BRAIN DYSCONNECTIVITY AS A BIOMARKER OF TREATMENT RESISTANCE IN SCHIZOPHRENIA

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“The environment is somehow different – not to a gross degree – perception is unaltered in itself but there is some change which envelops everything with a subtle, pervasive and strangely uncertain light.” – Karl Jaspers
ABSTRACT

BACKGROUND
Pharmacological intervention is the most effective means for controlling the symptoms of schizophrenia but is ineffective in a proportion of individuals. This thesis attempted to identifying biomarkers of treatment resistance in people with schizophrenia, using structural and functional magnetic resonance imaging (MRI).

METHODS
Two cross-sectional studies were included in this thesis: the TRS study (including first-line responders (FLR), individuals with treatment-resistant schizophrenia (TRS), individuals with ultra-treatment-resistant schizophrenia (UTRS) and healthy controls) and the CloRes study (including FLR and those who were eligible for clozapine). A prospective arm of the CloRes study included clozapine-eligible participants who were later diagnosed with TRS or UTRS following a three-month trial of clozapine. Functional connectivity patterns were compared across groups in both studies using independent components analysis. Structural connectivity in FLR and those who were clozapine-eligible was assessed using diffusion tensor imaging (DTI) and probabilistic tractography. Graph theory was used to examine network organisation and communication in participants from the TRS study. As a final experiment, we investigated whether structural and functional connectivity could predict response to clozapine using the NeuCube spiking neural network algorithm.

RESULTS
ICA revealed increased functional connectivity within the language network of individuals with UTRS compared with healthy controls, specifically in the left paracingulate gyrus. In addition, graph theoretical analysis revealed large disruptions in modularity, connection strength and network organisation in people with schizophrenia, particularly those with UTRS. Those who were clozapine-eligible showed increased connectivity between areas of the sensorimotor network and the precuneus compared with FLR. A structural connectivity analysis identified white matter abnormalities in the corpus callosum and respective branching tracts in those who were eligible for clozapine compared with FLR. The NeuCube algorithm achieved 74.9% accuracy for classification of TRS study data.

CONCLUSIONS
These data provide evidence to support a role for both structural and functional dysconnection in the resistance to antipsychotics. Dysconnectivity was greatest in those with UTRS but also present in FLR and those who were eligible for clozapine. The nature of dysconnection varied between response subtypes, suggesting that the mechanisms responsible for dysconnectivity may dictate an individual’s susceptibility to the effects of antipsychotics.
DEDICATION

For my participants, my parents and my sister.
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<td>5-HT</td>
<td>Serotonin</td>
</tr>
<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
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<tr>
<td>AD</td>
<td>Axial diffusivity</td>
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<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>ASSIST</td>
<td>Alcohol, Smoking and Substance Involvement Screening Test</td>
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<td>$B_0$</td>
<td>Direction of the main magnetic field</td>
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<td>$b_0$</td>
<td>Non diffusion-weighted image</td>
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<td>Blood oxygen-level dependent</td>
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<td>Ben's spiker algorithm</td>
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<td>Centre for Advanced MRI</td>
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<tr>
<td>CloRes study</td>
<td>Clozapine Response study</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>DAN</td>
<td>Dorsal attention network</td>
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<td>DLPFC</td>
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<td>Default mode network</td>
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<td>DSM</td>
<td>Diagnostic and Statistical Manual of Mental Disorders</td>
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<tr>
<td>DTI</td>
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<td>Fractional anisotropy</td>
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<td>$^1$H-MRS</td>
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<td>NMV</td>
<td>Net magnetisation vector</td>
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<tr>
<td>Abbreviation</td>
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<tr>
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<td>PANSS</td>
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<td>Spiking neural network</td>
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<td>SNNc</td>
<td>Spiking neural network cube</td>
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<td>SS-EPI</td>
<td>Single-shot echo-planar imaging</td>
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<td>Spike-time dependent plasticity</td>
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<td>Support vector machine</td>
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<td>TE</td>
<td>Echo time</td>
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<td>THC</td>
<td>Tetrahydrocannabinol</td>
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<td>TI</td>
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<td>Repetition time</td>
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Chapter 4 - Functional network connectivity as a biomarker of treatment response in schizophrenia

| Nature of contribution by PhD candidate | Carolyn McNabb (with assistance from Bruce Russell) was responsible for seeking collaborations for assistance with connectomics analysis. She performed all graph-theory-based analysis and network-based statistics, in addition to interpretation of results. All chapter content was written by Carolyn McNabb. |
| Extent of contribution by PhD candidate (%) | 89% |

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<td>Dr Roger Tait (University of Cambridge) was responsible for pre-processing of fMRI data and creation of wavelet correlation matrices for use in further analysis.</td>
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The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate’s contribution to this work, and the nature of the contribution of each of the co-authors; and
- that the candidate wrote all or the majority of the text.

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**Chapter 6 - Personalised, predictive modelling of treatment outcome using a spiking neural network-based approach**

Content regarding the integration of directional information into the NeuCube was adapted from:

Integrating Space, Time and Direction in Spiking Neural Networks: A Case Study on Multimodal Brain Data Modelling (currently unpublished) - Neelava Sengupta, Carolyn McIabb, Bruce Russell, Nikolai Kasabov

| Nature of contribution by PhD candidate | Carolyn McIabb sought assistance from engineers at the Knowledge Engineering and Discovery research institute to aid with predictive modelling of discrete response using MRI data. She made contributions to the creation of the algorithm used in this study during discussions with Neelava Sengupta to ensure that the model utilised meaningful data. She was responsible for pre-processing all MRI and DTI data and for interpretation of findings. Carolyn designed and implemented the CloRea study including ethics applications, participant recruitment, study visits and data processing. All content within the chapter was written by Carolyn McIabb, with the assistance of some figures and explanations modified from a paper written in conjunction with Neelava Sengupta (see above). |
| Extent of contribution by PhD candidate (%) | 70% |

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<td>Neelava Sengupta (PhD Candidate, Auckland University of Technology) created algorithms for incorporation of directional information from DTI and time-series data from MRI for use in the NeuCube. Discussions were held with Carolyn McIabb to ensure that the most relevant and meaningful data were incorporated into algorithms. He was also responsible for running the algorithm on pre-processed MRI data to obtain prediction accuracy rates. Neelava Sengupta has checked the introduction and method for correctness and provided some figures for inclusion in the method.</td>
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- the above statement correctly reflects the nature and extent of the PhD candidate’s contribution to this work, and the nature of the contribution of each of the co-authors; and

- that the candidate wrote all or the majority of the text.

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Last updated: 19 October 2015
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Chapter 1

1. INTRODUCTION TO SCHIZOPHRENIA, ANTIPSYCHOTICS AND BIOMARKERS

In 2013, the Global Burden of Disease Study estimated that 23 million people, worldwide, were living with schizophrenia (1). Accounting for less than 0.01% of the total population (though McGrath et al. previously reported prevalence rates of between 0.3% and 0.7% (2)), schizophrenia was a leading cause of years lived with disability and accounted for 3.7% of the global burden of disease (1). In a systematic review, the annual national cost of schizophrenia was estimated to range between USD 92 million and USD 102 billion, with indirect costs contributing to between 50% and 85% of the total expenditure (3).

A leading cause of disability in individuals aged between 15 and 44 years (4), schizophrenia most commonly emerges during adolescence or early adulthood (5). The disorder is characterised by relapsing episodes of delusions, hallucinations and diminished emotional expression, as well as persistent cognitive dysfunction and a number of other disruptive symptoms that impair an individual’s ability to participate normally in society and severely degrade their quality of life (5). Pharmacological intervention is the most effective means for controlling the symptoms associated with schizophrenia and is effective in the majority of instances (6). However, there exists a small proportion of individuals who remain symptomatic despite best-practice. Estimates range from as few as 5% to as many as 60% of the population affected with schizophrenia are resistant to first-line treatment (7-12). Accounting for some of the highest rates of hospitalisation and impaired functioning in mental health (13, 14), resistance to first-line antipsychotic therapy is estimated to require USD 34 billion per annum in direct healthcare costs in the US alone (15). The atypical antipsychotic clozapine is effective for treating between 30% and 70% of individuals who fail first-line treatment, relieving them of their most debilitating symptoms (7, 16-19). However, the serious and potentially life-threatening side-effects associated with clozapine limit its use in the clinic. No current method exists for identifying who will respond to treatment with clozapine, or for identifying who will fail to respond to first-line therapy. This thesis attempts to address these issues by searching for markers of treatment response and resistance in people with schizophrenia, using structural and functional magnetic resonance imaging (MRI).

1.1. THESIS OUTLINE

As an introduction to the following chapters, this section will first discuss the categorisation of schizophrenia according to how the disorder responds to pharmacological intervention, in
addition to current theories on the aetiology of schizophrenia and heterogeneity of treatment response that are relevant to this thesis. The use of biomarkers for response prediction will then be addressed, accompanied by the hypotheses and objectives of this thesis. Lastly, this chapter will introduce the two studies included in this thesis, providing a brief outline of each. For an introduction to the MRI techniques used in these studies, the reader is directed to chapter 2, which gives detailed descriptions of the physics and applications of MR technology for use in brain research; more experienced readers may disregard this chapter.

This thesis contains data from two cross-sectional studies, both conducted to investigate structural and functional biomarkers of treatment resistance in schizophrenia. The Treatment Resistant Schizophrenia (TRS) study included participants from four cohorts, including healthy controls, first-line responders (FLR), those with treatment-resistant schizophrenia (TRS) and those with ultra-treatment-resistant schizophrenia (UTRS). The Clozapine Response (CloRes) study included FLR and those who were eligible for clozapine. A prospective arm of the CloRes study included clozapine-eligible participants who were diagnosed with TRS or UTRS after a three-month trial of clozapine.

Chapter 3 first briefly introduces resting-state functional MRI (rs-fMRI) and describes various functional abnormalities identified in those with schizophrenia. A case for the use of rs-fMRI for identifying differences in connectivity between individuals of different response subtypes is then made. The remainder of chapter 3 describes the methods and results of independent components analyses (ICA), used to compared functional connectivity patterns across response groups in both studies. Discussions, implications and limitations are provided after the results section of each study.

In chapter 4, graph theory-based analytical techniques were used to examine the consequences of functional dysconnectivity on network organisation and communication and to determine whether these outcomes could be further utilised to distinguish responders from non-responders to treatment. The chapter first introduces important concepts of graph theory, including measures for both anatomical connectomes and functional connectivity. Findings from graph theoretical analysis in individuals with schizophrenia are then discussed, followed by a rationale for using graph theory to identify differences between response subtypes of the disorder. The chapter then describes methods and results of an investigation of connection strength, diversity, modularity and network connectivity in individuals from the TRS study cohort. Discussions, implications and limitations follow.
Chapter 5 consists of a structural connectivity analysis, comparing white matter connectivity between cohorts from the CloRes study. The chapter first describes the likely cause of white matter disruption in schizophrenia, as well as findings from studies comparing individuals with schizophrenia and healthy controls. The rationale for investigating white matter connectivity as a biomarker for treatment response is discussed, followed by methods and results of an investigation comparing fractional anisotropy (FA), radial diffusivity (RD), axial diffusivity (AD) and probabilistic tracts between FLR and those who are eligible for clozapine. A discussion of the results, probable implications and limitations follow.

As a final experiment, chapter 6 examines whether structural and functional connectivity can predict response to clozapine in clozapine-eligible individuals. The chapter opens with a discussion of machine learning techniques and their suitability for predicting treatment outcomes in psychiatric populations. It then introduces spiking neural networks and the novel architecture of the NeuCube spiking neural network. A description of the method used to create a predictive model of clozapine response, as well as the results of this model follow. Lastly, a discussion of the results and limitations is included.

This thesis concludes with an overall discussion of the studies reported in previous chapters and highlights possible implications, limitations and directions for future research.

1.2. DIAGNOSIS AND CLASSIFICATION OF SCHIZOPHRENIA

1.2.1. CURRENT DIAGNOSTIC CRITERIA

Diagnostically, schizophrenia is heterogeneous, permitting two individuals with distinct, non-overlapping symptoms to receive the same diagnosis (5). The Diagnostic and Statistical Manual of Mental Disorders – version V (DSM-5) of the American Psychiatric Association requires that a diagnosis of schizophrenia be made if an individual presents with delusions, hallucinations or disorganised speech, either in combination with each other or with grossly disorganised or catatonic behaviour, diminished emotional expression or avolition (5). As such, no one symptom is pathognomonic in schizophrenia. The heterogeneity of the disorder has led some authors to suggest that schizophrenia constitutes multiple disorders, which they refer to as the schizophreniaes (20). Separating this polythetic disorder into biologically meaningful subtypes has proven difficult. Until recently, schizophrenia was classified according to clinical features, with subtypes including simple, hebephrenic, catatonic, paranoid, undifferentiated, schizoaffective, childhood and chronic (21). However, the low stability, poor inter-rater reliability, minimal clinical utility and limited validity of these subtypes (22, 23) has led to their exclusion in the most
recent version of the DSM (24). A similar revision has been proposed for the International Classification of Diseases (ICD-11) (25).

1.2.2. SYMPTOM DOMAINS IN LIEU OF CLASSIFICATION
Although the removal of schizophrenia subtypes in the latest DSM is well supported, a primary criticism of the current diagnostic criteria is that it still holds little biological validity (26). There is a paucity of evidence in support of a unitary pathophysiological process resulting in the manifestation of schizophrenia and research into the underpinnings of the disorder should reflect this (26). Recently, a dimensional approach to clinical assessment was proposed (27). This approach sees individuals evaluated in eight domains, including hallucinations, delusions, disorganised thought, disorganised/abnormal motor behaviour, negative symptoms, cognition, depression and mania (24, 27). These domains were proposed in lieu of the former subtypes; advocates reasoning that schizophrenia is best treated when clinicians attend to the meaningful variation in symptoms, the severity of which may predict other important aspects of the disorder (26, 27). However, others have argued that there is insufficient evidence to suggest that a dimensional approach to diagnosis is superior or even equal to previous categorical nosology (28). Further arguments assert that, even in the absence of a unitary pathophysiological process, schizophrenia may still derive from a common pathway and that evaluating severity in individual domains would neither increase diagnostic validity nor inform treatment efforts (24). Hence, a more biologically relevant means for classifying individuals with schizophrenia is required (29).

1.2.3. CLINICAL STAGING AS A CLASSIFICATION SCHEME
The success of clinical staging used to define the extent of disease progression in other medical conditions led McGorry and colleagues to propose a similar strategy for the classification of psychotic disorders (30, 31). This staging model proposes that severe mental disorders, such as schizophrenia and severe mood disorders, develop from initial nonspecific symptoms associated with a background of specific and nonspecific risk factors (31). Worsening of symptoms and acquisition of new symptoms is proposed to accompany progressive neurobiological abnormalities that eventually result in a clear-cut mental disorder (31). Further worsening of symptoms and illness progression is possible, but not inevitable, and may result in frequent relapses or chronic impairment (31). The proposed model classifies individuals into one of six stages, from which they may progress if symptoms worsen or persist; the most severe stage relates to individuals with established schizophrenia, reflecting severe, persistent and unremitting symptoms (31). This model holds merit in some clinical settings; however, as schizophrenia is classified as a single (ultimate) stage in this model, individuals already diagnosed with the disorder may benefit little from such a paradigm.
1.2.3. TREATMENT RESPONSE AS A CLASSIFICATION SCHEME

In practice, clinicians observe three distinct subtypes of schizophrenia that are equally diverse in phenotype and currently impossible to distinguish at the onset of the disorder. These subtypes reflect the responsiveness of psychotic symptoms to treatment with antipsychotic agents, and include responsive, resistant and ultra-resistant forms (32). Upon commencement of treatment with a typical or atypical (non-clozapine) antipsychotic, between 60% and 80% of individuals with schizophrenia experience a positive clinical response (33, 34), only a small proportion of which will achieve complete remission (the reduction of symptoms to a level that no longer interferes with psychosocial functioning) (35). Although some authors estimate figures to be greater or smaller than those reported above, the general consensus is that clinical response to antipsychotics is varied and uncertain (33). In individuals who fail to respond to a first trial of antipsychotic treatment, response rates to a second non-clozapine antipsychotic are markedly lower (34). Agid et al. observed that only 16% of individuals experiencing a suboptimal response to an initial trial of olanzapine or risperidone achieved a response when their treatment was switched (34). Remarkably however, switching individuals who fail two trials of antipsychotics to the atypical antipsychotic clozapine results in a distinctly higher response rate (7, 16-19). Agid et al. reported that of those individuals who agreed to a trial of clozapine, 75% experienced a clinical response (34). There then remains a group of individuals who have failed to respond to first-line antipsychotics as well as clozapine (those with UTRS); at present, there is no recommended treatment strategy for this cohort (36, 37).

Building on the clinical staging hypothesis of McGorry et al. (30, 31), and centred on the observation that schizophrenia can be divided into responsive and non-responsive subtypes, Farooq et al. recently proposed that schizophrenia be classified according to treatment response (29, 38). Based on the patterns of response discussed above, these authors recommended subtypes of ‘antipsychotic responsive’, ‘clozapine responsive’ and ‘clozapine resistant’ (29), which correspond to ‘first-line responders’ (FLR), those with ‘treatment-resistant-schizophrenia’ (TRS) and those with ‘ultra-treatment-resistant schizophrenia’ (UTRS), respectively, in the current thesis. Despite concerns that ‘TRS’ is ambiguous (may be interpreted as ‘those who are eligible for clozapine’ as well as ‘those who are responsive to clozapine’ (38)), the term is becoming increasingly utilised in the context of clozapine response. As such, in this thesis, the term ‘TRS’ will be used to refer to those individuals who have failed two trials of first-line therapy yet responded to a trial of clozapine. Individuals who have failed two trials of first-line therapy but have not had a trial of clozapine will be referred to hereafter as ‘clozapine-eligible’. Clozapine eligibility in the current thesis is defined as failure to achieve full remission of positive
or negative symptoms or satisfactory clinical improvement despite at least two trials with different antipsychotic drugs (one of which must be an atypical antipsychotic), each at the recommended dosage for a treatment period of at least six-to-eight weeks (39-41).

Evidence suggests that treatment-resistance can occur from the outset, with early lack of response consistently predicting poor treatment outcome and clozapine eligibility (34, 42, 43). Shifting the focus of research to investigate underlying differences between response subtypes in schizophrenia should provide a clearer view of the disorder, addressing at least some of its heterogeneity.

1.3. HYPOTHESES OF SCHIZOPHRENIA AND THE ROLE OF ANTIPSYCHOTIC DRUGS – TARGETS, MECHANISMS AND RESPONSE

1.3.1. THE DOPAMINE HYPOTHESIS

Without exception, all currently licenced antipsychotic drugs, whether through antagonism, inverse agonism or partial agonism, block dopamine D_{2} receptors (44). Together with research demonstrating that amphetamine- and methylphenidate-mediated increases in extracellular dopamine can induce psychotic symptoms, this observation led to the initial “dopamine hypothesis” of schizophrenia (45-48).

The dopamine hypothesis has since seen two major revisions (45, 49). In the first, Davis et al. proposed that schizophrenia is characterised by frontal hypodopaminergia that results in striatal hyperdopaminergia; frontal hypodopaminergia was proposed to account for the negative symptoms of schizophrenia and striatal hyperdopaminergia for the positive symptoms of the disorder (49). Much of the evidence for this updated hypothesis was derived from animal and lesion studies, with no direct evidence for diminished dopamine levels in the frontal cortex and limited evidence for elevated striatal dopamine function (45).

Since that time, elevated presynaptic striatal dopamine has become the most widely replicated brain dopaminergic abnormality in schizophrenia (50). Modest (10%-20%) elevations in striatal D_{2/3} receptor density (51-53) as well as increased baseline occupancy of D_{2} receptors by dopamine (54) (corresponding to increased levels of dopamine in the synapse) have also been identified. Dysfunction of D_{1} receptors, which mediate dopaminergic transmission in the prefrontal cortex, has since been linked to cognitive impairment and negative symptoms in schizophrenia (55); however, Howes and Kapur argued in their 2009 revision of the dopamine hypothesis (the second major revision) that the evidence for hypofrontality in schizophrenia was weak (45). At that time, three studies had investigated the relationship between D_{1} receptor
levels and cognitive or negative symptoms in unmedicated individuals with schizophrenia and had found opposing results (56-58). Howes and Kapur lent particular emphasis to the study by Abi-Dargham et al., which reported increased binding of the D₁ receptor ligand [¹¹C]NNC ((+)-5-(7-Benzofuranyl)-8-chloro-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine) in the dorsolateral prefrontal cortex (DLPFC) of unmedicated individuals with schizophrenia that directly correlated with poor performance in a working memory task (56). Abi-Dargham and colleagues attributed this finding to an upregulation of D₁ receptors in the DLPFC, secondary to sustained deficiency in mesocortical dopamine function and subsequent low levels of dopamine in the prefrontal cortex (56). More recently, Slifstein et al. reported generalised blunting of dopamine release in most extrastriatal regions, including the DLPFC, following administration of amphetamine, which correlated with working memory-related activation in this region (59). Gurvich et al. have since found an association between the D₁ receptor gene DRD1 and negative schizotypy in a non-clinical population, supporting a role for D₁ dysfunction in the negative symptoms of schizophrenia (60).

A lack of consistent evidence with respect to frontal dopaminergic dysfunction in schizophrenia at the time led Howes and Kapur to omit this component from their revised hypothesis, which instead proposed a link between dopamine dysregulation and “psychosis”, rather than schizophrenia (45). Other key features of the revised dopamine hypothesis were: a series of “hits”, including genetic and environmental risk factors, resulting in a final common pathway of dopamine dysregulation; primary dopamine dysfunction at the presynaptic control level as opposed to the D₂ receptor level; and a dopamine-induced adjusted appraisal of stimuli, mediated through a process of aberrant salience (45). Within this context, increased levels of dopamine in the striatum, which normally accompany learning of reward or avoidance, become chronic (61). Whereas dopamine normally mediates the process of salience, the stimulus-independent, chronically elevated levels of dopamine seen in psychosis usurp the normally contextually driven attribution of salience and lead to a misattribution of significance to external objects and internal representations (61, 62). Thereby, dopamine becomes a creator of salience (61). Consequently, individuals with psychosis attribute inappropriate salience to non-salient stimuli, resulting in a misinterpretation of significance to normally mundane objects and experiences (61-63). Antipsychotics, especially D₂ receptor antagonists, are proposed to improve symptom severity in schizophrenia by reducing dopamine-mediated aberrant salience (63).
Given recent findings about the dysregulation of dopamine in the frontal cortex, the dopamine hypothesis may be revised yet again to accommodate the potential effects of dopamine on negative and cognitive symptoms.

1.3.2. Clozapine’s unique pharmacological profile

The unique effectiveness of clozapine in TRS, in accessory to its broad pharmacological binding profile, confers a role for non-dopaminergic neurotransmission in the aetiology of treatment-resistance. Initially, clozapine’s superiority over traditional antipsychotic drugs was credited to its actions at serotonin (5-HT) receptors, specifically, its greater affinity for the 5-HT2A receptor in contrast to the D2 receptor (64-66). Based on this hypothesis, a multitude of antipsychotics were developed to mimic the high 5-HT2A:low D2 receptor binding properties of clozapine (65, 67). These antipsychotics were termed ‘atypical’, branding all antipsychotics that came before them (i.e. those with equal affinity for D2 and 5-HT2A receptors) ‘typical’. In early human work and animal studies, atypical antipsychotics provided additional benefits over typical antipsychotics, most notably with respect to their lower extrapyramidal side effects, but also because they were thought to improve negative and cognitive symptoms (68-70). Large-scale randomised studies have refuted claims that atypical antipsychotics provide reliable improvements over typical antipsychotics for negative and cognitive symptoms (71-73); however, Meltzer calls attention to substantial flaws in their study designs that may invalidate such conclusions (68). Regardless, no typical or atypical antipsychotic to date has rivalled clozapine’s efficacy for TRS (17, 37), indicating that the drug’s superior efficacy profile is not attributable to its differing properties at D2 and 5-HT2A receptors alone.

Notwithstanding its actions at D2 and 5-HT2A receptors, clozapine possesses affinity for a plethora of other targets (74). These include additional dopamine and 5-HT receptors (D1, D3, D4, 5-HT1B/D, 5-HT2B, 5-HT2C, 5-HT1E, 5-HT3, 5-HT6, 5-HT7), adrenoceptors (α1, α2A, α2B, α2C), muscarinic acetylcholine receptors (M1, M2, M3, M4), the noradrenaline transporter (NET), serotonin transporter (SERT) and the histamine H1 receptor (74). Other targets may also exist (74). Many atypical antipsychotics share affinity for some of these receptors (67), yet none have demonstrated comparable efficacy to clozapine for TRS (despite some reports that olanzapine, clozapine’s closest structural relative, may provide benefits for some individuals) (17, 37). As such, it is yet unclear which of these targets account for clozapine’s success.

1.3.3. The glutamate (NMDA receptor hypofunction) hypothesis

Central to Farooq et al.’s proposed classification scheme (29) is the concept that individuals with schizophrenia who respond differently to treatment with antipsychotics do so due to differences
in the underlying pathophysiology of their disorder (32). This is supported by early work demonstrating that responders and non-responders to the typical antipsychotic haloperidol exhibit comparable indices of antipsychotic uptake and binding (75), implying that the underlying cause of differential response is pharmacodynamic. This is not a novel concept; thus far however, few studies have sought to investigate structural, functional or neurochemical differences between FLR, those with TRS and those with UTRS (32).

A key discovery to date has been the differential activation of dopaminergic and glutamatergic systems between FLR and those who are eligible for clozapine (76-78). Employing a combination of positron emission tomography (PET) and proton magnetic resonance spectroscopy (1H-MRS), Demjaha et al. established that striatal dopamine synthesis capacity was elevated in FLR but not in those who were clozapine-eligible, and that glutamate levels in the anterior cingulate were elevated in clozapine-eligible individuals but not FLR (76). These cross-sectional findings were validated in a prospective study by Szulc et al., who demonstrated that off-treatment ratios of Glx/Cr (reflecting a combination of glutamate, glutamine and y-amino butyric acid (GABA) scaled to creatine) were higher in individuals who subsequently failed to respond to antipsychotic treatment compared to those who did respond (79). These findings lend support to the hypothesis that schizophrenia develops from abnormal dopaminergic signalling in FLR but is more likely attributable to other mechanisms, possibly glutamatergic abnormalities, in those who are clozapine-eligible (44, 80, 81). Glutamatergic dysfunction has long been implicated in the pathophysiology of schizophrenia (82-84), and its specific involvement in resistance to first-line antipsychotics may account for at least some of the variation observed in this disorder.

The glutamate hypothesis arose from observations that NMDA (N-methyl-d-aspartate) receptor antagonists such as phencyclidine and ketamine, which inhibit glutamate-mediated calcium (Ca2+) influx, induce psychotic, negative and cognitive symptoms that closely resemble those seen in schizophrenia (83, 84). The intensity with which these drugs induce psychotomimetic effects directly reflects their potency at NMDA receptors (84), and although several versions of the glutamate hypothesis in schizophrenia have been proposed, the prevailing hypothesis is for NMDA receptor hypofunction (85).

Contrary to early reports that individuals with schizophrenia exhibit reduced levels of glutamate in the cerebrospinal fluid (CSF), it has been increasingly demonstrated that hyper- rather than hypo-glutamatergic function is responsible for the symptoms of schizophrenia (86). This is purported to be mediated via the activation of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and is supported by work demonstrating that co-
administration of AMPA receptor antagonists can block the neurotoxic effects of NMDA receptor antagonists in rats (87). Hyperglutamatergia associated with NMDA receptor antagonism may result from disinhibition of pyramidal neurons (88). Homayoun and Moghaddam demonstrated that antagonism of NMDA receptors predominantly decreased the activity of GABAergic interneurons, shortly after which the firing rate of pyramidal neurons increased (88). This may be attributable to lower voltage thresholds of interneurons compared with pyramidal cells (89). Consequently, voltage-dependent NMDA receptor antagonists may bind to receptors on GABAergic interneurons at lower concentrations than those required to bind to receptors on glutamatergic cells, resulting in the inhibition of GABAergic transmission and subsequent disinhibition of glutamatergic signalling (86).

Whether a similar mechanism is responsible for the increased glutamate levels observed in the frontal cortex of individuals with schizophrenia remains to be seen. Evidence to date points toward aberrant glutamate receptor localisation, possibly driven by abnormal NMDA receptor trafficking (90-92). Abnormalities in genes coding for NMDA receptor signalling (specifically GRIN2A and SRR) in people with schizophrenia support this claim (93). If these aberrations refer specifically to NMDA receptors on GABAergic interneurons, then a similar mechanism to that observed with NMDA receptor antagonists is feasible.

Although NMDA receptors are primarily localised to postsynaptic dendritic terminals, they also occupy presynaptic terminals, as well as growth cones of oligodendrocytes (94). Activation of NMDA receptor signalling is crucial for the differentiation of and myelination by oligodendrocytes, however excessive signalling can trigger excitotoxicity (95-97). NMDA receptor hypofunction could therefore be accountable for the well-replicated white matter abnormalities observed in schizophrenia, either through disinhibition and subsequent excitotoxicity or via under-stimulation, and poor successive differentiation, of oligodendritic precursors.

Consistent with the dopamine hypothesis of schizophrenia, the NMDA receptor hypofunction hypothesis converges on a final common pathway of dopamine hyperactivity (44). Via disinhibition of pyramidal neurons, increased glutamate signalling is thought to increase activation of midbrain dopaminergic neurons, leading to psychotic symptoms (44). However, this integrated hypothesis fails to account for clozapine-eligible individuals who are resistant to dopaminergic interventions (44). The development of negative and cognitive symptoms is also poorly accounted for.
The development of glutamatergic interventions for the treatment of schizophrenia has met with limited success. Although agents such as minocycline, bitopertin and NMDA receptor modulators have demonstrated initial efficacy in small clinical trials, none have progressed to a clinical setting (44). This may be attributable to poor participant selection, given that recent studies have identified glutamate abnormalities in only a select group of individuals with schizophrenia (76, 78). Importantly, augmentation of clozapine with lamotrigine (an inhibitor of glutamate release) has been shown to demonstrate efficacy in individuals with UTRS (98). Clozapine is also known to possess glutamatergic properties (99), which may account for a portion of the drug’s effectiveness when taken by those who fail treatment with antipsychotic agents. More work is needed to determine who might benefit from treatment with glutamatergic drugs and establish systems for identifying these individuals at first contact.

1.3.4. THE DYSCONNECTION HYPOTHESIS
NMDA receptors are also implicated in the dysconnection (or dysconnectivity) hypothesis of schizophrenia, which ascribes the structural, functional and phenotypic characteristics of the disorder to abnormal NMDA receptor-mediated synaptic plasticity (100). Similar to the NMDA receptor hypofunction hypothesis of schizophrenia, the dysconnection hypothesis asserts that synaptic dysfunction arises from irregular trafficking, phosphorylation or subunit expression of NMDA receptors (100). However, whereas aberrant NMDA receptor function is the primary malefactor in the NMDA receptor hypofunction hypothesis, the dysconnection hypothesis attributes abnormal NMDA receptor function to the modulatory properties of dopamine, serotonin and acetylcholine (100).

The strength of glutamatergic synapses is regulated by the NMDA receptor-dependent exocytosis and endocytosis of AMPA receptors, resulting in long-term potentiation (LTP) and long-term depression (LTD), respectively (101-104). By modulating the activity of NMDA receptors through various mechanisms, neurotransmitters such as dopamine, serotonin and acetylcholine can therefore influence the plasticity of synapses (105). For instance, dopamine and serotonin can influence the trafficking and insertion of NMDA receptors in the cell membrane as well as regulate their endocytosis (90). The phosphorylation status and subsequent conductance properties of NMDA receptors are also controlled by these neurotransmitters (106-109). In addition, serotonin and acetylcholine have been shown to impact the relative expression of NMDA receptor subunits, altering their molecular structure and electrophysiological properties (110-112).
NMDA receptor dysfunction is believed to account for abnormalities in GABAergic and dopaminergic function observed in schizophrenia (113, 114); however, it is also plausible that NMDA receptor dysfunction arises from a primary dysfunction in dopaminergic transmission (100). Hence, dopamine dysfunction may exist as both the cause and consequence of NMDA receptor dysfunction; though, as noted above, other neurotransmitters may also participate in this process (100). Within this framework, the potential for phenotypic, aetiological and pharmacological heterogeneity in schizophrenia is realised. Individuals whose symptoms improve when taking anti-dopaminergic agents may experience NMDA receptor dysfunction resulting from dopaminergic disruptions, whereas those who are clozapine-eligible may possess different underlying disruptions.

The dysconnection hypothesis posits that aberrant synaptic plasticity could disrupt functional coupling (115), leading to a disruption in self-monitoring and ultimately the positive, negative and cognitive symptoms associated with schizophrenia (100). Structural correlates of abnormal synaptic plasticity are also anticipated, likely emerging as changes in the morphology or distribution of dendritic spines as well as deviations in receptor capacity, composition and phosphorylation status (100). Such disruptions have been noted in post-mortem studies, which identified reductions in dendritic field size and in the number of dendritic spines of cortical neurons (116-119). Given the role of functional coupling in developmental pruning (120), such irregularities could also affect the survival of long-range connections in the developing brain (100). This may account for the white matter disruptions often described in those with schizophrenia (100, 121-123).

The mechanism by which disrupted synaptic plasticity gives rise to delusions and hallucinations is hypothesised to involve corollary discharge (also referred to as efference copy signalling), specifically under conditions that require integration of contextual or environmental factors (100, 124). In this context, corollary discharge mechanisms are governed by spike-timing dependent plasticity, a form of synaptic plasticity relying critically on the actions of NMDA receptors and regulated by dopamine, serotonin and acetylcholine (125-128). Authors of the dysconnection hypothesis propose that experiences of passivity and hallucinations in individuals with schizophrenia arise from an imbalance between expectation and experience (100, 129). Under healthy conditions, this relationship is governed by communication between ‘top-down’ and ‘bottom-up’ processes, whereby neuronal representations in higher cortical levels generate predictions about incoming stimuli (mediated by corollary discharge (a copy of the motor command) in the case of motor actions), which are then compared with representations from
lower-level regions about the sensations of these stimuli (129). If sensory inputs deviate from a prediction, an error signal is generated and the prediction is adjusted (129). This exchange of signals is thought to suppress prediction error to provide a hierarchical explanation for sensory inputs (129). Stephan et al. and Friston et al. propose that, in individuals with schizophrenia, the precision of prior expectations is abnormally high (100, 129). This aberrant emphasis on prior expectations leads to false inferences about the cause of perceptual states (i.e. everything becomes surprising) (100, 129). This is not too dissimilar from Kapur’s hypothesis of aberrant salience in schizophrenia (61), and may provide a link between the dysconnectivity and dopamine hypotheses of schizophrenia.

With respect to negative and cognitive symptoms, Stephan and colleagues implicate NMDA receptor-mediated disturbances in learning and memory (100). Specifically, failure of operant (modification of behaviour) and emotional learning during social interactions may render an individual incapable of learning the adaptive and motor processes required for successful social exchange (100). Subsequently, social interactions become frustrating and unpredictable, leading to social withdrawal and apathy (100). Deficits in learning would also affect cognition, with loosening of associations potentially resulting in thought disorder, altered speech and reasoning deficits (100). Friston has also proposed that cognitive dysmetria (difficulty in prioritising, processing, coordinating and responding to information (130)) could arise from mechanisms similar to those responsible for delusions of passivity (i.e. abnormal connectivity leading to a disintegration of neuronal dynamics) (131).

1.2.4.1. SUPPORT FOR A ROLE OF DYSCONNECTION IN SCHIZOPHRENIA

Evidence to support a dysconnectivity hypothesis of schizophrenia is abundant and partly attributable to the numerous in vivo techniques available for the detection of functional and structural abnormalities. These include electroencephalography (EEG), magnetoencephalography (MEG), functional MRI (fMRI), diffusion-weighted imaging (DWI), 1H-MRS and structural MRI. Using EEG, investigators have identified robust reductions in an event-related potential (ERP) associated with predictive coding, referred to as the mismatch negativity (MMN), in people with schizophrenia (132-134). The MMN is elicited by a deviant, unexpected stimulus that interrupts an ongoing sequence of otherwise identical stimuli (usually auditory) (132). Under healthy conditions, a deviation from the predicted input elicits an error, or mismatch, that initiates an exchange of signals that ‘explain away’ that error (133). This process is purportedly under glutamatergic control and is critically dependant on NMDA receptor-mediated synaptic plasticity (133). Both acetylcholine and serotonin have been shown to alter
MMN, most probably via their effects on the NMDA receptor (135, 136). Dampening of the MMN in schizophrenia therefore reflects a deficiency in predictive coding, possibly mediated by NMDA receptor dysfunction.

Grey matter abnormalities corresponding to changes in dendritic length and spine density can be visualised in vivo using voxel-based morphometry (VBM) and other MRI analysis techniques (137, 138). Bora et al. performed a multimodal voxel-wise meta-analysis and meta-regression analysis and found lower grey matter density in individuals with schizophrenia compared with healthy controls (139). Grey matter reductions were more severe in individuals with chronic schizophrenia compared to those in their first episode, suggesting that abnormalities may worsen with ongoing symptom progression (139). Alternatively, the chronic cohort could represent those more likely to be clozapine-eligible, suggesting a bi-modal severity index of grey matter loss.

