in preclinical trials can make it difficult to interpret inconsistent reports on clinical outcomes, as is the case for treatments that aim to increase synaptic glycine concentration in schizophrenia. The primary auditory pathway is one neurobiological system that allows unique insight into the integrity of excitatory neurotransmitter functioning within the brain. Mismatch Negativity (MMN) is a measure of auditory change detection and is a potential biomarker to index *N*-methyl-D-aspartate receptor (NMDAr) hypofunction in schizophrenia. There is need to determine the nature of the relationship between MMN and NMDAr function, in order to inform the utility of MMN to index neuronal integrity in brain regions and pathways underlying core refractory symptoms.

The following chapter defines MMN as an event-related potential indexing deviance detection and discusses the pharmacology of its generation, with particular focus on neuronal networks and brain regions relevant to the pathophysiology of schizophrenia. Discussions on the hierarchical structure of the primary auditory pathway and networks involved in processing frequency and duration sound features, provide a framework to discuss the contribution of excitatory and inhibitory networks involved in MMN generation. This chapter reviews MMN findings within schizophrenia and proposes MMN as a potential biomarker to stratify NMDAr dysfunction within the disorder. Further, this chapter concludes that changes in MMN may be useful to index alterations in NMDAr

function following neuronal target engagement of pharmacological treatments.

2.2 Mismatch Negativity Defined

MMN indexes the brain's pre-attentive ability to detect stimulus change in sensory memory. Auditory MMN is a negative deflection of the event-related potential (ERP), elicited above the threshold of discrimination between a deviant stimulus and a pattern of sounds forming a sensory memory trace [174, 175]. Deviant stimuli may vary from the memory trace, also referred to as standards, in differing complexity, such as change in spectral, temporal, or higher order features [176, 177] (higher order constructs such as phonetic structure, sequence pattern and stimulus omission are not discussed here). Typically, MMN is calculated by subtracting the ERP to standards from the ERP to deviants, creating a difference waveform (see section 2.2.1). The negative potential observed in the difference waveform is thought to index additional excitatory processing required for deviance detection. As MMN is a pre-attentive measure of attention and is responsive to neurobiological change, it is a candidate biomarker for translational clinical research.

2.2.1 Electroencephalographic Measurement

MMN is elicited approximately 100-200ms after the onset of a deviant stimulus [178]. It is typically measured by recording ongoing spontaneous neuronal activity via electroencephalograph (EEG) at frontal electrode sites, compared to a relatively neutral reference electrode such as the nose or linked mastoids [179].

The EEG recording at each electrode indexes the voltage signal of many neurons working together across time-varying domains. The latency of the ERP indexes both the degree of neural activity required to process the stimulus and ongoing neural activity that is non-specific to the stimulus. In order to remove the non-specific or irrelevant neural activity, multiple ERP trials to the same stimulus presentation are averaged together. This method assumes that neural activity generated in response to the stimulus will be most prominent in the averaged waveform due to its consistent temporal presentation across trials, while the non-specific activity is 'averaged out' along the temporal domain of the ERP recording.

2.2.2 Oddball Paradigm

Typically, much research has utilised an auditory oddball paradigm to measure MMN, whereby deviant tones are presented intermittently within a background of identical standard tones (Figure 2.1). The number of standard tones is presented at differing train lengths to allow the deviant stimulus to be presented at unexpected time intervals and of an unknown probability. The deviant tone may be characterised by, but not limited to, changes in duration, frequency, intensity, or spatial location of a sound. The average response to all standard tones is subtracted (separately) from the averaged response to each type of deviant tone, creating a difference MMN waveform for each deviant type.

2.2.3 Roving Paradigm

In a roving MMN paradigm, each stimulus type functions as both a standard and

deviant throughout the MMN sequence and is randomly repeated in blocks of differing train lengths. The first stimulus in each block functions as a deviant stimulus, due to the relative change in sound properties from the preceding block (Figure 2.2a). Rather than reverting back to the same standard stimulus, as is typical in an oddball paradigm, the roving design continues to repeat the 'deviant'. After two-to-three presentations, the deviant stimulus is processed as a new series of standards (Figure 2.2b). Therefore, the relative presentation order of a stimulus defines it as a deviant or standard (Figure 2.2c). MMN waveforms are calculated for each stimulus type separately, in order to control for sound properties. This is achieved by subtracting the averaged standard ERP from the averaged deviant ERP across stimuli with identical sound features (Figure 2.2c).

2.3 Mismatch Negativity Generators

MMN has traditionally been defined as an index of functioning in auditory cortical networks required for deviance detection. The most consistently reported generators of MMN include the temporal and frontal cortices [176, 180, 181]. Functional magnetic resonance imaging (fMRI) has shown increased activation in the superior temporal gyrus of the auditory cortex (AC) in response to deviant tones [182]. The AC is proposed to detect sound features and establish a memory trace to which incoming stimuli are compared [174]. Cerebral blood flow, EEG and scalp current density analyses have provided evidence for an additional frontal generator in the inferior frontal gyrus (for further review, see [183]), with evidence to suggest that duration deviants predominately activate the left inferior frontal

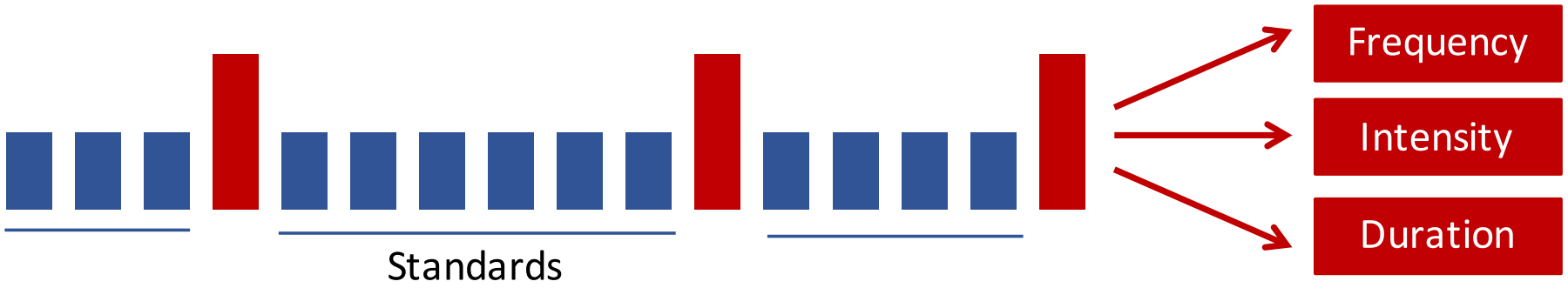

Figure 2.1. Mismatch negativity oddball paradigm. In an oddball mismatch negativity paradigm, a series of standard stimuli [blue] are presented at differing train lengths to establish a memory trace in auditory sensory memory. Deviant stimuli [red] (i.e. deviating from the standard in frequency, intensity or duration), are interspersed within the train of standards at *unexpected* time intervals.