Functional dysconnection of disparate brain regions in schizophrenia has been visualised using fMRI and a range of analytical techniques. Findings include both increases and decreases in functional connectivity in schizophrenia (140), suggesting a generalised disorganisation of network communication. However, while there is consensus that dysconnectivity exists in schizophrenia, studies may disagree about the site of dysconnections within specific networks (140). Considering the heterogeneous nature of schizophrenia, it is conceivable that these discrepancies in functional dysconnectivity are attributable to differences between FLR and those with TRS or UTRS. This hypothesis is examined further in this thesis.

The dysconnectivity hypothesis originally stemmed from the notion that schizophrenia results from pathological interactions between brain regions that arise from anatomical disruption to association fibres (141). Although the current hypothesis steers away from a primary structural aetiology, a large body of evidence supports an eventual disruption of white matter in schizophrenia (139, 142). Similar to investigations of functional connectivity in schizophrenia, a consensus on the site of white matter abnormalities has not been met (142). However, abnormalities in a number of major white matter tracts all point toward a reduction in white matter integrity in schizophrenia (139, 142). The role of structural connectivity in deciphering individuals who respond to antipsychotics from those who do not, as well as the relationship between structural and functional connectivity, will be discussed further in the following chapters.
As a supplement to traditional structural and functional connectivity analyses, graph theory can provide further insight into brain network characteristics. This technique can be used to elucidate key organisational features of the brain’s architecture and formulate theories about the role of network elements and characteristics in brain function (143). Crossley and colleagues conducted a meta-analysis of graph theory-based studies in schizophrenia and identified under-activations and over-activations in a wide range of areas that were more likely to arise in regions corresponding to core elements of the connectome (144). Once again, the diverse nature of their findings emphasises the heterogeneous character of schizophrenia. If different core elements are affected in different subtypes of the disorder, graph theory could provide a means for identifying individuals who are clozapine-eligible at an earlier stage of the disorder.

The dysconnection hypothesis provides the most integrated approach to describing the pathology of schizophrenia and allows for investigation using a number of non-invasive techniques. Work to date has consistently supported the concept of dysconnection in the disorder; however, results have been widespread and changeable. Given the theorised roles of dopamine, serotonin, acetylcholine and NMDA receptors in this model, different dysconnection profiles are likely to reflect different response subtypes of the disorder. The current thesis has capitalised on a multi-modal approach to investigation and used a variety of MRI acquisition and analysis techniques to explore the utility of dysconnection as a biomarker of treatment resistance in schizophrenia.

1.4. INVESTIGATING TREATMENT RESISTANCE THROUGH BIOMARKER DETECTION

A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (145). The National Institutes of Health (NIH) Biomarkers Definition Working Group proposed that, among other functions, biomarkers may be used as indicators of disease prognosis and for the prediction and monitoring of clinical response to an intervention (145). A biomarker that could identify individuals with TRS or UTRS would serve both these purposes.

Biomarkers have been used to guide treatment intervention in many non-psychiatric disorders (e.g. clinicians know to treat HER2 receptor-positive breast cancer with a HER2-targeted therapy such as trastuzumab (146)). However, despite decades of effort, no biomarker has been identified that contributes in a meaningful way to treatment of any major psychiatric disorder (147). The MMN ERP has long been shown to be impaired in individuals with schizophrenia
(147); however, even this biomarker does not possess enough specificity to endorse its unaccompanied use for the diagnosis of schizophrenia in clinical practice.

MRI offers a safe, non-invasive technique for reliably assessing aspects of neuroanatomy, physiology, chemistry and pathology. As such, this approach may have potential for identifying biomarkers of disease prognosis and response in psychiatry. This technique may be especially effective for identifying biomarkers of treatment response in schizophrenia, owing to the supposed role of dysconnection in the disorder and the numerous methods available for measuring brain connectivity using MRI.

1.5. HYPOTHESES AND OBJECTIVES OF THIS THESIS

In this thesis, it was hypothesised that those who were clozapine-eligible or diagnosed with TRS or UTRS would exhibit different structural and functional abnormalities to those who responded to first-line therapy. Although it was expected that those in the more resistant groups would demonstrate more severe degrees of dysconnection to treatment-responders, it was also expected that some biomarkers of response and resistance would be unique to individual cohorts, perhaps accounting for the often conflicting results identified in research to date. To investigate these hypotheses, structural and functional methods of MRI, including DWI and fMRI, were employed and data analysed using a range of techniques. The resolve to utilise numerous analytical techniques to query the same dataset was guided by an interest in determining whether different techniques could confirm the findings of others and whether utilising any particular method would provide greater insight into the distinctions between response cohorts.

The final experiment set out to determine whether structural and functional connectivity could be used to predict a response to clozapine in clozapine-eligible individuals. To achieve this, a machine-learning algorithm was designed specifically for dealing with spatio-temporal brain data. Although only a small number of participants were included in the final model, the outcome of this experiment provides a platform for future work in the area.

The results of this thesis have been obtained from two cross-sectional studies of people with schizophrenia who have responded to varying degrees of treatment intervention, as well as a prospective study of clozapine-eligible individuals who received a three-month course of clozapine monotherapy. Details of the studies are presented below.
1.6. THE TREATMENT RESISTANT SCHIZOPHRENIA (TRS) STUDY

The TRS study was conducted at the University of Auckland between June 2009 and December 2013. Study design, ethics approval, recruitment and data acquisition were handled by Associate Professor Bruce Russell, Professor Rob Kydd, Dr Meghan McIlwain (MM) and Dr Valerie Anderson. A selection of results has been published previously (148-150). Although the study utilised a number of neuroimaging techniques and involved a wide range of measures, only material relevant to the current thesis will be provided in the description below.

1.6.1. PARTICIPANT INCLUSION AND EXCLUSION CRITERIA

The TRS study recruited healthy controls, FLR, individuals with TRS and those with UTRS with the explicit intention of identifying neuroimaging and other biological markers of treatment response and resistance. Individuals with a diagnosis of schizophrenia according to the DSM-IV were recruited from inpatient and outpatient mental health clinics within the Waitemata and Counties Manukau districts of Auckland, New Zealand.

Inclusion criteria required participants to be aged between 18 and 45 years, present with clinically stable symptoms for at least six weeks prior to study inclusion, and be receiving at least one atypical antipsychotic for the treatment of schizophrenia.

Based on treatment history and current antipsychotic regimen, participants were enrolled into one of three study arms: those who were responding well to first-line atypical antipsychotic monotherapy (FLR), those who had failed at least two previous six-to-eight-week trials of atypical antipsychotics and were now receiving clozapine (TRS), and those who had failed at least two previous six-to-eight-week trials of atypical antipsychotics as well as a trial of clozapine monotherapy (UTRS). All medication regimens past and present were confirmed by a registered pharmacist (MM).

Twenty psychiatrically healthy control participants were recruited from the same geographic location. These individuals were required to have no history of psychiatric illness, established based on medication history and self-report, and be between 18 and 45 years of age.

Exclusion criteria for all participants consisted of history of traumatic brain injury resulting in loss of consciousness greater than three minutes, other significant physical disorders that were uncontrolled and could affect brain structure or function, active substance dependence and contraindications for MRI such as a pacemaker, brain aneurysm clip, injury to the eye with a metallic object or fragment and ferrous metal in the body. Current or previous diagnosis of any
other axis I disorder was also an exclusion criterion. This was determined by careful review of psychiatric notes, a medication review and discussion with the treating clinician.

Participants were asked to provide a urine sample for drug screening during the study visit, though recreational substance use was not an exclusion criterion. Urine was screened for the presence of amphetamine, methamphetamine, benzodiazepines, cocaine, opiates and tetrahydrocannabinol (THC).

The study was approved by the Northern X Regional Ethics Committee and all participants gave informed written consent (see appendix 8.1 for ethics approval documentation and appendices 8.2, 8.3 and 8.4 for participant information sheets and consent form).

1.6.2. STUDY VISIT AND MRI ACQUISITION
Study visits took place at the University of Auckland Medical and Health Sciences Campus in Grafton, Auckland, New Zealand. Participants were required to attend two sessions, the second of which included an MRI scan at the Centre for Advanced MRI (CAMRI), University of Auckland.

Duration of psychosis, Positive and Negative Syndrome Scale (PANSS) scores (151) and past and present substance abuse (evaluated using the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST; World Health Organisation) scale) were assessed by MM at study entry. Duration of psychosis was calculated as the interval between first contact with psychiatric services and study assessment. Only four participants had been unwell for less than three years (three FLR and one individual with TRS).

Details of MRI acquisition are provided in each relevant chapter. Images were acquired using a 3 Tesla (3T) Siemens Magnetom Skyra MRI scanner and included structural, rs-fMRI and DWI scans. 1H-MRS was also conducted at this time but results have been published elsewhere (150). A VBM analysis of structural data has also been published (148).

Although some data from the TRS study has been analysed previously, the study provided a rich dataset that included many aspects that were not examined previously. The current thesis has utilised just two components of the dataset, namely rs-fMRI and DWI. Results of the rs-fMRI analysis using ICA as well as graph-theory and network based statistics are presented in chapters 3 and 4, respectively. Data from the rs-fMRI and DWI were also used to create the prediction model used in chapter 6.
1.7. THE CLOZAPINE RESPONSE (CLORes) STUDY

Evidence from the TRS study supported the utilisation of MRI for the identification of response-related biomarkers in schizophrenia. However, the cross-sectional nature of the study and subsequent variation in antipsychotic treatment regimens between the cohorts hindered efforts to draw definitive conclusions from the data. The CloRes study was designed specially to address this issue. In addition to recruiting only individuals who were receiving the same class of antipsychotic drugs, the CloRes study employed a novel MRI acquisition technique, known as simultaneous multi-slice acquisition (multibanding, see chapter 2 for details), which has been shown to improve sensitivity during fMRI analysis (152). This technique also allows for faster acquisition of DWI, improving study conditions for this highly vulnerable cohort.

Study design, ethics approval, recruitment and data acquisition were handled by the PhD candidate (Carolyn McNabb) under the supervision of Associate Professor Bruce Russell and Professor Rob Kydd. Psychiatric interviews for all clozapine-eligible participants and some FLR were conducted by Professor Rob Kydd (RK), Dr Frederick Sundram (FS) and Dr Ian Soosay (IS). Helen Duyvestyn (research nurse) assisted with recruitment, psychiatric screenings and data acquisition during 2016.

1.7.1. PARTICIPANT INCLUSION AND EXCLUSION CRITERIA

Participants were recruited from inpatient and outpatient mental health clinics within the Waitemata, Counties Manukau and Auckland District Health Boards of New Zealand as well as from mental health support groups and social media. Two cohorts of participants were recruited, including a group of FLR and a group of clozapine-eligible individuals.

Participants in the FLR group were required to have a current diagnosis of schizophrenia according to the DSM-5 or a history of a psychotic episode. Additional inclusion criteria required that they be between 18 and 45 years of age, have no history of treatment with clozapine, and be clinically stable on a first-line antipsychotic drug with a total PANSS score of less than 50. Our justification for including individuals who had not received a diagnosis of schizophrenia was attributable to the diagnosing habits of psychiatrists and other practitioners within New Zealand mental health services. The diagnosis of schizophrenia is associated with a considerable degree of stigma and is sometimes circumvented to prevent undue harm if an individual responds well to treatment. Although we realise this is a limitation, it was a necessary concession given the attitudes of our participants and their carers, as well as the community mental health clinics involved in the study.
Participants in the clozapine-eligible group were recommended for the study by their treating clinician, who must have made the decision to switch their client to clozapine. A participant’s eligibility for clozapine had to be unanimously approved by two psychiatrists, including the participant’s treating psychiatrist and one of the study psychiatrists (RK, FS or IS). Clozapine-eligible participants were required to be between 18 and 45 years of age, meet DSM-5 criteria for schizophrenia as confirmed by one of the study psychiatrists, have failed at least two six-week trials with first-line antipsychotic drugs of which they must still be receiving treatment with at least one, be able to give informed written consent (determined by their treating clinician) and present with persistent positive or negative symptoms contributing to a PANSS score of at least 50 during screening.

Exclusion criteria for both groups included diagnosis of another psychiatric disorder, co-morbid neurological illness, self-reported low treatment adherence to current antipsychotic medication, claustrophobia, history of traumatic brain injury resulting in loss of consciousness greater than three minutes, active substance dependence and contraindications to MRI. Clozapine-eligible participants should not have had a trial of clozapine within three months of the screening visit.

Participants were asked to provide a urine sample for drug screening during the study visit, though recreational substance use was not an exclusion criterion. Urine was screened for the presence of amphetamine, methamphetamine, benzodiazepines, cocaine, opiates and THC.

The study was approved by the Northern A Regional Ethics Committee (ref 14/NTA/103/AM11) and all participants gave informed written consent (see appendix 8.5 for ethics approval documentation and appendices 8.6 and 8.7 for participant information sheets and consent forms). One participant from the FLR cohort of the CloRes study was also included as a FLR in the TRS study. No other participant participated in both studies.

1.7.2. STUDY VISITS AND MRI ACQUISITION

Prior to study entry, all participants were screened by a CloRes study psychiatrist or research nurse. Screening consisted of a semi-structured interview to confirm diagnosis, combined with a PANSS assessment. Diagnosis was later confirmed with the treating psychiatrist or general practitioner.

Eligible participants were invited to the University of Auckland Medical and Health Sciences Campus where they underwent an MRI scan using a 3T Siemens Magnetom Skyra. Details of MRI acquisition for the CloRes study are provided in chapters 3 and 5. When possible, clozapine-eligible participants were scanned within one month before clozapine titration; however, due to
conflicting wishes of some participants, as well as space restrictions in some inpatient units, this was not always viable.

Clozapine-eligible participants were initiated on clozapine by their treating psychiatrist or under the supervision of an inpatient mental healthcare team. Participants were followed up three months after treatment initiation to determine their response to treatment. Treatment response was defined as a $\geq 25\%$ reduction of the PANSS total score from baseline. Based on attainment of this endpoint, individuals were assigned to TRS or UTRS groups. This component of the study is ongoing; however, an interim analysis of the data (evaluated for its potential to predict clozapine response) is provided in chapter 6.

1.8. UPCOMING MATERIAL

Successive chapters of this thesis will examine the potential applicability of functional and structural brain connectivity as biomarkers of treatment response or resistance in schizophrenia. After an introduction to the MRI techniques employed in the TRS and CloRes studies (chapter 2), functional connectivity, identified using ICA, will be assessed as a marker of treatment resistance in chapter 3. Chapter 4 investigates the network characteristics of treatment response and resistance using data from the TRS study and chapter 5 employs DWI analysis techniques including tract-based spatial statistics and probabilistic tractography to further elucidate the underlying physiology of dysconnectivity in participants from the CloRes study. The final results chapter (chapter 6) uses data from the TRS study to build a classification tool for discriminating individuals with TRS from those with UTRS and then incorporates prospective data from the CloRes study to evaluate the applicability of this tool for prospectively predicting clozapine response.
2. INTRODUCTION TO MRI: PHYSICS, SEQUENCES AND DISTORTIONS

The objective of this chapter is to familiarise the reader with the basic concepts of nuclear magnetic resonance imaging (MRI), providing a platform from which an understanding of the acquisition sequences and processing pipelines applied in this thesis can be appreciated. The chapter first introduces some fundamental concepts in MRI physics, including spin properties, radiofrequency pulses, gradients and $k$-space. Some MR sequences employed in the current thesis are then discussed. An introduction to common distortions observed in MR images comes next, followed lastly by a brief discussion of the 2016 article by Eklund et al. (153), which brought to light an exceedingly high rate of false positives associated with functional MRI (fMRI) research.

Material related to the arrangement of $k$-space, gradients and composition of MR sequences in this chapter is adapted from lectures given by Dr Michael Lipton, Albert Einstein College of Medicine, New York, New York, USA.

2.1 BASIC PHYSICS OF MRI

MRI utilises the electromagnetic properties of protons in biological tissue to create three- and four-dimensional images of the human body. A brief explanation of how the MR signal is formed is provided below.

2.1.1. MAGNETIC PROPERTIES OF PROTONS

The principles of MRI depend on the spinning motion of atomic nuclei in biological tissue. Subatomic pairs of protons and neutrons spin at the same rate in opposing directions. For atoms with equal numbers of protons and neutrons, this results in a nucleus with no net spin. However, for atoms with unequal numbers of protons and neutrons, the spins of these particles fail to cancel each other out and result in nuclei that possess what is termed ‘net spin’ or ‘angular momentum’.

When a charged particle moves (e.g. spins), it creates a magnetic field, giving each proton a North and a South pole. The axis of these poles is represented by a magnetic moment and it is the magnetic moments of protons that are measured in MRI. In the absence of an external magnetic field, these magnetic moments will be randomly oriented (as shown in Figure 1).
When placed in a strong static external magnetic field (denoted $B_0$), such as that generated by an MRI scanner, the magnetic moments of protons will align either parallel or antiparallel to the direction of the external magnetic field. The direction of the magnetic moment will depend on the energy possessed by each proton/nucleus. Low-energy nuclei will align parallel with $B_0$ (termed spin-up nuclei) and high-energy nuclei will align antiparallel with $B_0$ (termed spin-down nuclei) (see Figure 2). The stronger the external magnetic field, the more energy required to oppose this field; therefore, in a scanning environment, the magnetic moments of most nuclei will align parallel with $B_0$ (see Figure 3).

The net magnetisation vector (NMV) represents the sum of the magnetic moments of spin-up and spin-down nuclei. As a result, greater magnetic field strengths will produce larger NMVs. In a static magnetic field, the NMV will run parallel with $B_0$.

2.1.2. PRECESSION OF MAGNETIC MOMENTS AND THE USE OF RADIOFREQUENCY PULSES TO TARGET HYDROGEN ATOMS

Though the magnetic moments in Figure 2 and Figure 3 are aligned with $B_0$, in reality, these magnetic moments precess around $B_0$, as shown in Figure 4. The frequency of this precession is directly dependent upon the strength of $B_0$ and is the same for all nuclei of a single element in the same magnetic field.
FIGURE 2. WHEN PLACED IN A STRONG EXTERNAL MAGNETIC FIELD, LOW-ENERGY NUCLEI WILL ALIGN PARALLEL WITH $B_0$ (A) AND HIGH-ENERGY NUCLEI WILL ALIGN ANTIPARALLEL WITH $B_0$ (B).

FIGURE 3. MAGNETIC MOMENTS OF PROTONS IN PRESENCE OF STRONG STATIC EXTERNAL MAGNETIC FIELD ($B_0$).
FIGURE 4. THE INFLUENCE OF $B_0$ CAUSES THE MAGNETIC MOMENT OF EACH NUCLEUS TO PRECESS IN A CIRCULAR PATH ‘AROUND’ $B_0$.

Because different elements possess different precessional frequencies, it is possible to ‘excite’ particles of a single element by applying pulses that oscillate at the same frequency as that element. When nuclei are excited, they gain energy and become high-energy (spin-down) nuclei (see Figure 2b). This is referred to as resonance. Simultaneously, the magnetic moments of these nuclei begin to precess in phase with one another; that is, their positions on the precessional path align (Figure 5).

FIGURE 5. IN A STATIC EXTERNAL MAGNETIC FIELD, MAGNETIC MOMENTS WILL PRECESS OUT OF PHASE BUT AT THE SAME FREQUENCY (A). APPLYING A PULSE AT THE SAME FREQUENCY AS THE PRECESSIONAL FREQUENCY OF THE NUCLEI WILL CAUSE THE MAGNETIC MOMENTS OF THESE NUCLEI TO PRECESS IN PHASE (B).
MRI exploits the electromagnetic properties of the hydrogen isotope protium, which possesses a single proton and no neutrons. This isotope of hydrogen is readily abundant in the human body, most commonly found in water (bound to oxygen) and fat (bound to carbon).

When placed in the external magnetic field of an MR scanner, the magnetic moments of hydrogen nuclei precess at a frequency corresponding to the radiofrequency band ($10^6$-$10^8$ Hz) of the electromagnetic spectrum. Radiofrequency (RF) pulses (which oscillate at hydrogen’s exact precessional frequency) can be induced by RF coils in the scanner (or head coil, as shown in Figure 6) to excite hydrogen protons, converting them to spin-down nuclei and causing their magnetic moments to precess in phase (Figure 7). Other MR-active nuclei such as carbon and oxygen (among others) do not absorb energy at this frequency and so are unaffected.

The strength of the RF pulse determines what proportion of hydrogen nuclei gain energy and is named for the degrees to which it shifts the NMV from $B_0$. A 90° RF pulse excites approximately 50% of nuclei (Figure 8) and shifts the NMV to lie 90° to $B_0$, whereas a 180° RF pulse excites most nuclei in a slice of tissue (Figure 9) and shifts the NMV to lie 180° to $B_0$. The effect of the RF pulse on the NMV not only depends on the proportion of nuclei in the high-energy state but also on the precessional coherence of these nuclei; it is the sum of longitudinal and transverse components of magnetisation (shown in Figure 7).
FIGURE 7. APPLICATION OF AN RF PULSE EXCITES LOW-ENERGY ‘SPIN-UP’ PROTONS (SHOWN IN PURPLE), CONVERTING THEM TO HIGH-ENERGY ‘SPIN DOWN’ NUCLEI (SHOWN IN GOLD). THIS AFFECTS THE LONGITUDINAL MAGNETISATION OF THE TISSUE (PURPLE ARROW) AS WELL AS THE TRANSVERSE MAGNETISATION (GOLD ARROW).

FIGURE 8. WHEN A 90° RF PULSE IS APPLIED TO PROTONS IN A STATIC MAGNETIC FIELD, APPROXIMATELY HALF OF THE PROTONS WILL GAIN ENERGY AND BECOME ‘SPIN-DOWN’ NUCLEI.
2.1.3. RELAXATION, DECAY AND THE MR SIGNAL

Once an RF pulse is switched off, excited nuclei begin to release energy back into surrounding tissue and return to a low-energy spin-up state. Consequently, as more nuclei become spin-up nuclei, the longitudinal magnetisation increases (or recovers). This process is referred to as T1 recovery (Figure 10). Concurrently, magnetic moments that are precessing in phase during the RF pulse begin to dephase. Dephasing of magnetic moments results in a ‘shortening’ or reduction in transverse magnetisation. This process is referred to as T2 decay (Figure 11).

As the longitudinal magnetisation recovers (T1 recovery), the transverse magnetisation decays (T2 decay) and it is the rate at which these two processes occur that determines the strength of the MR signal. As such, when hydrogen nuclei recover and decay at different rates, as occurs between fat and water (among other tissue types), the strength of the signal varies. It is this property that generates contrast within an MR image (see Figure 12).

By adjusting the time between RF pulses (repetition time; TR), the influence of T1 recovery on tissue contrast can be manipulated. A long TR, which allows for T1 recovery in both fat and water before delivery of a successive RF pulse, eliminates the influence of T1 recovery on the MR signal. To emphasise the difference in T1 recovery between the two tissue types, a short TR can be employed. The quick succession of RF pulses takes advantage of the faster T1 recovery of fat compared with water, meaning that when the successive RF pulse is delivered, the NMV of fat
becomes stronger than water in the transverse plane, generating a larger MR signal in the receiver coils (RF coils; Figure 6). This technique produces a T1-weighted image and is illustrated in Figure 13.

Just as the effects of T1 recovery can be manipulated by adjustment of the TR, the effects of T2 decay can be influenced by adjusting the length of time between the 90° RF pulse and sampling of the MR signal by the receiver coils, known as echo time (TE). Following an RF pulse, the magnetic moments of hydrogen nuclei in fat dephase faster than those in water. By employing a long TE, the difference in decay times of the two tissue types can be accentuated, resulting in larger transverse magnetisation (and therefore signal) in water compared with fat. This produces a T2-weighted image. If the TE is short, the influence of T2 decay on the MR signal is diminished.

**FIGURE 10. T1 RECOVERY: AS THE HIGH-ENERGY SPIN-DOWN NUCLEI RELEASE ENERGY INTO SURROUNDING TISSUE, THE MAGNETIC MOMENTS OF THESE NUCLEI ALIGN MORE CLOSERLY WITH $B_0$ (TOP OF FIGURE). THIS PRODUCES AN INCREASE IN LONGITUDINAL MAGNETISATION (SHOWN ON Y AXIS AT BOTTOM OF FIGURE) REFERRED TO AS T1 RECOVERY.**
FIGURE 11. T2 DECAY: DURING AN RF PULSE, THE MAGNETIC MOMENTS OF NUCLEI SPIN IN PHASE WITH EACH OTHER, PRODUCING A LARGE TRANSVERSE COMPONENT OF MAGNETISATION (A). AS THE MAGNETIC MOMENTS BEGIN TO DEPHASE, THE TRANSVERSE COMPONENT OF MAGNETISATION DIMINISHES (B). THIS OCCURS IN CONCERT WITH T1 RECOVERY, MEANING THAT TRANSVERSE MAGNETISATION DIMINISHES AS LONGITUDINAL MAGNETISATION GROWS.
FIGURE 12. T1 RECOVERY (A) OF FAT (GOLD) OCCURS FASTER THAN THAT OF WATER (BLUE). BY INCREASING OR DECREASING THE LENGTH OF TIME BETWEEN RF PULSES (REPETITION TIME; TR), THE CONTRAST BETWEEN FAT AND WATER CAN BE DECREASED OR INCREASED, RESPECTIVELY. T2 DECAY (B) OCCURS FASTER IN FAT (GOLD) COMPARED WITH WATER (BLUE). BY INCREASING OR DECREASING THE LENGTH OF TIME BETWEEN EXCITING NUCLEI WITH AN RF PULSE AND COLLECTING SIGNAL (ECHO TIME; TE), THE CONTRAST BETWEEN FAT AND WATER CAN BE INCREASED OR DECREASED, RESPECTIVELY.
FIGURE 13. FOLLOWING AN INITIAL 90° RF PULSE (TOP OF FIGURE), THE T1 RECOVERY OF FAT (GOLD) OCCURS AT A FASTER RATE THAN THAT OF WATER (BLUE). BEFORE EITHER TISSUE HAS RECOVERED, DELIVERY OF A SECOND (AND EACH SUCCESSIVE) 90° RF PULSE (BOTTOM OF FIGURE) PUSHES BOTH NMVS BEYOND THE TRANSVERSE PLANE, RESULTING IN PARTIAL SATURATION OF THE VECTORS. AS THE NMV OF FAT HAD RECOVERED MORE LONGITUDINAL MAGNETISATION THAN THE NMV OF WATER AFTER THE FIRST RF PULSE, AFTER THE NMV IS FLIPPED ANOTHER 90° BY THE SECOND RF PULSE, THE TRANSVERSE COMPONENT OF MAGNETISATION (AND THEREFORE MR SIGNAL) OF FAT BECOMES GREATER THAN THAT OF WATER.

2.1.4. GRADIENTS, COILS AND SLICE SELECTION
In a homogenous magnetic field, all hydrogen nuclei precess at the same frequency regardless of their location in space. If an RF pulse is applied under such conditions, all nuclei regardless of their three-dimensional locations will be equally affected. If the MR signal were to be sampled thereafter, the signal from two separate locations would be indistinguishable. It is therefore necessary in the scanning environment to introduce a gradient of magnetisation that adjusts the frequency of precession depending on the spatial location of nuclei (spatial encoding). This is achieved using gradient coils (coils of wire through which current is passed).

Magnetic field strength is proportional to current strength and by adjusting the current within a coil it is possible to control the intensity of the magnetic field. By passing current in opposing
directions along gradient coils lying perpendicular to x, y and z planes within the scanner it is possible to manipulate the magnetic field strength of \( B_0 \) along these planes (see Figure 14).

![Gradient Coils Diagram](image)

**FIGURE 14. GRADIENT COILS PERPENDICULAR TO THE Z (RED LOOPS), Y (BLUE LOOPS) AND X (YELLOW LOOPS) PLANES EACH CONDUCT OPPOSING CURRENTS WHICH GENERATE MAGNETIC FIELD GRADIENTS (BLUE TO RED ARROWS) ALONG THE DIRECTION OF THE MAIN MAGNETIC FIELD (\( B_0 \)). THE STRONGER THESE CURRENTS, THE STEEPER THE GRADIENTS.**

Figure 15 illustrates the effect of each gradient on \( B_0 \). Switching the gradient on in the z direction changes magnetic field strength in the z plane, causing a reduction in field strength ventral to the magnetic isocentre and an increase in field strength dorsal to the isocentre (Figure 15a). This affects the precessional frequency of nuclei along this plane. An RF pulse can then be tuned to excite nuclei in only a single slice of the z plane, that is, those nuclei precessing at a frequency equal to that of the RF pulse (Figure 16a). After excitation, the nuclei precess in phase. Brief activation of the y gradient (known as the phase-encoding gradient; Figure 15b) accelerates
precession of nuclei anterior to the magnetic isocentre and slows precession of nuclei posterior to the isocentre (or vice versa). As the $y$ gradient is switched on only momentarily, the nuclei return to an equal precessional frequency but remain out of phase (known as phase shift; see Figure 16b). Subsequent activation of the $x$ gradient (known as the frequency-encoding gradient; Figure 15c) accelerates precession of nuclei to the right of the magnetic isocentre and slows precession of nuclei to the left of the isocentre (or vice versa).

FIGURE 15. MAGNETIC FIELD STRENGTH OF $B_0$ WHILE $Z$ (A), $Y$ (B) AND $X$ (C) GRADIENTS ARE SWITCHED ON INDIVIDUALLY. BLUE INDICATES LOWER FIELD STRENGTH AND RED INDICATES HIGHER FIELD STRENGTH.

FIGURE 16. A) SLICE SELECTED THROUGH $Z$ PLANE ACCORDING TO ELECTROMAGNETIC GRADIENT PRODUCED BY $Z$ COILS (RADIFREQUENCY PULSE TARGETED TO RESONANT FREQUENCY OF PROTONS IN THAT SLICE). B) PHASE-ENCODING ($G_y$) GRADIENT SWITCHED ON FOR SHORT PERIOD OF TIME SLOWS DOWN PRECESSION FREQUENCY AT BOTTOM AND SPEEDS UP PRECESSION FREQUENCY AT TOP OF $Y$ AXIS BY INCREASING MAGNETIC GRADIENT ALONG $Z$ AXIS (PHASE SHIFT). C) FREQUENCY-ENCODING ($X$) GRADIENT SWITCHED ON AND REMAINS ON - SLOWS DOWN PRECESSION FREQUENCY ON LEFT SIDE AND SPEEDS UP PRECESSION FREQUENCY ON RIGHT SIDE OF $X$ AXIS BY INCREASING MAGNETIC GRADIENT ALONG $Z$ AXIS. SIGNAL IS COLLECTED FROM THE WHOLE SLICE SIMULTANEOUSLY; EACH FREQUENCY IN THE SIGNAL CONTAINS THE INFORMATION FROM ALL VOXELS IN ONE COLUMN (REPRESENTED BY THE GREEN BAR).
Each spatial location within the slice is now encoded based on the phase and precessional frequency of nuclei (Figure 16c). At the same time as the frequency-encoding gradient is turned on, signal from the whole slice is read by the receiver (RF) coils; information from each column is embedded as a separate frequency within the main signal. Each frequency contains information from all voxels in one column. This process is repeated for each slice in the image.

2.1.5. K-SPACE AND THE FOURIER TRANSFORM

Signal from the slice is read into a raw data file, which is used to produce a single row of time-domain data in what is known as k-space (see Figure 17a). This single row of k-space \((k_i)\) represents data from a single slice acquired following application of a single phase-encoding gradient \((G_{γ1})\). The signal is acquired as multiple samples either side of TE (when signal amplitude is greatest), resulting in maximal signal amplitude (seen as white pixels in Figure 17) in the midline of each \(k_i\) and lower signal amplitude (seen as dark grey pixels in Figure 17) nearer to the edges. To fill additional lines of k-space, more phase-encoding steps, applying different phase-encoding gradients (e.g. \(G_{γ2}\); see Figure 17b) are required. As the phase-encoding gradient becomes stronger, adjacent spins experience different magnitudes of magnetic field strength and precess at different frequencies, diminishing the net transverse magnetisation and reducing the signal. To capitalize on the increased signal acquired with lower phase-encoding gradients, these rows \(k_i\) of data are placed in the centre of k-space, with lower-signal data from higher phase-encoding gradients placed near the periphery (top and bottom); this results in a matrix similar to that shown in Figure 18a.

Each pixel of k-space contains information from all columns and all rows of the original slice (referred to hereafter at columns \(s_0\) and rows \(s_o\)). Each column \(s_0\) is encoded by a different frequency, which can be separated using a Fourier transform (see Figure 17 and Figure 18b). The one-dimensional (1D)-Fourier transform produces a matrix of rows \((s_{01})\) and columns \((s_{10})\) in which the columns \(s_{10}\) represent signal acquired from an entire column \(s_0\) of the original slice (i.e. the information from rows \(s_o\) (individual voxels) is still imbedded within the signal) and rows \(s_{01}\) represent signals acquired at different phase-encoding gradients (see Figure 18c). A second Fourier transform is then required to extract the signal from each voxel \((s_{0i})\) from the columns \(s_{10}\) of the 1D-Fourier transform matrix.

The mechanism by which the second Fourier transform separates the signal from individual voxels within a column \(s_0\) depends on the spatial distance of these voxels along the phase-encoding gradient and the subsequent change in phase that the protons in these voxels experience when the magnitude of the gradient is changed (see Figure 18d). Voxels closer to
isocentre will experience the smallest change in phase from one gradient strength to the next, whereas protons in voxels further away from isocentre will experience a much larger change in phase. This is attributable to the steepness of the gradients, which produce much larger differences in magnetic field strength nearer the top and bottom (or right and left) of the scanning environment compared with the isocentre. The second Fourier transform uses information about the change in phase from one phase-encoding gradient strength to the next to tease apart the signals in the 1D-Fourier transform and place the signal information from each voxel in its correct position (Figure 18e).

**FIGURE 17. THE SIGNAL FROM A SINGLE EXCITED SLICE IS READ INTO K-SPACE. APPLICATION OF A PHASE-ENCODING GRADIENT (Gγ) FOLLOWED BY A FREQUENCY-ENCODING GRADIENT AND SIMULTANEOUS READOUT BY THE RF COILS PRODUCES A SIGNAL THAT IS SAMPLED MULTIPLE TIMES AT TE. EACH SAMPLE IS ENTERED AS A PIXEL IN A SINGLE ROW OF K-SPACE, ARRANGED IN THE ORDER FROM WHICH THE SAMPLES WERE ACQUIRED (TIME-DEPENDENT). EVERY VOXEL CONTAINS INFORMATION FROM ALL COLUMNS (1-7) OF THE IMAGE (OR SCANNER SPACE), WHICH CAN BE SEPARATED USING A FOURIER TRANSFORM. SIGNALS FROM WEAK PHASE-ENCODING GRADIENTS (Gγ1) ARE READ INTO THE MIDDLE ROWS OF K-SPACE (A), WHEREAS STRONGER GRADIENTS (Gγ2) PRODUCE SIGNALS THAT ARE READ INTO MORE PERIPHERAL LINES OF K-SPACE (B).**
FIGURE 18. FOURIER TRANSFORMS FROM K-SPACE TO 1-DIMENSIONAL AND 2-DIMENSIONAL TRANSFORMS. A) DEPICTION OF K-SPACE WITH SIGNAL FROM WEAKER PHASE-ENCODING GRADIENTS IN THE CENTRE AND STRONGER GRADIENTS AT THE TOP AND BOTTOM OF THE MATRIX. FROM LEFT TO RIGHT, PIXELS REPRESENT SAMPLES COLLECTED IN TIME, WITH SAMPLES COLLECTED AT TE IN THE MIDDLE. B) A FOURIER TRANSFORM SEPARATES THE FREQUENCY SIGNALS ASSOCIATED WITH EACH COLUMN FROM EACH K-SPACE VOXEL. C) THE 1-DIMENSIONAL FOURIER TRANSFORM CONTAINS SIGNALS FROM EACH COLUMN OF THE ORIGINAL SLICE (COLUMNS) ACCORDING TO THE STRENGTH OF THE PHASE-ENCODING GRADIENT FROM WHEN THEY WERE COLLECTED (ROWS). D) AFTER APPLICATION OF A PHASE-ENCODING GRADIENT, PROTONS NEARER THE PERIPHERY OF THE MAGNETIC FIELD EXPERIENCE GREATER DEPHASING THAN THOSE IN THE ISOCENTRE, WITH STEEPER GRADIENTS PRODUCING MORE DEPHASING THAN SHALLOWER GRADIENTS. PROTONS CLOSE TO ISOCENTRE EXPERIENCE LITTLE DIFFERENCE IN DEPHASING BETWEEN DIFFERENT GRADIENT STRENGTHS; HOWEVER, PROTONS FARther AWAY FROM ISOCENTRE EXPERIENCE LARGE DIFFERENCES IN THE AMOUNT OF DEPHASING ASSOCIATED WITH STEEP AND SHALLOW GRADIENTS. THE DIFFERENCE IN DEPHASING BETWEEN GRADIENT STRENGTHS (ΔPHASE) CONTAINS INFORMATION ABOUT THE SPATIAL LOCATION OF VOXELS AND IS USED BY THE SECOND FOURIER TRANSFORM TO CORRECTLY POSITION SIGNALS FROM EACH VOXEL IN THE 2D FOURIER TRANSFORM IMAGE (E).
Every pixel in k-space contains information about the entire image slice. As signal is greatest in the centre of k-space, these pixels contain information about contrast in the image but have little spatial resolution (see yellow box, Figure 19). The spatial resolution of an image is instead stored in the peripheral pixels of k-space (see blue box, Figure 19), which contain data collected at higher gradient strengths and temporally further from TE.