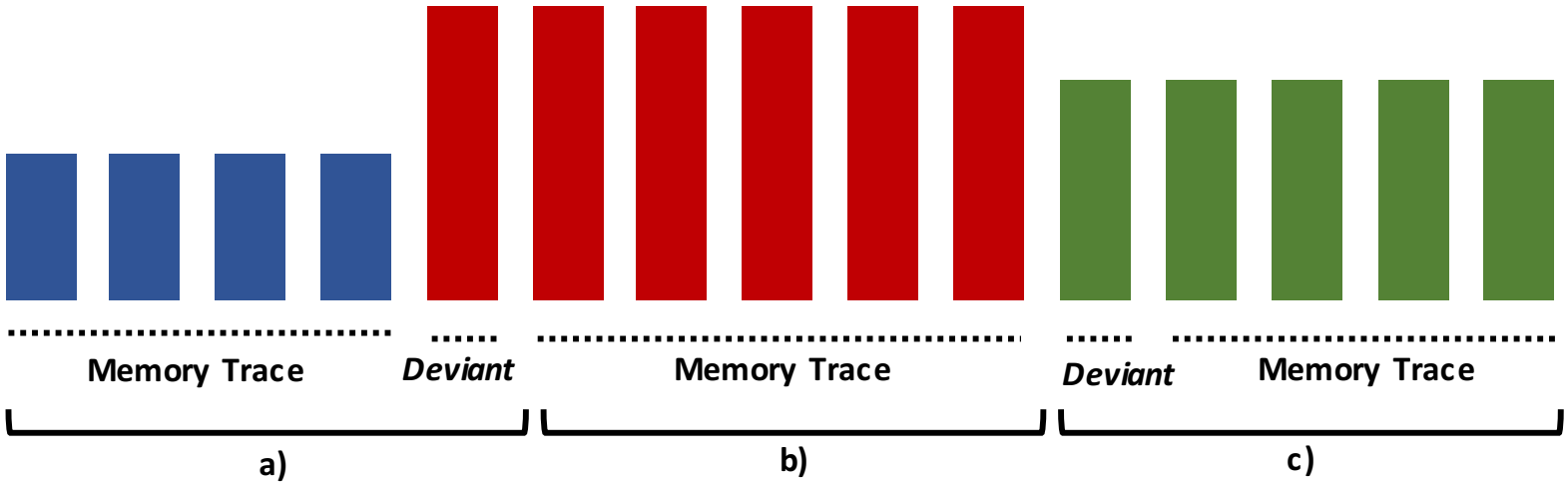

Figure 2.2. Mismatch negativity roving paradigm. In a roving mismatch negativity (MMN) paradigm, different stimuli are presented in blocks of varying train length: a) The first tone in the new block functions as a deviant (red deviant), due to the change in sound properties relative to the preceding memory trace (blue memory trace); b) The deviant tone is repeated in order to establish a new memory trace of identical sound features (red memory trace); c) The MMN difference waveform is calculated for each stimulus type separately, whereby the event-related potential (ERP) of the standards (for example, the average of all green memory traces) is subtracted from the ERP of corresponding deviant stimuli (for example, the average of all green deviants).

gyrus [184], while frequency deviants activate the right inferior frontal gyrus [182, 184]. Frontal activation is related to an involuntary switch in attention that is required for higher-level deviance detection when comparing a stimulus to an established memory trace [184-186].

2.4 Stimulus-Specific Adaptation

The generation of MMN is thought to result from a series of deviance detection processes occurring in both subcortical and cortical structures of the auditory pathway. Stimulus-specific adaptation (SSA) is a form of short-term plasticity in response to a repeated stimulus. When the same stimulus is repeated, such as standard tones in an oddball paradigm (see section 2.3.1), the neuronal activation in response to the stimulus is reduced. Following the presentation of a deviant stimulus, the firing rate of the same neurons are significantly increased, releasing the suppression of neuronal firing imposed on the repeated stimulus [187]. This finding supports the separation of the SSA response from an independent model of neuronal fatigue. SSA in response to deviant stimuli are well developed in regions of the midbrain [188], suggesting that SSA contributes proportionally to deviance detection processes generated higher in the auditory pathway, including MMN. While SSA contributes to deviance detection via bottom-up processing of sound features, corticofugal projections from the AC help to modulate the firing rate of neurons in midbrain structures [189] (corticofugal projections supporting SSA are discussed further in section 2.6).

2.5 Prediction Error Encoding

The magnitude of the MMN is dependent on the strength of the standard pattern in echoic memory, implicating an important role of synaptic plasticity in MMN generation. The detection of a deviant stimulus requires on-line modification of the established memory trace [190], such that the brain receives bottom–up thalamic inputs which inform current stimulus properties and allow the brain to adjust top–down predictions [191, 192]. A model of prediction error defines MMN as the difference between these thalamic inputs and NMDAr spike timing of a dependent synaptic plasticity discharge, which occurs when information is fed-back to predictive neurons. This model suggests reduced efficiency in having learnt the regularity of the predictive rule (the standard memory trace), or impairment in detecting or communicating the response to an unexpected deviant stimulus. In the latter case, MMN is considered a prediction error signal of the acoustic environment [193, 194].

2.6 Auditory Processing Hierarchy

Processing of auditory sound features begins in the cochlear nucleus. Neuronal signals ascend through the inferior colliculus (IC) to the medial geniculate body (MGB; part of the auditory thalamus). The cytoarchitecture of the MGB suggests that spectral and temporal properties are deconstructed, prior to being processed in core areas of the primary (AI) and secondary (AII) AC [195]. The frontal cortex tracks the violation of expected sounds by comparing change in stimulus features, generating low frequency activity in response to a prediction error [196].

The descending cortico-thalamic and cortico-collicular pathways assist in neuronal adaptation and deviance detection at the level of subcortical structures [189]. Descending projections to the MGB and IC primarily extend from layer V and VI of the AI [197-199]. The MGB has a high ratio (10:1) of corticofugal projections compared to the corresponding ascending pathways [200, 201]. These projections demonstrate strong stimulus-specific adaptation, indexing a gain control mechanism of the AC to MGB neurons. Corticofugal projections update sensory representation of sounds, via synaptic depression, which is then projected back to higher order cortical areas [202, 203]. Sounds features are then integrated in the belt and parabelt areas surrounding the AI and AII, before projecting to the frontal and parietal cortices for higher order processing required for deviance detection [195].

2.6.1 Frequency Sound Processing

Tonotopic maps are the spatial arrangement of different laminae or bands sensitive to differing sound frequencies. These maps ascend through the auditory pathway in a bottom-up fashion, allowing the processing of sound frequency to be communicated directly to corresponding bands in the next level of the ascending auditory pathway [204]. Projections extending from the central nucleus of the IC, to the ventral division of the medial geniculate body (MGBv) and AI, form the leminiscal pathway of the auditory system. Neurons in the leminiscal pathway are of short latency, sharp tuning curve, and have a consistent neuronal response to differing sound frequencies [205]. There is evidence to suggest this tonotopic

organisation in the leminiscal pathway also extends to belt areas of the AC [206-208].

2.6.2 Duration Sound Processing

Within the brainstem, neurons are non-selective to the duration of a sound, having a sustained response to the duration of all auditory stimuli. Within the IC, further along the ascending auditory pathway, duration-tuned neurons have a neurophysiological response characterised by specific stimulus durations. These duration-tuned neurons have also been reported in areas of the auditory thalamus and AC in mammals [195, 209]. The selective activation of duration-tuned neurons occurs via excitatory inputs corresponding to the onset and offset of a stimulus, while temporally offset inhibitory inputs supress the excitatory response occurring at neurons of non-corresponding durations [195, 210]. Projections from the IC to the medial (MGBm) and dorsal (MGBd) divisions of the MGB and belt areas of the AC are less tonotopically organised, forming the non-leminiscal pathway of the auditory system. In the MGBd, neurons are typically of broad tuning curve, while the MGBm appears to have both broad and narrow-tuned neurons to allow more accurate discrimination between different sound durations [205]. The leminiscal and non-leminiscal pathways are not mutually exclusive, rather, temporal and spectral processing occurs parallel to allow complex integration of differing sound properties. Corticofugal projections from the AC descend primarily to the non-leminiscal subcortical structures of the MGB and IC [211]. The processing of duration sound features requires the complex decomposition of the sound and

connectivity across a range of ascending and descending neuronal projections between subcortical and cortical regions.