Acquiring data to fill all of k-space requires that the phase-encoding gradients be applied in both directions (e.g. left to right then right to left). This produces a matrix that is essentially symmetrical across the diagonal plane (Figure 20a). As a result, an image of almost equal quality can be obtained by acquiring only half of k-space and using signal information from that portion to interpolate the rest of the data (Figure 20b). Other methods may also be applied, including initial acquisition of a single matrix of k-space followed by multiple fast acquisitions of just the middle rows, (Figure 20c) during contrast imaging. The peripheral rows from the original matrix can then be used to ‘fill in’ the missing information and produce high-quality sequential images within a biologically relevant timeframe. Likewise, if only information about contrast is required, just the centre of k-space can be acquired (Figure 20d).

2.2. MR SEQUENCES
In addition to the magnetic field inhomogeneities that are caused by gradients, the static magnetic field of the scanner possesses inhomogeneities of its own (see Figure 21). These inhomogeneities cause rapid dephasing of nuclei, which degrades the transverse magnetisation before either T1 relaxation or T2 decay can occur (154) (The combined effect of T2 decay and dephasing due to magnetic inhomogeneities is termed T2*). To take advantage of the T1 and T2 properties of different tissue types, the signal must be regenerated. This can be achieved with either an additional 180° RF pulse or via the switching of gradients (154).
FIGURE 21. A HOMOGENEOUS STATIC MAGNETIC FIELD WITH ALL NUCLEI PRECESSING AT THE SAME FREQUENCY (A) AND A SECOND INHOMOGENEOUS MAGNETIC FIELD (B), WHERE AN INCREASE IN STRENGTH OF THE MAGNETIC FIELD AT THE BOTTOM RIGHT CAUSES AN INCREASE IN PRECESSIONAL FREQUENCY OF NUCLEI IN THIS REGION.

2.2.1. SPIN-ECHO PULSE SEQUENCE
Sequences that employ a 180° RF pulse to regenerate the MR signal are known as spin-echo pulse sequences. Following an initial 90° RF pulse, nuclei begin to dephase, diminishing the transverse magnetisation of the tissue. Application of a second, 180° RF pulse flips the magnetic moments of these nuclei through 180° in the transverse plane. As the nuclei continue on their precessional paths, they momentarily realign (termed re-phasing) and the transverse magnetisation regains its strength (Figure 22). The signal (representing either T1 recovery or T2 decay) can then be acquired. An example of a typical spin-echo pulse sequence is provided in Figure 23.

2.2.2. GRADIENT-ECHO PULSE SEQUENCE
A typical gradient-echo pulse sequence is shown in Figure 24. Unlike spin-echo pulse sequences, gradient-echo pulse sequences do not compensate for inhomogeneities in the magnetic field and consequently suffer from magnetic susceptibility artefact. However, without the time constraints of a refocusing 180° RF pulse, gradient-echo pulse sequences are able to acquire signal much quicker than spin-echo pulse sequences.

In the absence of a refocusing RF pulse, the gradient-echo pulse sequence utilises the frequency encoding gradient to dephase and then re-phase the magnetic moments of nuclei to enhance
the MR signal at TE (shown in Figure 25). The steepness and duration of this gradient determines the TR, with steeper and quicker gradients permitting shorter TRs.

Another feature of the gradient-echo pulse sequence is its variable flip angle ($\alpha^\circ$). By utilising a flip angle smaller than 90°, it is possible to eliminate the influence of T1 recovery on the MR signal without the requirement for long TRs. Whereas a flip angle of 90° permits greater T1 recovery of fat compared with water and subsequently greater transverse magnetisation after the next TR (Figure 13), a flip angle of 30° is sufficiently small that there is insufficient time for the T1 properties of the tissues to emerge before all longitudinal magnetisation is recovered (Figure 26). This results in an MR signal that is representative of T2 (or more correctly T2*) decay only.

**FIGURE 22. SPIN ECHO PULSE SEQUENCE (ADAPTED FROM (154)). FOLLOWING THE INITIAL 90° RF PULSE, THE TRANSVERSE MAGNETISATION DIMINISHES AS A RESULT OF DE-PHASING (REPRESENTED BY ARROWS AT BOTTOM OF FIGURE). APPLICATION OF A 180° RF PULSE FLIPS THE MAGNETIC MOMENTS AND RESULTS IN REPHASING AND SUBSEQUENT RESTORATION OF THE SIGNAL.**

FIGURE 24. GRADIENT-ECHO PULSE SEQUENCE. $\alpha^\circ$ REPRESENTS A VARIABLE FLIP ANGLE.
FIGURE 25. EFFECT OF FREQUENCY-ENCODING GRADIENT ON MR SIGNAL. AFTER THE INITIAL RF PULSE, THE FREQUENCY-ENCODING GRADIENT IS SWITCHED ON IN THE REVERSE DIRECTION TO DECAY THE SIGNAL. WHEN THE FREQUENCY-ENCODING GRADIENT IS SWITCHED BACK ON IN THE OPPOSING DIRECTION, THE SIGNAL IS RESTORED MOMENTARILY. THE SIGNAL IS READOUT AT THE TIME WHEN IT IS AT ITS PEAK. THE POLARITY OF THE INITIAL FREQUENCY ENCODING GRADIENT IS REVERSED COMPARED TO THE SPIN-ECHO PULSE SEQUENCE BECAUSE THE 180° RF PULSE IS NOT PRESENT TO FLIP THE MAGNETIC MOMENTS.

FIGURE 26. EFFECT OF SMALL FLIP ANGLE (30°) ON LONGITUDINAL AND TRANSVERSE MAGNETISATION.

2.2.3. ECHO-PLANAR IMAGING
Echo-planar imaging (EPI) is a form of gradient-echo pulse sequence where the phase-encoding gradient is adjusted multiple times in a TR (blipped; filling multiples lines of $k$-space), accompanied by fluctuating polarity of the frequency-encoding gradient (Figure 27). Each
sequential shift in the frequency-encoding gradient dephases or re-phasates the magnetic moments and the MR signal is read out. Spin-echo EPI uses an additional 180° RF pulse to increase signal after the initial T2* decay (Figure 28). For gradient-echo and spin-echo EPI, the rate at which T2* decays and the strength and duration of the frequency-encoding gradients, determine the amount of k-space that can be filled within a single TR. If all of k-space is filled following a single RF pulse, the pulse sequence is referred to as single-shot (SS) EPI, whereas multi-shot EPI refers to EPI where more than one RF pulse is required to fill k-space.

All gradient pulses produce velocity-dependent phase shifts, characterised by adjustments in nuclear precession that are more commonly associated with changes in blood flow velocity (154). SS-EPI produces these velocity-dependent phase shifts but unlike multi-shot EPI, the phase shifts are smooth rather than discontinuous or periodic (155). Phase-shifts caused by SS-EPI can therefore be corrected to avoid artefact from pulsatile cerebrospinal fluid (CSF) and blood flow velocity (156), making SS-EPI an essential technique in the acquisition of fMRI and diffusion MRI (155) (discussed next).

![FIGURE 27. GRADIENT ECHO ECHO-PLANAR IMAGING SEQUENCE](image-url)
2.2.4. FUNCTIONAL MRI

Functional MRI (fMRI) takes advantage of magnetic susceptibility artefacts caused by deoxygenated haemoglobin in the brain. Magnetic susceptibility is a measure of the magnetic properties of a material and in this case refers to the interaction between a tissue or other substance and the in-scanner magnetic field strength. Any material with magnetic susceptibility will perturb the homogeneity of a magnetic field: materials with negative magnetic susceptibility are referred to as diamagnetic, and those with positive magnetic susceptibility are referred to as paramagnetic. The effects of these substances on a homogenous magnetic field are illustrated in Figure 29. Introduction of a paramagnetic substance such as deoxyhaemoglobin into the scanner magnetic field causes variability in field strength, spin dephasing, geometric distortion and loss of signal; fMRI exploits this property by measuring changes in the relative ratio of oxygenated (diamagnetic) to deoxygenated (paramagnetic) haemoglobin in the blood.
FIGURE 29. MAGNETIC SUSCEPTIBILITY ARTEFACTS. THE HOMOGENEOUS MAGNETIC FIELD IS REPRESENTED BY PARALLEL LINES (TOP), INDICATING EQUAL MAGNETIC FIELD STRENGTH ALONG THE ENTIRE FIELD. INTRODUCTION OF A DIAMAGNETIC SUBSTANCE OR TISSUE WILL DECREASE THE MAGNETIC FIELD STRENGTH WITHIN ITS VICINITY AND CAUSE AN INHOMOGENEITY IN THE MAGNETIC FIELD (MIDDLE); HOWEVER, THIS PERTURBATION IS ONLY SMALL. INTRODUCTION OF A PARAMAGNETIC SUBSTANCE SUBSTANTIALLY INCREASES THE MAGNETIC FIELD STRENGTH AROUND THAT SUBSTANCE AND CAUSES A LARGE INHOMOGENEITY IN THE MAGNETIC FIELD (BOTTOM).

FMRI measures the haemodynamic response to neuronal excitation and is therefore a secondary measure of neuronal activity. As the metabolic demands of neurons increase (as observed during task performance), astrocytes are signalled to produce prostaglandin E₂ and epoxyeicosatrienoic acids, which diffuse to arteriolar smooth muscle and cause vasodilation (157). Independently, adenosine, a breakdown product of adenosine triphosphate (ATP) produced during periods of high metabolic demand signals pericytes (contractile cells surrounding capillaries) to relax, permitting increased blood flow through capillaries (157). This increased blood flow delivers high concentrations of oxygenated haemoglobin and glucose to the activated region (see Figure 30), increasing the ratio of oxygenated to deoxygenated haemoglobin. As discussed above, deoxyhaemoglobin is paramagnetic and causes dephasing and signal loss in the MR image. Increasing the ratio of oxyhaemoglobin to deoxyhaemoglobin reduces signal loss, because oxyhaemoglobin is diamagnetic. It is this decreased signal loss, corresponding to the peak of the haemodynamic response function (HRF), that is measured during fMRI experiments. This is referred to as the blood oxygen-level dependent (BOLD) signal. Importantly, the HRF produces only a 1%-2% change in signal following a single stimulus; for this reason, it is required that data be collected over a long period of time so that the signal to noise ratio can be improved.

FMRI may be acquired during performance of a cognitive task or during rest. During rest, the brain exhibits patterns of spontaneous activity that coincide with those present during task
performance (158), making resting-state fMRI (rs-fMRI) an excellent tool for investigating functional brain connectivity.

FIGURE 30. HAEMODYNAMIC RESPONSE FUNCTION. ONSET OF A STIMULUS INCREASES NEURONAL ACTIVITY, CAUSING AN INITIAL DIP IN OXYGENATED HAEMOGLOBIN CONCENTRATION. INCREASED NEURONAL ACTIVATION TRIGGERS VASODILATION AND BLOOD FLOW TO THE AREA IS INCREASED, RESULTING IN A PEAK CONCENTRATION OF OXYGENATED HAEMOGLOBIN APPROXIMATELY 6 SECONDS AFTER THE INITIAL STIMULUS. THIS PEAK IS FOLLOWED BY A POST-STIMULUS UNDERSHOT, WHICH THEN RECOVERS TO BASELINE.

2.2.5. DIFFUSION MRI: DIFFUSION TENSOR IMAGING AND TRACTOGRAPHY

Diffusion-weighted imaging (DWI) uses immense gradient amplitudes together with spin-echo or gradient-echo EPI sequences to provide a measure of the relative diffusion of molecules in tissue. In any pulse sequence, movement of molecules in the direction of a gradient causes a greater phase shift and therefore greater loss of signal than movement along orthogonal planes to the gradient. Equally, molecules that are stationary experience less phase shift and therefore cause less signal loss than those that are moving parallel to the gradient. DWI exploits this phenomenon by applying gradients at extremely high amplitudes to enhance phase shifting and signal loss. The amplitude and the duration of gradients affects the amount of phase shifting and it is the combination of these two parameters that determines the b value (higher b values correspond to greater gradient amplitude/duration). By applying gradients along the x, y and z planes at different strengths and in different combinations, it becomes possible to measure the signal loss along many directions (diffusion-encoding directions, termed b vectors; Figure 31a). This technique is referred to as diffusion tensor imaging (DTI).

In any diffusion-weighted pulse sequence, only molecular displacements along the direction of the gradient are visible (159). By applying more diffusion-encoding directions by increasing the
number of gradient combinations (see Figure 31b) a greater uniformity and minimised directional bias may be achieved (159). Likewise, adjusting the b value can influence the angular contrast of a diffusion-weighted image (160). Increasing the b value provides greater angular contrast; permitting greater differentiation of diffusion directions within a single voxel and reducing the uncertainty associated with estimating fibre orientations (160). However this comes at a cost of decreased signal-to-noise. Subsequently, to profit from both contrast and signal, it is beneficial to utilise a range of carefully positioned b values (multi-shell acquisition) when acquiring diffusion data (160). At least one image acquired in the absence of any gradients \( (b_0) \) is also an essential component of DWI. The \( b_0 \) image contains information about T2 decay in the absence of diffusion encoding and provides a reference to which all diffusion-encoded images must be normalised.

![Figure 31](image.png)

**FIGURE 31.** APPLICATION OF X, Y AND Z GRADIENTS AT DIFFERENT AMPLITUDES CAUSES LOSS OF SIGNAL IN DIFFERENT DIFFUSION DIRECTIONS (A). THE NUMBER OF DIRECTIONS (B VECTORS; B) MAY BE INCREASED TO PROVIDE GREATER DIFFUSION DETAIL WITHIN THE IMAGE. FIGURE ADAPTED FROM ACOSTA-CABRONERO AND NESTOR (2014) (161).
The diffusion of hydrogen within a voxel is described by the diffusion tensor (D, shown in Equation 1). D is a reciprocal matrix, with six independent scalar elements (D_{xx}, D_{xy}, D_{xz}, D_{yx}, D_{yy}, and D_{yz}). This equation can also be expressed in terms of orthogonal eigenvectors (e) and their respective eigenvalues (λ). These variables describe the elements of the diffusion ellipsoid, shown in Figure 32.

**EQUATION 1.** THE DIFFUSION OF HYDROGEN IN A VOXEL (D) IS DESCRIBED BY A 3X3 SECOND-ORDER TENSOR THAT DEFINES THE PROPERTIES OF AN ELLIPSOID IN 3D SPACE (162).

\[
D = \begin{pmatrix}
xx & xy & xz \\
yx & yy & yz \\
zx & zy & zz
\end{pmatrix} = \begin{pmatrix}
e_1 \\
e_2 \\
e_3
\end{pmatrix} \begin{pmatrix}
\lambda_1 & 0 & 0 \\
0 & \lambda_2 & 0 \\
0 & 0 & \lambda_3
\end{pmatrix} \begin{pmatrix}
e_1 \\
e_2 \\
e_3
\end{pmatrix}
\]

**FIGURE 32.** THE DIFFUSION ELLIPSOID, DESCRIBED BY THE PRINCIPLE, SECONDARY AND TERTIARY DIFFUSION DIRECTIONS (EIGENVECTORS) (A) AND THE PRINCIPLE, SECONDARY AND TERTIARY EIGENVALUES (B).

When the diffusion within a voxel is isotropic, the diffusion ellipsoid is represented by a sphere with all eigenvalues equal (Figure 33a). Conversely, if diffusion is restricted due to tissue boundaries such as those observed in white matter (axons) the diffusion ellipsoid will appear as in Figure 33b, with a greater eigenvalue coupled to the principle diffusion direction compared with those corresponding to the secondary and tertiary diffusion directions.

From the tensor equation depicted in Equation 1, a number of metrics corresponding to the properties of diffusion in the voxel can be derived. The eigenvalue (λ_1) corresponding to the primary direction of diffusion (principle eigenvector; e_1) is referred to as the axial diffusivity (AD). Radial diffusivity (RD) is the mean of λ_2 and λ_3 and reflects the diffusion behaviour transverse to the axonal path (161). Mean diffusivity (MD) is a measure of combined AD and RD and describes the net diffusion of hydrogen within a voxel. Both AD and RD are used as measurements of
diffusion in chapter 5; however, given its dependence on other measures, MD is not discussed further.

**FIGURE 33. ELLIPSOID REPRESENTATIONS OF FREE DIFFUSION (A) AND RESTRICTED DIFFUSION (B) WITHIN A SINGLE VOXEL.**

Fractional anisotropy (FA) is a measure of the shape of an ellipsoid and provides information about the degree of anisotropy in a voxel. It is calculated using the equation described in Equation 2. High FA values represent areas of high anisotropy, signifying restricted water movement as in Figure 33b, whereas low FA values represent areas of low anisotropy, where water molecules diffuse freely (as in Figure 33a). Care must be taken when interpreting low FA values, however, as they are also known to correspond with areas of crossing fibre architecture. This is further addressed in chapter 5.

**EQUATION 2. FORMULA FOR DETERMINING THE FRACTIONAL ANISOTROPY WITHIN A VOXEL.**

\[
FA = \sqrt[2]{\frac{1}{2} \left( \frac{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \right)^2}
\]
Diffusion tensor information can also be used to reconstruct white matter bundles in the brain. This technique is termed tractography. Tractography uses diffusion orientation information from tensor imaging to calculate the direction of fibre bundles in vivo. In deterministic, or streamline tractography, the local tract direction is defined by the major eigenvector of the diffusion tensor (163). This causes issues in voxels with crossing, kissing or splitting fibres, however, as the algorithm is only capable of estimating one fibre orientation (163). Probabilistic tractography addresses these issues by estimating the orientations of two or three different fibre populations within a single voxel (164). Thereafter, at every voxel, the algorithm estimates the most probable fibre orientation but also provides a distribution representing the probability that every other orientation lies along that fibre. This process is repeated many times, each time using a slightly different orientation (according to its likelihood). The integration of all estimates provides a collective measure of connection probability along each tract (164, 165).

2.2.6. PARALLEL IMAGING
Parallel imaging is a technique that enhances the efficiency of image acquisition by under-sampling the MR signal (i.e. acquiring fewer lines of k-space) at multiple coils and using the spatial information contained within each coil (sensitivity profile) to unalias (tease apart) the resulting images (Figure 34). Several techniques exist, including GRAPPA and SENSE.

The more coils available to sample the MR signal, the fewer lines of k-space required and therefore the shallower the phase-encoding gradient needed. As the magnitude of the phase-encoding gradient is less, the resulting signal loss due to dephasing (T2*) is also less. A disadvantage of parallel imaging is the reduction in signal to noise ratio; however, with greater magnetic field strengths (e.g. 3T) this is not a significant issue.

2.2.7. MULTIBAND RADIOFREQUENCY EXCITATION
First proposed by Larkman et al in 2001 (166), multiband RF excitation utilises a series of RF pulses from numerous coils to accelerate MR image acquisition by a factor much greater than is possible with EPI or parallel imaging alone. By tuning multiple RF pulses to match the precessional frequencies of nuclei along the slice-encoding gradient, multiband RF excitation is able to simultaneously excite (and later acquire signal from) multiple slices of tissue (Figure 35). Used in combination with gradient-echo EPI (simultaneous multi-slice EPI; see Figure 36) and parallel imaging, multiband RF excitation is able to accelerate image acquisition by up to 16 times (167).

Analogous to the aliasing that occurs during parallel imaging, multiband RF excitation produces interslice aliasing. Blipped Controlled Aliasing in Parallel Imaging (blipped-CAIPI) employs a sequence of polarity- and amplitude-modulated blips in the slice-encoding gradient (see $z$ gradient in Figure 36) that coincide with activation of the phase-encoding gradient to introduce phase modulation during readout and create an interslice image shift between simultaneously acquired slices (168). Adjusting the polarity of blips to periodically refocus (rewind) prevents phase accumulation which would otherwise cause voxel tilt and blurring (168).
FIGURE 35. MULTIBAND RF EXCITATION. PURPLE, RED AND ORANGE RF PULSES ARE TUNED TO EXCITE PROTONS IN DIFFERENT SLICES ALONG THE SLICE-ENCODING GRADIENT SIMULTANEOUSLY.

FIGURE 36. MULTIBAND RF EXCITATION COUPLED WITH GRADIENT-ECHO EPI. Z GRADIENT REPRESENTS THE SLICE-ENCODING GRADIENT, WHICH IN THIS INSTANCE IS USED TO UNALIAS THE SIGNAL FROM SIMULTANEOUS SLICES. THE BLACK GRADIENT PRECEDING OTHER SLICE-ENCODING GRADIENTS IS USED TO FURTHER REDUCE MINOR GHOSTING ARTEFACT OF THE BLIPPED CAIPI SEQUENCE.
2.3. DISTORTIONS, MOTION AND DRIFT

Once the MR image has been generated, it may still contain a number of artefacts caused by various distortions or properties of the scanning environment. A few important artefacts relevant to the current thesis are covered below.

2.3.1. MOTION – SUBJECT MOTION AND PHYSIOLOGICAL MOTION

A common distortion is motion artefact, caused by voluntary or involuntary (physiological; e.g. heartbeat and respiration) motion within the tissue. Despite careful instruction, some participant populations, such as those used in the current studies, may find it difficult to remain still or comply with instructions. This can be of greatest detriment during long scanning acquisitions such as during fMRI.

Head motion is a prominent concern in the field of rs-fMRI, as even small amounts of movement can produce spurious but spatially structured patterns of connectivity (169). These structured artefacts arise because head motion adds false variance to ‘true’ timeseries (170). As the variance is most similar in nearby voxels, the correlations between BOLD timeseries are most greatly increased in these voxels (170).

Several techniques have been developed to decrease the impact of subject motion on BOLD timeseries data, including, but not limited to, nuisance regression, spike regression, motion scrubbing (169), FMRIB’s ICA (independent components analysis)-based Xnoiseifier (ICA-FIX (171)) and ICA-based Automatic Removal of Motion Artifacts (ICA-AROMA (172)). In addition to linear and non-linear image registration, the current thesis employs ICA-AROMA, an ICA-based technique developed by Pruim et al. that identifies and removes independent components specifically related to head motion (172). The algorithm implements a classifier that assesses each independent component in light of its correlation with realignment parameters, edge fraction, CSF fraction and high-frequency content (172). A component is classified as motion-related if it exceeds at least one of three criteria, including exceeding a decision boundary combining the edge fraction and maximum realignment parameter correlation, exceeding a CSF fraction of 10%, or exceeding a high-frequency content of 35% (172). This technique has been shown to minimise the impact of head motion while preserving the signal of interest and increasing the reproducibility of resting-state networks (173). This technique is also able to remove some physiological noise from the data (172).
Physiological noise may also be minimised by recording pulse and respiratory data during scanning and regressing this out of the data; however, this method was not available at the University of Auckland at the time the studies included in this thesis were launched.

2.3.2. MAGNETIC FIELD DISTORTIONS
As discussed in sections 2.2. and 2.2.4., the magnetic field within the scanner suffers from inhomogeneities caused by the scanner environment itself and the magnetic susceptibilities of tissue and other materials. These inhomogeneities directly impact the MR signal and may cause geometric distortions in the MR image. Fortunately, maps of the magnetic field inside the scanner can be acquired and used downstream to counteract these distortions. Three images are acquired: two magnitude images (one for each echo) and a phase difference image. During preprocessing, the phase image and one magnitude image are merged to create a fieldmap (Figure 37), which is used to warp any resulting images based on how much each voxel was affected by magnetic field inhomogeneities.

![Figure 37. Fieldmap image constructed from magnitude and phase images. Contains information about the relative disruptions in phase caused by inhomogeneities in the scanner magnetic field. A=anterior, P=posterior, R=right, L=left.](image)

2.3.3. EDDY CURRENTS
DWI requires strong, rapidly switching diffusion-encoding gradients (as described in section 2.2.5.) to increase dephasing and signal loss in protons moving along these gradients. These rapidly changing gradients induce eddy currents (localised electrical currents) in conductors within the bore, which in turn induce an opposing magnetic field, known as an eddy current-induced off-resonance field (174). The resulting change in magnetic field strength affects
dephasing and, subsequently, signal loss. As a result, eddy current correction is a necessary step in the preprocessing pipeline of DWI data and is included in the current thesis.

2.4. ISSUES REGARDING FALSE POSITIVES IN fMRI RESEARCH

In 2016, Eklund et al. published an article claiming that over 40,000 fMRI studies may be invalid due to inflated false-positive rates well above the nominal familywise error rate of 5% (153). A correction to this statement was later published, omitting details of the quantity of results affected (175); however, the essence of the argument was maintained. These authors found that across three popular software tools (FSL (176), SPM (177) and AFNI (178)), clusterwise inference (which provides an estimate of significance based on the spatial extent of an activated region (179)) for one- and two-sample t-tests gave an astonishingly high degree of false positives (up to 70%) (153). However, when clusterwise inference was subjected to permutation testing, this rate of false positives diminished to the expected 5% level (153). Permutation testing, such as that employed in FSL’s randomise tool, is now commonplace and has been employed in the current thesis. Therefore, the results presented here should not suffer from inflated false positives.

The following chapters of this thesis utilise the MR pulse sequences and preprocessing steps outlined in the current chapter. Additional details relevant to the MR acquisition and preprocessing used for each study are provided within the methods sections of those chapters.
3. INDEPENDENT COMPONENTS ANALYSIS OF RESTING-STATE FUNCTIONAL MRI FROM THE TRS AND CloRes STUDIES

3.1. INTRODUCTION

Post-mortem and in vivo studies of schizophrenia have provided overwhelming evidence for the hypothesis that schizophrenia is fundamentally a disorder of dysconnectivity (129, 180-184). Dysconnectivity hypotheses of schizophrenia posit disruption of large-scale brain networks (130), resulting in abnormal functional integration among spatially disparate brain regions (100).

Resting-state functional magnetic resonance imaging (rs-fMRI) provides an excellent means for identifying patterns of spontaneous brain activity in disorders such as schizophrenia, where diminished cognitive capacity might bias results from task-based imaging studies (180). Functional networks that are active during rest have been shown to correspond to those present during cognitive task performance (158, 180, 185), allowing for investigation of complex functional networks in a task-free environment. These resting state networks (RSNs) also correspond well with the known anatomical networks observed in studies of white matter connectivity (186).

rs-fMRI studies of schizophrenia have revealed abnormalities both within and between functional RSNs (181, 182, 187, 188). Connectivity of the default mode network (DMN; named for its enhanced activation during rest and diminished activation during periods of increased cognitive demand) is altered in people with schizophrenia; however, findings are varied. Selected studies report reduced DMN connectivity while others report increased connectivity both within DMN and between DMN and non-DMN RSNs (180). Whitfield-Gabrieli and colleagues identified abnormally enhanced connectivity within the DMN in individuals with schizophrenia and their first-degree relatives at rest, as well as reduced deactivation during task performance (189). Another study demonstrated increased spatial recruitment to the DMN as well as increased frequency fluctuations, accompanied by a reduction in activity within the medial prefrontal cortex (190). Woodward and colleagues also showed altered spatial topography of the DMN in individuals with schizophrenia, noting greater connectivity between the posterior cingulate cortex and left inferior frontal gyrus, left middle frontal gyrus and left middle temporal gyrus (187).
Although the DMN has held much of the attention in schizophrenia research, abnormalities in other RSNs are also apparent and could be equally influential. Reduced connectivity within the fronto-parietal network (FPN) has repeatedly been shown in studies comparing individuals with schizophrenia and healthy controls. Tu et al. demonstrated cortico-subcortical dysconnection within the FPN in addition to increased functional connectivity between the FPN and regions belonging to default mode and sensorimotor networks (191). Likewise, Baker and colleagues found psychosis-related reductions in FPN connectivity in people with schizophrenia and psychotic bipolar disorder (192).

The salience network, comprising the dorsal anterior cingulate cortex (ACC) and bilateral insulae, has also demonstrated dysfunction in schizophrenia (193). Manoliu et al. reported increased functional connectivity within the dorsal ACC alongside weaker connectivity in bilateral insulae (194) and White and colleagues found significantly weaker functional connectivity between multiple regions of the salience network in people with schizophrenia compared with healthy controls (193). Others have reported similar findings, concluding that disruption in the salience network is associated with impaired deactivation of the DMN in individuals with schizophrenia (195).

Dysfunction of other networks has also been reported (140, 187, 188), including aberrant connectivity within the language network of those affected with schizophrenia (196). Previous studies have found lower within-network connectivity between the left frontal and temporal regions (197), in addition to reduced lateralisation of language processing (198-200) and increased connectivity between language and attention networks (197). An association between reduced language network or frontotemporal connectivity and auditory verbal hallucinations has been described in several studies (201-204), while others have reported no significant change in connectivity between individuals with first-episode psychosis who have developed auditory verbal hallucinations and healthy controls (205).

Evidence suggests that functional dysconnectivity in schizophrenia could arise from the abnormal regulation of synaptic plasticity (100). In particular, disrupted synaptic plasticity could be attributed to the downstream effects of dopamine, acetylcholine and serotonin on N-methyl-D-aspartate (NMDA) receptor-mediated synaptic function (100). NMDA receptors mediate long-term potentiation (LTP) and long-term depression (LTD) via their effects on the functional state and number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors at synaptic junctions (90, 100, 103). Modulating the activity or transport of NMDA receptors is
therefore likely to affect LTP and LTD, causing downstream changes to brain connectivity (100). This is discussed in depth in the introductory chapter of this thesis.

Given the large body of literature identifying disruptions in RSNs in schizophrenia, the modulatory effects of these neurotransmitters on synaptic plasticity and overall functional connectivity might explain how antipsychotic drugs (D₂ and 5-HT₂A receptor antagonists) attenuate symptoms of the disorder. However, while there is general consensus that dysconnectivity is a hallmark of schizophrenia, several studies disagree about the location and direction of dysconnections within specific networks (140). Considering the heterogeneous nature of schizophrenia, it is conceivable that these discrepancies in functional dysconnectivity could be attributed to disrupted neurotransmission. If the functional network connectivity and therefore pathophysiology of schizophrenia is different amongst individuals with the disorder, the likelihood of a single antipsychotic agent or class displaying clinical efficacy for all individuals is improbable. In fact, what we observe is a division of schizophrenia into different response subtypes, with first- and second-generation antipsychotics providing relief for ~70% of individuals (34) and clozapine (the gold-standard treatment for those who fail to respond to first-line therapy) providing relief for only 30% to 70% of its recipients (7, 16-18). Farooq and colleagues proposed subtyping schizophrenia according to treatment response, suggesting that division into subgroups, especially within the scope of research and drug development, could help us better understand and thereby treat this often disabling disorder (29, 38). This concept is supported by work demonstrating differences in dopaminergic and glutamatergic transmission between FLR and individuals who fail to respond to treatment (44, 76).

In this chapter, RSNs were compared between different cohorts of treatment responders from two independent studies using independent components analysis (ICA). The TRS study is described in section 3.2. and the CloRes study in section 3.3. of this chapter. In the TRS study (section 3.2.), RSNs were compared between people with schizophrenia responding to first- or second-generation antipsychotics, clozapine monotherapy or augmented antipsychotic treatment to establish whether differences in RSNs between response groups could act as a potential biomarker for treatment response. It was hypothesised that the three different subgroups would possess unique functional signatures that would distinguish them from the overall cohort.

In the CloRes study (section 3.3.), RSNs were compared between people with schizophrenia who had failed treatment with first-line antipsychotic therapy and were eligible for clozapine and people who had responded well to antipsychotics following diagnosis with a psychotic episode.
or schizophrenia. Based on findings from the TRS study, no differences in RSN connectivity were expected between the two response groups. However, it was necessary to establish whether the results obtained from the initial (TRS) study would persist when participants were receiving the same class of antipsychotic.

### 3.2. Treatment Resistant Schizophrenia (TRS) Study

#### 3.2.1. Methods

**3.2.1.1. Participants**

Details about the recruitment of participants have been described previously (148). Briefly, individuals with a diagnosis of schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) were recruited from inpatient and outpatient mental health clinics within the Waitemata and Counties Manukau districts of Auckland, New Zealand. Participants were required to be between 18 and 45 years of age, clinically stable for at least six weeks prior to study inclusion and receiving atypical antipsychotics for the treatment of schizophrenia. Twenty psychiatrically healthy control participants were also recruited. Exclusion criteria consisted of current or previous diagnosis of any other axis I disorder, history of traumatic brain injury resulting in loss of consciousness greater than three minutes, other significant physical disorders that were uncontrolled and may have impacted brain structure or function, active substance dependence and contraindications for MRI (see appendix 8.8. for MRI safety and consent form). Participants were also immediately excluded from analysis if their functional image or supporting fieldmap image was exceedingly corrupted by motion. The study was approved by the Northern X Regional Ethics Committee and all participants gave informed written consent.

Based on their treatment history and current antipsychotic regimen, participants were enrolled into one of three study arms. Those who were responding well to first-line atypical antipsychotic monotherapy were assigned to the “first-line responder” (FLR) group. Those who had failed at least two previous six-to-eight-week trials of atypical antipsychotics and were now receiving clozapine were assigned to the “treatment-resistant” (TRS) group (see (39, 41, 206) for discussions about the criteria for diagnosing TRS). Finally, participants who had failed at least two previous six-to-eight-week trials of atypical antipsychotics and had also failed a trial of clozapine monotherapy were assigned to the “ultra-treatment-resistant” (UTRS) group. Participants within the UTRS group were all receiving a combination of two antipsychotic drugs; 79% were receiving clozapine as part of an augmented treatment strategy.
Duration of psychosis, Positive and Negative Syndrome Scale (PANSS) scores (151) (see appendix 8.9. for PANSS score sheet) and past and present substance abuse (evaluated using the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST; World Health Organisation) scale) were all assessed at study entry.

Participant demographics were compared across cohorts using IBM SPSS Statistics Version 22. Variables that satisfied assumptions of homoscedasticity (Brown-Forsythe test for equality of variances) and normality (Shapiro-Wilk test for normality) were analysed using a one-way analysis of variance (ANOVA) followed by post-hoc t-tests with correction for multiple comparisons (Bonferroni correction) in cases where the ANOVA demonstrated statistically significant differences between cohorts. For those variables that violated assumptions of normality and/or homoscedasticity, the Kruskal-Wallis (non-parametric) test with pairwise comparisons was employed.

3.2.1.2. DATA ACQUISITION
Structural and resting-state functional magnetic resonance images were acquired using a Siemens Magnetom Skyra 3T scanner located at the Centre for Advanced MRI, University of Auckland, New Zealand. All but 4 of the participants were imaged using a 32-channel head coil. Those with a larger head size who found the 32-channel coil too restricting were imaged using a larger, 20-channel head coil (2 FLR and 2 UTRS). Structural T1-weighted images were acquired using a magnetization-prepared 180-degrees radio-frequency pulses and rapid gradient-echo (MPRAGE) sequence. Acquisition parameters were as follows: Repetition time (TR) 1900 ms; echo time (TE) 2.39 ms; inversion time (TI) 900 ms; flip angle 9°; repetition 1; acceleration (parallel imaging; GRAPPA) factor of 2; field of view (FOV) 230 mm; matrix 256 x 256; voxel size 0.9 x 0.9 x 0.8 mm.

Resting-state functional images were acquired using echo-planar imaging (EPI) with the following parameters: TR 3000 ms, TE 30 ms; echo spacing 0.65 ms (0.62 ms for last 7 participants, following software upgrade); phase-encode direction A >> P; slices 54; volumes 160; FOV 192 mm; acceleration factor of 2; matrix 64 x 64; voxel size 3.0 x 3.0 x 3.0 mm. Participants were asked to lie still with eyes open and concentrate on a fixation cross presented on a screen in front of the scanner. Participants were instructed to think of nothing in particular. Gradient distortion images for functional data were acquired using a gradient echo pulse sequence with the following parameters: TR 655 ms; TE1 4.92 ms; TE2 7.38 ms; voxel size 3.4 x 3.4 x 2.4 mm; phase-encode direction A >> P; FOV 220 mm.
3.2.1.3. IMAGE PREPROCESSING

Image preprocessing and analysis were performed using FMRIB’s software library (FSL) version 5.0.7 (176, 207). Structural images were reoriented to a standard template and brain tissue was extracted from raw image files using FSL’s brain extraction tool (BET) (208). If automatic brain extraction failed to eliminate all non-brain tissue, the excess was removed manually. Magnitude images were subjected to the same process, after which brain-extracted images were eroded to ensure that no voxels containing non-brain tissue remained. Fieldmaps were then created using the fsl_prepare_fieldmap function. Functional image registration to high resolution structural and Montreal Neurological Institute (MNI152) standard space was performed using FMRIB’s Expert Analysis Tool (FEAT). Preprocessing parameters in FEAT were as follows: motion correction = MCFLIRT; b0 unwarping = on; echo spacing = 0.325 (0.31 for last 5 participants); TE = 30; spatial smoothing = 5 mm; global intensity normalisation = on; temporal filtering = off; MELODIC = off; registration to structural image = boundary-based registration; registration to MNI152_2mm = non-linear; warp resolution = 10 mm. Subject motion was assessed and if motion parameters exceeded absolute motion 1.5 mm or root mean square relative motion 0.2 mm, the participant’s data were excluded. Likewise, if registration failed between structural, functional and MNI spaces and could not be amended, participants’ data were removed from further analysis.

ICA-based Automatic Removal Of Motion Artifacts (ICA-AROMA) was used to remove motion artefacts from the fMRI data utilising FSL’s FEAT output as input. (172, 173). White matter and cerebrospinal fluid (CSF) maps were segmented from high resolution structural images using FSL’s FAST (209) and warped to functional space using linear registration to FEAT output (FSL’s FLIRT (210, 211)). Nuisance timeseries were generated from ICA-AROMA output (denoised functional data) using CSF and white matter maps as input. A general linear model (GLM) of residual activity was then generated from the denoised functional data and nuisance timeseries using FSL’s GLM. A temporal mean file of denoised functional data was created, to which highpass temporal filtering (sigma=16.7) was applied, in addition to removal of residual activity attributed to CSF and white matter. Filtered, denoised functional data were then warped to standard space for use in further analysis steps.