2.7 Pharmacology of Mismatch Negativity

The pharmacological underpinnings of MMN have been investigated by modulating different neurotransmitter system functioning [212]. Auditory sensory memory, like other areas of working memory, involves a complex interaction of excitatory and inhibitory processes. The most robust and consistent pharmacological modulation of MMN has been demonstrated by altering NMDAr function (for further review, see [213, 214]). Minimal effects have been reported following modulation of dopaminergic [215], serotonergic [214, 216, 217] and gamma-aminobutyric acid (GABA) type A [218] receptor modulators. MMN appears heavily dependent on glutamatergic function, the main excitatory neurotransmitter system in the brain, and more specifically on excitatory pyramidal neurons [87].

2.7.1 Glutamate

Several studies have reported MMN reductions in humans following acute ketamine administration, an NMDAr antagonist, for both frequency and duration deviants [214, 219-222]. Two studies failed to replicate these findings: Oranje and colleagues [223] suggest that their negative findings may reflect low plasma level of ketamine at 158ng/ml, compared to 426ng/ml reported in an earlier positive study [221]; Roser and colleagues [224] also failed to find an effect of ketamine

and reported similar low plasma levels (133.8±58.2ng/ml) comparable to previous negative findings. Further support for a critical role of NMDAr function in generating MMN comes from administration of other NMDAr antagonists including Nitrous Oxide (N20) gas [199] and memantine [200]. At low doses, memantine increased MMN amplitude, potentially due to the ability of memantine to increase glycine affinity when administered at low doses [201].

Contrary to expectations that glycine would increase MMN amplitude, high-dose glycine reduced duration MMN amplitude in healthy controls [225]. In that study, participants were administered 0.8g/kg of glycine, the dose typically reported for clinical benefits in schizophrenia. The observed reduction in MMN amplitude may be indicative of a worsening in pre-attentive change detection in individuals with *intact* glutamatergic function prior to glycine administration. This interpretation is supported by animal models reporting cognitive impairment beyond optimal levels of synaptic glycine concentration [226]. These findings suggest a dose-dependent relationship between glycine and NMDAr function prior to glycine administration, whereby glycine may increase MMN amplitude in those with relatively low baseline NMDAr function, such as in schizophrenia, while reducing MMN amplitude in those with normal baseline functioning. However, the nature of this relationship has not been examined directly.

2.7.2 Dopamine

Modulating dopamine signalling has yielded a weak association with MMN generation. Studies in healthy controls have shown no effect of bromide, a

dopamine type-II (D_2) receptor agonists, or pergolide, a dopamine receptor type-I (D_1) and D_2 receptor agonist, on MMN amplitude [215, 227]. No changes in MMN were observed following growth hormone response to apomorphine (non-selective dopamine agonist) or clonidine (an ą$_2$ adrenergic agonist) [228], providing indirect evidence that MMN generation is not dependant of dopaminergic function. In addition, inhibiting the reuptake of dopamine and norepinephrine under methylphenidate did not affect MMN [229]. Of three studies that have investigated the effects of haloperidol, a dopamine D_2 receptor antagonist and antipsychotic treatment for schizophrenia (see section 1.5), two studies reported no effects on MMN amplitude [230, 231]. Only one study reported that haloperidol reduced MMN amplitude in healthy controls, in addition to increasing other selective and non-selective components of the ERP [232].

2.7.3 Cannabinoid

Altered synaptic plasticity in regular cannabis users [148, 233] is thought to occur, in part, due to cannabinoid type-1 (CB_1) receptor-mediated downregulation of NMDArs [147, 148]. An acute administration study of exogenous cannabinoids found Δ^9-Tetrahydrocannabinol (Δ^9-THC) did not affect MMN amplitude in healthy controls, while co-administration of Δ^9-THC and cannabidiol (CBD) increased MMN amplitude [234]. These effects of Δ^9-THC have shown to be mediated by the neuregulin 1 gene [235, 236], while in a separate study, the CB_1 receptor agonist rimonabant reduced MMN amplitude [237]. Roser and colleagues [237] reported no group differences between cannabis users and controls overall for duration or

frequency MMN, but long-term and heavy cannabis users showed smaller frequency MMN amplitude at frontal sites when compared to shorter-term and lighter cannabis users. Rentzsch and colleagues [238] reported attenuated frequency MMN amplitude in abstinent users and while no differences were found in that study between chronic cannabis users with and without schizophrenia, both patient groups had smaller MMN amplitude compared to controls.

Interestingly, group differences in each of these studies were primarily highlighted for the MMN component elicited by a frequency deviant; although both studies included a duration deviant condition, neither study reported any group differences for duration MMN. Pesa and colleagues [239] reported an altered pattern of duration MMN in first-episode psychosis patients who used cannabis, relative to patient nonusers. In that study, increased quantity and frequency of recent cannabis use was associated with smaller duration MMN amplitude. More recently (and since the publication of our findings in chapter four), Impey and colleagues [240] reported reduced duration MMN amplitude in nicotine naïve cannabis users. Together, these findings suggest that longer periods of heavy cannabis use may reduce MMN amplitude. Given the prevalence of cannabis use in patients within schizophrenia [133] and the effects of the endocannabinoid system in regulating NMDAr function [217], further clarification of the relationship between current regular cannabis use and MMN is required (see section 1.7.2 for a review of endocannabinoid-mediated regulation of NMDAr function).

2.8 Mismatch Negativity in Schizophrenia

Reduced MMN amplitude in schizophrenia is a robust phenotype, with a meta-analysis of studies reporting a large mean effect size (Cohen's *d*>1) [241]. An earlier meta-analysis separately reported a large effect size for duration MMN (*d*=1.01) and a medium effect size for frequency MMN (*d*=0.47) [242]. Smaller MMN amplitude has been associated with impaired daily functioning [243] and cognitive deficits [244]. Todd and colleagues [212] propose that MMN is more likely to index stable features of schizophrenia, however the relationship between MMN and discrete clinical symptoms or functional outcomes has not been consistently reported (for further review, see [89]). Clarifying the nature of the relationship between MMN, NMDAr function and discrete phenotypes in schizophrenia, may inform mechanisms of core clinical features and changes in neurobiological function underlying clinical treatment efficacy.

As is the case in MMN more broadly, the degree of MMN generation in schizophrenia is influenced by stimulus features, such as the degree of perceptual discrimination between standards and deviants and the predictability of a deviant presented within a standard memory trace [245]. Greater differentiation between a deviant stimulus and the memory trace typically elicits a larger MMN response [174, 192]. What is thought to be deficient in schizophrenia is the ability to produce larger MMN amplitudes with increasing stimulus deviance, as the MMN amplitude appears to plateau earlier compared to controls along a continuum of increasing stimulus discrepancy [246]. Therefore, greater differences between patients and controls become more prominent with increasing differences in

stimulus features.

Reduced MMN to duration deviants is a robust finding in patients with early onset schizophrenia and has been shown to be impaired in the prodromal phase of illness and those at risk of developing psychosis [247-249]. Longitudinal evidence provides further support for MMN as a translational biomarker, with smaller MMN amplitudes predicting conversion to schizophrenia [247, 248]. In contrast, attenuated frequency MMN amplitude has been associated primarily with chronic schizophrenia illness [250]. These findings of frequency MMN deficits are thought to relate to the tonotopic organisation of the AC and alterations in plasticity with disease progression [250-253]. Smaller MMN generation in schizophrenia is unlikely due solely to the generation of MMN in the frontal cortex, as MEG studies have also demonstrated deficient MMN generation in patients and this measure is insensitive to frontal cortical activation [254]. While differences for duration versus frequency MMN do not offer clear discrimination of neurotransmitter functioning involved in the stage or severity of illness [255], MMN overall appears sensitive to changes in NMDA neurotransmitter function throughout the disorder.

2.8.1 Antipsychotic Medication

The use of antipsychotic medications does not significantly impact MMN amplitude in schizophrenia patients. Both clozapine and haloperidol have thus far failed to consistently modulate MMN amplitude, while clozapine consistently increased P300 in the same studies [256-258]. Once MMN deficits are observed,

they tend to persistent despite ongoing antipsychotic medication. This is contrary to the pattern of sensory gating deficits, which are more reliant on dopaminergic function and typically resolve following reduced psychotic symptoms treated with antipsychotics [259]. These findings indicate that MMN is related to neurochemical imbalances independent of dopaminergic functioning and may be a useful tool for examining pharmacological underpinnings of the disorder in relation to glutamatergic models [260].

2.8.2 Glutamatergic Treatments

A 60-day trial of N-acetyl-cysteine (NAC), a glutathione precursor, significantly increased MMN in schizophrenia, without affecting the P300 component. It is unclear in this study whether the effect of increased MMN amplitude is from cysteine properties enhancing NMDAr function, or other mechanisms such as redox-sensitive transcription factors (or both) [261]. Magnetic resonance spectroscopy findings have reported that smaller duration MMN amplitude is associated with reduced glutathione levels in the posterior medial prefrontal cortex and further associated with increased negative symptoms in schizophrenia [262]. Using structural equation modelling, MMN has also been reported as an intermediary biomarker between glutamate dysfunction and verbal learning memory deficits in patients [263]. This study found smaller duration MMN was associated with reduced glutamate, GABA and glutamate-to-glutamine ratios in the medial prefrontal and anterior cingulate regions in schizophrenia. Together, these findings support the role of NMDAr hypofunction in the generation of

smaller MMN amplitudes in schizophrenia and support the utility of MMN as a biomarker to index change in NMDAr function following treatment administration.

2.9 Chapter Summary

MMN may be a useful biomarker of functional target engagement to help clarify the relationship between NMDAr-mediated treatments and therapeutic efficacy in schizophrenia. However, few studies have investigated the pharmacology of altered MMN in schizophrenia directly, particularly following administration of NMDAr-mediated treatments. Findings from Leung and colleagues [225] suggest that high-doses of glycine (0.8g/kg) may reduce (rather than increase) MMN in those with *intact* baseline NMDAr function, supporting the presence of an Inverted-U dose-response relationship between synaptic glycine concentration and cognitive performance in humans. Determining the nature of this relationship is particularly important due to the heterogeneity of NMDAr dysfunction within schizophrenia [53-55].

Given the regulatory mechanism of endocannabinoids on NMDAr activation via pre- and post-synaptic mechanisms (see section 1.7.2), it is reasonable to assume that cannabis exposure reduces MMN following regular use, and may prevent the efficacy of increased synaptic glycine concentration to improve NMDAr function. Early findings suggest that cannabis does not alter the observed MMN deficit in schizophrenia [238], while prolonged and heavier cannabis use in healthy controls [237], and more frequent and heavier use in first episode psychosis [239], has been associated with smaller MMN amplitude. It may

be that in healthy controls the effects of cannabis use are more pronounced, while a floor effect of reduced MMN in patients restricts observation of further impairment in MMN following exposure to cannabis. Such findings may confound the utility of MMN to index neurobiological function related to the pathophysiology of schizophrenia and may modulate the neuronal response to NMDAr-mediated treatments.

Chapter Three

Outline of the Current Thesis

3.1 Literature Summary

Increasing synaptic glycine concentration may assist in treating core refractory symptoms in schizophrenia. However, inconsistent reports of therapeutic efficacy have raised doubt as to the benefit of such treatments. Endocannabinoid-mediated alterations in NMDAr excitability may modulate neuronal functioning in target pathways of NMDAr-mediated treatments. Cannabinoid receptor type-I (CB_1) agonism, following repeated cannabis use, may lead to increased inhibition of NMDArs and potentially reduce the clinical efficacy of increasing synaptic glycine concentration in schizophrenia. Peripheral markers, such as plasma glycine or Δ^9-Tetrahydrocannabinol (Δ^9-THC) concentrations, are limited in informing neuronal functioning within the brain. Utilising biomarkers that index neurotransmitter functioning in brain networks involved in the pathophysiology of schizophrenia may help clarify the neurobiological relationship between neural target engagement and therapeutic efficacy following pharmaceutical intervention.

The primary auditory pathway is one neurobiological system which may inform changes in neuronal functioning following administration of NMDAr-mediated treatments. Specifically, MMN may inform the neuronal integrity of NMDArs following increased synaptic glycine concentration in schizophrenia. Few studies have examined the direct pharmacological modulation of MMN using NMDAr agonists in patients, while early evidence suggests that the relationship between synaptic glycine concentration and NMDAr function is characterised by an Inverted-U dose-response relationship. Therefore, the therapeutic efficacy of

glycine-mediated treatments may depend on NMDAr function prior to treatment administration. MMN may be a useful biomarker to stratify neurobiological dysfunction of NMDArs in schizophrenia and index mechanisms of improved clinical symptoms following neuronal target engagement of NMDAr-mediated treatments.

3.2 Thesis Aims

The aim of the current thesis is to determine the nature of the relationship between MMN and NMDAr function, in order to inform the utility of MMN as a biomarker to stratify NMDAr dysfunction and index neuronal target engagement of NMDAr-mediated treatments in schizophrenia. To achieve this aim, we investigated the relationship between MMN and altered NMDAr function in three independent studies, following: prolonged periods of regular cannabis use in otherwise healthy individuals; acute and chronic administration of glycine in schizophrenia patients; and a glycine dose-dependence trial in healthy controls. There was no participant overlap between studies described in this thesis.

In *chapter four* we investigate the effects of regular cannabis use on MMN by comparing current regular cannabis users against a sample of healthy age- and gender-matched nonuser controls. Given the role of the endocannabinoid system in regulating NMDAr function and glutamate release within the brain, it is important to know whether regular cannabis use alters neuronal functioning in target pathways of novel NMDAr-mediated treatments. Based on models of NMDAr-mediated structural and cognitive dysfunction following repeated

exposure to exogenous cannabinoids, we hypothesise that individuals with prolonged and heavy cannabis use will have smaller MMN amplitudes compared to matched controls.

In *chapter five* we investigate whether an acute dose and repeated administration of glycine increases MMN amplitude in schizophrenia patients. Further, we investigate whether baseline and changes (from baseline to post-glycine) in MMN amplitude are associated with change in clinical symptoms following 6-weeks of adjunct glycine treatment. Given inconsistent reporting of therapeutic benefits following NMDAr-mediated treatments in schizophrenia, there is need to establish biomarkers, such as MMN, to index neuronal target engagement and further inform the mechanisms of therapeutic efficacy. We hypothesise that acute glycine (0.2g/kg) and adjunct glycine treatment (0.6g/kg/day; 6-weeks) will increase MMN amplitude in schizophrenia. We also hypothesise that smaller baseline MMN amplitude (indicating poorer NMDAr functioning) will be associated with greater improvements in clinical symptoms following adjunct glycine treatment.