3.2.1.4. INDEPENDENT COMPONENTS ANALYSIS AND DUAL REGRESSION

FSL’s Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) was used to decompose data from all participants into different spatial and temporal components using ICA (212). Parameters for MELODIC were as follows: no brain extraction, brain/non-brain threshold = 10, TR = 3, mixture modelling = 0.5. A factor effects GLM was set up
to compare the mean of each group with the average mean across all groups. A post-hoc t-test was designed to determine the direction of the difference between groups for any statistically significant results arising from the ANOVA.

Using FSL’s dual regression function (213), spatial maps from the group-average analysis were used to generate individualised versions of the spatial maps and associated timeseries. An ANOVA comparing group differences over 5000 non-parametric permutations with threshold-free cluster enhancement was performed using FSL’s randomise permutation testing tool following dual regression (213). The threshold for statistical significance was set at $p=0.05$ [family-wise error corrected] adjusted for multiple comparisons by using the null distribution of the maximal voxelwise test statistic.

3.2.2. RESULTS

3.2.2.1. PARTICIPANT DEMOGRAPHICS

MRI scans were obtained from 54 individuals with schizophrenia and 20 healthy controls. Data from three participants were excluded because of excessive movement during scanning and six datasets were excluded because of poor registration. One dataset was excluded because of a corrupted fieldmap image. Of the remaining participants, 15 were healthy controls, 17 were FLR, 14 were responding to clozapine (had TRS) and 16 had failed clozapine and were responding to augmented therapy (had UTRS).

Participant demographics for each cohort are presented in Table 1. There were no statistically significant differences between the four participant groups in terms of age or sex, or between the three treatment groups in terms of duration of illness, or PANSS total or sub-scores. One participant in the TRS cohort failed to complete the ASSIST. Although ASSIST scores were higher in the UTRS and FLR cohorts compared with healthy controls, no significant difference in the proportion of positive drug screens was observed between any of the cohorts. Participants in the UTRS cohort were receiving significantly higher doses of antipsychotics (chlorpromazine equivalents) compared with the other two treatment groups ($p<0.05$).

3.2.2.2. INDEPENDENT COMPONENTS ANALYSIS

ICA identified 38 components from resting-state fMRI, from which 19 were identified as resting-state networks and 19 as noise. After correction for multiple comparisons using false discovery rate (FDR), ANOVA revealed a significant effect of IC10 ($p<0.05$, corrected), identified as the language network (Figure 38).
TABLE 1. PARTICIPANT DEMOGRAPHICS FOR TRS STUDY COHORT, SHOWING MEAN (STANDARD DEVIATION) FOR PARAMETRIC AND MEDIAN (INTERQUARTILE RANGE) FOR NON-PARAMETRIC COMPARISONS. COMPARISONS WERE MADE USING PARAMETRIC TESTS, UNLESS DENOTED BY A †.*ONE PARTICIPANT DID NOT COMPLETE THE ASSIST INTERVIEW; STATISTICAL COMPARISONS ARE PROVIDED ONLY FOR THOSE PARTICIPANTS WHO COMPLETED THE QUESTIONNAIRE.

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=17)</th>
<th>First-line responders (n=17)</th>
<th>Treatment-resistant (n=18)</th>
<th>Ultra-treatment-resistant (n=13)</th>
<th>F and p values</th>
<th>Kruskal-Wallis test statistic and p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age† (years)</td>
<td>36.7 (14.4)</td>
<td>29.2 (10.6)</td>
<td>32.4 (18.2)</td>
<td>33.4 (13.3)</td>
<td>KW=1.81; p=0.61</td>
<td></td>
</tr>
<tr>
<td>Gender† (% female)</td>
<td>17.6</td>
<td>17.6</td>
<td>22.2</td>
<td>23.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of illness† (years)</td>
<td>-</td>
<td>7.3 (7.4)</td>
<td>11.2 (12.1)</td>
<td>13.5 (7.0)</td>
<td>KW=5.38; p=0.07</td>
<td></td>
</tr>
<tr>
<td>PANSS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive†</td>
<td>-</td>
<td>12.0 (11.0)</td>
<td>10.0 (6.3)</td>
<td>10.0 (7.5)</td>
<td>KW=1.61; p=0.45</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>17.4 (5.8)</td>
<td>18.2 (7.0)</td>
<td>19.3 (7.8)</td>
<td>F=0.28; p=0.76</td>
<td></td>
</tr>
<tr>
<td>General psychopathology</td>
<td>-</td>
<td>29.6 (5.5)</td>
<td>28.2 (6.5)</td>
<td>29.1 (4.2)</td>
<td>F=0.26; p=0.77</td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>-</td>
<td>60.5 (10.5)</td>
<td>57.8 (14.8)</td>
<td>60.9 (10.8)</td>
<td>F=0.31; p=0.73</td>
<td></td>
</tr>
<tr>
<td>Current prescribed antipsychotic</td>
<td>-</td>
<td>Risperidone = 6</td>
<td>Clozapine = 18</td>
<td>Clozapine + amisulpride = 4</td>
<td>KW=10.63; p=0.005: p&lt;0.05 for UTRS vs FLR and TRS</td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine equivalents†</td>
<td>-</td>
<td>430.0 (235.8)</td>
<td>391.2 (311.9)</td>
<td>679.3 (596.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASSIST score†</td>
<td>24.0 (28)</td>
<td>46.0 (33)</td>
<td>36.0 (21)*</td>
<td>43.0 (34.5)</td>
<td>KW=15.404; p=0.002</td>
<td>p&lt;0.01 for controls vs FLR and UTRS</td>
</tr>
<tr>
<td>Drug Screen† (% THC positive)</td>
<td>0</td>
<td>17.6</td>
<td>5.6</td>
<td>15.4</td>
<td>KW=3.98; p=0.26</td>
<td></td>
</tr>
</tbody>
</table>
Post-hoc t-tests showed significantly greater connectivity within the paracingulate gyrus (peak coordinates -8 44 26 mm) in people with UTRS compared with healthy controls (p<0.05, corrected; Figure 39). No other comparisons reached statistical significance.

As changes in the language network could affect conceptual disorganisation and auditory verbal hallucinations, symptom scores for PANSS items P2 Conceptual disorganisation and P3 Hallucinations were compared between treatment groups. Non-parametric analysis revealed no significant differences between any of the treatment groups (Kruskal Wallis test: p=0.61 for P2 and p=0.23 for P3).

3.2.3. DISCUSSION
The results of this investigation provide evidence of aberrant spatial connectivity underlying response to antipsychotics in people with schizophrenia. Individuals who failed to respond to both first-line antipsychotics and clozapine monotherapy exhibited increased functional
connectivity within the language network compared with healthy controls. Specifically, connectivity was increased in the left paracingulate gyrus of those with UTRS, possibly reflecting an expansion of the language network compared with healthy controls. No other statistically significant differences were observed between treatment groups.

Disruptions in the language network of those affected with schizophrenia are well recognised (196, 214), with evidence from a familial high-risk study suggesting that abnormalities may be present in unaffected relatives also (215). The observation that individuals with UTRS but not those with TRS or treatment-responsive schizophrenia exhibit aberrant language network connectivity could suggest that disruptions in the language network reflect (or could even predict) poor treatment response. Alternatively, language network abnormalities may increase with worsening prognosis and, given the relatively small sample size, the study may have been underpowered to detect smaller changes in FLR and TRS groups.

In addition to abnormalities in functional connectivity, data from several studies have shown disruptions in structural connectivity within the language network of those with schizophrenia (142, 216-219). Unpublished work from our lab revealed lower fractional anisotropy in people with TRS compared with healthy controls (and those with UTRS) but no significant differences in people with UTRS or in FLR (220). These findings in combination with those of the current study suggest that the changes observed in functional connectivity of the language network in those with UTRS are unlikely to be due to abnormalities in the structural connectivity of this network.

Within the language network, functional connectivity was increased within the left paracingulate gyrus. A recent meta-analysis of fMRI studies investigating the functional organisation of the medial frontal cortex suggested the area corresponding to the paracingulate gyrus is important for reward-driven decision-making and working memory (221). Another review reported that it may be involved in the conceptual representation of language (196). Cognitive data from this study have been published previously (149) and revealed deficits in several domains, including working memory, in people with schizophrenia compared to healthy controls. However, no significant differences between FLR, those with TRS and those with UTRS were found in any cognitive domain (149). The battery of tasks used to interrogate cognitive function in the current study was not specifically designed to investigate decision-making or language functioning, so it is difficult to determine whether the differences observed in the language network are reflected in the functioning of those with UTRS. More work is needed to understand the implications of disturbances in the language network and how these impact treatment response.
Despite alterations in functional connectivity within the language network, differences in PANSS scores for conceptual disorganisation or hallucinations were not observed. These findings are supported by those of Benetti et al. who found no association between auditory verbal hallucinations and structural or functional connectivity within the Perisylvian language network in people with first episode psychosis (205). Similarly, a study by Liemburg et al. revealed no significant correlation between the severity of conceptual disorganisation or hallucinations and connectivity between Broca’s area and the ACC which represent language and attention networks, respectively (197). Given the absence of any association between PANSS scores and network connectivity in the current study, increased connectivity in the language network could serve as a biomarker for UTRS.

As with all fMRI studies, the changes in signal observed do not directly represent changes in neuronal activation but instead represent changes in blood flow, a secondary measure of neuronal activity. It is therefore possible that the effects observed do not represent changes in connectivity but rather changes in synchronisation of blood flow to different regions of the brain. To validate the current findings, a similar investigation using electroencephalography (EEG) or magnetoencephalography (MEG), which provide more direct measures of neuronal activation, would be necessary. Functional connectivity would then be determinable based on the extent of phase synchronisation across regions of the language network.

Another limitation of the current study is its cross-sectional design, which prevents definitive inferences about the link between language network connectivity and antipsychotic response. Specifically, as treatment groups were defined based on clinical responses to, and thereby receipt of different classes of antipsychotic, it becomes difficult to determine whether the changes observed are a result of underlying pathophysiology or drug effects. A meta-analysis of three longitudinal studies investigating the effects of antipsychotic treatment on cerebral blood flow (i.e. fMRI) reported antipsychotic-induced increases in blood flow in the left caudate and decreases in the medial frontal gyrus, cerebellum and right thalamus (222). Given that the effect observed in the current analysis was in the medial frontal gyrus, antipsychotic dose or class may be a contributing factor and must be considered. Further work is needed to confirm whether increased connectivity within the language network is present before treatment with antipsychotics and to establish whether this could be used to predict treatment outcome in antipsychotic-naïve individuals.

Acute administration of δ9-tetrahydrocannabinol (δ9-THC) has been shown to influence functional connectivity in healthy subjects (223). Recreational drug use was not an exclusion
criterion in the current study, though it was assessed using the ASSIST screening test. Statistical comparison revealed significant differences in drug-taking behaviour between controls and FLR as well as those with UTRS. One participant failed to complete the ASSIST questionnaire; however, urine drug screening on the day of testing revealed no significant differences in active drug use between the cohorts, suggesting that results of the present analysis are unlikely to be a consequence of recreational drug (most notably, THC) exposure.

Although no significant differences in duration of illness were observed between the treatment groups, average duration was longest in those with UTRS and may have had some influence on the current findings. Little work has been conducted investigating the effects of illness duration on functional connectivity using traditional analytical methods (180); however, when investigated using graph theoretical techniques, duration of illness was negatively correlated with short-range regional functional connectivity strength in a cohort of individuals with minimally-treated schizophrenia (224). Reduced connectivity among core hubs of the brain has also been associated with longer duration of illness (225). In the current study, duration of illness appeared to lengthen with worsening prognosis. Given the known delays in initiating individuals on clozapine (226) and the additional time period associated with diagnosis of UTRS, the longer duration of illness in this cohort is not unexpected. Moreover, as illness duration and prognosis are unlikely independent, it may be difficult to tease apart the effect of each in the current study. In order to investigate the effect of illness duration on functional connectivity and its impact on the language network, a prospective study may be required.

These findings highlight a novel distinction in language network connectivity between individuals with schizophrenia who require treatment augmentation and healthy controls. Further longitudinal research is needed to determine whether the increase in connectivity observed in those with UTRS is present prior to initiation of treatment with clozapine and eventually to determine whether these markers are present at first-episode.
3.3. CLOZAPINE RESPONSE (CLORES) STUDY

Though no difference in functional connectivity was observed between FLR and those with TRS in the original TRS study, the CloRes study sought to investigate potential differences between responders and non-responders to first-line antipsychotics using a more sophisticated acquisition technique, known as multibanding (see chapter 2). This technique has been shown to improve sensitivity during ICA (152, 171, 227), mainly attributable to the improved separation of signal from noise during data cleaning.

The CloRes study also benefited from similarities in treatment regimens between cohorts. Whereas the TRS study recruited FLR receiving first-line antipsychotic drugs and people with TRS receiving clozapine, all participants in the CloRes study were receiving first-line antipsychotics at baseline. Participants were allocated into FLR and clozapine-eligible groups depending on symptom severity and eligibility for clozapine.

3.3.1. METHODS

3.3.1.1. PARTICIPANTS

Fifteen individuals who were eligible for clozapine and ten FLRs were recruited from inpatient and outpatient mental health clinics within the Waitemata, Counties Manukau and Auckland District Health Boards of New Zealand as well as from mental health support groups and social media (FLR only). Participants in the FLR group were required to be between 18 and 45 years of age, have a history of schizophrenia or a psychotic episode, no history of treatment with clozapine, and be clinically stable on a first-line antipsychotic drug, with a PANSS score of <50 during screening. Participants in the clozapine-eligible group were required to be between 18 and 45 years of age, meet DSM-5 criteria for schizophrenia as confirmed by one of the study psychiatrists, have failed at least two six-week trials with first-line antipsychotic drugs, still be receiving treatment with at least one of the aforementioned antipsychotics, be able to give informed written consent (determined by their treating clinician), and present with persistent positive or negative symptoms contributing to a PANSS score of ≥50 during screening. Exclusion criteria for both groups included diagnosis of another psychiatric disorder, co-morbid neurological illness, self-reported low treatment adherence to current antipsychotic medication, claustrophobia, history of traumatic brain injury resulting in loss of consciousness greater than three minutes, active substance dependence (recreational substance use was not an exclusion criterion) and contraindications to MRI such as a pacemaker, brain aneurysm clip, injury to the eye with a metallic object or fragment and ferrous metal in the body. Participants in the clozapine-eligible group should not have had a trial of clozapine within three months of the
screening visit. The study was approved by the Northern A Regional Ethics Committee (ref 14/NTA/103/AM11) and all participants gave informed written consent.

Participants in the clozapine-eligible group were screened by one of the CloRes study psychiatrists prior to study entry. Screening consisted of a semi-structured interview to confirm diagnosis as well as a PANSS assessment. Participants in the FLR group were screened by a study psychiatrist or the study nurse using the PANSS. Diagnosis was confirmed with the treating clinician or general practitioner. Participants were requested to provide a urine sample for drug screening (Medix Pro-Split Integrated Cup, Multi Drug Screening Test; Sobercheck Ltd) during the study visit. Urine was screened for the presence of amphetamine, methamphetamine, benzodiazepines, cocaine, opiates and THC. One participant in the clozapine-eligible group refused drug screening.

Participant demographics were compared across cohorts using IBM SPSS Statistics Version 22. Variables that satisfied assumptions of homoscedasticity (Brown-Forsythe test for equality of variances) and normality (Shapiro-Wilk test for normality) were analysed using a Student’s t-test. For those variables that violated assumptions of normality and/or homoscedasticity, the Mann-Whitney U test was employed. Z scores were calculated for demographics that were better described using proportions.

3.3.1.2. DATA ACQUISITION
Structural and resting-state functional magnetic resonance images were acquired using a Siemens Magnetom Skyra 3T scanner at the Centre for Advanced MRI, University of Auckland, New Zealand. All participants were imaged using a 32-channel head coil. A structural T1-weighted image was acquired using an MPRAGE sequence with TR 2000 ms; TE 3.48 ms; TI 1010 ms; flip angle 9°; repetition 1; acceleration (GRAPPA) factor of 2; FOV 230 mm; voxel size 0.9 x 0.9 x 0.9 mm.

Gradient distortion (fieldmap) images were acquired using a gradient echo pulse sequence with the following parameters: TR 655 ms; TE1 4.92 ms; TE2 7.38 ms; voxel size 3.4 x 3.4 x 2.4 mm; phase-encode direction A >> P; FOV 220 mm.

Resting-state functional images were acquired using a multiband gradient-echo EPI pulse sequence (University of Minnesota (167)) with TR 735 ms, TE 39 ms; multiband acceleration factor 8; flip angle 52°; echo spacing 0.64 ms; EPI factor 92; phase-encode direction A >> P; slices 64; volumes 410; FOV 220 mm; parallel imaging off; matrix 92 x 92; voxel size 2.4 x 2.4 x 2.4 mm.
Participants were asked to lie still with eyes open and concentrate on a fixation cross presented on a screen in front of the scanner. Participants were instructed to think of nothing in particular.

3.3.1.3. IMAGE PREPROCESSING
Image preprocessing and analysis were performed using FSL version 5.0.7 (176, 207). Structural images were reoriented to a standard template and brain tissue was extracted from raw image files using FSL’s BET (208). If automatic brain extraction failed to eliminate all non-brain tissue, the excess was removed manually. Magnitude images were subjected to the same process, after which brain-extracted images were eroded to ensure that no voxels containing non-brain tissue remained. Fieldmaps were then created using the fsl_prepare_fieldmap function. Functional image registration to high resolution structural and MNI152 standard space was performed using FEAT. Preprocessing parameters in FEAT were as follows: motion correction = MCFLIRT; b0 unwarping = on; echo spacing = 0.64; TE = 39; spatial smoothing = 5 mm; global intensity normalisation = on; temporal filtering = off; MELODIC = off; registration to structural image = boundary-based registration; registration to MNI152_2mm = non-linear; warp resolution = 10 mm. Slice order correction was also performed at this stage (see appendix 8.10. for determination of slice order from multiband sequence). Subject motion data was recorded; however, no subject was excluded due to excess motion. Instead, ICA-AROMA was used to remove motion artefacts from the fMRI data utilising FSL’s FEAT output as input. (172, 173). White matter and CSF maps were segmented from high resolution structural images using FSL’s FAST (209) and warped to functional space using linear registration to FEAT output (FSL’s FLIRT (210, 211)). Nuisance timeseries were generated from ICA-AROMA output (denoised functional data) using CSF and white matter maps as input. A GLM of residual activity was then generated from the denoised functional data and nuisance timeseries using FSL’s GLM. A temporal mean file of denoised functional data was created, to which highpass temporal filtering (sigma=16.7) was applied, in addition to removal of residual activity attributed to CSF and white matter. Filtered, denoised functional data were then warped to standard space for use in further analysis steps.

3.3.1.4. INDEPENDENT COMPONENTS ANALYSIS AND DUAL REGRESSION
FSL’s MELODIC was used to decompose data from all participants into different spatial and temporal components using ICA (212). To prevent overfitting of the data, the number of components was limited to 20. Parameters for MELODIC were as follows: no brain extraction, brain/non-brain threshold = 10, TR = 0.735, mixture modelling = 0.5. A cell means GLM was set up to compare the means of each group. Though differences in the proportion of male and female participants between groups were not statistically significant, gender was added as a
covariate to account for the low number of female participants included in the clozapine-eligible group.

Using FSL’s dual regression function (213), spatial maps from the group-average analysis were used to generate individualised versions of the spatial maps and associated timeseries. T-tests comparing group differences over 5000 non-parametric permutations with threshold-free cluster enhancement were performed using FSL’s randomise permutation testing tool (213). The threshold for statistical significance was set at $p=0.05$ [family-wise error corrected] adjusted for multiple comparisons by using the null distribution of the maximal voxelwise test statistic.

3.3.2. RESULTS

3.3.2.1. PARTICIPANTS
Fifty individuals with schizophrenia or psychotic disorder were screened for inclusion and 25 (10 FLR and 15 who were eligible for clozapine) completed the study protocol. Participant demographics are presented in Table 2.

Three participants in the FLR group and two participants who were eligible for clozapine tested positive for THC. One member of the clozapine-eligible group refused to provide a sample for drug screening and so results have not been included in the participant demographics table.

Mean (standard deviation) absolute and relative root mean squared values for in-scanner motion were 0.29 mm (±0.21) and 0.14 mm (±0.06), respectively, for FLR and 0.27 mm (±0.20) and 0.11 mm (±0.09), respectively, for those who were clozapine-eligible. No significant differences in motion were observed between response groups ($p=0.79$ and $p=0.37$ for absolute and relative motion, respectively). Two participants in the FLR group and one participant in the clozapine-eligible group had relative motion greater than 0.2 mm; however, due to small study numbers, they were not excluded from the analysis.

3.3.2.2. INDEPENDENT COMPONENTS ANALYSIS
Individuals who were eligible for clozapine exhibited enhanced functional connectivity within IC13, identified as the sensorimotor network (SMN), compared with FLR ($p<0.05$, corrected). This network comprised areas of the motor cortices, primary somatosensory cortices, bilateral insulae, central opercular cortices, bilateral crus VI of the cerebellum and left thalamus (Figure 40).
TABLE 2. PARTICIPANT DEMOGRAPHICS FOR CLORES STUDY PARTICIPANTS, SHOWING MEAN (STANDARD DEVIATION) FOR PARAMETRIC AND MEDIAN (INTERQUARTILE RANGE) FOR NON-PARAMETRIC COMPARISONS. COMPARISONS WERE MADE USING PARAMETRIC TESTS, UNLESS DENOTED BY A †.

<table>
<thead>
<tr>
<th></th>
<th>FLR (n=10)</th>
<th>Clozpine-eligible (n=15)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years; mean(sd))</td>
<td>29.1 (8.35)</td>
<td>25.4 (5.1)</td>
<td>t=1.253; p=0.231, equal variances not assumed</td>
</tr>
<tr>
<td>Gender (% female)†</td>
<td>40</td>
<td>13.3</td>
<td>Z score=-1.529; p=0.126</td>
</tr>
<tr>
<td>Duration of illness (years)†</td>
<td>5.5 (5.9)</td>
<td>5.1 (4.4)</td>
<td>Mann Whitney U=72; p=0.892</td>
</tr>
<tr>
<td>Age of onset (years)†</td>
<td>23.9 (5.5)</td>
<td>21.4 (3.4)</td>
<td>Mann Whitney U=95; p=0.285</td>
</tr>
<tr>
<td>PANSS score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive†</td>
<td>8 (3)</td>
<td>19 (11)</td>
<td></td>
</tr>
<tr>
<td>Negative†</td>
<td>11 (6)</td>
<td>20 (17)</td>
<td></td>
</tr>
<tr>
<td>General psychopathology</td>
<td>20.9 (2.8)</td>
<td>37.7 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40.1 (6.3)</td>
<td>79.7 (17.0)</td>
<td></td>
</tr>
<tr>
<td>Current prescribed antipsychotic</td>
<td>Amisulpride=1</td>
<td>Amisulpride=1</td>
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<tr>
<td></td>
<td>Aripiprazole + olanzapine (low dose)=1</td>
<td>Aripiprazole=2</td>
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<tr>
<td></td>
<td>Olanzapine=4</td>
<td>Olanzapine=3</td>
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<tr>
<td></td>
<td>Quetiapine=1</td>
<td>Olanzapine + quetiapine=1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Risperidone=3</td>
<td>Palideridone=3</td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine equivalents†</td>
<td>375.3 (304.2)</td>
<td>508.6 (377.7)</td>
<td>Mann Whitney U=46; p=0.115</td>
</tr>
</tbody>
</table>

Those who were clozapine-eligible had additional connectivity in the precuneus (probabilistic anatomical label, Harvard-Oxford Cortical Structure Atlas: 29% precuneus cortex, 24% intracalcarine cortex, 22% supracalcarine cortex, 10% lingual gyrus, 3% cuneal cortex, 1% cingulate gyrus, posterior division) compared with FLR (cluster size: 11 voxels; peak MNI coordinates -2, -66, 14 mm; Figure 41). No other comparisons reached statistical significance and no effect of gender was observed.
3.2.3. DISCUSSION
Results of the present analysis revealed a difference in SMN connectivity between people who were eligible for clozapine and FLR. Specifically, those who were clozapine-eligible showed increased connectivity between areas of the SMN and the precuneus compared with FLR. These findings are supported by previous work by Kaufmann et al. (228), which described an increase in connectivity between SMN and DMN components in people with schizophrenia compared with healthy controls. Of particular relevance to the current finding was their observation of increased connectivity between a component of the DMN comprising the precuneus and one of the SMN comprising the motor and sensory cortices (228). The precuneus is a functional core of the DMN (229-231) and (along with the posterior cingulate cortex) is thought to be the most densely connected functional hub of the brain during rest (232). It is involved in a number of cognitive tasks, including deductive reasoning, mental imagery and navigation, music processing,
episodic memory retrieval and, most interestingly, self-processing and social stimulus processing (233, 234). With regard to processing of first-person versus third-person perspective, the precuneus shows stronger activation when individuals imagine third-person simulations (reviewed in (233)). Its increased functional connectivity with the SMN in people who are eligible for clozapine could represent increased crosstalk between the DMN and SMN in this population and reflect a misattribution of self-generated thoughts or feelings to external influences.

Among other functions, the SMN is involved in the production of speech (235-237), somesthesis (158) and somatosensory gating (238), all of which may be affected in schizophrenia. One hypothesis suggests that the symptoms observed in individuals with schizophrenia could be attributed to disruptions in efference copy signals, which predict reafferent sensory feedback and modulate responses of the sensory cortex (124). Under normal conditions, efference copy signals releasing glutamate (239) allow sensory reafferents from motor and other outputs to be recognised as self-generated (e.g. hearing one’s own voice or feeling one’s own arm move and recognising that these experiences are self-induced) (124, 240, 241). Failure of these copy signals to warn the sensory cortex of incoming information in people with schizophrenia is proposed to account for the auditory hallucinations and delusions of passivity experienced by some individuals (242, 243). As part of this mechanism, the cerebellum is thought to generate internal predictions of feedback and, when input is congruent to these predictions, modify responses in the parietal cortex (124, 244). Connections between the cerebellum and posterior parietal cortex are well documented (245) and may specifically involve lobule VI of the cerebellum (246) as was observed in the current study. Therefore, the present observation of increased functional connectivity between the SMN and the precuneus in people who were eligible for clozapine compared with FLR could result from disrupted efference copy signalling causing changes to cerebellar connectivity with the parietal cortex. Whitford et al. suggested that abnormalities in myelination could result in disturbances in efference copy signalling (247), though of the frontal fasciculi as opposed to more posterior or thalamic tracts. This hypothesis as well as the likely impact of structural connectivity on the functional results will be further explored in chapter 5.

Given that the comparison group of FLR in the current study were experiencing little or no symptoms at the time of participation, the difference in SMN connectivity observed here is suggestive of a feature specific to resistant symptoms of schizophrenia. As those who are eligible for clozapine have failed to respond to treatment with atypical antipsychotic drugs, all of which target D₂ (and 5-HT) receptors, these results are suggestive of a malfunction in some neurotransmitter system other than dopamine. It may instead be a consequence of primary
disruptions in NDMA receptor function or abnormal modulation of synaptic plasticity by acetylcholine or some other neurotransmitter system in those with clozapine-eligible schizophrenia. This provides support for the hypothesis of hyperdopaminergic and normodopaminergic subtypes of schizophrenia proposed by Howes & Kapur (81). Alternatively, these findings may represent differing patterns of functional specialisation between cohorts. Woodward et al. previously reported differences in clustering of regions after ICA between healthy controls and those with schizophrenia and showed that the increased connectivity they observed within the DMN of those with schizophrenia overlapped with the executive control network in healthy controls (187). As such, increased connectivity in the present study could represent an alteration in the spatial topography of the SMN, reallocating part of the precuneus to this network.

The current study benefited from several strengths, including similarities in duration of illness, age of onset, drug dosage (measured in chlorpromazine equivalents) and drug class between cohorts. These features reduced the demographic variability between groups, increasing the likelihood that the observed differences in functional connectivity were due to underlying differences in pathophysiology. Due to the cross-sectional nature of the study, however, we were unable to determine whether differences observed were a result of the successful response to antipsychotic treatment in FLR (and not in those who were eligible for clozapine) or whether these differences existed prior to treatment initiation. Nevertheless, the effect of antipsychotics on functional connectivity should not be an issue here, given the similarity in drug class and chlorpromazine equivalents at the time of scanning.

The study also employed advanced imaging techniques, specifically 3T MRI and multiband image acquisition, to improve data cleaning and ICA (152, 171, 227). The utilisation of multiband imaging may account for the differences in functional connectivity observed between FLR and those who were clozapine-eligible in the current study while no differences were found in the previous (TRS) study.

No differences in absolute or relative motion were observed between the response groups; however, no participants were excluded due to excess motion as they were for the previous study. This was due to small study numbers, where exclusion of data would have been costly to the overall analysis. Instead, ICA-AROMA was used to remove motion-related noise (172). ICA-AROMA has been shown to successfully remove components associated with motion, with minimal effect to brain-related activation (172, 173). Consequently, these findings are unlikely to be due to differences in motion between the two cohorts.
Though no psychiatrically healthy controls were included in the CloRes study, the inclusion of such a cohort would have added a confounding factor of antipsychotic drug exposure that would have been impossible to separate from history of psychotic episodes or schizophrenia. As an alternative, a group of FLR who had little or no symptoms at the time of scanning was included, providing a better comparison for the group who were receiving first-line antipsychotics but remained symptomatic.

Due to difficulties with recruitment, the ratio of male to female participants was not matched between cohorts. As a result, the FLR group had more females than the clozapine-eligible group (40% versus 13% female). Although gender was added as a covariate in the GLM and showed no significant effect on connectivity, previous work by Zhang and colleagues (248) demonstrated differences between male and female subjects with regard to connectivity of the precuneus and must not be ruled out as a potential rationale for the findings presented in this chapter. Recruitment of participants in both groups is ongoing and should eliminate any potential gender bias affecting the current analysis.

Acute administration of δ9-THC has been shown to influence functional connectivity in healthy subjects, particularly within the SMN and dorsal visual stream (223). Of specific concern to the current study, administration of δ9-THC increased connectivity between the cerebellum and SMN (223). Within the current cohort, three individuals in the FLR group and two in the clozapine-eligible group tested positive for THC; although, one individual in the clozapine-eligible group refused screening so a direct comparison was not made. Given that the difference observed in connectivity of the SMN was in the opposite direction to that found in (223), it is unlikely that the results presented here are a consequence of THC exposure, though long-term exposure cannot be ruled out as a confounding factor.

These findings highlight a difference in SMN connectivity between FLR and people who are eligible for clozapine, lending support for the dysconnectivity hypothesis of schizophrenia, in addition to theories of abnormal efference pathway connectivity in this population. Further research is needed to determine whether the differences observed in the current study are present in drug-naive individuals and whether connectivity within the SMN can be used to predict outcome to first-line treatment in people with first-episode psychosis or schizophrenia.

Further discussion of the results presented in this and the following chapters (including an interpretation of the results en bloc) is provided in chapter 7.
4. FUNCTIONAL NETWORK CONNECTIVITY AS A BIOMARKER OF TREATMENT RESPONSE IN SCHIZOPHRENIA (DATA FROM THE TRS STUDY)

In chapter 3, it was established that independent components analysis (ICA) is a useful tool for identifying differences in functional connectivity between subtypes of schizophrenia based on the success of drug treatments. Nevertheless, there may be information stored within the resting-state functional data that traditional (low-dimensional) ICA is unable to identify. By shifting away from low-dimensional ICA and seed-based correlation methods and toward high-dimensional graph theory-based analysis, a richer examination of the network connections is attainable (249). In this chapter, graph theory-based techniques were used to investigate whether functional connectivity on a global network level could distinguish between responders and non-responders to antipsychotic medication, using data from the Treatment Resistant Schizophrenia (TRS) study.

Special thanks to Dr Roger Tait, University of Cambridge, United Kingdom, for image preprocessing and generation of the correlation matrices.

4.1. INTRODUCTION

Functional connectivity can be defined as the statistical association, or temporal correlation, between spatially remote neurophysiological events (or timeseries) (250, 251). Using graph theory to decipher the complex mathematical relationships between functionally distinct brain regions (nodes) and their associated functional connections (edges), the new field of functional connectomics endeavours to map the functional connectivity of the human brain (249).

In contrast to the more traditional methods of functional magnetic resonance imaging (fMRI) analysis, connectomics considers the brain as a network, permitting investigation of the brain as an integrated system, rather than a collection of individual components (252). Consistent with many other networks (such as social networks and transport networks) the human brain demonstrates properties of small-worldness, modularity and hierarchy (253-256) and is heavily influenced by basic network characteristics such as number of nodes and edges and the number of edges attached to each node (degree) (255). Within the architecture of a network, selected nodes become more highly connected than others. These nodes can be identified as hubs (illustrated in Figure 42), defined as nodes with many edges or with edges that place them in central positions to facilitate communication within a network (257). These hubs are essential
for integrating information between densely linked groups of nodes, known as clusters or modules (see Figure 43) (143, 255).

**FIGURE 42.** REPRESENTATION OF NODES (SPHERES) AND EDGES (LINES) SHOWING FEATURES OF DIFFERENT TYPES OF HUB NODES. HUBS, SHOWN IN GREEN, YELLOW AND BLUE, ARE THOSE NODES THAT JOIN MANY EDGES AND PROVIDE PATHWAYS FOR COMMUNICATION ACROSS THE NETWORK. PROVINCIAL HUBS (BLUE) POSSESS MANY LINKS WITH NODES IN THEIR OWN MODULE (SEE FIGURE 43 FOR ILLUSTRATION OF MODULES); CONNECTOR HUBS (YELLOW) LINK WITH NODES IN THEIR OWN MODULE AS WELL AS OTHER MODULES. IN THE BRAIN, SOME HUBS (RICH CLUB HUBS; GREEN) ARE MORE DENSELY INTERCONNECTED THAN PREDICTED ON THE BASIS OF THEIR DEGREE ALONE. THESE HUBS ACCOUNT FOR A DISPROPORTIONATELY LARGE SHARE OF THE BRAIN'S TOTAL WIRING LENGTH DESPITE THEIR SMALL NUMBERS (143). THIS MAKES THEM ‘COMPUTATIONALLY’ EXPENSIVE, GRANTING THEM THE APTLY CHOSEN NAME, ‘RICH CLUB’.

**FIGURE 43.** DEPICTION OF NODES ORGANISED INTO MODULES (LEFT) AND THE CONTRIBUTION OF INTRA AND INTERMODULAR CONNECTIONS TO THE PARTICIPATION COEFFICIENT (RIGHT). NETWORK MODULES ARE GROUPS OF DENSELY CONNECTED NODES WITH ONLY SPARSE CONNECTIONS TO NODES IN OTHER MODULES. THE RELATIVE PROPORTIONS OF INTRA AND INTERMODULAR CONNECTIONS A NODE MEDIATES DETERMINES ITS PARTICIPATION COEFFICIENT (144).
Modules represent the segregation of nodes within networks, as nodes within a module are very highly connected to other nodes within the same module but not to those in other modules (143, 255). Within the human brain, these modules exhibit hierarchical characteristics, whereby a ‘top-level’ module may consist of numerous lower level sub-modules, containing fewer nodes with strong links (256).

The connectivity of hubs to other nodes can be described in terms of a participation coefficient (Figure 43). Hubs that primarily link nodes within their own module have a low participation coefficient and are known as provincial hubs, whereas hubs that primarily link nodes across different modules have a high participation coefficient and are known as connector hubs (143). The existence of hubs supports integration of information within and between modules and contributes to the small-world characteristics of the human brain (143). Small-worldness refers to a pattern of network configuration in which nodes without direct links to each other are likely to be connected to the same neighbouring node. These networks are significantly more clustered than random networks yet possess approximately the same path length (a measure of integration, defined as the length of a sequence of nodes and links between a pair of regions), making them simultaneously highly segregated and integrated (see Figure 44) (255). The anatomical brain network (connectome) possesses both of these characteristics and is said to have small-world topology; the functional brain network, however, is less well integrated and therefore has weaker small-world attributes (255).

![Random network vs Small-world network](image)

**FIGURE 44. RANDOM VERSUS SMALL-WORLD CONNECTIVITY.** WITHIN A RANDOM NETWORK, THE NUMBER OF LINKS PER NODE AS WELL AS THE PROXIMITY OF NODES THAT LINK IS RANDOM. SMALL-WORLD NETWORKS POSSESS MAINLY CLOSE-RANGE LINKS IN ADDITION TO A SMALL NUMBER OF LONG-DISTANCE CONNECTIONS AND MORE CLOSELY RESEMBLE NETWORKS IN THE REAL WORLD.
Network analyses of the human brain have consistently identified anatomical hubs in the precuneus, anterior and posterior cingulate cortex, insular cortex, superior frontal cortex, temporal cortex and lateral parietal cortex (143, 258, 259). Functional hubs also exist and focus mainly in the ventral and dorsal precuneus, posterior and anterior cingulate gyrus, ventromedial frontal cortex and inferior parietal cortex (143, 232, 257). These hubs have been found to coincide with anatomical lesions in a number of brain disorders, including schizophrenia (260). With specific regard to schizophrenia, functional abnormalities identified during task-based fMRI analyses, including both over- and under-activations compared with healthy controls, have been shown to coincide with hubs, particularly those belonging to the rich club (see Figure 42 for a description of rich club hubs) (144).