In *chapter six* we investigate the dose-response relationship between glycine and MMN in an independent sample of healthy controls. Following the outcomes of *chapter five* and further evidence suggesting glycine may only exert therapeutic benefits within an optimal range of synaptic glycine concentration, we compare the effects of placebo and three different glycine doses (0.2g/kg; 0.4g/kg; 0.8g/kg) on MMN generation. We hypothesise an Inverted-U dose-response relationship between increasing glycine dose and MMN amplitude. In *chapter seven* the

findings of previous chapters are summarised and discussed, as well as their limitations and directions for future research.

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Chapter Four

Chronic Effects of Cannabis Use on the Auditory Mismatch Negativity

Running Head: Cannabis and MMN

Greenwood L-M, Broyd SJ, Croft RJ, Todd J, Michie P, Johnstone S, Murray R, Solowij N. (2013). Chronic effects of cannabis use on the auditory mismatch negativity. *Biological Psychiatry*, *75*(6), 449-458.

DOI: 10.1016/j.biopsych.2013.05.035.

Chapter Five

Acute and Chronic Effects of Glycine on Auditory Mismatch Negativity in Chronic Schizophrenia

Running Head: Schizophrenia and MMN

Greenwood L-M, Leung S, Michie PT, Green A, Nathan PJ, Fitzgerald P, Johnston P, Solowij N, Kulkarni J, Croft RJ. (2018). The effects of adjunct glycine treatment on auditory mismatch negativity in schizophrenia. *Schizophrenia Research, 191*, 61-69.

DOI: 10.1016/j.schres.2017.05.031.

Chapter Six

Dose-Response Relationship between Glycine and Mismatch Negativity in Healthy Controls

Running Head: Glycine Dose-Response

Greenwood L-M, Michie, P, Leung S, Nathan P, Fitzgerald P, Johnston P, Solowij N, Croft RJ. (2018). Dose-response relationship between glycine and mismatch negativity in healthy controls. *In Preparation*.

Chapter Seven

Summary and Discussion

7.1 Scope of the Thesis

The aim of the current thesis was to determine the nature of the relationship between mismatch negativity (MMN) and alterations in *N*-methyl-D-aspartate receptor (NMDAr) function. Understanding this relationship is important as it informs the utility of MMN to stratify NMDAr dysfunction in schizophrenia and to index functional target engagement following NMDAr-mediated treatments. In order to achieve this overarching aim of the thesis, three independent empirical studies were performed: The first study (chapter four) aimed to determine whether MMN is smaller in regular cannabis users compared to controls; the second study (chapter five) aimed to determine the effects of acute and repeated glycine administration on MMN in chronic schizophrenia patients and its relation to treatment outcomes; the third study (chapter six) aimed to determine the nature of the dose-response relationship between glycine and MMN in healthy controls.

The empirical studies in this thesis were supported by two separately funded research schemes. Chapter four, examining MMN in cannabis users, was part of a project scheme investigating vulnerability markers in the association between cannabis use and schizophrenia. Chapters five and six, examining the effects of glycine in schizophrenia and in healthy controls, were part of a scheme investigating the efficacy of glycine as a therapeutic adjunct treatment in chronic schizophrenia patients. While these studies were part of a broader series of projects, they are complementary in clarifying the relationship between NMDAr function and MMN generation.

7.2 Summary of Findings

In chapter four, we examined duration and frequency MMN processing in a sample of forty-two regular cannabis users, compared to forty-four age- and gender-matched non-user controls. Within this sample, we then examined MMN in shorter- and longer-term cannabis users relative to their matched control counterparts. Frequency MMN amplitude was smaller in the overall sample of cannabis users, with this finding evident in both short- and long-term user groups. Smaller duration MMN amplitude was more pronounced in long-term users compared to controls and shorter-term users, and was associated with more prolonged and heavier cannabis use, particularly daily use, across the entire sample of regular users.

In chapter five, we examined the effects of acute glycine administration (0.2g/kg) and chronic glycine treatment (increased to 0.6g/kg) as an adjunct to ongoing antipsychotic medication in chronic schizophrenia. In a sample of twenty-two schizophrenia (or schizoaffective disorder) out-patients we compared the effects of glycine to that of placebo, utilising a randomised, double-blind, between-groups design. In this study, duration MMN amplitude at baseline was smaller in schizophrenia compared to age- and gender-matched controls. Acute administration of glycine increased duration MMN amplitude compared to placebo, while no between-group differences in MMN were found after 6-weeks of repeated glycine administration. Smaller duration MMN amplitude at baseline was associated with greater negative symptoms assessed using the Positive and Negative Syndrome Scale (PANSS) and predicted a trend-level improvement in

negative symptoms following 6-weeks of adjunct glycine treatment (improvement defined as significantly reduced symptom scores from baseline to post-6-week treatment). No changes in negative symptoms were found following placebo.

In chapter six, we examined the dose-response relationship between glycine and MMN in an independent sample of twenty healthy controls. In this study, we report a quadratic relationship between increasing glycine dose and change in MMN amplitude, providing evidence for an Inverted-U dose-response relationship following acute glycine administration. High-dose glycine (0.8g/kg) reduced MMN amplitude compared to low-dose (0.2g/kg) and medium-dose (0.4g/kg), while low-dose glycine increased MMN amplitude (at trend level) compared to placebo. Further, baseline MMN amplitude was linearly associated changes in MMN (from baseline to post-drug) following glycine administration, whereby larger baseline amplitudes were associated with reduced MMN and smaller baseline amplitudes were associated with increased MMN.

7.3 General Discussion

This thesis reports findings of smaller MMN amplitudes in regular cannabis users without psychiatric history, suggesting that regular use alters the sensitivity of MMN to index NMDAr functioning directly related to the pathophysiology of core schizophrenia phenotypes. Although the effects of regular cannabis use were not examined in schizophrenia patients, chapter four aimed to determine the nature of the relationship between regular cannabis exposure and MMN in otherwise healthy subjects. These findings were used to infer the effects of regular cannabis

use in altering endocannabinoid-mediated regulation of NMDAr excitability. Smaller MMN amplitudes in regular cannabis users (chapter four) support previous studies reporting cannabinoid type-I (CB_1) receptor-mediated inhibition of NMDArs [156-158]. These findings suggest that prolonged cannabis use disrupts CB_1-NMDAr regulatory mechanisms, thereby reducing NMDAr activation in cortical and subcortical networks important for MMN generation.

In terms of clarifying the nature of the relationship between cannabis use and MMN, this thesis replicated previous findings of smaller frequency MMN amplitude [237] in a larger sample of heavier (average of 15.6 versus 8.8 joints per week) and more protracted (average 9.6 versus 3.0 years of regular use) cannabis users. Roser and colleagues [237] reported smaller frequency MMN amplitude in a sub-group of heavier and longer-term users, and a linear association between smaller frequency MMN amplitude and longer durations of cannabis use. Contrary to these findings, we did not report any relationship between frequency MMN and the duration or quantity of cannabis use. Instead, the development of frequency MMN deficits may be a less sensitive index of cumulative exposure to cannabis in our sample of heavier and more protracted users. These findings support the view that heavy and prolonged cannabis use results in pathophysiological and functional brain changes similar to the robust pattern of smaller frequency MMN amplitudes reported in chronic schizophrenia patients [337].