Analysis of functional networks differs from that of anatomical networks. This is due to the global integration of functional networks, whereby every node is connected to every other node in the network. Consequently, measures such as degree and participation coefficient are unable to accurately describe these networks. Instead, connection strength and connection diversity are employed as measures of centrality in functional networks (261). These measures have clear parallels to, but also important distinctions from, their anatomical counterparts. Whereas within-module degree can describe the relative importance of a node within its own module in an anatomical network, the global integration of functional networks means that within-module strength does not afford the same insight (261). Rather, centrality of functional networks is best described using the total strength of all correlations associated with a node, whereby the strengths of positive and negative correlations are weighted differently (261). In a similar way, participation coefficient cannot accurately describe the functional integration of a node with other communities; therefore connection diversity is used to describe nodal integration, taking into consideration the strength of a node within its own module (261).

Graph theoretical measurements of anatomical brain networks in schizophrenia have revealed that they are less hierarchical, less clustered, less efficiently wired and exhibit less small-world topology than those of healthy controls (181, 205, 252, 262, 263). Network analysis has also revealed reduced strength and greater diversity of functional connectivity in those with schizophrenia, associated with less clustered and hub-dominated topology (181). The schizophrenia network is, however, associated with greater robustness to random attack (removal of nodes in descending order of degree), suggesting that there may be some evolutionary benefit to the architecture of this network (181).
Network-based statistics (described in detail in section 4.2.5.) provide another useful tool for investigating the functional organisation of the human brain (264) and have been used to investigate differences between healthy controls and people with schizophrenia. Zalesky et al. reported a sub-network of 40 pairwise functional connections that were significantly weaker in those with schizophrenia compared with healthy controls (264). This sub-network comprised fronto-temporal, occipito-temporal, supplementary motor area-temporal and -occipital connections as well as connections within the cingulum (264), consistent with previously reported abnormalities in these regions (265-267). A study by Cocchi et al. employing the same analytical technique identified three sub-networks exhibiting differential connectivity in people with schizophrenia and reported that although structure-function relationships were disrupted in one sub-network (lower correlation between functional connectivity and white matter integrity), the remaining two sub-networks exhibited no such disruption (268).

Although graph theory is a comparatively new approach to examining structural and functional connectivity, findings have proven invaluable to our understanding of the human brain as a network. The observation that brain architecture bears resemblance to other biological and non-biological networks yet exhibits distinct differences in terms of rich-club organisation (269) has increased our understanding of the importance of specific connections within the biology of health and disease. Network organisation is likely to be influenced by disturbances in structural or functional connectivity and may therefore differ between individuals exhibiting different forms of disruption. Modulation of NMDA receptor-mediated synaptic plasticity by neurotransmitters such as dopamine, serotonin and acetylcholine is hypothesised to account for the functional dysconnectivity observed in individuals with schizophrenia (100). Should the underlying mechanisms responsible for this modulation differ between response subtypes of the disorder, network organisation may be affected to varying degrees or in a different manner in those who respond to antipsychotics compared to those who do not (i.e. are resistant). Given the growing body of literature indicating disrupted network connectivity in people with schizophrenia, in addition to the differential connectivity observed between response subtypes in chapter 3, it was hypothesised that network connectivity, as assessed by connection strength, diversity and network-based statistics, would detect differences between response subtypes of schizophrenia and healthy controls. As functional connectivity (assessed using ICA) was disrupted in individuals with UTRS but not in those with treatment-resistant schizophrenia (TRS) or first-line responders (FLRs), it was anticipated that those with ultra-treatment-resistant schizophrenia (UTRS) would exhibit the greatest degree of dysconnectivity, potentially in regions
associated with the language network; although disruptions to network organisation in FLR and those with TRS were also expected.

4.2. METHODS

4.2.1. PARTICIPANTS
Recruitment details and inclusion/exclusion criteria for the TRS study are presented in chapter 3. Briefly, 20 psychiatrically healthy controls, 20 FLR, 20 individuals with TRS receiving clozapine and 20 individuals with UTRS receiving augmented antipsychotic therapy were enrolled in the study. The study was approved by the Northern X Regional Ethics Committee and all participants gave informed written consent.

Participant demographics were compared across cohorts using IBM SPSS Statistics Version 23. Variables that satisfied assumptions of homoscedasticity (Brown-Forsythe test for equality of variances) and normality (Shapiro-Wilk test for normality) were analysed using a one-way analysis of variance (ANOVA) followed by post-hoc t-tests with correction for multiple comparisons (Bonferroni correction) in cases where the ANOVA demonstrated statistically significant differences between cohorts. For those variables that violated assumptions of normality and/or homoscedasticity, the Kruskal-Wallis (non-parametric) test with pairwise comparisons was employed.

4.2.2. DATA ACQUISITION
Details of structural and resting-state (rs) MRI acquisition parameters for the TRS study are reported in chapter 3. Structural T1-weighed images were acquired using an MPRAGE sequence and resting-state functional images were acquired using an EPI sequence with parallel imaging factor 2. All scans were conducted using a 3T Siemens Magnetom Skyra scanner.

4.2.3. IMAGE PREPROCESSING
Structural data were processed with the Advanced Normalization Toolkit (270). Processing steps included initial N4 bias correction of raw structural images; brain extraction using a hybrid segmentation/template-based strategy; construction of a study-specific template and segmentation priors based on all participants in the cohort; alternation between study-specific prior-based segmentation and “pure tissue” posterior probability weighted bias correction using Atropos and N4; DiReCT-based cortical thickness estimation; normalization to a study-specific template and cortical parcellation using the AT116 anatomical parcellation template.

Preprocessing of functional data was conducted using the BrainWavelet Toolbox (speedypp; www.brainwavelet.org) (271). Preprocessing steps have previously been reported (272) and
included slice time correction using seventh order Lagrange polynomial interpolation; rigid-body head movement correction to the first volume using fifth order polynomial interpolation to estimate the realignment parameters (three displacements and three rotations); affine co-registration to the skull-stripped structural image using a grey matter mask; registration to the MNI152_T1_1mm template in Talairach space (TT_N27); and spatial smoothing (6 mm full width at half maximum). Data were then subjected to additional processing for the correction of head motion. This included unsupervised timeseries despiking in the wavelet domain; signal regression of the six motion parameters estimated during rigid-body head movement correction, their first order temporal derivatives and the cerebrospinal fluid (CSF) signal; high pass frequency filtering above 0.02 Hz.; and spatial smoothing (6 mm full width at half maximum Gaussian kernel).

Motion-corrected fMRI data were subjected to parcellation and divided into 116 parcels based on the AFNI TT N27 EZ ML atlas. For each individual, the mean timeseries was extracted from each of the 116 anatomically parcellated regions (nodes). The extracted blood oxygen-level dependent (BOLD) signals were decomposed into four frequency bands by wavelet transform (251): scale 1, 0.125–0.25 Hz; scale 2, 0.06–0.125 Hz; scale 3, 0.03–0.06 Hz; scale 4, 0.02–0.03 Hz (273). Based on evidence from previous rs-fMRI studies demonstrating most salient differences between healthy controls and people with schizophrenia at frequencies in the range of 0.06 to 0.125 Hz (181, 273), the scale 2 wavelet was selected for comparison in the current study.

The strength of a connection between two nodes was the Pearson’s correlation coefficient of the wavelet coefficients. Weighted, undirected correlation matrices were derived and further analysis was undertaken using Matlab 2015a (MathWorks, USA). All self-connections were removed from correlation matrices prior to analysis. Examples of correlation matrices from individual participants are depicted in Figure 45a. Weights represent the magnitude of correlational interaction between nodes, with positive weights (red) representing positive functional correlations and negative weights (purple) representing anticorrelations between nodes (255).

4.2.4. GRAPH-THEORETICAL ANALYSIS

Functional networks may be characterised by their modular (or community) structure, revealed by subdividing the network into groups of nodes that share maximum within-group links and minimum between-group links (see Figure 43) (274). The degree to which a network can be segregated into such modules is termed the modularity (255).
Connection strength is the sum of all functional connections associated with a node and provides information about the influence of a node in its network. The integration (and possible influence) of a node with those outside of its module is measured using the diversity coefficient. Nodes with high diversity coefficients tend to facilitate global intermodular integration; whereas nodes with low diversity coefficients are characterised by more intramodular connections. Both positive and negative diversity can be determined, providing information about correlated and anticorrelated functional connectivity, respectively.

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**FIGURE 45. PREPROCESSING STEPS FOR DETERMINING CONNECTION DIVERSITY FOR EACH PARTICIPANT AT EACH NODE.**

Community structure, modularity, connection strength and diversity for each individual in the current dataset were computed in Matlab 2015a using the Brain Connectivity Toolbox ([brain-connectivity-toolbox.net](http://brain-connectivity-toolbox.net)) (255). Total and nodal connection strengths for positive and negative weights were determined using the strengths algorithm for signed networks, as outlined in Rubinov and Sporns (261). Group comparisons were conducted using IBM SPSS Statistics Version 23. Data violated assumptions for normality, so the non-parametric Kruskal Wallis test with

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pairwise comparisons was employed. Corrections for multiple comparisons were performed within each test, using the false discovery rate (FDR). Post-hoc corrections for multiple comparisons were performed using Bonferroni’s correction.

Community structure was established by subdividing the network into non-overlapping groups of nodes that maximised the number of within-module edges and minimised the number of between-module edges for each participant (see Figure 45b). Modularity was also determined at this time. An algorithm designed to designate unequal importance to positive and negative weights, thereby reducing the contribution of negative weights to the modularity/partition equation, was employed (261). This technique has been shown to improve the accuracy of modularity output in simultaneously positively and negatively weighted networks (261). For illustrative purposes, the value of gamma (γ), which regulates the size of modules, was adjusted over a range of values; however, only data for γ=1 was used for determining connection diversity. Data violated assumptions of normality and so modularity values for each group were compared using the Kruskal Wallis test for non-parametric measures in SPSS. Post-hoc pairwise comparisons were performed, using Bonferroni’s correction for multiple comparisons.

Connection diversity for each node was established by applying the community structure to the weighted, undirected correlation matrix. Both positive and negative connection diversity values were determined at each node for every participant (Figure 45c) using the normalised Shannon entropy (261). Group comparisons were made using SPSS. Homoscedastic variables were assessed using an analysis of variance (ANOVA). Variables that violated assumptions of homoscedasticity were assessed using the Kruskal Wallis test for non-parametric measures. Corrections for multiple comparisons were performed individually across positive and negative diversity measures using the FDR. Post-hoc pairwise comparisons were corrected for multiple comparisons using Bonferroni’s correction.

4.2.5. NETWORK-BASED STATISTICS
In addition to investigating graph theoretical measures, this chapter sought to determine whether response to antipsychotic treatment can be described on a network level using network-based statistics (264). This technique allows for identification of pairwise associations (manifesting as links or connections between nodes) that differ between groups (264), making it an ideal tool for the current study.

Comparisons of functional network organisation were performed in Matlab 2015a using the Network Based Statistic (NBS) Toolbox (264). NBS seeks to identify arrangements of node-to-node connections (structures) formed by links that surpass a given threshold (264). The
topological extent of each structure is then used to determine its significance (264). Permutation testing (using random assignment of each subject to a group) then ascribes a p value (controlled for the family-wise error; FWE) to each structure based on its size (264). The total number of permutations for which the size of the permuted structure is greater than the size of the actual structure determines the p value for that arrangement of connections (264). Using NBS, a one-way ANOVA with contrasts (α≤0.05) was performed to establish whether any difference existed between the groups. Data were permuted 5000 times using the network-based statistics method, applying a threshold of 4.9. A value of 4.9 was selected as it showed a substantial degree of dysconnection while still granting partition of dysconnections into meaningful sub-networks. Networks were determined based on their extent (i.e. the number of connections they comprised). Post-hoc one-tailed t-tests (α≤0.05, corrected for 2 multiple comparisons using the FDR) were performed to reveal the directionality of any differences established during an ANOVA (again, the network-based statistic method with 5000 permutations was employed). Brain networks were visualized with the BrainNet Viewer (http://www.nitrc.org/projects/bnv/) (275).

4.3. RESULTS
4.3.1. PARTICIPANT DEMOGRAPHICS
Data from 17 healthy controls, 18 FLR, 18 individuals with TRS and 16 individuals with UTRS are included in the analysis. Due to variations in preprocessing between this study and that reported in chapter 3 (ICA of the TRS study cohort), participant inclusion varies between the two chapters and care should be taken when extrapolating results across techniques.

Demographic data are presented in Table 3. One individual in the TRS cohort did not complete the ASSIST assessment. No difference was observed between groups for any other demographic characteristic.

4.3.2. GRAPH THEORETICAL MEASURES
Node positions are shown in Figure 46. The strength of functional connections was compared using both positive and negative weights, representing correlations and anticorrelations, respectively. Across the entire network, individuals with UTRS exhibited weaker positive connectivity and stronger negative connectivity than healthy controls (see Table 4). A similar pattern emerged when comparing positive and negative connection strengths of individual nodes across treatment groups. Individuals with UTRS had lower positive connection strength than healthy controls at many frontal and parietal nodes, in addition to a select few cerebellar nodes. In addition, one cerebellar node was associated with lower positive connection strength.
in all schizophrenia cohorts compared with controls (Figure 47). Negative connection strength was greater in many nodes of the frontal lobe, left parietal cortex and cerebellum in those with UTRS compared with healthy controls (Figure 48). FLR also had greater negative connection strength at frontal and cerebellar nodes. The only node demonstrating differences in connection strength between schizophrenia cohorts was a right frontal node, for which individuals with UTRS exhibited greater negative connection strength than those with TRS.

Functional networks from all participants demonstrated modular community structure, with networks from all groups partitioning into a median of three modules. When considering functional networks across different individuals, partitions varied considerably, though tended to result in posterior (including cerebellar and occipital nodes), frontal and temporal/parietal communities. The degree to which each network could be subdivided into clearly delineated non-overlapping modules was assessed using the modularity index Q* (261). Non-parametric analysis revealed higher modularity scores in people with UTRS and FLR compared with healthy controls (Q* (IQR) = 0.09 (0.03), 0.16 (0.18), 0.13 (0.16), 0.22 (0.14) for controls, FLR, TRS and UTRS, respectively; KW=13.1, p=0.004; UTRS vs controls p=0.004, FLR vs controls p=0.035). Modularity values determined by lowering the value of y resulted in lower modularity scores for those with UTRS compared with controls and are provided in appendix 8.11.

The community structure was further described using connection diversity, a centrality measure for functional networks that depicts the extent to which each node participates in intra- and inter-modular functional connectivity (261). No differences in diversity were observed between any of the groups when calculated using positive weights. However, node 82, located in the parietal cortex, exhibited greater diversity of negative connections in those with UTRS compared with healthy controls (Figure 49).
**TABLE 3. DEMOGRAPHIC CHARACTERISTICS FOR TRS STUDY POPULATION. WHERE DATA MET ASSUMPTIONS OF NORMALITY AND HOMOSCEDASTICITY, MEANS AND STANDARD DEVIATIONS ARE REPORTED. DATA VIOLATING EITHER OF THESE ASSUMPTIONS ARE DENOTED BY A †. MEDIANS AND INTERQUARTILE RANGES ARE REPORTED FOR THESE VARIABLES.**

*ASSIST SCORE WAS NOT COMPLETED FOR ONE PARTICIPANT; STATISTICAL COMPARISON IS PROVIDED FOR ALL REMAINING PARTICIPANTS.*

<table>
<thead>
<tr>
<th>Health controls (n=17)</th>
<th>First-line responders (n=18)</th>
<th>Treatment-resistant (n=18)</th>
<th>Ultra-treatment-resistant (n=16)</th>
<th>F and p values</th>
<th>Kruskal-Wallis test statistic and p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age† (years)</td>
<td>32.7 (11.5)</td>
<td>30.0 (12.3)</td>
<td>34.5 (15.4)</td>
<td>1.187; p=0.756</td>
<td>KW=1.187; p=0.756</td>
</tr>
<tr>
<td>Gender† (% female)</td>
<td>11.8</td>
<td>22.2</td>
<td>27.8</td>
<td>1.432; p=0.698</td>
<td>KW=1.432; p=0.698</td>
</tr>
<tr>
<td>Duration of illness† (years)</td>
<td>-</td>
<td>7.5 (9.3)</td>
<td>11.2 (12.5)</td>
<td>3.486; p=0.175</td>
<td>KW=3.486; p=0.175</td>
</tr>
<tr>
<td>PANSS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive†</td>
<td>-</td>
<td>13.0 (11.3)</td>
<td>10.5 (7.5)</td>
<td>1.804; p=0.406</td>
<td>KW=1.804; p=0.406</td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
<td>16.2 (5.3)</td>
<td>18.3 (7.1)</td>
<td>1.208; p=0.308</td>
<td></td>
</tr>
<tr>
<td>General psychopathology</td>
<td>-</td>
<td>28.8 (5.9)</td>
<td>28.7 (6.4)</td>
<td>0.015; p=0.985</td>
<td></td>
</tr>
<tr>
<td>Total score</td>
<td>-</td>
<td>58.9 (10.6)</td>
<td>58.7 (15.0)</td>
<td>1.187; p=0.756</td>
<td>KW=1.187; p=0.756</td>
</tr>
<tr>
<td>Current prescribed antipsychotic</td>
<td>-</td>
<td>Risperidone = 6</td>
<td>Clozapine = 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amisulpride = 1</td>
<td>Clozapine + amisulpride = 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Olanzapine = 9</td>
<td>Risperidone + quetiapine = 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aripiprazole = 2</td>
<td>Clozapine + aripiprazole = 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Quetiapine + aripiprazole = 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clozapine + quetiapine = 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Clozapine + risperidone = 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine equivalents</td>
<td>-</td>
<td>112.5 (32.5)</td>
<td>105.6 (24.0)</td>
<td>0.998; p=0.376</td>
<td>KW=0.998; p=0.376</td>
</tr>
<tr>
<td>ASSIST score†</td>
<td>25.0 (26.0)</td>
<td>46.0 (34.0)</td>
<td>36.0 (21.0)*</td>
<td>11.335; p=0.010</td>
<td>KW=11.335; p=0.010</td>
</tr>
<tr>
<td>Drug Screen† (% THC positive)</td>
<td>0</td>
<td>16.7</td>
<td>5.6</td>
<td>4.410; p=0.220</td>
<td>KW=4.410; p=0.220</td>
</tr>
</tbody>
</table>
TABLE 4. TOTAL CONNECTION STRENGTH OF THE NETWORK (ACROSS ALL NODES), CALCULATED FROM POSITIVE AND NEGATIVE WEIGTHS. VALUES ARE REPORTED AS MEDIAN (INTERQUARTILE RANGE); *P VALUES ≤ 0.05 WERE CONSIDERED STATISTICALLY SIGNIFICANT; **P VALUES ≤ 0.05, CORRECTED FOR MULTIPLE COMPARISONS USING THE BONFERRONI CORRECTION, WERE CONSIDERED STATISTICALLY SIGNIFICANT FOR POST-HOC PAIRWISE COMPARISONS.

<table>
<thead>
<tr>
<th>Post-hoc pairwise comparisons (PW)</th>
<th>Test statistic and P values**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls vs FLR</td>
</tr>
<tr>
<td></td>
<td>Controls vs TRS</td>
</tr>
<tr>
<td></td>
<td>Controls vs UTRS</td>
</tr>
<tr>
<td></td>
<td>FLR vs TRS</td>
</tr>
<tr>
<td></td>
<td>FLR vs UTRS</td>
</tr>
<tr>
<td></td>
<td>TRS vs UTRS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total strength from positive weights</th>
<th>Controls</th>
<th>FLR</th>
<th>TRS</th>
<th>UTRS</th>
<th>Kw=10.6</th>
<th>Kw=8.5</th>
<th>PW vs FLR</th>
<th>PW vs TRS</th>
<th>PW vs UTRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4756 (964)</td>
<td>3757 (1329)</td>
<td>3989 (1835)</td>
<td>3378 (1842)</td>
<td>PW=13.3</td>
<td>PW=-14.3</td>
<td>PW=-19.6</td>
<td>PW=10.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>97 (105)</td>
<td>184 (280)</td>
<td>117 (254)</td>
<td>344 (491)</td>
<td>PW=-0.304</td>
<td>PW=-0.212</td>
<td>PW=-0.301</td>
<td>PW=4.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total strength from negative weights</th>
<th>Controls</th>
<th>FLR</th>
<th>TRS</th>
<th>UTRS</th>
<th>Kw=8.5</th>
<th>Kw=8.5</th>
<th>PW vs FLR</th>
<th>PW vs TRS</th>
<th>PW vs UTRS</th>
</tr>
</thead>
<tbody>
<tr>
<td>97 (105)</td>
<td>184 (280)</td>
<td>117 (254)</td>
<td>344 (491)</td>
<td>PW=-14.3</td>
<td>PW=-10.3</td>
<td>PW=-9.3</td>
<td>PW=9.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>97 (105)</td>
<td>184 (280)</td>
<td>117 (254)</td>
<td>344 (491)</td>
<td>PW=-0.212</td>
<td>PW=-0.780</td>
<td>PW=-9.3</td>
<td>PW=9.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 46. SAGITTAL, AXIAL AND CORONAL PROJECTIONS OF NODES COLOURED ACCORDING TO BRAIN REGION. FIGURE ADAPTED FROM SIMAS ET AL. 2015 (272).

FIGURE 47. NODES EXHIBITING GREATER STRENGTH CALCULATED FROM POSITIVE WEIGHTS IN HEALTHY CONTROLS COMPARED TO PARTICIPANTS WITH UTRS, TRS AND FLR; P<0.05, CORRECTED FOR MULTIPLE COMPARISONS.

FIGURE 48. NODES EXHIBITING GREATER STRENGTH CALCULATED FROM NEGATIVE WEIGHTS IN THOSE WITH UTRS/FLR COMPARED TO CONTROLS AND THOSE WITH TRS; P<0.05, CORRECTED FOR MULTIPLE COMPARISONS.
FIGURE 49. GRAPHICAL REPRESENTATION OF NODE 82 (GREEN), EXHIBITING GREATER DIVERSITY CALCULATED FROM NEGATIVE WEIGHTS IN THOSE WITH UTRS COMPARED WITH HEALTHY CONTROLS; P<0.05, CORRECTED FOR MULTIPLE COMPARISONS USING FDR. MEDIAN (IQR) DIVERSITY VALUES AT NODE 82 FOR EACH GROUP ARE AS FOLLOWS: CONTROLS 0.00 (0.19), FLR 0.00 (0.63), TRS 0.00 (0.65), UTRS 0.58 (0.60). KW P=0.009, PW UTRS VS CONTROLS P=0.009, CORRECTED FOR MULTIPLE COMPARISONS USING BONFERRONI’S CORRECTION.

4.3.3. NETWORK-BASED STATISTICS
Network organisation across groups was compared using network-based statistics (264). Analysis revealed a statistically significant difference in network organisation between healthy controls and individuals with UTRS (p<0.05). Post-hoc t-tests identified significantly greater connectivity in healthy controls (p<0.05, corrected) that extended primarily from cerebellar and parietal regions to the frontal cortex.

A map of all dysconnections (with an arbitrary threshold of 4.8 applied for ease of visualisation; showing greater strength in healthy controls compared to those with UTRS) is illustrated in Figure 50. Dysconnections could be divided into three separate structures (sub-networks), representing cerebellar-frontal dysconnections (Figure 51, sub-network 1), cingulo-frontal-temporal dysconnections (Figure 51, sub-network 2) and fronto-parietal dysconnections (Figure 51, sub-network 3). No differences in network connectivity were observed between controls and FLR or those with TRS or between any of the schizophrenia cohorts.
FIGURE 50. NODES (GREY SPHERES) AND EDGES (RODS) REPRESENTING FUNCTIONAL DYSCONNECTION IN PEOPLE WITH UTRS COMPARED WITH HEALTHY CONTROLS. GREATER DIFFERENCES BETWEEN GROUPS ARE SHOWN IN RED (THICKER EDGES) AND WEAKER DIFFERENCES ARE SHOWN IN DARK BLUE (THINNER EDGES); MINIMUM THRESHOLD=4.8.

FIGURE 51. SUB-NETWORKS EXHIBITING DIFFERENCES BETWEEN HEALTHY CONTROLS AND THOSE WITH UTRS. THREE NETWORKS WERE IDENTIFIED, EACH DEMONSTRATING GREATER CONNECTIVITY IN CONTROLS VS UTRS. THRESHOLD = 4.9; P<0.001, SUB-NETWORK 1; P=0.003, SUB-NETWORK 2; P=0.003, SUB-NETWORK 3.
4.4. DISCUSSION

The current analysis sought to determine whether individuals with schizophrenia who fail to respond to treatment with first-line (TRS) or second-line (UTRS) antipsychotics exhibit different functional network structure from those who do respond (FLR) and to determine whether any of these groups differ from psychiatrically healthy controls. Results demonstrated large disruptions in modularity, connection strength and network organisation in people with schizophrenia, particularly those with UTRS. These findings corroborate earlier findings of functional network disruption in UTRS and may add new insights into the underpinnings of treatment failure.

Previous work by Lynall et al. reported reduced connection strength and increased diversity in individuals with schizophrenia that was accompanied by less clustered and less hub-dominated topology (181). Findings from the current study support and extend upon these results, demonstrating decreased connection strength of positive correlations and increased connection strength of anticorrelations in individuals with UTRS compared with healthy controls. Assessing the strength of individual nodes, people with UTRS had weaker positive connections in frontal, parietal and cerebellar regions, while connection strength was increased over frontal, left occipital and left cerebellar regions for negative weights. In addition to those abnormalities observed in the group with UTRS, a single node in the cerebellum exhibited weaker positive connectivity in all schizophrenia cohorts compared with healthy controls. This same node demonstrated greater negative connection strength in FLR and those with UTRS compared to controls. Likewise, FLR showed greater negative connection strengths at a number of frontal and cerebellar nodes compared with healthy controls. These findings support previous work identifying disruptions in cerebellar, parietal and frontal communication in individuals with schizophrenia (276-278) and correspond well with changes in network organisation observed in this cohort (see below).

Only one node displayed significant differences in connection strength between cohorts with schizophrenia, suggesting that the difference observed may not be sufficiently robust to serve as a biomarker for treatment response. However, the observed differences between those with UTRS and healthy controls and between FLR and healthy controls suggest there may still be different disruptions in connectivity that define response subtypes of the disorder. An overall reduction in correlated connection strength and an increase in anticorrelated connection strength observed in those with UTRS, and to a lesser degree FLR, indicates a shift in network communication, whereby areas usually working in sync begin to function independently from one another. Such reorganisation could ultimately lead to disruptions in everyday functioning,
potentially leading to the psychological and cognitive symptoms observed in schizophrenia. Alternatively, this reorganisation of communication could be acting merely as a compensatory mechanism, offsetting other underlying disruptions.

The degree of modularity associated with functional networks was dependent on the relative size of \( \gamma \), indicating the preferred size of modules. At a classic \( \gamma \) value of 1, individuals with UTRS and FLR exhibited greater modularity than healthy controls, though this relationship was reversed when the value of \( \gamma \) was shifted closer to 0. This observation of higher modularity is at odds with previous work; however, work by Alexander-Bloch et al., which reported reduced modularity in people with childhood-onset schizophrenia that was consistent across a number of different methodologies, found that this was only true for a narrow range of costs when applied to weighted graphs (279). Adjusting the value of \( \gamma \) to reflect the range of costs that reportedly resulted in lower modularity in those with schizophrenia, similar effects were observed. However, the quantity of networks associated with these costs in the present study (between one and two modules only) did not reflect previous reports that the human functional network generally subdivides into between three and five modules (280-282). As such, more work is needed to determine the circumstances under which the schizophrenia brain network becomes less modular to validate the reproducibility of such findings.

No node demonstrated differences in diversity calculated from positive weights, despite previous reports that diversity was increased in orbitofrontal, insular and parietal association cortices in individuals with schizophrenia compared with healthy controls (181). Diversity calculated from negative weights (i.e. anticorrelations), however, was considerably larger in individuals with UTRS compared with all other cohorts (though only statistically significant compared with healthy controls) at one node corresponding to an area of the left medial-parietal lobe. The position of this node corresponds with parietal regions of the DMN (158), which have been shown to possess anti-correlative relationships with prefrontal-based motor planning and control circuits (283). While nodal diversity does not provide information about specific node-to-node functional connections, the greater diversity of this node in people with UTRS might suggest a less coordinated mode of connectivity (181). Despite greater diversity in this node, however, no corresponding difference in strength of connectivity was observed at this site. This finding suggests that, although the role of this node in intermodular communication has changed, the overall connection strength, indicative of global influence, has not (261). The consequence of such a change may therefore be slight.
Investigating differences in functional connectivity on a more global network level, three sub-networks that exhibited weaker connectivity in people with UTRS compared with healthy controls were identified. Previous work by Cocchi et al. described similarly dysconnected networks in people with schizophrenia (268); however, their exclusion of cerebellar nodes (a key distinction between this study and previous network-based statistics studies in schizophrenia) prevented identification of any potential cerebellar dysconnections. An earlier study by Zalesky et al. (264) identified only a single dysconnected network in 12 people with schizophrenia, although the locations of dysconnections were similar to those reported by Cocchi et al. (268). In the current study, dysconnected sub-networks were identified only in participants with UTRS and not FLR or those with TRS. This is consistent with findings from ICA (see chapter 3), which showed no differences in FLR or those with TRS compared with healthy controls in the TRS study cohort. In contrast with results from the low-dimensional ICA, however, network-based statistics identified weaker associations between regions in those with UTRS compared with healthy controls. This discrepancy between traditional and more high-dimensional methods is not surprising given the different manners by which they handle data. It does, however, emphasise how the choice of analytical technique influences the outcome of hypothesis testing.

In contrast to previous studies employing network-based statistics, a large sub-network consisting primarily of interhemispheric dysconnections between cerebellar and prefrontal nodes was identified. This follows results from voxel-based morphometry analysis in the same cohort of individuals that identified, among other disruptions, a reduction in grey matter density in the left cerebellum of individuals with UTRS compared with healthy controls (148). Post-mortem studies indicate that grey matter reductions observed in schizophrenia are attributable to reductions in cortical neuropil, comprising the axons, dendrites, pre- and post-synaptic terminals of cortical neurons (137). Therefore, a loss of synaptic communication within the cerebellum may be responsible for the weakened functional connectivity observed between the cerebellum and prefrontal cortex in the current study. Alternatively, functional dysconnectivity in this group could be mediated by disruptions in prefrontal-thalamic-cerebellar projections (284), which have been suggested as a mechanism for ‘cognitive dysmetria’, a well-known hypothesis for the disorder (130). Cognitive dysmetria is expressed as disruptions in prioritising, processing, coordinating, and responding to information (130) and is posited to arise from aberrant regulation of NMDA receptor function by modulatory neurotransmitters (100, 131); this leads to disruptions in reinforcement, emotional and/or perceptual learning.
Although dysconnections in the current study do not provide information about effective connectivity, evidence from imaging studies infers a causative role for the cerebellum in these dysconnections. Lawyer et al. and Okugawa et al. have shown abnormalities in both the grey and white matter of the cerebellar vermis in individuals with schizophrenia (285, 286). In addition, Demertas-Tatlidede et al. showed improved cognitive and emotional symptoms following transcranial magnetic stimulation of the cerebellar vermis in people with treatment-refractory schizophrenia (287). Disruptions in cerebellar-thalamic white matter projections, as reported by Magnotta et al. (288), may also contribute to this dysconnection. However, given that data have revealed grey matter loss but not white matter deficits (220) along this pathway in those with UTRS, it is more likely that the dysconnectivity observed between cerebellar and prefrontal nodes is a consequence of disturbances in synaptic function rather than structural dysconnectivity.

The second sub-network to demonstrate dysconnectivity in those with UTRS included connections between temporal, cingulate and prefrontal nodes. Similar to sub-network 1, regions of sub-network 2, including middle temporal gyri, anterior cingulate gyrus and ventromedial prefrontal cortices, exhibited decreased grey matter density in people with UTRS compared with healthy controls (148). Within this sub-network, the cingulate node was the most extensively connected, linking to multiple nodes in the frontal cortex and left temporal lobe. Work by Fletcher et al. also identified abnormalities in this network, describing decreased cingulate modulation of fronto-temporal connectivity in people with schizophrenia (266). Other studies have since corroborated their finding of altered fronto-temporal connectivity in the disorder (202, 258, 289). Although Fletcher and colleagues’ primary hypothesis supported a failure of cingulate modulation of prefrontal cortical influence over the superior temporal cortex (266), the results presented in this chapter suggest that disruption of this network is more likely to arise from reduced prefrontal influence over cingulo-temporal connections, or alternatively, decreased cingulate modulation of temporal to frontal connections. While reduced fronto-temporal connectivity is well supported in studies of schizophrenia, a study by Allen et al. (290) failed to identify differences in task-based activation of fronto-temporal connections compared with healthy controls in individuals at high risk for the disorder. This group did, however, show greater engagement of the anterior cingulate gyrus and caudate during task performance (290). Together with these findings, results from the current analysis suggest altered modulation of cingulo-frontal-temporal connectivity may be specific to individuals with UTRS, and that cingulate dysconnectivity alone is reflective of the prodromal stage of the disorder. Although other studies have reported alteration in fronto-temporal or cingulo-frontal-temporal
connectivity in schizophrenia as a collective disorder, no disruptions in either FLR or participants with TRS were identified. This may be a reflection of the influence of UTRS subtypes on the overall data in previous work, or suggestive of a weaker effect in FLR and those with TRS that could not be identified in the number of participants included in this study.

Comparing results of the current analysis with those of low-dimensional ICA (in chapter 3), nodes within sub-network 2 lie in close proximity to regions involved in the language network (IC10), although ICA identified greater connectivity in this network in people with UTRS compared with healthy controls. The discrepancy in altered connectivity between these regions may reflect expansion of this network to encompass a larger area (seen with ICA) to compensate for dysconnections identified by network-based statistics. Indeed, this hypothesis is more consistent with previous data showing reduced structural and functional connectivity in the language network of individuals with schizophrenia (214). Disruption to this network in UTRS may lead to a misperception of spoken words (potentially via aberrant efference copy signalling; see chapter 3), as well as misinterpretation of assigned meanings, leading to delusional thoughts (196). Formal thought disorder and problems in verbal fluency may also develop from such dysconnections (291). Further investigation into the mechanisms underlying dysconnections in this sub-network in those with UTRS is required.

Sub-network 3 also included dysconnections involving the prefrontal cortex, this time to nodes within the medial parietal cortex. Unlike nodes within the first two sub-networks, however, these regions were not associated with areas of grey matter loss in UTRS (148). Likewise, no differences in structural connectivity, as measured using tract-based spatial statistics, were observed in individuals with UTRS compared with controls (220). Research by Cocchi et al. into the underpinnings of dysconnectivity as measured by network-based statistics revealed that functional dysconnections correlated with reductions in structural integrity in only some cases (268). Further work is needed to determine the source of these dysconnections in those with UTRS.

Nodes within sub-network 3 correspond closely to regions in the frontoparietal network (FPN) identified by Smith et al. (158). The FPN is posited to play a role in the selection of relevant environmental information (priority coding) by mediating the spatial and non-spatial orienting of attention and interruption of current cognitive activity (292). Sensory- and goal-driven signals representing bottom-up and top-down control of cognitive processing, respectively, are transferred between prefrontal and parietal cortices within the network (293). Disrupted connectivity between these areas could upset priority coding and result in the inappropriate
transfer of attention or disrupted goal-directed cognition. Goal-directed cognition is thought to be mediated by the FPN through modulation of the DMN and dorsal attention network (DAN) (294). Its neuroanatomical position and ability to flexibly couple between these networks means the FPN is important for mediating a dynamic balance between the DMN and DAN activity (294). Baker and colleagues suggested that decreased connectivity within the FPN in people with schizophrenia and other psychotic disorders might lead to inappropriate activation of other networks and consequent blurring of internally and externally oriented processing (192). In support of this hypothesis, Tu et al. found reduced connectivity within the FPN (between the caudate and other regions of the network) alongside increased connectivity between several regions in the FPN and regions belonging to the DMN and another network involved in primary sensory processing (191).

Network disruptions exclusive to those with UTRS highlight the importance of subtyping in studies of schizophrenia. Pooling of subtypes may add statistical weight to the power of a study but subsequently diminishes opportunities for detecting clinically relevant information. Therefore, future subtyping of participants based on response to treatment and subsequent recruitment into discrete study cohorts should be considered. The absence of any network dysconnections in FLR and those with TRS in the current study indicates normative functional connectivity in these treatment groups. However, studies utilising network-based statistics in the past have indicated some level of dysconnectivity in participants not subtyped for response (264, 268). Analysis of a larger cohort of participants, providing more study power to detect a small difference between subtypes, may aid the testing of this hypothesis.

This study benefits from a well characterised cohort of participants, demonstrating similar degrees of symptom severity, duration of illness and prescribed antipsychotic dose (measured by chlorpromazine equivalents) as well as similar ratios of male to female participants. One individual in the cohort did not complete the ASSIST assessment; statistical comparison of remaining participants revealed higher rates of drug-taking behaviour in FLR and those with UTRS compared with healthy controls. However, no statistically significant differences in positive drug screen on the day of testing were observed between groups, suggesting that current exposure to recreation drugs was unlikely to account for the differences observed between groups in the current study.

The study’s cross-sectional design does impart some degree of uncertainty about the underlying cause of observed differences. Future work is required to establish whether differences between
controls and individuals with UTRS exist at treatment onset. If so, functional connectivity may become a useful predictive biomarker of treatment resistance in schizophrenia.