Findings of smaller frequency MMN amplitude in regular cannabis users in this thesis are based on a cross-sectional design and do not offer direct causal evidence that repeated cannabis exposure reduces MMN amplitude. It may be,

for example, that cannabis users in chapter four had reduced frequency MMN amplitudes prior to the onset of use and that the results instead reflect a vulnerability to use cannabis. There is yet to be a longitudinal investigation to determine whether cannabis users, without history of psychosis, transition from normal frequency MMN (relative to non-users) prior to the onset of use, to smaller MMN amplitudes following prolonged exposure to cannabis. However, this interpretation is less likely given that Roser and colleagues [237] report longer durations of cannabis exposure with smaller frequency MMN amplitudes, suggesting MMN deficits develop with ongoing use. Rather than smaller frequency MMN amplitude indexing a pre-onset vulnerability to use cannabis, it is more likely that findings in chapter four index the impairing effects of prolonged cannabis exposure on frequency MMN processing.

Smaller frequency MMN amplitudes may index increased gyrification of the tonotopic organisation of the auditory cortex (AC) following repeated exposure to exogenous cannabinoids. This view is supported by previous findings of abnormal gyrification and cortical thinning in cannabis users, who were of similar age and duration of regular cannabis use to participants in chapter four [306]. Frequency MMN deficits (and to a lesser extent, duration MMN) in chronic schizophrenia patients has shown to be correlated with grey matter loss in the auditory and frontal cortices [286]. These findings are consistent with a model of increased age-related fractional anisotropy [367], cognitive decline [24] and altered synaptic plasticity [250-253], with increasing disease progression in schizophrenia patients. Following this, smaller frequency MMN amplitude in

cannabis users in this thesis may index the effects of repeated cannabis exposure in down-regulating CB_1 receptors, resulting in NMDAr-mediated abnormal gyrification of the auditory cortex, similar to that reported in chronic schizophrenia (for example, see [286]).

Smaller duration MMN amplitudes may index functional impairment that is more sensitive to the degree of cannabis exposure following prolonged periods of use. The pattern of smaller duration MMN amplitude in regular users in this thesis (chapter four) has since been replicated in a sample of tobacco-naïve regular cannabis users [240]. Exogenous cannabinoids disrupt experience-dependent alterations in neuronal excitation [322] and synaptic integration across brain regions [325], which are necessary for neuronal plasticity and deviance detection. In regular cannabis users, ongoing exposure may lead to NMDAr-mediated alterations in cortico-thalamo networks in non-leminiscal pathways of the auditory system, which are required for duration MMN processing [195, 210, 246, 313]. Smaller duration MMN amplitudes in this thesis (chapter four) were found in the long-term user subgroup only, with smaller amplitudes being associated with longer periods of daily (and regular) use in the overall sample of regular cannabis users. These findings are consistent with patterns of smaller duration MMN amplitude being associated with increased duration and frequency of cannabis use in schizophrenia patients [215]. Further, they support the view that smaller MMN amplitudes in response to duration deviants is more profound following protracted and heavier patterns of cannabis use.

The nature of duration MMN deficits in regular cannabis users (chapter

four) is contrary to the pattern of findings reported in schizophrenia. In chapter four, smaller duration MMN amplitudes were associated with more protracted cannabis use, while smaller duration MMN amplitudes in schizophrenia are primarily found in individuals at risk for developing psychosis or early in the prodrome [247, 249, 284]. It is unlikely that smaller duration MMN amplitude in cannabis users in this thesis (chapter four) index a premorbid psychosis vulnerability, as users had no history of psychosis despite their protracted use, and instead are likely to represent a sample of cannabis users without existing vulnerability. Differences in the pattern of duration MMN between long-term cannabis users and schizophrenia suggest a common functional deficit with different underlying neuropathology. Smaller duration MMN amplitudes have been shown to index lower thalamic glutamate plus glutamine levels in the prodromal stages of the illness [53], while our finding of smaller duration MMN amplitude in chronic schizophrenia patients (chapter five) was associated with greater negative symptoms, which is also thought to be mediated by hypofunctional NMDArs [54]. Together, these findings suggest MMN is a sensitive index of NMDAr hypofunction throughout the course of illness and further clarification is required to determine differences in underlying mechanisms of reduced MMN amplitude (as an index of NMDAr function), as well as their relation to clinical symptoms, during different stages of the disorder.

Smaller duration MMN amplitude in long-term cannabis users (chapter four) was associated with increased psychotic-like symptoms while intoxicated, suggesting that ongoing endocannabinoid-mediated alterations in NMDAr

function leads to downstream effects in neurotransmitter pathways such as dopamine. These findings are consistent with the glutamatergic hypothesis outlining a preliminary NMDAr hypofunction which leads to excessive dopamine release in the mesolimbic pathway [53-55]. CB_1 receptors have been show to mediate the inhibition of glutamate release at excitatory neurons in the ventral tegmental area [368]. Findings of acute intoxication symptoms in this thesis (chapter four) are consistent with previous reports of smaller duration MMN amplitude being associated with increased psychotic symptoms in non-clinical individuals [369] and NMDAr antagonist models that induce psychotic symptoms in individuals without psychiatric history [51, 56]. Although cannabis users in chapter four did not develop psychosis (discussed above), these findings suggest a common neurochemical mechanism for which long-term cannabis use might lead to schizophrenia-like changes in the brain, particularly those associated with conversion to psychosis [178, 248].

In determining the neurochemistry associated with smaller MMN amplitudes in schizophrenia, findings in chapter five confirm that acute glycine administration increases MMN amplitude in chronic patients. This suggests that glycine crosses the blood-brain barrier to increase NMDAr neurotransmission and supports the view that NMDAr hypofunction underlies robust MMN deficits previously reported in schizophrenia [241]. The same low-dose glycine administered in healthy controls (chapter six) favoured a trend towards increased MMN amplitude, but this difference was not significant in the overall sample when compared to placebo. A likely explanation for these reported differences is that

some individuals in chapter six had no, or little, benefit from receiving glycine. Although these studies are not directly comparable, together they support the view that increasing synaptic glycine concentration is more beneficial in the context of remediation, whereby those with lower baseline NMDAr function (indexed by MMN), such as in schizophrenia, benefit from increased excitatory neurotransmission following glycine administration.

MMN may be useful in stratifying neurobiological function as a predictor of treatment response. The reported linear associations between baseline and changes in MMN amplitude following low- and high-dose glycine (chapter six) are consistent with the view that baseline NMDAr function mediates the effect of glycine. Almost all participants in chapter six had reduced MMN amplitudes following high-dose glycine, while findings were mixed (increases versus decreases) following low-dose. It is possible this linear relationship indexes a dose-dependent effect of glycine, mediated by baseline levels of NMDAr functioning prior to treatment administration. However, caution is required when interpreting this linear relationship due to potential bias towards the mean. It may be that individuals with more extreme MMN amplitude values at baseline regress towards the mean for post-glycine measurements, thereby biasing the change score. Further research is required to confirm the effects of baseline NMDAr function in mediating functional outcomes following neuronal target engagement of glycine.

Findings in chapter six confirm the nature of the relationship between glycine and MMN is that of an Inverted-U dose-response curve. High-dose glycine reduced MMN compared to small- and medium-doses, suggesting reduced

efficacy of high-dose glycine to increase NMDAr neurotransmission in healthy controls. This pattern is consistent with previous studies reporting decreased cognitive performance [226, 365] and reduced NMDAr currents [342] at higher synaptic glycine concentrations. Impaired pre-pulse inhibition, which is thought to be mediated by dopaminergic function, has also been reported following higher doses of glycine in patients with chronic schizophrenia [225, 359]. Contrary to previous reports [225], high-dose glycine in chapter six did not reduce MMN compared to placebo, suggesting that the high-dose condition did not 'impair' MMN generation in this study, but did reduced NMDAr function (indexed by smaller MMN amplitudes) when compared to low- and medium-doses. Further clarification of the functional significance of smaller MMN amplitude following higher (compared to low and medium) doses of glycine is needed in order to inform the potential risks of exceeding optimal synaptic glycine concentrations within a therapeutic context.

Previous reports on the efficacy of increasing synaptic glycine concentration in schizophrenia have been inconsistent, with supporting evidence coming primarily from smaller independent trials [117, 122, 123] and mixed findings reported in recent Phase-II versus Phase-III clinical trials [128]. Since the publication of chapter five, two phase-III multi-centre trials of bitopertin were reported in a cumulative sample of 1199 schizophrenia patients [370]. Following 24-weeks of treatment, bitopertin improved negative symptoms but did not show superior efficacy when compared to placebo. Contrary to these findings, glycine improved negative symptoms compared to placebo in this thesis, suggesting the

need for continued investigation into the conditions for optimal treatment efficacy. The sample of schizophrenia outpatients in chapter five were relatively low on positive symptoms compared to previous studies [128] and may index a more homogenous sample in relation to symptom profiles, particularly compared to larger trials. Inconsistencies across studies suggest NMDAr-mediated treatments may be beneficial in a subgroup of patients; however, categorising patients based on broad diagnostic features does not appear to adequately dissociate individuals who may benefit from increasing synaptic glycine concentrations. Further to this, Beck and colleagues [2] raise concerns of secondary negative symptoms inflating a placebo effect following NMDAr-mediated treatments, particularly in chronic patients. Utilising a placebo and treatment-as-usual or waitlist group may help to further clarify the efficacy of NMDAr-mediated treatments in schizophrenia.

Clinical findings in schizophrenia patients in this thesis (chapter five) report improved PANSS-Total, PANSS-Negative and PANSS-General symptoms, and favoured a trend towards improved depressive symptoms on the Calgary Depression Rating Scale (CDRS) following 6-weeks of glycine treatment. These findings support previous studies reporting improved symptoms following glycine [115-117, 121-123], D-Serine [118, 119] and NAC [120] in schizophrenia. While a full dissociation between primary and secondary negative symptoms is not feasible in this study design, scores on the CDRS and PANSS-General symptom scales were not associated with baseline or changes in MMN amplitude following treatment. These findings provide indirect support for an independent association

of primary and secondary negative symptoms within a model of NMDAr hypofunction, but confirmatory research is needed to verify this dissociation. Following glycine treatment, there were no changes in functional impairment as assessed by the Work and Social Adjustment Scale, suggesting that changes in negative symptoms were not secondary to change in social functioning in this thesis. It may be that longer trials of NMDAr agents are required to facilitate more gross functional changes that are secondary to negative symptom improvement. The current findings support the need to investigate the nature of more global improvements in schizophrenia following increased glutamatergic function.

In determining the relationship between MMN and clinical symptoms in schizophrenia in this thesis, smaller baseline MMN amplitude was found to be associated with greater severity in negative symptoms (chapter five). MMN has previously been associated with illness duration and premorbid, cognitive and psychosocial functioning [243, 284, 371], and in some studies with improved clinical symptoms [257, 339]. This finding is in line with the glutamatergic hypothesis and pathophysiological model of NMDAr hypofunction [53-55] involved in the generation of negative symptoms. In further support of this view, baseline duration MMN amplitudes predicted (at trend level) the degree of improved negative symptoms following 6-weeks of adjunct glycine treatment (chapter six). Together, these findings support the use of MMN as an index of NMDAr deficit severity related to the pathophysiology of negative symptoms and suggest the need to stratify patients based on neurobiological dysfunction in order to achieve optimal treatment efficacy.

Findings of an Inverted-U relationship between glycine dose and MMN (chapter six) suggest there may be an optimal window for clinical benefits following NMDAr-mediated treatments. The state of NMDAr hypofunction [54] and reduced synaptic glycine concentrations [357] in schizophrenia may allow greater margin for increasing synaptic concentrations, before reaching saturation. This view suggests that the optimal dose to increase NMDAr function is higher in schizophrenia compared to controls. However, given the heterogeneity of NMDAr function within schizophrenia, these findings also suggest that higher doses may lead to more-varied treatment outcomes; higher doses may be beneficial in restoring NMDAr hypofunction in patients with low NMDAr neurotransmission, while the same dose administered in patients with relatively normal NMDAr functioning may lead to saturation of glycine at the synapse. If this is the case, only a subset of patients would be expected to benefit from higher doses of NMDAr-mediated treatments. This view is further supported by findings of smaller duration MMN amplitudes predicting greater improvements in negative symptoms in schizophrenia patients (chapter five), whereby those with smaller MMN amplitudes experienced greater clinical benefits in negative symptoms following glycine treatment.

MMN appeared sensitive to alterations in NMDAr function following acute glycine administration in schizophrenia (chapter five) and healthy controls (chapter six) in this thesis, however no differences in MMN were found following 6-weeks of glycine treatment. This finding suggests that the efficacy of glycine to increase glutamatergic function did not extend to long-term plasticity changes in

auditory cortical networks involved in MMN generation, as was inferred from findings in regular cannabis users in chapter five. An alternative explanation is that changes in duration MMN amplitude are a more sensitive index to state changes in NMDAr function, while improved clinical symptoms index the cumulative effects of glycine. It is noteworthy that glycine was not administered on the day of 6-week follow-up testing in this thesis. It is possible that although acute glycine administration altered NMDAr function, repeated administration of adjunct glycine treatment may not lead to long-term plasticity changes over this time period. If so, this would mean that the therapeutic benefits reported in chapter five are due to cumulative exposure or possibly secondary effects on other neurochemical systems, rather than long-term changes to the NMDAr system itself. Consistent with this interpretation, altered duration MMN processing was associated with more prolonged and heavier periods of cannabis use in chapter five, whereby group differences in duration MMN amplitude were reported for the long-term user group only.

7.4 Limitations and Future Direction

Progressive decline in MMN amplitude, particularly in earlier stages of cannabis use, may overlap with models of advanced age-related decline reported in schizophrenia (for example, see [250]). Findings in regular cannabis users in this thesis are based on a cross-section design and are limited in offering direct causal evidence between repeated cannabis exposure and reduced MMN amplitude. Future studies could profitably utilise longitudinal methods to investigate the

biological mechanisms of repeated exposure to exogenous cannabinoids on progressive structural alterations contributing to MMN deficits reported in regular cannabis users. Given emerging evidence that increased cannabis dependence severity is associated with dopaminergic dysregulation [372] in neuronal pathways implicated in the pathophysiology of schizophrenia, future studies could control for changing severity of cannabis dependence. These findings may inform underlying mechanisms of positive and negative symptoms, and similarities in NMDAr-mediated structural and functional alterations between schizophrenia and regular cannabis users.