The use of other parcellation schemes, with bigger or smaller regions of interest, may also help to corroborate these findings (295). Some authors recommend that parcellation be based on cortical surface models rather than high-resolution structural images, due to the expected misalignment of cortical functional areas across participants (249). These are considerations that can be taken into account in future studies.

Results have shown distinct differences in graph theory measures and functional connectivity in FLR and individuals with UTRS compared with healthy controls. Future work must determine whether these changes occur prior to the onset of treatment and if they can be used to predict a response to antipsychotics during first-episode psychosis. Work is currently underway to address these questions.
5. DIFFERENCES IN STRUCTURAL CONNECTIVITY BETWEEN FLR AND THOSE WHO ARE ELIGIBLE FOR CLOZAPINE, MEASURED USING TBSS

As discussed in chapter 3, evidence exists to support the hypothesis that schizophrenia is a disorder of abnormal connectivity, characterised by impaired N-methyl-d-aspartate (NMDA) receptor-dependent synaptic plasticity that leads to failures in self-monitoring (100, 129). As an extension of this hypothesis, in chapter 3, the influence of functional connectivity on the response to antipsychotic treatment was considered, revealing encouraging results. To investigate whether the functional dysconnection identified in clozapine-eligible participants was accompanied by disruptions in structural connectivity, white matter integrity was compared between clozapine-eligible individuals and FLR using tract-based spatial statistics (TBSS). Results of this investigation, in addition to an exploration of probable tracts arising from the disrupted region, are presented in this chapter.

5.1. INTRODUCTION

The dysconnectivity hypothesis of schizophrenia refers specifically to aberrant modulation of synaptic efficacy via interactions between modulatory neurotransmitters, such as dopamine and serotonin, and NMDA receptors (129). This in turn is purported to affect functional connectivity in the brain via disruptions in efference copy signalling (100) and predictive coding (129), which produce the symptoms associated with schizophrenia (129). A large number of studies have identified disruptions in structural connectivity, not only in those with chronic symptoms of schizophrenia but also in treatment-naïve individuals with first-episode psychosis (121, 123, 139, 296). In addition, structural findings are often reported in conjunction with abnormal functional connectivity (297). Given that a number of these studies report low white matter anisotropy in individuals with schizophrenia, an argument for abnormal myelination as the primary aetiology of schizophrenia (247) seems reasonable. However, Friston et al. suggested that, because signs and symptoms of schizophrenia can be elicited by psychomimetic drugs such as ketamine and phencyclidine (NMDA receptor antagonists), anatomical characteristics of schizophrenia must be consequences of the underlying pathophysiology and do not have an aetiological role in the disorder. Irrespective of whether white matter loss is a cause or consequence of schizophrenia, there may be differences in white matter connectivity between FLR and those who are clozapine-eligible that may further explain the functional connectivity findings reported in chapter 3 and provide further insight into the aetiology of treatment resistance.
White matter is named for the phospholipid processes of oligodendrocytes, known as myelin, that wrap around axons and provide insulation for electrical signals traveling between neurons (298). Myelination is vital to the high-speed conduction of action potentials over long distances and plays an important role in neurodevelopment (298). Within the brain, myelinated axons connecting spatially disparate brain regions bundle together to form fasciculi, which can be easily seen using modern neuroimaging techniques (Figure 52).

FIGURE 52. MAJOR WHITE MATTER FIBRES OF THE BRAIN, DIVIDED INTO ASSOCIATION FIBRES (TOP), THALAMIC RADIATIONS (MIDDLE) AND PROJECTION FIBRES (BOTTOM); REPRODUCED FROM COX ET AL. (299) NATURE PUBLISHING GROUP.

One such technique, recently adopted by a large number of studies, is diffusion tensor imaging (DTI; discussed in chapter 2). DTI employs large magnetic gradients to measure the movement of water molecules in tissue. Adjusting the strength of each gradient changes the so-called gradient direction, allowing for the measurement of water diffusion from many angles. The result is an array of values for each voxel that represents the movement of water orthogonal to each gradient direction. These values are combined to give a matrix of tensors signifying diffusion in each plane and represented by an ellipsoid. Isotropic ellipsoids represent areas where the movement of water is equal in all directions, signifying unhindered diffusion. Anisotropic ellipsoids, in contrast, represent those areas where diffusion is restricted in one or more
directions, such as in fasciculi, where fibres possess a high degree of collinearity (300). Fractional anisotropy (FA) is a measure of the shape of an ellipsoid (discussed in chapter 2), providing information about the degree of anisotropy in a voxel. High FA values represent areas of high anisotropy, and consequently restricted water movement, whereas low FA values represent areas of low anisotropy, where water molecules diffuse freely. Related measures of diffusion are axial diffusivity (AD) and radial diffusivity (RD), which represent diffusion in the primary (principal) direction (ε₁) and perpendicular directions (ε₂ and ε₃), respectively. Though in vivo measures of white matter integrity possess limitations in terms of interpretation, especially with regard to low FA measurements in areas of crossing or kissing fibres, it is believed that FA represents the degree of diffusion orientation coherence, AD, the degree of axonal shrinkage and RD, the degree of myelination within a voxel.

Disruptions in white matter architecture are a common feature of schizophrenia, yet inconsistencies about the site and nature of abnormalities arise. A meta-analysis of studies investigating recent-onset schizophrenia and clinical high-risk individuals failed to identify a specific underlying neurobiological deficit in white matter, despite reporting reliable differences between these cohorts and healthy controls (121). The most consistent findings were those of white matter deficits in the frontal, fronto-temporal and fronto-limbic regions, including the superior longitudinal fasciculus, cingulum, uncinate fasciculus, and corpus callosum (CC) (121). Other studies in individuals with chronic or first-episode schizophrenia have identified white matter abnormalities in the genu and splenium of the CC, forceps, fronto-occipital fasciculus, corticospinal tract, anterior thalamic radiation and inferior longitudinal fasciculus, in addition to those tracts mentioned above (123, 301, 302). These findings suggest wide-spread white matter deficits in schizophrenia but fail to identify any pathway that is strongly correlated with the disorder. As discussed in chapter 3, discrepancies in the literature to date may be a result of poor patient subtyping and may be rectified by classifying individuals according to their treatment response (29, 38, 148, 149).

Zeng et al. investigated white matter microstructure before and after eight weeks of antipsychotic treatment in first-episode schizophrenia and reported a correlation between changes in the left superior longitudinal fasciculus and improved positive symptoms (302). These authors did not, however, examine features of pre-treatment white matter structure that might predict clinical outcome. An earlier study by Mitelman et al. categorised participants with schizophrenia according to whether they had good outcome or poor outcome, based on their ability for self-care (303). Those with poor outcome had deficits in white matter in both
hemispheres compared with healthy controls, compared to only lateralised deficits in those with good outcome (303). More recently, Reis Marques et al. conducted an investigation in first-episode psychosis to determine whether pre-treatment FA could distinguish responders from non-responders to a 12 week course of antipsychotics (304). They identified lower FA in non-responders compared with responders in several white matter tracts, including the uncinate, stria terminali, superior frontal-occipital tract, CC, internal and external capsule and corona radiata (304). Though their study did not follow participants for long enough to confirm treatment-resistance, their findings support the hypothesis that pre-treatment FA could predict a progression to clozapine eligibility. The current analysis is designed to determine whether individuals with established resistance to first-line antipsychotics exhibit different white matter properties to FLR prior to initiating clozapine. Though the design is cross-sectional and therefore not as robust as the prospective study by Reis Marques et al., the results highlight differences in FA between these two populations and explore whether differences in FA reflect patterns of functional connectivity identified in chapter 3.

In addition to the standard measures of FA, RD and AD that provide information about white matter structure, diffusion-weighted imaging can be used to predict fibre directions using probabilistic diffusion tractography (see chapter 2) (164, 165). This technique utilises local fibre direction information from the diffusion tensor model to estimate global connectivity (the probability that a connection exists between two points) in the brain (165). Here, probabilistic tractography was employed to identify regions of the cortex that may be affected by disruptions in FA and to determine whether the spatial distribution of fibres is different in FLR and those who are eligible for clozapine. It was anticipated that these findings would help to explain the differences in functional connectivity observed between these groups.

To obtain a large number of diffusion directions while minimising opportunities for in-scanner head motion, a multiband diffusion acquisition sequence was used (167). This shortened scanning time to six minutes, while still permitting the use of 105 diffusion-encoding directions.

5.2. METHODS

5.2.1. PARTICIPANTS
Recruitment details and inclusion/exclusion criteria for the CloRes study are reported in chapter 3. Briefly, 10 individuals with a history of a psychotic episode or diagnosis of schizophrenia who responded to treatment with first-line antipsychotics (FLR) and 15 individuals with a diagnosis of schizophrenia who failed to respond to two independent trials of first-line antipsychotic drugs
(clozapine-eligibility) were recruited. The study was approved by the Northern A Regional Ethics Committee (ref 14/NTA/103/AM11) and all participants gave informed written consent.

Participant demographics were compared across cohorts using IBM SPSS Statistics Version 22. Variables that satisfied assumptions of homoscedasticity (Brown-Forsythe test for equality of variances) and normality (Shapiro-Wilk test for normality) were analysed using a Student’s t-test. For those variables that violated assumptions of normality and/or homoscedasticity, the Mann-Whitney U test was employed. Z scores were calculated for demographics that were better described using proportions.

5.2.2. DATA ACQUISITION

Structural and diffusion-weighted magnetic resonance images were acquired on a Siemens Magnetom Skyra 3T scanner at the Centre for Advanced MRI, University of Auckland, New Zealand. All participants were imaged using a 32-channel head coil. A structural T1-weighted image was acquired using an MPRAGE sequence with repetition time (TR) 2000 ms; echo time (TE) 3.48 ms; inversion time (TI) 1010 ms; flip angle 9°; repetition 1; acceleration (GRAPPA) factor of 2; field of view (FOV) 230 mm; voxel size 0.9 x 0.9 x 0.9 mm.

Gradient distortion (fieldmap) images were acquired using a gradient echo pulse sequence with the following parameters: TR 704 ms; TE1 4.92 ms; TE2 7.38 ms; voxel size 3.4 x 3.4 x 2.0 mm; phase-encode direction A >> P; FOV 220 mm.

Diffusion-weighted images were acquired using a multiband gradient-echo pulse sequence (University of Minnesota (167)). One image without diffusion gradients (b=0 s/mm²) was acquired, in addition to 105 images with diffusion-encoding directions isotropically distributed in space at b values ranging from 5 to 2010 s/mm² (10 values in total). Seventy-two slices were acquired in the anterior to posterior direction, with a base resolution of 108 and the following parameters: TR 3600 ms; TE 92.4 ms; echo spacing 0.67 ms; EPI factor 108; multiband acceleration factor 3; flip angle 78°; FOV 220 mm, voxel size 2 x 2 x 2 mm.

5.2.3. IMAGE PREPROCESSING

Image preprocessing and analysis were performed using FSL version 5.0.9 (176, 207). Structural images were reoriented to a standard template and brain tissue was extracted from raw image files using FSL’s brain extraction tool (BET) (208). If automatic brain extraction failed to eliminate all non-brain tissue, the excess was removed manually. Magnitude images were subjected to the same process, after which brain-extracted images were eroded to ensure that no voxels containing non-brain tissue remained. Fieldmaps were then created using the
The gradient-free image was used to create a binary mask with BET. Data were then corrected for head movement and eddy current distortions, using eddy_openmp to align all volumes (174). Slices with average intensity at least four standard deviations lower than the expected intensity were interpolated with predictions made by the Gaussian Process (305). In-scanner head motion was determined for each slice and averaged across volumes to give a single mean for each participant. Gradient distortions were corrected using FSL’s fugue function and output registered to gradient-free images using the linear registration function (FLIRT) (210, 211). DTIfit was used to independently fit diffusion tensors to each voxel, limited to brain space using the binary brain mask. Output from DTIfit yielded voxelwise maps of FA, λ1 (AD), λ2 and λ3 (combined to give RD) for each participant.

5.2.4. TRACT-BASED SPATIAL STATISTICS

Voxelwise statistical analysis of the FA data was carried out using FSL’s TBSS (306). First, FA images were created by fitting a tensor model to the raw diffusion data using FDT, and then brain-extracted using BET. All subjects’ FA data were then aligned to a white matter (FMRIB58_FA) template and then to MNI152 1mm standard space using the nonlinear registration tool FNIRT (307, 308). The mean FA image was created and thinned to create a mean FA skeleton representing the centres of all tracts common to the group. A threshold of 2000 (corresponding to FA>0.2) was applied to exclude voxels containing grey matter and cerebrospinal fluid. Each subject’s aligned FA data was then projected onto the skeleton and fed into voxelwise cross-subject statistics. Between-group t-tests interrogating differences in FA over 5000 permutations were conducted using the Randomise tool (309). Though the difference in the proportion of male and female participants between groups was not statistically significant, gender was added as a covariate to account for the low number of female participants included in the clozapine-eligible group. Output contained statistical maps corrected for multiple comparisons (family-wise error corrected) at the cluster level using threshold-free cluster enhancement (310). These were further corrected for multiple comparisons between groups using the false discovery rate (FDR).

Mean FA within the significant cluster was determined for each participant and Glass’s delta (Δ) effect size for differences in FA (311) was calculated using the following equation:

\[ \Delta = \frac{\text{mean}_{\text{FLR}} - \text{mean}_{\text{Clozapine}}}{\text{standard deviation}_{\text{FLR}}} \]
To investigate the neural basis of a decrease in FA, the significant cluster from FA analysis (covaried for gender) was masked and back projected into native space for each participant using the TBSS Deproject tool. Individualised masks were applied to λ1, λ2 and λ3 output from DTIfit and mean values for masked regions obtained using FSL’s statistics tool. Mean λ1 from the masked region provided a mean value of AD for the significant cluster from FA analysis. Mean RD was determined by taking the combined mean of masked λ2 and λ3 maps. It was not appropriate to investigate MD, as it is not independent of AD and RD. Between-group t-tests were conducted in SPSS version 23 (IBM). This method was adapted from previous work by Bennett et al., who investigated the neural underpinnings of FA loss in aging adults (312).

5.2.5. PROBABILISTIC TRACTOGRAPHY
In addition to investigations of FA, RD and AD, probabilistic tractography was performed to determine the areas likely to be affected by differences in FA between the groups. First, crossing fibres were modelled using Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques (BEDPOSTX) (164). BEDPOSTX uses Markov Chain Monte Carlo sampling to create distributions on diffusion parameters at each voxel (164). Probabilistic tractography for each participant was implemented in FSL’s PROBTRACKX (164, 165), using the FA mask in MNI152 1mm space as a seed mask. PROBTRACKX repetitively samples from the distributions of voxel-wise principal diffusion directions, computing a streamline through these local samples to estimate the location of the true streamline (164, 165). Tracts were then constrained to a minimum threshold of 2000 to increase confidence in tractography results for each participant. The randomise function in FSL was then used to create a single mean for each group (FLR and clozapine-eligibility), which was overlaid on an MNI152 1mm brain for viewing purposes. No further statistics were performed on tractography output.

5.3. RESULTS
5.3.1. PARTICIPANT DEMOGRAPHICS
One participant from the clozapine-eligible group was excluded from analysis due to excessive head motion during scanning (Figure 53). After exclusion of this participant, mean movements from the first volume and from the previous volume were calculated. No significant differences in head movement were found between response groups (Table 5). Participant demographics for the remaining participants are reported in Table 6.
5.3.2. TRACT-BASED SPATIAL STATISTICS

Results of the skeleton-wise between-group t-test for FA are presented in Figure 54. Significantly lower FA was observed in the body of the CC in individuals who were clozapine-eligible compared to FLR. Glass’s Δ was 0.353 for the difference in FA between groups (FLR=0.87 versus clozapine-eligibility=0.83), representing a small to moderate effect size. Post-hoc investigation of AD and RD in this region revealed a significant increase in mean RD in those who were clozapine-eligible (2.1x10^-4) compared with FLR (1.6x10^-4; p<0.001). No difference in mean AD was observed for this region.
TABLE 6. DEMOGRAPHIC DATA FOR PARTICIPANTS ENROLLED IN THE CLOZAPINE STUDY. VALUES ARE PRESENTED AS MEAN (STANDARD DEVIATION), UNLESS DENOTED BY A †, INDICATING A NON-PARAMETRIC STATISTICAL COMPARISON FOR WHICH RESULTS ARE PRESENTED AS MEDIAN (INTERQUARTILE RANGE). AGE OF ONSET WAS DEFINED AS AGE AT FIRST RECORDED CONTACT WITH MENTAL HEALTH SERVICES. THC=TETRAHYDROCANNABINOL

<table>
<thead>
<tr>
<th></th>
<th>FLR (n=10)</th>
<th>Clozapine-eligible (n=14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.1 (8.4)</td>
<td>25.3 (5.3)</td>
<td>t=1.372; p=0.184,</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>40.0</td>
<td>14.3</td>
<td>Z score=-1.434</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.153</td>
</tr>
<tr>
<td>Duration of illness (years) †</td>
<td>5.5 (5.9)</td>
<td>5.3 (4.5)</td>
<td>Mann Whitney U=66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.841</td>
</tr>
<tr>
<td>Age of onset (years) †</td>
<td>23.9 (5.5)</td>
<td>21.1 (3.4)</td>
<td>Mann Whitney U=90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.259</td>
</tr>
<tr>
<td>PANSS score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>8.0 (3.0)</td>
<td>19.5 (10.0)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>11.0 (6.0)</td>
<td>20.0 (17.0)</td>
<td></td>
</tr>
<tr>
<td>General psychopathology</td>
<td>20.9 (2.8)</td>
<td>37.3 (8.1)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40.1 (6.3)</td>
<td>79.1 (17.4)</td>
<td></td>
</tr>
<tr>
<td>Current prescribed antipsychotic</td>
<td>Amisulpride=1</td>
<td>Aripiprazole=2</td>
<td>Mann Whitney U=41</td>
</tr>
<tr>
<td></td>
<td>Aripiprazole + olanzapine (low dose)=1</td>
<td>Aripiprazole + olanzapine + quetiapine=1</td>
<td>p=0.096</td>
</tr>
<tr>
<td></td>
<td>Olanzapine=4</td>
<td>Olanzapine=3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quetiapine=1</td>
<td>Palideridone=3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Risperidone=3</td>
<td>Risperidone=3</td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine equivalents †</td>
<td>438.9 (304.2)</td>
<td>663.7 (382.5)</td>
<td></td>
</tr>
<tr>
<td>Positive drug screen (THC; % participants)</td>
<td>30</td>
<td>14.3</td>
<td>Z score=-0.935</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.352</td>
</tr>
</tbody>
</table>

5.3.3. PROBABILISTIC TRACTOGRAPHY
Results from probabilistic tractography are presented in Figure 55. Mean probable tracts for the clozapine-eligible group extended farther than those of the FLR group; however, patterns of connectivity were similar. Major areas of probable connectivity included pre- and post-central...
gyri, precuneus cortices, superior frontal gyri, thalamic nuclei, areas of the frontal cortex including cingulate and paracingulate gyri and the subcallosal cortex (clozapine-eligible group only), parahippocampal gyri (clozapine-eligible group only) and brainstem (clozapine-eligible group only).

FIGURE 54. TBSS RESULTS SHOWING FA DIFFERENCE IN RED-ORANGE (FLR > CLOZAPINE-ELIGIBILITY; P < 0.05, FDR CORRECTED) IN THE BODY OF THE CC (PEAK MNI COORDINATE 5 -10 26 MM) OVERLAID ONTO MEAN FA SKELETON AND MNI152_1MM BRAIN.

FIGURE 55. MEAN TRACTS FOR FLR (RED-YELLOW) AND THOSE WHO WERE CLOZAPINE-ELIGIBLE (BLUE) OVERLAID ONTO MNI152 1MM BRAIN. FIGURE DEPICTS MEAN PROBABILISTIC TRACTOGRAPHY RESULTS FOR FLR OVERLAID ONTO CLOZAPINE-ELIGIBILITY (A) AND VICE VERSA (B) AT MNI COORDINATES -9 -17 30 MM.
5.4. DISCUSSION

This study examined whether variations in white matter microstructure could account for differences in the response to antipsychotic treatment in people with schizophrenia or psychosis. TBSS revealed significantly lower FA in the body of the CC in individuals who were clozapine-eligible compared to FLR. Post-hoc investigation of AD and RD in this cluster revealed greater RD in those who were eligible for clozapine compared with FLR, with no difference in AD observed between groups.

These results are in line with a number of studies reporting reduced FA in the CC of individuals with schizophrenia (123, 301, 304, 313-315). Though most studies focus on differences in the genu and splenium of the CC in those with schizophrenia compared with healthy controls (123, 301, 315) abnormalities in the callosal body have also been reported (304, 313). Of greatest significance to the current study are findings by Reis Marques et al., which demonstrated reduced FA in the body of the CC (among other regions) in non-responders compared with responders to 12 weeks of antipsychotic treatment (304). Whereas participants in the Reis Marques study (304) received 12 weeks of treatment only, participants in the current study were recruited into the clozapine-eligible group only if they had failed at least two six-week trials of antipsychotic drugs. Thereby, findings from this study expand on those by Reis Marques et al. (304) and suggest that low FA in the callosal body, specifically, is associated with clozapine-eligibility.

To investigate the neural underpinnings of lower FA in the clozapine-eligible group, AD and RD were measured in the cluster of voxels that exhibited a significant difference in FA between groups. Greater RD in the absence of any change in AD in the clozapine-eligible group was indicative of reduced myelination in this cohort compared with FLR (316). This hypothesis is supported by work demonstrating that myelin-deficient shiverer mice exhibit increased RD but unchanged AD compared with normal age-matched controls (317). A similar pattern of increased RD in healthy aging adults (312) as well as increased age-related FA decline in the CC of people with schizophrenia (318) suggest that the difference in RD observed could be attributable to accelerated age-related decline in those eligible for clozapine. Alternatively, these white matter deficits may be more static in nature and potentially due to disruptions in myelination that occur during adolescence (247).

It is currently unclear how deficits in white matter relate to treatment response; however, there may be an association with NMDA receptor function in oligodendrocytes. In addition to the role of NMDA receptors in synaptic plasticity and neuronal communication, NMDA receptor
signalling in oligodendrocytes is thought to play a crucial role in myelination and energy metabolism (94). Under normal circumstances, NMDA receptor activation may promote myelin induction (319) or be required to regulate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-dependent signalling with surrounding axons (320). NMDA receptor hypofunction (as discussed by Howes et al. (44)) in those eligible for clozapine but not (or to a lesser degree) in FLR may account for the lower FA and higher RD compared with FLR, in addition to the poor response to D₂ antagonists observed in this population. If NMDA receptor dysfunction is the root cause of these deficits, it is conceivable that the same may also be true for the aberrant functional connectivity observed in the clozapine-eligible group in chapter 3. Consequently, a hypothesis of abnormal efference copy signalling in those eligible for clozapine (which could be caused either by a deficit in NMDA function at synapses or reduced myelination leading to slowed action potentials) may still apply.

Although FA and RD provide a reliable measure of white matter integrity in areas of coherent fibre orientation, DTI studies suffer uncertainty in areas of crossing fibres (321). This problem can be minimised by employing large b values, many diffusion-encoding directions and smaller voxel size; however, the issue cannot be completely eliminated. As such, the decreased FA and increased RD observed in the clozapine-eligible group compared with FLR may be indicative of reduced axon packing density (322) or increased crossing fibres in the CC of those eligible for clozapine, rather than demyelination as previously discussed. This would denote a greater level of axon crossing in the clozapine-eligible group, potentially signifying greater disorganisation of structural connectivity. Though it was not possible to measure this conclusively in vivo, probabilistic tractography was employed to determine whether the destination of white matter tracts running through the cluster of low FA (CC cluster) was similar between the two groups. This also provided information related to the extent of connectivity in the clozapine-eligible group compared with FLR.

In both cohorts, probable tracts from the CC cluster terminated in areas of the sensorimotor network (SMN), including motor and sensory cortices and thalamic nuclei, in addition to other areas such as the cingulate and paracingulate gyri. Most interestingly, probable tracts from the CC cluster in the clozapine-eligible group, but not the FLR group, extended to the same region of the precuneus that demonstrated increased connectivity with the SMN in the functional connectivity analysis (chapter 3). This further strengthens the hypothesis that the SMN is somehow involved in the resistance to D₂ receptor (first-line) antipsychotics in people with schizophrenia.
In a recent study by Zeng et al., changes in FA following eight weeks of treatment with antipsychotic treatment were discovered in the left superior longitudinal fasciculus and correlated with changes in positive symptoms and processing speed (302). As discussed in chapter 3, the study’s cross-sectional nature prevents determination of causality with regard to the differences in FA observed here. However, the observation of decreased FA in the body of the CC in those eligible for clozapine is supported by prospective findings from Reis Marques et al. (304), suggesting these differences may be present prior to the onset of treatment. Further work is needed to determine whether FA can be used as a predictor of clozapine eligibility in drug-naïve patients.

The effect size for the difference in FA was small to moderate, though still provides evidence of lower FA in people eligible for clozapine compared to FLR. This modest effect size may relate to the small participant numbers included in the study. Small sample size also resulted in an unequal ratio of females to males (p>0.15) between the two groups. Differences in FA between healthy male and female participants have been reported in several regions of the brain, suggesting that the difference in gender ratios between groups could affect these results (323). As a precaution, gender was added as a covariate in the general linear model to reduce any likely effect on the results.

Three participants in the FLR group and two in the clozapine-eligible group tested positive for THC on the day of the MRI scan. Chronic cannabis use has been reported to reduce FA in several brain regions, including the CC, both in healthy adults and in people with early-phase schizophrenia (296). Given that a greater proportion of THC-positive urine screens were observed in the FLR group, it is unlikely that these results are confounded by the effects of cannabis use. However, it cannot be discounted that the effects of longer term cannabis use or lifetime exposure may have been greater in the clozapine-eligible group. Unfortunately, it would be impractical to measure lifetime exposure and self-reported use may be inaccurate.

These data reveal differences in diffusion measures between FLR and those eligible for clozapine and suggest that lower FA and greater RD in the CC may be a biomarker of treatment resistance in people with schizophrenia. In addition, they support the hypothesis that increased functional connectivity in the SMN of those eligible for clozapine is accompanied by increased structural connectivity within this network. More work is needed to determine whether the differences observed during treatment are present in treatment-naïve individuals and whether these differences are substantial enough to accurately predict clozapine eligibility. A more plausible
outcome is that a combination of factors, including FA or tractography, will be required to predict treatment outcome in schizophrenia.
6. PERSONALISED, PREDICTIVE MODELLING OF TREATMENT OUTCOME USING A SPIKING NEURAL NETWORK-BASED APPROACH

The preceding chapters of this thesis have revealed subtle differences in the structural and functional connectivity of individuals with schizophrenia who respond to antipsychotic therapy compared with those who are treatment-resistant. These findings contribute to the understanding of schizophrenia as a disorder of dysconnectivity and add weight to the argument that the disorder should be subtyped according to which drug class it responds. However, the immediate applicability of these findings to the clinical setting is indeterminate. An attempt to address this issue, using machine learning to create a personalised prediction model of clozapine response, is presented in this chapter.

Special thanks to Neelava Sengupta from the Knowledge Engineering and Discovery Research Institute, Auckland University of Technology, for his essential contribution to the development of the NeuCube (324) for the incorporation of multimodal brain data.

6.1. INTRODUCTION

Since the introduction of chlorpromazine in the mid-1950s, pharmacological intervention has been identified as an important and often essential element of recovery for people with schizophrenia (325). However, treatment with first-line antipsychotics is effective for only 60% to 80% of individuals (33, 34). Limited treatment options exist for those who fail to respond to first-line therapy, leaving many individuals without an effective means for controlling their symptoms. Evidence suggests that clozapine can successfully treat treatment-resistant schizophrenia (TRS) (19, 40, 326), producing dramatic improvements in quality of life and long-term functional outcomes in a proportion of individuals (16). However, clozapine’s potential to cause serious, life-threatening side effects, such as agranulocytosis, myocarditis and cardiomyopathy, has restricted its use and delayed access to this effective treatment for many individuals (226). Because of its remarkable potential to cause improvement, psychiatrists need dependable tools for predicting whether clozapine will be an effective and safe treatment option for clients.

There is strong evidence to suggest that schizophrenia is associated with disruptions to structural and functional connectivity (140, 142). Evidence from previous work also suggests that functional connectivity may differ between individuals who respond to clozapine and those who are ultra-treatment-resistant (UTRS) (327-329). Molina Rodriguez et al. identified lower
perfusion in the thalamus, left basal ganglia and right prefrontal regions in poor responders to clozapine prior to treatment (329) and found that individuals with high metabolic activity in the dorsolateral prefrontal cortex were more likely to experience improvements in negative symptoms following administration of clozapine (330). In another study, Knott et al. reported that improvement in the Positive and Negative Syndrome Scale (PANSS) correlated with pre-treatment inter- and intra-hemispheric spectral power asymmetry, measured using electroencephalography (EEG) (328). Findings from chapter 3 and 4 suggest that individuals with UTRS may exhibit differences in functional connectivity compared with healthy controls but these are not severe enough to distinguish these individuals from those with TRS. Furthermore, no biomarker of any nature identified using group-based univariate analysis has been successfully applied to the pre-treatment differentiation of TRS from UTRS in the clinical setting. For the personalised prediction of outcomes in individual consumers, machine learning may deliver a superior outcome.

Machine learning describes the use and development of algorithms with the ability to iteratively learn from data without explicit programming. These algorithms can be used to provide predictions about or respond to previously unseen instances (331), making them ideal tools for data classification in a clinical setting. The use of machine learning techniques may be especially advantageous in settings where current medical and scientific knowledge of a disorder does not provide clinicians with sufficient information to advocate an appropriate course of treatment. The pharmacological treatment of schizophrenia poses just such a problem; whereby, in the absence of any established biomarkers to predict treatment response, prescribing choices for first-line treatment rely on preference and experience, with rotation onto other possibilities following trial and error. This study sought to address this issue by investigating whether machine learning could be used to predict treatment outcomes in individuals initiating clozapine, using information gathered from people who had already responded or failed to respond to the drug. Structural and functional brain data hold promise for achieving such an endpoint, with studies suggesting that combining features from multiple sources can improve prediction accuracy (331-334).

To date, a number of studies have attempted to utilise machine learning techniques to predict treatment outcome in schizophrenia, with varying success. Lin et al. described the use of pharmacogenetic and clinical variables to predict clozapine response, reporting an overall prediction accuracy rate of 83% (335). Another study by Khodayari-Rostamabad et al. (334) utilised neurophysiological markers from EEG to obtain prediction accuracy rates of 85%.
However, no algorithm designed to predict response to clozapine has reached the clinical setting. This could be a result of underdeveloped algorithms. Although studies have incorporated data from different sources to improve classification (e.g. genetic data and fMRI (333)), no algorithm has utilised the information stored in one data source to facilitate learning of data from another. In this study, a machine learning algorithm capable of integrating data from diffusion magnetic resonance imaging (MRI) and functional MRI (fMRI) was first developed and then applied to the problem of response prediction in people initiating clozapine.

Machine learning is a rapidly developing area of research, within which exists both supervised and unsupervised learning. Supervised learning requires that data cases be labelled with their corresponding class. The algorithm then models the distribution of class labels based on a set of features, so that new, unlabelled instances can be assigned to a class based on those features (336). Where algorithms attempt to predict a discrete value (e.g. 0 or 1), this process is termed classification. Many algorithms exist for supervised classification, including logic-based (decision trees), perceptron-based (artificial neural networks), statistical-based (Bayesian networks) and instance-based algorithms (k-Nearest Neighbour), as well as support vector machines (336). Unsupervised algorithms also exist, where data are not labelled but are organised into classes based on characteristics that the algorithm itself identifies. This can be useful for identifying patterns in the data that were not otherwise immediately evident. An exhaustive review of machine learning techniques is beyond the scope of this thesis; however, a brief overview of some important algorithms and their respective strengths will be covered.

Decision trees (Figure 56a) are basic, easily understood algorithms, which attempt to classify instances based on a hierarchy of sequential rules that partition the data (337). The feature that best divides the data becomes the root of the tree and data are divided further and further until the same decision is reached for both branches (336). The simplicity and interpretability of these logical algorithms makes them ideal in instances where data can be divided orthogonally (336). However, these algorithms, like many learning algorithms, can be adversely affected by redundant and irrelevant attributes (338) and may suffer from overfitting when high-dimensional data are used. Feature selection is a potential means for alleviating this problem, whereby irrelevant and redundant features are removed to reduce the dimensionality of the data and prevent overfitting (339).

Instance-based learning is a category of statistical learning that requires less computation time than decision trees during training but greater computation time during classification (336). k-Nearest Neighbour (k-NN) is a lazy instance-based classifier based on the principle that instances
within a dataset will generally exist in close proximity to other instances in the same class (i.e. with the same properties) (340). This algorithm locates the \( k \) nearest instances to a new query instance and determines its class by identifying the most frequent class label in those \( k \) instances (340). Instances are considered as points in \( n \)-dimensional feature space, each dimension corresponding to one of \( n \) features used to describe the data (336). Neighbours are identified by the relative distance between instances, determined by a distance metric that must minimise the distance between two similarly classified instances and maximise the distance between instances of different classes (336). Despite disadvantages of higher computational effort and sensitivity to irrelevant features (336), Breiman reported that nearest neighbour classifiers are robust to small changes in training-testing splits, making them more stable than decision trees and some neural networks (341).

![Decision Tree and Logistic Regression Model](image)

**FIGURE 56. DEPICTION OF DECISION TREE (A) AND LOGISTIC REGRESSION MODEL (B) USED TO CLASSIFY INSTANCES IN CLASS 1 (PINK) AND CLASS 2 (PURPLE).**

Logistic regression is a simple discriminative classifier in which items from two classes (or more in the case of multi-class classification) are separated by a decision boundary determined by the relationship between two variables (see Figure 56b). A decision boundary can be expressed by a linear or non-linear equation but can only use information from at most two variables. This in turn limits the applicability of such algorithms when variability in the data cannot be explained by such a small number of features. Support Vector Machines (SVMs; Figure 57) address this problem by mapping the data into a higher dimension, explaining more of the variability with each additional dimension (342). SVM then generates a hyperplane that separates the two classes in high-dimensional feature space (342). The optimal hyperplane produces the greatest
margin separating the two classes (the space between each nearest case and the hyperplane will be maximised) (336).

Artificial neural networks (NNs) are biologically inspired computational models that consist of processing elements (neurons) and connections bound by weights (343). The single layer perceptron model, proposed by Pitts and McCulloch multiplies each input by its connection weight and then sums the weighted inputs (344, 345). The output class depends upon whether the sum is less than or greater than a predefined threshold (344, 345). The single-layer perceptron model possesses severe restrictions in its ability to learn and represent data; however, adding hidden layers to the network (multi-layer perceptron; see Figure 58) and using back-propagation (adjusting the weights of connections after misclassifications during training) to improve classification accuracy, many of these restrictions have been overcome (343, 346, 347). NNs can perform both supervised or unsupervised learning (343). Supervised NNs ‘learn’ to associate each training instance with its corresponding class and encode these examples in their internal structure; conversely, unsupervised NNs are presented with unclassified instances and ‘learn’ some internal feature of the whole dataset (including all training instances) (343).

Kotsiantis (336) reviewed a number of supervised machine learning algorithms and noted that the key question when dealing with machine learning classification is not whether any individual

FIGURE 57. GRAPHICAL DEPICTION OF SUPPORT VECTOR MACHINE (SMN). INSTANCES IN 'A' CANNOT BE CLASSIFIED BASED ON VARIABLES 1 AND 2 ALONE. SVM (B) TRANSFORMS DATA INTO A HIGHER DIMENSION (IN THIS CASE, ADDING A THIRD VARIABLE) AND A HYPERPLANE IS USED TO SEPARATE INSTANCES FROM DIFFERENT CLASSES. ONLY THREE DIMENSIONS ARE SHOWN IN ‘B’; HOWEVER, MANY MORE DIMENSIONS CAN BE INCORPORATED INTO THE MODEL.
algorithm is superior to others but rather under which circumstances an algorithm can significantly outperform others. Methods such as SVM and multi-layer perceptron have been used successfully to measure static brain data; however, they fail to capture the complex spatio-temporal relationships of fMRI, leading to less than optimal classification performance (324).

![Multi-layer Perceptron (NN) Diagram](image)


For the learning and classification of complex time-dependent data such as fMRI, compressing data into binary spike trains (designed to emulate action potentials (348)) can improve pattern recognition in contrast to techniques employing uncompressed, analogue data (349). Spiking NN (SNN) architecture is similar to that of traditional NNs, except that the processing elements (neurons) behave in a similar fashion to post-synaptic neurons in the brain (350). Specifically, they receive input from many neurons but only fire (spike) when the summed input reaches a given threshold (324). The Leaky-Integrate and Fire model is one such model and is illustrated in Figure 59. Incorporating another feature of biological neural networks, the weight of inter-neuronal connections can be updated according to how closely in time neurons in different layers of the network spike (351). This is referred to as the spike-time dependent plasticity (STDP) learning rule and is illustrated in Figure 60.
To incorporate the spatial information from fMRI data, Kasabov (324, 352) developed an SNN capable of dispersing spatially disparate input data (clusters or voxels) throughout a computational reservoir of artificial neurons (the NeuCube; see Figure 61). Within this framework, continuous fMRI timeseries data from each cluster or voxel are first encoded into spike trains and then input into the SNN using coordinates of the original timeseries to govern the position of input neurons in the reservoir (324, 352, 353). Unsupervised learning within the SNN reservoir identifies features in the data and generates weighted connections between temporally associated artificial neurons (324, 352). Connection weights are then encoded into spike trains and used by a supervised linear discriminator such as k-NN or SNN for classification (324, 352).

FIGURE 59. LEAKY INTEGRATE AND FIRE MODEL OF SPIKING NEURAL NETWORK. SPIKE TRAINS FROM INPUT NEURONS INCREASE THE MEMBRANE POTENTIAL OF THE POST-SYNAPTIC (OUTPUT OR HIDDEN) NEURON WITH EACH SPIKE. WHEN THE MEMBRANE POTENTIAL REACHES A CERTAIN THRESHOLD, THE POST-SYNAPTIC NEURON WILL SPIKE. LEAKAGE ALLOWS THE NEURON TO RETURN TO A LOWER MEMBRANE POTENTIAL WHEN SPIKES ARE TIMED TOO FAR APART TO BE MEANINGFUL. A REFRACTORY PERIOD AFTER THE SPIKE ENSURES THAT THE NETWORK DOES NOT BECOME OVERLOADED WITH ACTIVITY.

The NeuCube has demonstrated superiority over other techniques in classifying spatiotemporal brain data (354) and, unlike traditional NNs, is able to visualise the learned features of the unsupervised SNN. This added feature permits inspection of the characteristics associated with classification decisions. To further advance the functionality of the NeuCube in the current study, structural connectivity information from diffusion MRI was incorporated into the model to influence learning in the reservoir. Given the limited performance of classifiers to date to successfully translate into the clinical setting, it was anticipated that the addition of structural
directionality information would increase the robustness of the algorithm by increasing the strength of connections associated with corresponding diffusion and fMRI data and weakening connections associated with contradictory data. This study utilised structural and functional connectivity data from people with TRS and UTRS to predict response to clozapine in a group of clozapine-naïve individuals.

![STDP Learning Rule](image)

**FIGURE 60. STDP LEARNING RULE.** As the temporal difference ($t_{post} - t_{pre}$) between neuronal spikes decreases, the effect on weight updating increases, so that spikes timed closely together lead to greater increases in weight updating than spikes timed further apart. The order of spikes also affects weight updating. If the pre-synaptic neuron fires before the post-synaptic neuron consistently, then the weight of the connection between them will continue to increase; however, if the order switches, the weight is reduced.

![Neucube SNN Architecture](image)

**FIGURE 61. THE NEUCUBE, SNN ARCHITECTURE.** Temporal information from fMRI is encoded into spike trains and fed into the Neucube architecture using the source of the temporal signal as the input neuron location. The unsupervised SNN learns patterns associated with each sample and encoded spike trains are fed into the classifier.
6.2. METHOD

6.2.1. TRS STUDY – DEVELOPMENT OF A MODEL FOR CLASSIFYING TRS AND UTRS

6.2.1.1. PARTICIPANTS
Recruitment details and inclusion/exclusion criteria for the Treatment Resistant Schizophrenia (TRS) study are presented in chapter 3. For the purposes of this analysis, only individuals with TRS receiving clozapine and those with UTRS receiving augmented therapy were included. The study was approved by the Northern X Regional Ethics Committee and all participants gave informed written consent.

Participant demographics were compared across cohorts using IBM SPSS Statistics Version 24. Variables that satisfied assumptions of homoscedasticity (Brown-Forsythe test for equality of variances) and normality (Shapiro-Wilk test for normality) were analysed using the Student’s t-test. For those variables that violated assumptions of normality and/or homoscedasticity, the Mann-Whitney U tests or Z-score was employed.

6.2.1.2. DATA ACQUISITION
Structural, diffusion and resting-state (rs) functional magnetic resonance images were acquired using a Siemens Magnetom Skyra 3T scanner at the Centre for Advanced MRI, University of Auckland, New Zealand. Structural T1-weighted images were acquired using a magnetization-prepared 180-degrees radio-frequency pulses and rapid gradient-echo (MPRAGE) sequence. Acquisition parameters were as follows: Repetition time (TR) 1900 ms; echo time (TE) 2.39 ms; inversion time (TI) 900 ms; flip angle 9°; repetition 1; acceleration (GRAPPA) factor of 2; field of view (FOV) 230 mm; matrix 256 x 256; voxel size 0.9 x 0.9 x 0.8 mm.

Diffusion-weighted (DWI) images were acquired using an echo planar imaging (EPI) sequence with the following parameters: TR 8900 ms, TE 95 ms, FOV 240 mm, 122 x 122 matrix, 2.0 mm slice thickness, isotropic voxel size 2.0 x 2.0 x 2.0 mm. An acceleration factor of 2 was employed. Sixty-seven slices were acquired parallel to the anterior comissure-posterior comissure (A >> P) direction with diffusion-weighting factor b=1000 s/mm² in 64 gradient directions. Eight scans without diffusion weighting (b=0 s/mm²) were also acquired.

Gradient distortion (fieldmap) images for diffusion data were acquired using a gradient echo pulse sequence with the following parameters: TR 655 ms; TE1 4.92 ms; TE2 7.38 ms; voxel size 3.8 x 3.8 x 2.0 mm; phase encode direction R >> L; FOV 240 mm.

Rs functional images were acquired using EPI with the following parameters: TR 3000 ms, TE 30 ms; echo spacing 0.65 ms (0.62 ms for last 4 participants, following software upgrade); phase-
encode direction A >> P; slices 54; volumes 160; FOV 192 mm; acceleration factor of 2; matrix 64 x 64; voxel size 3.0 x 3.0 x 3.0 mm. Participants were asked to lie still with eyes open and concentrate on a fixation cross presented on a screen in front of the scanner. Participants were instructed to think of nothing in particular.

Gradient distortion images for functional data were acquired using a gradient echo pulse sequence with the following parameters: TR 655 ms; TE1 4.92 ms; TE2 7.38 ms; voxel size 3.4 x 3.4 x 2.4 mm; phase-encode direction A >> P; FOV 220 mm.

6.2.1.3. IMAGE PREPROCESSING – DIFFUSION DATA
DWI image preprocessing was performed using FSL version 5.0.7 (176, 207). Structural images were reoriented to a standard template and brain tissue was extracted from raw image files using FSL’s brain extraction tool (BET) (208). If automatic brain extraction failed to eliminate all non-brain tissue, the excess was removed manually. Magnitude images were subjected to the same process, after which brain-extracted images were eroded to ensure that no voxels containing non-brain tissue remained. Fieldmaps were then created using the fsl_prepare_fieldmap function. The gradient-free image was used to create a binary mask with BET. Gradient distortions were corrected using FSL’s fugue function and output registered to gradient-free images using the linear registration function (FLIRT) (210, 211). Data were then corrected for head movement and eddy current distortions using FSL’s eddy tool (174). Slices with average intensity at least four standard deviations lower than the expected intensity were interpolated with predictions made by the Gaussian Process (305). DTIfit was used to independently fit diffusion tensors to each voxel, limited to brain space using the binary brain mask. Crossing fibres were modelled using Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques (BEDPOSTX) (164). BEDPOSTX estimates of primary fibre orientations (dyads1) were then warped to a standard MNI template for use in the initial NeuCube construction.

6.2.1.4. IMAGE PREPROCESSING – FMRI
FMRI image preprocessing was performed using FSL version 5.0.7 (176, 207). Brain tissue was extracted from raw magnitude files using FSL’s BET (208), after which brain-extracted images were eroded to ensure that no voxels containing non-brain tissue remained. Fieldmaps were then created using the fsl_prepare_fieldmap function. Functional image registration to high resolution structural and MNI152 standard space was performed using FMRIB’s Expert Analysis Tool (FEAT). Preprocessing parameters in FEAT were as follows: motion correction = MCFLIRT; b0 unwarping = on; echo spacing = 0.325 (0.31 for last 5 participants); TE = 30; spatial smoothing = 5
ICA-based Automatic Removal Of Motion Artifacts (ICA-AROMA) was used to remove motion artefacts from the fMRI data utilising FSL’s FEAT output as input. (172, 173). White matter and cerebrospinal fluid (CSF) maps were segmented from high resolution structural images using FSL’s FAST (209) and warped to functional space using linear registration to FEAT output (FSL’s FLIRT (210, 211)). Nuisance timeseries were generated from ICA-AROMA output (denoised functional data) using CSF and white matter maps as input. A general linear model (GLM) of residual activity was then generated from the denoised functional data and nuisance timeseries using FSL’s GLM. A temporal mean file of denoised functional data was created, to which highpass temporal filtering (sigma=16.7) was applied in addition to removal of residual activity attributed to CSF and white matter. Filtered, denoised functional data was then warped to MNI standard space for use in further analysis.

6.2.1.5. MACHINE LEARNING FOR CLASSIFICATION

All learning and classification experiments were conducted in Matlab R2016 (Mathworks). A modified version of the NeuCube spiking neural network architecture for spatiotemporal brain data was developed to classify TRS study participants into ‘TRS’ and ‘UTRS’ response classes (324). Whereas previous versions of NeuCube have initialised connection weights within the reservoir using randomly weighted small-world connections (324), the availability of structural connectivity data from the current study allowed for a more personalised approach to learning. Specifically, the reservoir was created with equally-weighted small-world connections and the strength of these connections updated based not only on STDP but, importantly, the angle of the primary fibre orientation (dyads1) output from BEDPOSTX (see Figure 62). This effect was referred to as the angular influence and is depicted in Figure 63. This method has been validated using artificial data (unpublished, Sengupta, McNabb, Kasabov & Russell); however, the details of this validation are beyond the scope of this thesis.

FIGURE 63. GRAPHICAL DEPICTION OF ANGULAR INFLUENCE. THE PRIMARY FIBRE ORIENTATION (ANGULAR INFORMATION) AT NEURON j GOVERNS THE MAXIMUM CONNECTION STRENGTH ATTAINABLE BETWEEN NEURONS j AND i. (θ,Φ) REFERS TO THE ANGULAR DISTANCE.

The high spatial resolution of fMRI and DWI data used in this analysis, in combination with the limited computing capacity of Von Neumann architecture employed by traditional computers
required that data be condensed prior to unsupervised learning. A wrapper-based feature selection algorithm was employed to select a set of features (voxels from the fMRI data) that resulted in optimal classification performance. Wrapper methods consider feature selection as a search problem, creating different combinations of features and evaluating their performance using a predictive model. The wrapper algorithm used in the current study was the evolutionary genetic algorithm, which identifies the most appropriate set of features by mimicking genetic interactions and natural selection (see Figure 64). From the original pool of features (voxels), the algorithm arranged features into ‘chromosomes’ that contained a given number of genes (features) (356). Each chromosome was evaluated for its capacity to correctly classify instances; a selection operator then selected the chromosomes it would permit to reproduce, resulting in fitter chromosomes producing more offspring than less fit chromosomes (356). Crossover during reproduction led to the exchange of subparts of chromosomes, roughly mimicking biological recombination in haploid organisms (356). At this stage, inversion of subparts in some chromosomes and mutations at individuals genes may also have occurred (356). The process was repeated, assessing accuracy, selecting chromosomes for reproduction and crossing over, until the algorithm reached an optimal chromosome containing the best set of genes (features) for classifying instances in the training set.

The best feature subset was generated by a genetic algorithm with hyperparameters: population size=50, number of generations=200, offspring selection technique=roulette wheel, probability of crossover=0.3, probability of mutation=0.05. The fitness function used to evaluate the feature subset included prediction accuracy in the NeuCube classification algorithm and Cohen’s kappa. FMRI timeseries from the pre-selected voxels (identified during feature selection) were encoded into spike trains using Bens Spiker algorithm (BSA) (348). Spike-trains and angular information for each participant were then fed into individualised spiking neural network cubes (SNNc; i.e. one NeuCube per subject). This process is illustrated in Figure 65.

Each SNNc contained a set of 1000 computational spiking neurons, arranged randomly near the preselected feature set of input neurons. Within the SNNc, input spike trains were spatially distributed according to the position from which the timeseries originated and connection strengths were updated (learned) using the unsupervised SNN algorithm shown in Figure 62.
FIGURE 64. EVOLUTIONARY GENETIC ALGORITHM. FEATURES ARE SELECTED BASED ON A PROCESS SIMILAR TO NATURAL SELECTION. ‘CHROMOSOMES’ THAT PRODUCE THE HIGHEST CLASSIFICATION ACCURACY ARE MORE LIKELY TO REPRODUCE WITH OTHER WELL-PERFORMING CHROMOSOMES THAN POORLY PERFORMING CHROMOSOMES. THIS PROCESS IS REPEATED UNTIL THE ULTIMATE CHROMOSOME CONTAINING THE BEST SET OF FEATURES IS DETERMINED.

A second, high-dimensional spike train encoding the connection strengths within the SNNc was then produced, from which the supervised classifier was trained (see Figure 66). A lazy $k$-NN model was employed for supervised learning, using the Euclidean distance between the normalised weight vectors as the distance function. This algorithm ‘memorised’ the dataset and compared each new subject’s data to this memorised pool; it then classified the new subject based on their closest data ‘neighbours’ (i.e. which SNNc their connectivity most closely resembled). Classification performance was assessed with a grid-based hyperparameter search
using leave-one-out cross-validation (357, 358) whereby one participant at a time was sequentially removed from the training set and the accuracy of the model tested using that participant’s data as the test case. The overall performance scores reported are aggregate performance scores for all iterations of the leave-one-out cross-validation. Classification accuracy was described using proportions of true positives and true negatives. The sensitivity and specificity of the model was defined by the following equations:

\[
\text{Sensitivity} = \frac{\text{Number of true positives}}{\text{Number of true positives} + \text{Number of false negatives}}
\]

\[
\text{Specificity} = \frac{\text{Number of true negatives}}{\text{Number of true negatives} + \text{Number of false positives}}
\]

The performance of the model was also measured using Cohen’s kappa coefficient, a robust measure of performance that considers the probability that a reported success rate occurred by chance.

FIGURE 65. DEPICTION OF LEARNING AND CLASSIFICATION IN THE MODIFIED NEUCUBE (SNNC), INCORPORATING ANGULAR INFLUENCE FROM DWI AND SPATIAL INFORMATION FROM FMRI.
FIGURE 66. PERSONALISED MODELLING AND CLASSIFICATION USING SPATIAL, TEMPORAL AND DIRECTIONAL INFORMATION. DATA FOR EACH INDIVIDUAL UNDERGO UNSUPERVISED LEARNING IN THE PERSONALISED SNNC, WHICH CAPTURES INFORMATION REGARDING STRUCTURAL AND FUNCTIONAL CONNECTIVITY WITHIN ITS CONNECTION STRENGTHS. THE CONNECTION STRENGTHS OF EACH PERSONALISED SNNC MAP ARE THEN USED IN THE SUPERVISED MODEL (CLASSIFIER), WHICH LEARNS THE PATTERNS ASSOCIATED WITH EACH CLASS. NEW DATA IS PREDICTED BY THE CLASSIFIER ONCE IT HAS PASSED THROUGH THE UNSUPERVISED LEARNING ALGORITHM (SNNC).

6.2.2. MODEL CHALLENGE USING PREVIOUSLY UNSEEN DATA FROM THE CloRes STUDY
The primary objective of this study was to establish whether it is possible to predict response to clozapine in clozapine-naïve individuals. Therefore, the predictive accuracy of the response model was tested using previously unseen data from the Clozapine Response (CloRes) study.

6.2.2.1. PARTICIPANTS
This portion of the study was a prospective, longitudinal investigation of response to a three-month trial of clozapine in individuals with schizophrenia who had failed to respond to at least two six-week trials of first-line antipsychotic drugs. Recruitment details and inclusion/exclusion criteria for the CloRes study are reported in chapter 3, though only participants with TRS who initiated treatment with clozapine were included in the current analysis.

All participants met DSM–V criteria for schizophrenia and were receiving stable treatment with a first-line antipsychotic at baseline. Prior to clozapine initiation, all participants were interviewed by an academic psychiatrist, who confirmed the diagnosis of schizophrenia and resistance to first-line antipsychotics and provided a recommendation regarding whether the initiation of
clozapine seemed appropriate. This included an assessment of residual symptoms using the PANSS, for which a score of >50 was required to meet study conditions for treatment resistance. Individuals who did not meet all the above criteria were recommended a further trial of first-line treatment before inclusion in the study would be considered. The study was approved by the Northern A Regional Ethics Committee (ref 14/NTA/103/AM11) and all participants gave informed written consent.

Following baseline assessments, eligible participants underwent structural and functional MR scans. Each participant was then initiated on a three-month course of clozapine, the dosing regimen of which was at the discretion of their treating psychiatrist. After three months of treatment, participants were reassessed and their symptoms rated using the PANSS. Participants who improved by at least 25% from baseline were classed as responders (TRS) and those who failed to meet this threshold were classed as non-responders (UTRS). This threshold is in agreement with previous work demonstrating poorer magnitude of response in individuals with TRS compared with FLR (359). Adjustment of this threshold to 50% did not affect the partition between responders and non-responders.

As there were only a small number of participants who met criteria for inclusion in the follow-up study, between-group statistical comparisons of baseline demographics were not conducted. Demographic data are presented for the reader’s interest only.

6.2.2.2. DATA ACQUISITION
Structural, diffusion and rs-fMRI images were acquired using a Siemens Magnetom Skyra 3T scanner at the Centre for Advanced MRI, University of Auckland, New Zealand. All participants were imaged using a 32-channel head coil. A structural T1-weighted image was acquired using an MPRAGE sequence with TR 2000 ms; TE 3.48 ms; TI 1010 ms; flip angle 9°; repetition 1; acceleration factor of 2; FOV 230 mm; voxel size 0.9 x 0.9 x 0.9 mm.

Diffusion-weighted images were acquired using a multiband gradient-echo pulse sequence (University of Minnesota (167)). One image without diffusion gradients (b=0 s/mm²) was acquired, in addition to 105 images with diffusion-encoding directions isotropically distributed in space at b values ranging from 5 to 2010 s/mm² (10 values in total). Seventy-two slices were acquired in the A >> P direction, with a base resolution of 108 and the following parameters: TR 3600 ms; TE 92.4 ms; echo spacing 0.67 ms; EPI factor 108; multiband acceleration factor 3; flip angle 78°; FOV 220 mm, voxel size 2 x 2 x 2 mm.
Gradient distortion images for diffusion data were acquired using a gradient echo pulse sequence with the following parameters: TR 704 ms; TE1 4.92 ms; TE2 7.38 ms; voxel size 3.4 x 3.4 x 2.0 mm; phase-encode direction A >> P; FOV 220 mm.

Rs functional images were acquired using a multiband gradient-echo EPI pulse sequence (University of Minnesota (167)) with TR 735 ms, TE 39 ms; multiband acceleration factor 8; flip angle 52°; echo spacing 0.64 ms; EPI factor 92; phase-encode direction A >> P; slices 64; volumes 410; FOV 220 mm; parallel imaging off; matrix 92 x 92; voxel size 2.4 x 2.4 x 2.4 mm. Participants were asked to lie still with eyes open and concentrate on a fixation cross presented on a screen in front of the scanner. Participants were instructed to think of nothing in particular.

Gradient distortion images for functional data were acquired using a gradient echo pulse sequence with the following parameters: TR 655 ms; TE1 4.92 ms; TE2 7.38 ms; voxel size 3.4 x 3.4 x 2.4; phase-encode direction A >> P; FOV 220 mm.

6.2.2.3. IMAGE PREPROCESSING – DIFFUSION DATA
DwI preprocessing was performed using FSL version 5.0.9 (176, 207). Preprocessing steps were the same as for section 6.2.1.3., save the following: following BET and the creation of a fieldmap, data were corrected for head movement and eddy current distortions using eddy_openmp to align all volumes (174). Gradient distortions were corrected using FSL’s fugue function after eddy correction and output was registered to gradient-free images using FLIRT (210, 211).

6.2.2.4. IMAGE PREPROCESSING - FMRI
FMRI preprocessing and analysis were performed using FSL version 5.0.7 (176, 207). Preprocessing steps were the same as for section 6.2.1.4., save preprocessing parameters in FEAT, which were as follows: motion correction = MCFLIRT; b0 unwarping = on; echo spacing = 0.64; TE = 39; spatial smoothing = 5 mm; global intensity normalisation = on; temporal filtering = off; MELODIC = off; registration to structural image = boundary-based registration; registration to MNI152_2mm = non-linear; warp resolution = 10 mm.

6.2.2.5. MACHINE LEARNING FOR PREDICTION
Data were condensed first using feature selection (determined by features identified during model development with TRS data) and then with spike encoding. An SNNc was created for each participant in the CloRes study and high-dimensional spike trains encoding connection weights within each participant’s SNNc were used as input for the k-NN classifier (see Figure 67). Classification accuracy was described using proportions of false positives (specificity) and false negatives (sensitivity) and Cohen’s kappa.
6.3. Results

6.3.1. Development of a classification model using TRS study data

6.3.1.1. Participant demographics
MRI scans were obtained from 36 individuals with schizophrenia, of which 11 were excluded due to excessive head motion during either diffusion or rs-fMRI scans. Of the remaining participants, 14 had TRS and 11 had UTRS. Participant demographics are presented in Table 7. Individuals with UTRS were receiving higher doses of antipsychotics compared with the TRS cohort (p<0.01) and were more likely to test positive for tetrahydrocannabinol (THC; p<0.05).

![Image of a diagram showing the process of training a model and applying it to classify samples.]

**Figure 67. Testing of response model using CloRes study data. Samples are classified using the K-NN classifier developed with TRS study data.**

6.3.1.2. Classification performance
The best performing feature subset is shown in Figure 68. This subset included 21,998 voxels, concentrated mainly in regions of the brainstem, parietal, frontal, temporal and occipital cortices. Leave-one-out cross-validation in the personalised SNN and angular influenced STDP model provided an overall accuracy rate of 74.9%. The true positive (actual UTRS = predicted UTRS) rate (sensitivity) was 75% and the true negative (actual TRS = predicted TRS) rate (specificity) was 74%. The Cohen’s kappa value for the classification model was 0.37, indicating relatively good classification performance.
TABLE 7. DEMOGRAPHIC CHARACTERISTICS OF TRS STUDY COHORT SHOWING MEAN (STANDARD DEVIATION) FOR PARAMETRIC AND MEDIAN (INTERQUARTILE RANGE) FOR NON-PARAMETRIC COMPARISONS. COMPARISONS WERE MADE USING PARAMETRIC TESTS, UNLESS DENOTED BY A †.

<table>
<thead>
<tr>
<th>TRS study</th>
<th>Treatment-resistant (n=14)</th>
<th>Ultra-treatment-resistant (n=11)</th>
<th>T/Z/ Mann-Whitney U statistic and p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.0 (8.1)</td>
<td>34.2 (7.3)</td>
<td>t=0.682; p=0.502</td>
</tr>
<tr>
<td>Gender† (% female)</td>
<td>35.7</td>
<td>22.0</td>
<td>Z score=0.969; p=0.332</td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td>12.3 (7.2)</td>
<td>11.8 (5.4)</td>
<td>t=0.180; p=0.859</td>
</tr>
<tr>
<td>PANSS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive†</td>
<td>8.5 (8)</td>
<td>14 (8)</td>
<td>MWU=102.0; p=0.183</td>
</tr>
<tr>
<td>Negative</td>
<td>18.4 (7.7)</td>
<td>20.2 (7.3)</td>
<td>t=-0.577; p=0.569</td>
</tr>
<tr>
<td>General psychopathology</td>
<td>27.9 (6.3)</td>
<td>29.4 (4.0)</td>
<td>t=-0.728; p=0.474</td>
</tr>
<tr>
<td>Total score</td>
<td>56.9 (13.8)</td>
<td>63.0 (9.2)</td>
<td>t=-1.272; p=0.216</td>
</tr>
<tr>
<td>Current prescribed antipsychotic</td>
<td>Clozapine=14</td>
<td>Clozapine + amisulpride=2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clozapine + quetiapine=1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clozapine + aripiprazole=4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clozapine + risperidone=2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quetiapine + aripiprazole=1</td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine equivalents</td>
<td>396.6 (194.2)</td>
<td>863.2 (423.1)</td>
<td>t=-3.388; p=0.005</td>
</tr>
<tr>
<td>Drug Screen† (% THC positive)</td>
<td>0</td>
<td>27.2</td>
<td>Z score=-2.083; p=0.038</td>
</tr>
</tbody>
</table>
FIGURE 68. FIRST BEST FEATURE SELECTION, CONTAINING 21,998 VOXELS FROM REGIONS OF THE BRAINSTEM, PARIETAL, FRONTAL, TEMPORAL AND OCCIPITAL CORTICES

The next best performing feature subset is shown in Figure 69. This subset included 34,629 voxels, concentrated mainly in the parietal, frontal and temporal cortices, brainstem and subcortical tissue. Leave-one-out cross-validation in the personalised SNN and angular influenced STDP model provided an overall accuracy rate of 73.7%. Sensitivity and specificity were 73% and 74%, respectively.

FIGURE 69. SECOND BEST FEATURE SELECTION, CONTAINING 34,629 VOXELS FROM REGIONS OF THE PARIETAL, FRONTAL AND TEMPORAL CORTICES, BRAINSTEM AND SUBCORTICAL TISSUE.
6.3.2. Prediction of future response in the CloRes study

6.3.2.1. Participant demographics
Fifteen clozapine-eligible individuals were enrolled into the CloRes study and 11 initiated treatment with clozapine. Of those individuals, four discontinued treatment due to adverse events: three due to suspected myocarditis and one due to suspected pericarditis. Of the remaining participants, five achieved a reduction from baseline on the PANSS of ≥25% (TRS) and two failed to reach this target (UTRS). All individuals who achieved a response ≥25% also achieved a response ≥50%. Participant demographics are presented in Table 8. As only two participants had UTRS, no statistical comparisons were performed between groups.

**TABLE 8. Participant demographics for CloRes study cohort. All values are expressed as median (interquartile range)**

<table>
<thead>
<tr>
<th>CloRes study</th>
<th>Treatment-resistant (n=5)</th>
<th>Ultra-treatment-resistant (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23(9)</td>
<td>25</td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td>3.9(6.6)</td>
<td>5.2</td>
</tr>
<tr>
<td>PANSS (baseline)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>20 (9)</td>
<td>22</td>
</tr>
<tr>
<td>Negative</td>
<td>31(17)</td>
<td>17</td>
</tr>
<tr>
<td>General psychopathology</td>
<td>42(18)</td>
<td>36</td>
</tr>
<tr>
<td>Total score</td>
<td>82(28)</td>
<td>75</td>
</tr>
<tr>
<td>PANSS (follow up)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10(4)</td>
<td>18</td>
</tr>
<tr>
<td>Negative</td>
<td>17(5)</td>
<td>22</td>
</tr>
<tr>
<td>General psychopathology</td>
<td>20(4)</td>
<td>32</td>
</tr>
<tr>
<td>Total score</td>
<td>48(11)</td>
<td>72</td>
</tr>
<tr>
<td>PANSS score change from baseline</td>
<td>61.7(18.4)</td>
<td>20</td>
</tr>
<tr>
<td>Prescribed antipsychotic at baseline</td>
<td>Olanzapine + quetiapine (PRN)=1</td>
<td>Olanzapine=1</td>
</tr>
<tr>
<td></td>
<td>Olanzapine + aripiprazole=1</td>
<td>Risperidone=1</td>
</tr>
<tr>
<td></td>
<td>Risperidone=1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aripiprazole=1</td>
<td></td>
</tr>
<tr>
<td>Chlorpromazine equivalents</td>
<td>416.2(406.5)</td>
<td>652.3</td>
</tr>
<tr>
<td>Drug Screen (% THC positive)</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
6.3.2.2. **CLASSIFICATION PERFORMANCE**

Classification performance for the prospective cohort was poor. Overall accuracy was 44.4%; sensitivity and specificity were 0% and 44%, respectively. No participant in the UTRS cohort was correctly classified, leading to a low kappa value of -0.36.

6.4. **DISCUSSION**

This was the first attempt to integrate information from multiple imaging modalities into SNN architecture. Although classification accuracy did not surpass that of previously reported studies (334, 335), results support the rationality of combining fMRI and diffusion MRI data for the classification and prediction of clozapine response in individuals with schizophrenia. The NeuCube personalised SNN and angular influenced STDP model was able to accurately classify 75% of individuals with UTRS and 74% of individuals with TRS (cross-sectional data) using a leave-one-out cross-validation procedure. Application of the model using prospective data from seven individuals initiating clozapine provided only 44% accuracy and failed to correctly identify any individual with UTRS prior to clozapine initiation. This poor result was not unexpected, given the small number of participants included in this part of the study.

A major issue in machine learning applications is the risk of over-fitting, arising when an algorithm over-adapts to the specificities of the training set and subsequently fails to generalise to new data samples. The learning algorithm employed in this study was formulated in such a way that corresponding data from diffusion and functional MRI was favoured over contradictory data. This was dictated by the angular influence of directional information over the effects of spiking between neighbouring neurons in the SNN. Consequently, the model should be more resilient to over-fitting compared with more traditional models that simply overlay information.

This algorithm performed poorly in comparison to an earlier published algorithm by Khodayari-Rostamabad et al., which achieved classification accuracy above 85% in samples of 23 (training and classifier development) and 14 (validation set) individuals using pre-treatment EEG (334). However, despite addressing the requirement of a 25% improvement from baseline in symptom rating score for those classified as responders, the algorithm was trained using an arbitrary total symptom score to define treatment response (334). Experiments were performed using a range of scores to define treatment response and the best performing algorithm selected for further testing (334). Consequently, the training set may not be representative of a clinical population and so the algorithm may not translate well into clinical practice. To test the validity of the model created in the current study, data from a prospective, previously unseen cohort of individuals were subjected to classification. The model performed poorly, with only 44% of
individuals correctly classified and no correctly identified cases of UTRS. As the model was developed using cross-sectional data from individuals who were already receiving and responding to treatment with clozapine or augmented antipsychotic therapy, the generalisability of the model was likely affected. A classifier constructed using pre-clozapine fMRI and diffusion MRI data may therefore provide a more robust predictor of treatment response.

The objective of this study was to develop a predictive model of clozapine response using relevant features from diffusion and functional MRI. Although the derivation of neurological information was not a primary focus of the study, the results of feature selection may still be informative. In the two best performing feature sets, the genetic algorithm chose features in the brainstem, subcortical tissue, parietal, frontal, temporal and occipital cortices. Disruptions in frontal, temporal and parietal connectivity in those with UTRS have been demonstrated in the preceding chapters of this thesis. Regions of the occipital, frontal, temporal and parietal cortices were also found to host grey matter density changes in those with UTRS compared with healthy controls (148). Selection of features within these regions may reflect global disruptions in connectivity observed in individuals with UTRS, although disruptions in those with TRS may also be driving feature selection. The use of a feature selection method such as genetic algorithm eliminates selection bias that arises when a priori hypotheses drawn from previous work are used to constrain the feature set. However, this may hinder classification performance if noisy or useless features are selected by the algorithm. Despite supplemental motion correction using ICA-AROMA (172), the large set of features selected within the brainstem may represent voxels with excessive noise caused by voluntary or physiological motion (see chapter 2 for a discussion of head motion in fMRI). To improve classification performance it may be necessary to mask the brain prior to feature selection, excluding noisy voxels within the lower brainstem and edges of the cortex.

The small sample size relative to the number of features also may have affected classification performance. Classification algorithms are generally developed using hundreds or thousands of data samples, providing robust models that can generalise to future input. The current study has verified the feasibility of using fMRI and diffusion MRI data to predict clozapine response; however, a much larger dataset would be required to develop a robust, future-proof prediction tool. This would require a large-scale data collection programme, coupled with long-term follow-up and clinician participation. The OPTiMiSE (Optimization of Treatment and Management of Schizophrenia in Europe) study plans to conduct a large scale investigation of MRI markers associated with response to a four-week trial of amisulpride (360); however, no large scale
investigation of long-term antipsychotic response, or clozapine response specifically, has been initiated.

Groups in the TRS study differed in terms of positive drug screens and the dose of antipsychotic drug taken at the time of scanning. Both THC and antipsychotics have been shown to influence functional connectivity measured using fMRI (222, 223). Consequently, the model developed from this data may contain features that are specific to antipsychotic dose or THC use, which may distract from the model’s intended purpose of distinguishing between TRS and UTRS. This is just one of many complex issues that arise from working with a real-world clinical population. Exclusion of individuals following recreational drug use, however, would further narrow the applicability of any model developed for the clinic, causing additional problems with performance.

The prospective component of this study suffered from low participant accrual, with only 11 participants initiating treatment with clozapine. This was accompanied by high rates of clozapine-induced adverse events, specifically, suspected myocarditis (n=3) and pericarditis (n=1), leading to discontinuation of clozapine in four participants. The suspected rate of myocarditis in the current cohort was 27%, substantially higher than the 0.7%-1.2% of treated individuals reported in a retrospective analysis of cases in Australia in 2003 (361). All participants who discontinued clozapine due to suspected myocarditis in the current study were male and were aged 23 to 24 years; two were of Pacific Island descent and one of European descent. At present, it is difficult to establish why the rate of discontinuation due to myocarditis was so high in the present study. An ongoing study at the University of Auckland is investigating biomarkers of clozapine-induced myocarditis and cardiomyopathy (ethics number 14/NTB/86) and may provide answers.

Clozapine is an effective treatment that is markedly underused in the clinical setting due to its propensity to cause serious, life-threatening adverse effects. Development of an algorithm for predicting a response to clozapine would greatly assist clinicians in weighing up the associated risks and benefits. Integration of directional information from DWI and functional connectivity information from fMRI was able to achieve only moderate classification accuracy using the NeuCube SNN architecture. To improve model performance and generalisability, future developments should include prospective data acquired prior to clozapine initiation and a larger number of samples.
7. DISCUSSION AND CONCLUSIONS

The findings presented in this thesis reveal substantial disruptions to functional and structural connectivity in individuals with schizophrenia who are eligible for clozapine and in those with an ultra-resistant subtype of the disorder. These findings are consistent with the hypothesis that schizophrenia is a disorder of dysconnectivity and support the classification of individuals based on their response to antipsychotic medication. This chapter will review the major findings of the preceding chapters and discuss their implications with respect to the wider field of schizophrenia. Limitations of the work will also be addressed, followed by proposals for future work.

7.1. SUMMARY AND DISCUSSION OF FINDINGS

Major findings from this thesis and their proposed relationships to treatment response are included in this section.

7.1.1. FIRST-LINE RESPONDERS

First-line responders (FLR) to antipsychotic medication exhibited no significant differences in functional connectivity compared with healthy controls or any other schizophrenia cohort when measured using independent components analysis (ICA). However, FLR did demonstrate a moderate degree of dysconnectivity compared with healthy controls when assessed using graph theoretical measures.

Compared with healthy controls, FLR exhibited lower positively correlated connection strength at one cerebellar node and greater anticorrelated connection strength at two frontal and three cerebellar nodes. Both of these observations were shared with at least one other schizophrenia cohort, suggesting that the observed differences were likely associated with an overall diagnosis of schizophrenia as opposed to a probability of response to antipsychotic medication. However, the extent to which FLR exhibited increased strength of negatively weighted connections at cerebellar and frontal nodes was less than in those with ultra-treatment-resistant schizophrenia (UTRS). This suggests that in terms of anticorrelated connection strength, FLR may exhibit similar pathology to UTRS but to a lesser degree. A shift away from correlated functional activity and toward stronger anticorrelated activity may signify a loss of functional synchrony between structurally disparate brain regions. In the case of FLR, these regions may belong to different functional networks, which may explain why graph theoretical analysis was able to identify a
disruption in connectivity where ICA found none. This finding may therefore be attributable to a strengthened connection between anticorrelated functional networks. Spontaneous anticorrelations between functional networks have been demonstrated in healthy subjects (362) and disruptions in anticorrelated network activity have been described in individuals with schizophrenia (189, 191). As such, it may be useful to subject the data to further analysis using network modelling (e.g. FSLnets) or Granger causality so that the relationship between the networks identified during ICA can be established.

As the disruptions to functional connectivity observed in FLR were not unique to this cohort, it is difficult to determine whether there is any specific class of dysconnection that renders an individual receptive to the effects of antipsychotics. As individuals were responding to therapy at the time of the study, it may be possible that treatment with antipsychotic medication has dampened the severity of dysconnection. This could indicate that the aetiology of schizophrenia in FLR is more transient, possibly triggered by a dopamine-induced disruption to NMDA receptor function that is ameliorated upon treatment with D$_2$ receptor antagonists.

7.1.2. CLOZAPINE-ELIGIBLE PARTICIPANTS

All clozapine-eligible participants had failed to respond to treatment with two D$_2$ receptor antagonists, indicating that, unlike FLRs, any pathology observed in this group is unlikely to originate from a primary dysfunction in dopaminergic activity.

Individuals who met eligibility criteria for a trial of clozapine exhibited differences in both functional and structural brain connectivity compared with FLR. ICA revealed supplementary connectivity of the precuneus in a network otherwise comprising the motor cortices, primary somatosensory cortices, insulae, central opercular cortices, cerebellum and thalamus (identified as the sensorimotor network; SMN). This finding was accompanied by lower fractional anisotropy (FA), higher radial diffusivity (RD) and no difference in axial diffusivity (AD) in the corpus callosum (CC) compared with FLR. Increased RD in the absence of any change in AD is proposed to indicate a deficit in myelination (316). Insufficient myelination may be the result of NMDA receptor dysfunction, as normal NMDA receptor activation has been shown to promote myelin induction and regulate AMPA receptor-dependent axon signalling (319, 320).