The concentrations of exogenous cannabinoids in cannabis plant matter used by participants in this thesis were not measured or controlled. Smaller MMN amplitudes in regular cannabis users (chapter four) suggest an 'impairing' effect of cannabis on NMDAr function in otherwise healthy individuals. Increasing the concentration of CBD in cannabis plant matter may reduce these 'impairing' effects. The purported therapeutic benefits of CBD for schizophrenia may increase NMDAr function in patients, as was demonstrated in chapter five following low-dose glycine (indexed by changed in MMN). It was beyond the scope of the current thesis to examine the therapeutic efficacy of cannabis containing higher concentrations of CBD. Therefore, the direction of cannabis effects reported here should be interpreted with caution, particularly within the context of CBD as an alternative treatment for psychotic-related disorders.

This thesis (chapter five) failed to replicate smaller frequency MMN amplitude in chronic schizophrenia patients and was therefore unable to

generalise the effects of glycine across a broader framework of MMN deviance detection. Changes in experience-dependent plasticity has been demonstrated for frequency discrimination [350], whereby reorganisation of neuronal populations increase sensitivity to relevant stimuli and generate additional neuronal responses to trained frequencies. The malleability of this structural organisation is likely susceptible to pharmacological modulation, as was inferred from findings in regular cannabis users in chapter four. Future studies should investigate the effects of NMDAr-mediated treatments on frequency MMN processing, as it may be a more sensitive index (compared to duration MMN) of the cumulative effects following increased NMDAr function. Such findings may inform the differential patterns of each MMN deviant type throughout the chronicity of schizophrenia and the differential patterns of frequency versus duration MMN processing which were observed following regular cannabis use in this thesis.

The low number of positive symptoms endorsed by schizophrenia outpatients in chapter five may not adequately index the potential therapeutic benefits of increasing synaptic glycine concentrations in treating this symptom domain. Research is required to map narrowly defined symptoms within schizophrenia. Determining the relationship between MMN as a marker of NMDAr function and its relationship with narrowly defined symptoms, particularly negative symptom sub-domains, may inform the transdiagnostic utility of MMN to index treatment-specific targets, rather than relying on broader diagnostic categorisation (which may be too broad to be relevant). Neurocomputational models may better inform the mechanism of discrete symptom clusters, including

their primary and secondary nature, and facilitate greater accuracy in guiding early treatment interventions.

Evidence of an Inverted-U dose-response relationship between glycine and MMN amplitude in this thesis is based on single-dose administration. The effects of long-term repeated dosing of glycine is unclear and may alter the efficacy of glycine to increase NMDAr function following repeated administration. It would thus be important to clarify whether the acute Inverted-U dose-response relationship between glycine and MMN remains when administered repeatedly in a model of treatment efficacy. Further, the nature of the dose-response relationship between glycine and MMN in this thesis was not directly examined in schizophrenia. It is unclear whether the Inverted-U curve is present in schizophrenia patients, or whether there is a linear increase in MMN amplitude following increased glycine dose. Replicating the nature of the Inverted-U relationship in schizophrenia may better inform optimal treatment doses to increase NMDAr hypofunction in schizophrenia and further inform the heterogeneity of treatment outcomes at higher glycine doses.

A methodological limitation in our examination of the glycine dose-response effect in healthy controls (chapter six), is comparability to previous findings [225]. Our use of a change variable to examine the effects of glycine (change from baseline to post-drug administration) aimed to control for potential differences in baseline MMN amplitude across treatment sessions. Following high-dose glycine, we report reduced MMN amplitudes in individuals with higher baseline MMN. However, the use of a change score may have created a floor-type

effect in the low baseline group, while the longer testing period, including baseline and post-drug measures, may have created a regression towards the mean, reducing the sensitivity of our measure to index glycine-mediated changes in MMN. Future studies should investigate the significance of reduced MMN relative to other cognitive performance measures sensitive to functional outcomes in schizophrenia.

While this thesis informs the nature of the relationship between NMDAr function and MMN amplitude, these findings are limited in informing the mechanisms underlying changes in MMN generation. Utilising MRI brain structural analysis to support current source density mapping may be a useful way to examine the effects of pharmacological intervention and inform the mechanisms for different deviant types. A combined spectral decomposition analysis of the MMN waveform may inform the independent contributions of cortico-cortico and cortico-thalamic networks in altering MMN generation, particularly following increased synaptic glycine concentration. These methods may aid further understanding of the independent and overlapping pathways involved in frequency versus duration MMN processing, and inform differential findings reported throughout the chronicity of schizophrenia and following prolonged periods of cannabis use, as well as their sensitivity in predicting treatment outcomes.

A limitation of glycine and other NMDAr agonists, such as D-serine, is the variability they introduce from metabolic processes and the large doses required to cross the blood-brain barrier. It may be useful to replicate the MMN findings in

this thesis following administration of GT1-RIs, such as bitopertin and sarcosine. These treatments increase synaptic glycine concentrations by blocking the reuptake of glycine in the synapse. Determining whether GT1-RIs have a similar effect to that of glycine will provide important information as to the mechanism of their effect. It may be, for example, that GT1-RIs require sufficient endogenous glycine to be efficacious, and that lower endogenous glycine levels may limit their ability to improve NMDAr function. Such information would be important for tailoring effective treatments for the heterogeneity present in schizophrenia.

While this thesis aimed to determine the nature of the relationship between MMN and NMDAr function, it is limited in informing the mechanisms of change in neuronal functioning. This thesis concludes that altering NMDAr function may result in different neuronal functional outcomes and that such variabilities in neuronal response is likely involved in the heterogeneity of clinical treatment efficacy in schizophrenia following NMDAr-mediated treatments. However, the findings used to infer this relationship, including regular cannabis use and the dose-response effects of glycine, were not directly examined in schizophrenia patients. The nature of the relationship between MMN, regular cannabis use and glycine dose-dependence may differ within the context of NMDAr hypofunction in schizophrenia patients. Therefore, further studies are required to confirm the stability of these MMN findings in schizophrenia, in order to understand their implications for indexing neuronal target engagement and clinical efficacy following NMDAr-mediated treatments.

7.5 Conclusion

This thesis demonstrates that MMN is a sensitive biomarker to index the neurobiological state of NMDAr function. Chapter four provides indirect evidence of endocannabinoid-mediated alterations of NMDArs in auditory cortical networks important for MMN generation. Acute administration of glycine in chapter five increased MMN in schizophrenia patients and the same pattern was observed (at trend level) in chapter six following low-dose glycine administered in healthy controls. An Inverted-U dose-response relationship between glycine and MMN suggests there is an optimal therapeutic window for glycine to increase NMDAr function, beyond which treatment efficacy is reduced. Together, these findings indicate that changes in MMN index alterations in NMDAr function, which may arise from pre or post-synaptic mechanisms. Further, they support the utility of MMN to index changes in NMDAr excitability following neuronal target engagement of NMDAr-mediated treatments. Indexing change in NMDAr function following treatment may identify subgroups of patients who will (and won't) benefit from increasing synaptic glycine concentrations.

The efficacy of glycine to increase NMDAr function in this thesis appeared greater in the context of remediation, whereby increasing synaptic glycine concentration improved MMN in models of NMDAr hypofunction. Smaller duration and frequency MMN amplitudes were associated with intoxicated psychotic-like symptoms in long-term regular cannabis users, while smaller duration MMN amplitude in schizophrenia was associated with greater severity of negative symptoms and predicted negative symptom improvement (trend-level)

following glycine treatment. These findings suggest that MMN is a useful index to stratify core phenotypes based on biological dysfunction, rather than broad diagnostic criteria. It would be useful for future studies to replicate findings of an Inverted-U dose-response relationship for MMN following administration of glycine-reuptake inhibitors, such as bitopertin. Clarifying this relationship would assist in tailoring effective treatment and further inform the heterogeneity of clinical treatment response in schizophrenia.

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