Probabilistic tractography from the CC seed space identified probable tracts extending to the precuneus in the clozapine-eligible group but not in FLR. This finding corresponds well with the observed increase in functional connectivity between the SMN and precuneus in those who are clozapine-eligible. However, whether extended structural connectivity to the precuneus is the
primary pathology or is secondary to long-term aberrations in synaptic plasticity within the SMN or precuneus is currently indeterminable. Long-term aberrations in synaptic plasticity (possibly mediated by NMDA receptor dysfunction) could affect the survival of long-range structural connections during developmental pruning (100), thereby resulting in abnormal structural connectivity between the SMN and precuneus secondary to functional dysconnectivity. Equally however, a primary disruption in structural connectivity between the SMN and precuneus, or a reduction in myelination (noted above) leading to slower processing speed, could account for increased functional connectivity between these regions. Research has shown that disrupted white matter connectivity is present at the onset of schizophrenia (313, 315); however, this may be attributable to much earlier disruptions in synaptic plasticity (100) that manifest as both structural and functional deficits by the time symptoms emerge.

Previous studies have identified elevated levels of glutamate in individuals who were eligible for clozapine as well as in those who later became eligible for the drug (76, 79). These findings indicate that disruptions in the glutamatergic system, most likely attributable to abnormal NMDA receptor function, may be responsible for D2 receptor antagonist-resistance. Given the purported roles of NMDA receptors in synaptic plasticity and myelination, it is possible that disrupted NMDA receptor function may account for the structural and functional abnormalities observed in clozapine-eligible individuals in this study. However, other than to assume the deficit does not stem from a primary abnormality in dopamine signalling, it is unclear what mechanism is driving the proposed NMDA receptor-mediated dysconnectivity.

7.1.3. TREATMENT-RESISTANT SCHIZOPHRENIA

Individuals with treatment-resistant schizophrenia (TRS) exhibited a small degree of dysconnectivity compared with healthy controls; however, no feature was unique to this cohort (i.e. the same disturbances were also seen in FLR and/or those with UTRS). Graph theoretical analysis revealed lower correlated connection strength at one cerebellar node compared with healthy controls; however, this same node demonstrated lower correlated functional connection strength in FLR and those with UTRS. No other functional abnormality was observed using either ICA or graph theory-based analysis.

Previous analysis of the same cohort revealed significant reductions in the FA and AD of several major white matter tracts compared with controls (220). Low AD is indicative of axonal injury or shrinkage, which could reflect damage to neurofilaments within the axon (316). Damage to axons would presumably lead to eventual disruption in communication; however, no such
disruption was observed using ICA and very little disruption was found using graph theory-based analysis.

This cohort has also demonstrated reductions in grey matter density in the right central operculum and right inferior temporal gyrus compared with healthy controls (148), which indicates that there may be some functional dysconnection present at the synaptic or dendritic level. Individuals within the TRS cohort were receiving clozapine at the time of participation and were considered to be responding well to treatment. As this group showed numerous signs of structural aberration but no major functional dysconnectivity, it may be feasible that clozapine dampened the presence of any observable functional disturbance but had no effect on structural abnormalities, which may be less transient. Conversely, clozapine may cause damage to axons and dendrites such that the changes observed while participants are receiving treatment may reflect the effects of clozapine as opposed to the underlying pathology of the disorder. This would account for the observation that AD was decreased in those with TRS receiving clozapine whereas no such change was observed in those who were eligible for clozapine but receiving treatment with D2 antagonists. However, animal work showing that clozapine attenuates rather than accentuates white matter damage caused by the copper chelator cuprizone (363) suggests this is unlikely.

The absence of substantial disruptions to functional connectivity despite earlier reports of structural abnormalities in those with TRS suggests there is either an underlying dysfunction ameliorated by clozapine or the mechanism responsible for TRS (which responds to clozapine) is different to that of clozapine-eligibility (i.e. failure to respond to first-line antipsychotics). NMDA receptor dysfunction could potentially account for changes in grey matter density by increasing synaptic pruning (100); however, the effects on AD are less easily explained. Rather, TRS may develop from an alternative, as yet unidentified mechanism, manageable only by clozapine.

7.1.4. ULTRA-TREATMENT-RESISTANT SCHIZOPHRENIA

Individuals with UTRS demonstrated robust changes in functional connectivity using a range of analytical techniques. ICA revealed greater functional connectivity between the paracingulate gyrus and other regions of the language network, including temporal and parietal cortices, superior and middle frontal gyri, frontal orbital cortices, angular gyri and the precuneus in individuals with UTRS compared with healthy controls. Interestingly, temporal, cingulate and prefrontal nodes lying within close proximity to these regions formed one of the disconnected sub-networks identified by network-based statistics. This may indicate expansion of the language network to compensate for an otherwise reduced level of communication in nearby
regions. Alternatively, the method by which these two analysis techniques recognise functional networks may differ in such a way that changes in network communication manifest in opposing manners.

Two additional dysconnected sub-networks were identified by network-based statistics, the first extending between cerebellar and frontal nodes and the second between frontal and parietal nodes. Both demonstrated diminished functional connectivity in those with UTRS compared with healthy controls. Weakened functional connectivity between the cerebellar and frontal nodes is likely attributable to functional dysconnection at a synaptic level in cerebellar tissue. No aberrations in structural connectivity have been identified in this cohort (220). However, previously published voxel-based morphometry (VBM) results from this same cohort demonstrated disruptions to grey matter density in the cerebellum (148), indicative of dendritic loss or damage (137). Regions of grey matter loss also coincided with frontal and parietal nodes from the alternative sub-network, suggesting that those with UTRS may exhibit a global state of functional dysconnection caused by disruptions to cell communication at a synaptic level. This concept is further reinforced by findings that individuals with UTRS demonstrated weaker correlated (positive) connectivity strength compared with controls across the whole brain as well as at frontal, parietal and cerebellar nodes.

As with FLR, individuals with UTRS demonstrated stronger functional anticorrelations (negatively weighted connectivity) than healthy controls at frontal and cerebellar nodes. However, the extent of increased negative connection strength was more widespread (resulting in stronger whole brain negatively weighted connectivity) in those with UTRS, suggesting there may be a gradient of dysconnection associated with worsening prognosis of schizophrenia. Weaker correlated functional activity in conjunction with stronger anticorrelated activity may signify a temporal (phase) shift in inter-regional communication. Under such conditions, two regions typically activated simultaneously may experience a phase lag, or the activation phase of one region may become completely inverted with respect to the other. This may be further elucidated using network analysis or Granger causality, as discussed above.

A single node in the right frontal lobe exhibited greater negative connection strength in those with UTRS compared to those with TRS and healthy controls. This was the only node to demonstrate any difference in connection strength between different schizophrenia cohorts. Stronger anticorrelated connectivity with this node could represent alterations in synaptic plasticity between those who are resistant to clozapine and those who respond. More research would be required to determine whether this node exhibits differences between subtypes of
schizophrenia before the onset of treatment; however, the small scale of this finding suggests it may be too weak to rely upon as a biomarker for clozapine resistance.

Negative connection diversity, a description of negatively weighted integration, was increased at a single parietal node in individuals with UTRS compared with healthy controls. Greater diversity of this node in people with UTRS may be suggestive of a less coordinated mode of connectivity (181). However, given that the node’s connection strength (indicative of global influence (261)) was unchanged, the overall effect of increased diversity at this node may be trivial.

Work from this thesis provides overwhelming evidence for a disruption in functional connectivity in people with UTRS, lending support to an aetiological role for dysconnectivity in this subtype of schizophrenia. Robust changes in functional connectivity, in addition to evidence of widespread grey matter loss (148) and a lack of white matter disruption (220) signifies a mechanistic role for aberrant synaptic plasticity in UTRS. In view of the reported efficacy of lamotrigine (an inhibitor of glutamate release) augmentation in individuals with UTRS (98), glutamatergic dysfunction (likely driven by NMDA receptor-mediated synaptic disruption) may well be responsible for UTRS in selected individuals. As individuals with UTRS have already failed treatment with D₂ receptor antagonists and clozapine monotherapy, the source of NMDA receptor dysfunction is likely attributable to a non-dopaminergic mechanism dissimilar to that responsible for TRS. Rather, UTRS may arise from a fundamental defect in the NMDA receptor itself or from acetylcholinergic (100) or other neurotransmitter-related influences. Deeper investigation into the underpinnings of functional dysconnectivity in this group of individuals is understandably required.

7.1.5. PREDICTING CLOZAPINE RESPONSE

The NeuCube personalised spiking neural network (SNN) and angular influenced spike-time dependent plasticity (STDP) model was able to accurately classify 75% of individuals with UTRS and 74% of individuals with TRS using a leave-one-out cross-validation procedure. Feature selection identified a large number of voxels across all regions of the brain, suggesting that differences between TRS and UTRS are widespread and unlikely to converge in any particular region or network. The moderate performance of this model mirrors the paucity of between-group differences identified in other chapters of the thesis and suggests that more data will be needed to properly distinguish between these two subtypes of schizophrenia.
7.2. IMPLICATIONS OF THIS RESEARCH WITH REGARD TO THE CURRENT LITERATURE

Evidence of neurological abnormalities in clozapine-eligibility, TRS and UTRS has been systematically reviewed in two recent papers by Nakajima et al. (364) and Mouchlianitis et al. (365). In the more recent review by Mouchlianitis et al., 61 articles covering various fields of neuroimaging were included in a qualitative analysis, of which only 16 compared treatment-responsive and treatment-resistant subtypes of schizophrenia and 35 examined the effects of or response to clozapine (365). Data from the current thesis adds to this small pool of research by providing comparisons between responsive, resistant and ultra-resistant subtypes of the disorder and comparing responsive individuals to those who are eligible for clozapine. This work also provides the first graph theoretical examination of functional networks comparing the different subtypes of the disorder.

Available diffusion tensor imaging (DTI) data has identified reduced FA and increased RD in the corpus callosum and temporal lobe regions of cortico-cortical white matter tracts in clozapine-eligible individuals compared with healthy controls (366). These data suggest that the observation of reduced FA and increased RD in the CC of clozapine-eligible individuals compared to FLR in the current thesis is attributable to an abnormality in the clozapine-eligible subgroup and not FLR. Furthermore, a prospective analysis by Reis Marques et al. (304) identified lower FA in the CC (among other regions) of non-responders compared with responders to 12 weeks of treatment with first-line antipsychotics. As such, low FA in the CC could be a biomarker for resistance to D₂ receptor antagonists.

Findings from functional connectivity studies have been less consistent (365). To date, research has demonstrated both increases and decreases in functional connectivity across a range of networks in individuals with TRS compared to both healthy controls and FLR (203, 367, 368), failing to give rise to a coherent conclusion (365). This may be attributable to poorly-defined criteria for treatment response and resistance, with studies either failing to separate those receiving clozapine from those receiving first-line antipsychotics (368) or failing to identify whether any participant with TRS was receiving clozapine at the time of scanning (203, 367). The use of seed-based or hypothesis-driven approaches also may have hindered replicability of findings. In this thesis, resting-state fMRI data were analysed using both ICA and network-based approaches. Most importantly, functional connectivity outcomes of the same dataset differed according to which technique was employed. ICA revealed increases in functional connectivity, whereas graph-theory and network-based approaches revealed decreased correlated and increased anticorrelated connectivity and decreased network connectivity, respectively, in those...
with UTRS compared with healthy controls. This observation warns against combining results from studies that utilise different analytical techniques.

Taking an ICA approach to analysis, results demonstrated greater functional connectivity between the SMN and precuneus in clozapine-eligible participants compared with FLR. These data suggest that functional SMN-precuneus connectivity may provide a useful biomarker for distinguishing clozapine-eligible individuals from FLR once they have already failed to respond to $D_2$ receptor antagonists. Although this may assist some clinicians in making the decision to switch a client to clozapine, a more useful approach would be to analyse patients at first contact. This is planned for future work and will be discussed in the future directions section of this chapter.

ICA also revealed greater functional connectivity within the language network (specifically within the paracingulate gyrus) of individuals with UTRS compared with healthy controls. These findings provide useful insight into the functional dynamics of UTRS but, as no significant differences were observed between those with UTRS and those who responded to first-line antipsychotics or clozapine, they are unlikely to serve as a useful biomarker for treatment resistance. A similar conclusion may be drawn from the findings using graph-theoretical and network-based analyses. Numerous disruptions to network organisation were observed in those with UTRS and FLR compared with healthy controls; however no robust differences in organisation were observed between the subtypes of schizophrenia. These data suggested that FLR exhibit a weaker form of disorganisation than those with UTRS and that network organisation is most likely similar in individuals with TRS and healthy controls. This would indicate that treatment-responsive and ultra-treatment-resistant subtypes of schizophrenia derive from similar underlying mechanisms, while the pathophysiology of TRS is unique.

Poor participant accrual in the prospective arm of the CloRes study prevented successful validation of the classification algorithm developed for predicting clinical response to clozapine. However, the integration of directional information from diffusion-weighted imaging and functional connectivity information from fMRI was able to achieve moderate classification accuracy in the cross-sectional training set, suggesting it to be a viable modelling approach. These findings should encourage future work combining the fields of machine learning and psychiatry.
7.3. LIMITATIONS

Limitations specific to each study have been addressed in each relevant chapter. An examination of the broader limitations affecting this research will be addressed here.

The primary caveat of this work is the cross-sectional design of the TRS and CloRes studies. Inclusion of individuals receiving first-line antipsychotics (FLR), clozapine (TRS) or augmented therapy (UTRS) in the TRS study allowed for the division of participants into definitive response subtypes but introduced a potential confounder of drug class effect to the analysis. Antipsychotics have been shown to increase blood flow in various regions of the brain (222), potentially influencing the results of fMRI studies. As differences in functional connectivity were mainly identified between individuals with UTRS and controls, and those with UTRS were receiving the highest dose of antipsychotics (chlorpromazine equivalents) compared with other treatment groups, it cannot be completely discounted that these differences may be attributable to the effects of antipsychotics and not the underlying pathology of UTRS. To address this issue, the CloRes study was designed to assess functional and structural brain connectivity in clozapine-eligible individuals receiving treatment with first-line antipsychotics. Clozapine was initiated following the baseline assessment and participants were to be followed for three months to determine whether they had responded (TRS) or failed to respond (UTRS) to treatment. Regrettably, the CloRes study suffered from poor rates of accrual and high rates of clozapine discontinuation due to adverse events. Consequently, the prospective component of this study is currently incomplete. However, a comparison with FLR receiving first-line antipsychotics was possible, allowing for a cross-sectional analysis of treatment response and resistance, without the confounding influence of drug class effects. The prospective component of the study is ongoing and will be discussed further in the future directions section of this chapter.

No psychiatrically healthy controls were included for comparison in the CloRes study. Whether this is a limitation, however, is debatable. Comparison of individuals with schizophrenia and healthy controls introduces an additional confounder of antipsychotic drug exposure, from which healthy controls are naïve. Therefore, although it may be useful to observe the difference between healthy controls and FLR or clozapine-eligible individuals, a comparison between well responding FLR and those who are eligible for clozapine is much more indicative of the pathology associated with treatment resistance.

In a recent article by the Treatment Response and Resistance in Psychosis (TTRIP) working group, criteria for the inclusion of treatment-resistant individuals in schizophrenia research were
considered and consensus guidelines provided (369). Minimum criteria included: at least moderate symptom severity assessed using a standardised rating scale such as the Positive and Negative Syndrome Scale (PANSS); minimum 12 weeks duration of symptoms; at least moderate functional impairment; adequate assessment of past treatment response including pill counts, staff or carer reports and dispensing charts as well as current treatment adherence ≥80%; at least six weeks’ treatment duration at a therapeutic dosage to the midpoint of the target range for that antipsychotic or equivalent to ≥600 mg/day chlorpromazine; at least two previous trials of antipsychotic; and for those with UTRS, failure to respond to clozapine administered by the same criteria mentioned above (369). Although the author of this thesis was involved in the assembly of these guidelines, meetings took place after the initiation of both studies. Consequently, conditions for inclusion in the TRS and CloRes studies may not meet all criteria mentioned above. Specifically, although most individuals in the CloRes study were within close proximity of the recommended target dosage, median chlorpromazine equivalents were 508 mg/day, slightly lower than those recommended by TRRIP guidelines (369). The recommended target of 600 mg/day chlorpromazine equivalents is intended as an indication of suitable dosage and does not assure response in non-resistant individuals. Although the degree of D₂ receptor occupancy is a strong predictor of clinical improvement in FLR (370), therapeutic drug monitoring has had limited success in predicting antipsychotic efficacy in the clinical setting. In future studies, higher dosage targets may influence inclusion criteria; however this small discrepancy is unlikely to influence results from the present analysis.

Finally, total PANSS scores associated with treatment response differed between TRS and CloRes studies. This may be attributable to the high rates of forensic history in the TRS cohort, which may have influenced the degree of severity on many items of the PANSS. Despite higher PANSS scores, however, participants in both studies were deemed to be responding well to treatment by a mental health care provider, suggesting that this should not have influenced the outcomes of the work.

7.4. FUTURE DIRECTIONS

7.4.1. ONGOING RESEARCH

The prospective component of the CloRes study, investigating biomarkers of clozapine response and resistance in clozapine-eligible schizophrenia, is ongoing. To date, only seven participants have reached follow-up. This is attributable to a 36% drop-out rate due to suspected clozapine-induced adverse events (primarily myocarditis), a 27% drop-out rate prior to clozapine initiation and poor participant accrual. Only 26 potential participants were recommended for the
prospective portion of the study and only 58% (n=15) of those agreed to participate. Moreover, a large number of individuals were approached by their treating psychiatrist and never put forward for the study for various reasons. The rate of consent in individuals with psychosis in the CloRes study is therefore considerably lower than the 65% consent rate recently reported by Patel et al. (371). This may be a consequence of the more severely unwell target population in the CloRes study, or may be an indication of poor patient education and limited clinical support surrounding medical research in New Zealand. To produce a clinically reliable prediction model of clozapine response, many more participants are required. The CloRes study was designed to follow up approximately 60 participants, intending to provide a 3:2 split of responders and non-responders to clozapine. Recruitment will continue at the University of Auckland, with an additional centre at the University of Otago, Dunedin, a possibility for the near future. Data from the prospective study will be used to improve the prediction model described in chapter 6; however, a much larger cohort will be required to develop a model fit for the clinical setting. This ambitious project will require cooperation from multiple centres in order to accrue a suitable cohort of participants that generalise well to the New Zealand, or even the global, schizophrenia population.

Both cross-sectional and prospective components of the CloRes study included an EEG component, in addition to proton magnetic resonance spectroscopy (1H-MRS) and structural MRI. These data have already been collected and information from the cross-sectional component of the study will be analysed shortly. VBM analysis of the structural MRI data should provide additional insight into the structural and functional dysconnectivity already observed in the clozapine-eligible cohort. Disrupted grey matter density could represent disturbances in synaptic or dendritic structure (138), which would support a role for NMDA receptor dysfunction in this subtype of the disorder. A GABA-targeted 1H-MRS sequence was also included in the study, measuring relative levels of GABA in the basal ganglia. Given the purported hypofunction of NMDA receptors on GABAergic interneurons specifically, it will be interesting to observe whether concentrations of GABA are lower in either schizophrenia cohort.

Previous work has identified robust reductions in mismatch negativity (MMN) in people with schizophrenia (132, 134), yet this phenomenon is not consistent enough to verify its use as a biomarker for the disorder (147). This may be due to variability introduced by inclusion of individuals from numerous response subtypes of the disorder. MMN is associated with predictive coding, purported to be under glutamatergic control (133). Should NMDA receptor dysfunction differ between FLR and those who are eligible for clozapine, MMN may act as a
viable biomarker of response. The CloRes study has collected data from an auditory oddball task, eliciting the MMN ERP. Future analysis will assess whether MMN is different between the two CloRes study cohorts, assessing its practicality as a biomarker of treatment response.

Analysis of TRS study data using graph-theory based approaches elicited different results to those obtained by ICA. To further investigate the functional and structural properties of clozapine eligibility, data from the CloRes study will also be examined using a connectomics approach. Structural connectivity data will first be used to determine appropriate connectomes (structural organisation) for FLR and clozapine-eligible cohorts, followed by an investigation of functional connectivity as a measure of how each connectome operates (255). This approach should provide even further insight into the underpinnings of treatment resistance.

7.4.2. PROPOSED RESEARCH
The cross-sectional studies included in this thesis have permitted the examination of structural and functional differences between responders and non-responders to antipsychotic therapy, providing information on the dysconnectivity associated with each cohort after they have responded or failed to respond to treatment. To determine whether features identified in the current studies are predictors of response or resistance and not the result of antipsychotic effects or symptom progression, a prospective investigation of treatment-naïve, first-episode schizophrenia is required. Based on the results of TRS and CloRes studies, functional and structural MRI may provide useful tools for investigating predictors of treatment response.

Structural connectivity findings from the CloRes study suggest that low FA in the CC could be a biomarker for resistance to D2 receptor antagonists. As interhemispheric functional connectivity is likely to be affected by disrupted CC white matter integrity or organisation, it may be interesting to investigate this using a tool such as the Poffenberger paradigm (372). The Poffenberger paradigm measures interhemispheric transfer time by comparing the delay between a 4 ms visual stimulus presented to one eye and a physical response from the lateral or contralateral hand (372). Contralateral responses should be delayed compared with lateral responses, as the contralateral response requires transmission through additional synapses (372). If interhemispheric communication is affected in clozapine-eligible individuals, this could account for deficits in sensory-motor integration, attention, language, or memory in this population (373). Although no deficit in interhemispheric information transfer has been reported in individuals with schizophrenia compared with healthy controls (374), this paradigm may still be able to expose differences between response subtypes of the disorder.
The differences in functional and structural connectivity observed between different subtypes of schizophrenia in the current thesis suggest there may be different mechanisms underlying each form of dysconnection, which may influence the response to antipsychotics. NMDA receptor dysfunction is thought to play a causal role in schizophrenia via its effects on synaptic plasticity (80, 100) and thus may be responsible for some of the dysconnectivity observed in these cohorts. Stephan et al. hypothesised that NMDA receptor dysfunction in schizophrenia may be attributable to the effects of modulatory neurotransmitters, and proposed that different neurotransmitters likely have different effects on NMDA receptor function (100). To investigate the downstream structural and functional consequences of NMDA receptor dysfunction, it would be appealing to manipulate the trafficking, signalling and composition of NMDA receptors in vivo, via mutations in the NMDA receptor gene itself or in genes involved in transmission of dopamine or other modulatory transmitters. If the structural and functional deficits observed in living individuals with schizophrenia could be replicated by manipulating NMDA receptor function, it may be possible to determine how each response subtype emerges and therefore provide new targets for pharmacotherapy. An in silico approach to NMDA receptor manipulation may also be feasible. By modelling the interactions between NMDA receptors, synaptic plasticity and structural and functional connectivity, it may one day be possible to test the effects of different receptor disruptions on whole-brain connectivity and use this to examine the consequences of treatment with different antipsychotics to better understand how they achieve their clinical effects.

7.5. OVERALL CONCLUSIONS

Schizophrenia is a heterogeneous and poorly categorised disorder frequently associated with poor treatment outcomes and diminished quality of life. Several hypotheses converge on a core deficit in brain connectivity caused by NMDA receptor-mediated synaptic dysfunction, and work to date supports a role for dysconnection in schizophrenia. However, despite general consensus regarding aberrant connectivity, studies have failed to provide consistent evidence of a unitary disruption in schizophrenia. This may be attributable, at least in part, to the inappropriate inclusion of multiple response subtypes of the disorder in single comparisons. NMDA receptors are under modulatory control by neurotransmitters such as dopamine, serotonin and acetylcholine, all of which are targets for the majority of antipsychotics. Should different antipsychotic classes modulate synaptic plasticity via different mechanisms, the underlying pathology of schizophrenia should be determinable by the antipsychotic to which it responds. To gain a deeper understanding of the underlying mechanisms responsible for treatment response
and resistance in individuals with schizophrenia, the body of work in this thesis compared structural and functional connectivity between different response subtypes of the disorder. Evidence supported the hypothesis that brain connectivity differs between FLR and those who are eligible for clozapine and showed large disruptions in functional connectivity in those with UTRS (and to a lesser degree FLR) compared with healthy controls. An absence of dysconnectivity was noted in the TRS cohort, despite disruptions in structural and functional connectivity in those who were eligible for clozapine. These findings indicate that treatment-responsive, clozapine-eligible and ultra-treatment-resistant subtypes of schizophrenia derive from differently mediated forms of functional dysconnectivity, while the pathophysiology of TRS is unique. The application of machine learning techniques may assist in the prediction of treatment response in future; however, classification accuracy in the current study was not high enough to warrant immediate transfer to a clinical setting.
8. APPENDIX

8.1. ETHICS APPROVAL – TREATMENT RESISTANT SCHIZOPHRENIA (TRS) STUDY
Dear Bruce

NTX/09/05/042  Investigation of the mechanisms responsible for ultra treatment resistant schizophrenia: PIS/Cons V#3, 05/02/09
Principal Investigator: Dr Bruce Russell
Co-Investigators: Ms Meghan Murphy, Prof Rob Kydd, Mr Himadri Seth, Dr. Sandy Simpson
University of Auckland, Waitemata DHB

Thank you for your letter received 16 June 2009 attaching the Committee's requirements. The above study has been given ethical approval by the Northern X Regional Ethics Committee.

Approved Documents
- Information Sheet/Consent Form (Electroencephalography Study) V#2 dated 24 May 2009
- Participant Information Sheet/Consent Form (Genetic Sub-Study) V#3 dated 24 May 2009
- Questionnaire (undated)

Certification
The Committee is satisfied that this study is not being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which the trial is being carried out.

Accreditation
The Committee involved in the approval of this study is accredited by the Health Research Council and is constituted and operates in accordance with the Operational Standard for Ethics Committees, April 2006.

Progress Reports
The study is approved until 1 June 2012. However, the Committee will review the approved application annually and notify the Principal Investigator if it withdraws approval. It is the Principal Investigator's responsibility to forward a progress report covering both sites prior to ethical review of the project on 22 June 2010. The report form should be forwarded to you prior to this date but if not received, it is available on http://www.ethicscommittees.health.govt.nz (forms - progress reports). Please note that failure to provide a progress report may result in the withdrawal of ethical approval.

Final Report
A final report is required at the end of the study. The report form is available on http://www.ethicscommittees.health.govt.nz (progress reports) and should be forwarded along with a summary of the results. If the study will not be completed as advised, please forward a progress report and an application for extension of ethical approval one month before the above date.

Requirements for SAE Reporting
Title of Project: AN INVESTIGATION OF POTENTIAL BIOMARKERS FOR TREATMENT RESISTANCE IN PEOPLE WITH SCHIZOPHRENIA.

Researchers: Dr Bruce Russell, Meghan McIlwain, Valerie Anderson and Prof. Rob Kydd

You are invited to participate in this research, which is being carried out in conjunction with the parent trial in which you have consented to participate.

The first part of this study will involve a review of your clinical notes, looking at which medicines you have been prescribed for the treatment of schizophrenia. This will be done by a qualified pharmacist. If it is discovered that your medication therapy is different from the recommended treatment guidelines or could be improved, this will be discussed with your doctor.

Brain imaging
This study uses a method of determining human brain function and structure called magnetic resonance imaging (MRI). This involves you lying quietly in an MRI scanner which is able to generate pictures of your brain which can be studied.

During the scan you will be asked to lie on a table which slides into the MRI scanner (which is like a short tunnel) in the scan room, and the investigator will continuously monitor you through the window of the control room. It takes about 10 minutes to set you up in the MRI scanner; the total time involved per scan session is approximately 60 minutes. The scanner is quite noisy when it is operating so you will be given headphones to lessen the noise and through which the researchers can talk to you and music can be played.

Some people don’t like being in small spaces and may feel claustrophobic in the scanner, however you will be constantly monitored and will be given a button to press should you feel distressed and wish to come out of the scanner.
If you take part in this study you will receive a fifty dollar ($50.00) clothing voucher upon completing the MRI session. Your participation may also help improve treatment for others with schizophrenia.

To help us understand the results of the MRI we will also ask you to complete a form about any recreational drugs you have used, and provide a urine sample which will be tested for the presence of certain drugs. If you are female this urine sample will also be tested to confirm that you are not pregnant, as we are unable to scan people who are pregnant.

Risks/benefits
It is not expected that you will obtain any personal benefit from taking part in this study.

In the unlikely event that a clinical abnormality is detected through performing an MRI scan on you, you will be informed of this and referred to an appropriate medical specialist. Results will not routinely be sent to your GP, however this can be arranged at your request.

Because the images are not routinely reviewed by a radiologist we are unable to perform diagnostic scans for medical purposes of areas where you have known abnormalities.

Personal information
Your participation is entirely voluntary (your choice). You do not have to take part in this study. If you do agree to take part, you are free to withdraw at any stage of the testing (between now and the end of the study (1/10/12)). You do not have to give any reason for your decision and there will be no penalty of any sort for withdrawing. If you choose to withdraw from the study your data will be destroyed.

Any participants in this research may have access to the information collected about them including the results of the testing and the final published report of the study. You may contact the researchers if you wish to receive a summary of the findings at the end of the study period.

Results may be used for future research related to schizophrenia for which further consent will be obtained from an accredited New Zealand Ethics Committee.

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not automatic and your case will need to be assessed by ACC according to the provisions of the 2002 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue
the investigators. If you have any questions about ACC, contact your nearest ACC office or the investigator.

The study will be carried out in the Centre for Advanced Magnetic Resonance Imaging (CAMRI) at the Faculty of Medical and Health Sciences, University of Auckland, 85 Park Road, Auckland.

Please feel free to contact the researchers if you have any questions about this study. Further information can be obtained from Dr Bruce Russell or Professor Rob Kydd. The Head of the School of Pharmacy is Professor John Shaw 09 373 7599 Ext 83778

If you have any queries or concerns regarding your rights as a participant in this study, you can contact an independent Health and Disability Advocate. This is a free service provided under the Health and Disability Commissioner Act:
Telephone: 0800 555 050
Free Fax: 800 2787 7678 (0800 2 SUPPORT)
Email: advocacy@hdc.org.nz

This study has received ethical approval from the Northern X Regional Ethics Committee.
Reference NTX/09/05/042
February 2011

CONTROL PARTICIPANT INFORMATION SHEET

MRI Study

Title of Project: AN INVESTIGATION OF POTENTIAL BIOMARKERS FOR TREATMENT RESISTANCE IN PEOPLE WITH SCHIZOPHRENIA.

Researchers: Dr Bruce Russell, Meghan McIlwain, Valerie Anderson and Prof. Rob Kydd

Contact Details:
Dr Bruce Russell, School of Pharmacy Ph 09 3737599 Ext 86429
Meghan McIlwain, School of Pharmacy Ph 09 3737599 Ext 88468
Valerie Anderson, School of Pharmacy Ph 09 3737599 Ext 81914
Professor Robert Kydd, Dept. of Psychological Medicine 09 3737599 Ext 83774

You are invited to participate in this research, which is being carried out in conjunction with the parent trial in which you have consented to participate.

Brain imaging
This study uses a method of determining human brain function and structure called magnetic resonance imaging (MRI). This involves you lying quietly in an MRI scanner which is able to generate pictures of your brain which can be studied.

During the scan you will be asked to lie on a table which slides into the MRI scanner (which is like a short tunnel). The investigator will continuously monitor you through the window of the control room. It takes about 10 minutes to set you up in the MRI scanner; the total time involved per scan session is approximately 60 minutes. The scanner is quite noisy when it is operating so you will be given headphones to lessen the noise and through which the researchers can talk to you and music can be played.

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If you take part in this study you will receive a fifty dollar ($50.00) clothing voucher upon completing the MRI session. Your participation may also help improve treatment for people with schizophrenia.

To help us understand the results of the MRI we will also ask you to complete a form about any recreational drugs you have used, and provide a urine sample.
which will be tested for the presence of certain drugs. If you are female this urine sample will also be tested to confirm that you are not pregnant, as we are unable to scan people who are pregnant.

Risks/benefits
It is not expected that you will obtain any personal benefit from taking part in this study.

In the unlikely event that a clinical abnormality is detected through performing an MRI scan on you, you will be informed of this and referred to an appropriate medical specialist. Results will not routinely be sent to your GP, however this can be arranged at your request.

Because the images are not routinely reviewed by a radiologist we are unable to perform diagnostic scans for medical purposes of areas where you have known abnormalities.

Personal information
Your participation is entirely voluntary (your choice). You do not have to take part in this study. If you do agree to take part, you are free to withdraw at any stage of the testing (between now and the end of the study (1/10/12)). You do not have to give any reason for your decision and there will be no penalty of any sort for withdrawing. If you choose to withdraw from the study your data will be destroyed.

Any participants in this research may have access to the information collected about them including the results of the testing and the final published report of the study. You may contact the researchers if you wish to receive a summary of the findings at the end of the study period.

Results may be used for future research related to schizophrenia for which further consent will be obtained from an accredited New Zealand Ethics Committee.

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not automatic and your case will need to be assessed by ACC according to the provisions of the 2002 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. If you have any questions about ACC, contact your nearest ACC office or the investigator.

The study will be carried out in the Centre for Advanced Magnetic Resonance Imaging (CAMRI) at the Faculty of Medical and Health Sciences, University of Auckland, 85 Park Road, Auckland.
Please feel free to contact the researchers if you have any questions about this study. Further information can be obtained from Dr Bruce Russell or Professor Rob Kydd. The Head of the School of Pharmacy is Professor John Shaw 09 373 7599 Ext 83778

If you have any queries or concerns regarding your rights as a participant in this study, you can contact an independent Health and Disability Advocate. This is a free service provided under the Health and Disability Commissioner Act:
Telephone: 0800 555 050
Free Fax: 800 2787 7678 (0800 2 SUPPORT)
Email: advocacy@hdc.org.nz

This study has received ethical approval from the Northern X Regional Ethics Committee.
Reference NTX/09/05/042
8.4. CONSENT FORM – TRS STUDY

Title of Project: AN INVESTIGATION OF POTENTIAL BIOMARKERS FOR TREATMENT RESISTANCE IN PEOPLE WITH SCHIZOPHRENIA

Researchers: Dr Bruce Russell, Meghan McIlwain, Valerie Anderson and Prof Rob Kydd

Name of Subject: ____________________________  Age: _____ years

Subject Number: _____

I have read and I understand the information sheet dated February 2011 for volunteers taking part in the study designed to investigate markers for treatment response in people with schizophrenia. I have had the opportunity to discuss this study, and I am satisfied with the answers I have been given. I have also had time to consider whether to take part.

I understand my right to receive a copy of the results of this study and that the results may be used for future research related to the cognitive markers associated with schizophrenia and/or other psychiatric conditions for which further consent will be obtained by an accredited New Zealand ethics committee.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time up until the end of the study 1/10/12.

I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports of this study.

I understand that the testing procedure will be stopped if I am in any discomfort.

I __________________________ (full name) hereby consent to take part in this study.

Signature: ______________________
Date: ______________________

Project Explained By: Bruce Russell/Meghan McIlwain/Valerie Anderson/Rob Kydd

Project Role: Principal Investigator/Co-investigator

Signature: ______________________
Date: ______________________

This study has received ethical approval from the Northern X Regional Ethics Committee.

Reference NTX/09/05/042
8.5. Ethics Approval – Clozapine Response (CloRes) Study

13 August 2014

Dr Bruce Russell
School of Pharmacy, University of
Auckland Private Bag 92019
Grafton,
Auckland 1142

Dear Dr Russell

<table>
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<tr>
<th>Re:</th>
<th>Ethics ref:</th>
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<tr>
<td></td>
<td>Study title:</td>
<td>Development of a personalised model to predict clinical response to clozapine in people with treatment-resistant schizophrenia.</td>
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I am pleased to advise that this application has been approved by the Northern A Health and Disability Ethics Committee. This decision was made through the HDEC-Full Review pathway.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study’s sponsor, to ensure that these conditions are met. No further review by the Northern A Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at any locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at a given locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

After HDEC review

Please refer to the Standard Operating Procedures for Health and Disability
Statement of compliance

The Northern A Health and Disability Ethics Committee:

— is constituted in accordance with its Terms of Reference
— operates in accordance with the Standard Operating Procedures for Health and Disability Ethics Committees, and with the principles of international good clinical practice (GCP)
— is approved by the Health Research Council of New Zealand’s Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
— is registered (number 00008714) with the US Department of Health and Human Services’ Office for Human Research Protection (OHRP).

List of members

<table>
<thead>
<tr>
<th>Name</th>
<th>Category</th>
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<tbody>
<tr>
<td>Dr Brian Fergus</td>
<td>Lay (consumer/community perspectives)</td>
<td>01/07/2012</td>
<td>01/07/2015</td>
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<td>Dr Karen Bartholomew</td>
<td>Non-lay (intervention studies)</td>
<td>01/07/2013</td>
<td>01/07/2016</td>
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<tr>
<td>Ms Susan Buckland</td>
<td>Lay (consumer/community perspectives)</td>
<td>01/07/2012</td>
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<tr>
<td>Ms Shamim Chagani</td>
<td>Non-lay (health/disability service provision)</td>
<td>01/07/2012</td>
<td>01/07/2015</td>
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<td>Dr Christine Crooks</td>
<td>Non-lay (intervention studies)</td>
<td>01/07/2013</td>
<td>01/07/2015</td>
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<tr>
<td>Mr Kerry Hiini</td>
<td>Lay (consumer/community perspectives)</td>
<td>01/07/2012</td>
<td>01/07/2015</td>
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<tr>
<td>Ms Michele Stanton</td>
<td>Lay (the law)</td>
<td>01/07/2012</td>
<td>01/07/2015</td>
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http://www.ethics.health.govt.nz
This study is investigating predictive markers of treatment response in people with schizophrenia. You are invited to take part as a control participant in this study. You are eligible to take part because you are a person who has experienced a psychotic episode and who has responded well to treatment that includes first-line antipsychotic drugs. Whether or not you take part is your choice. If you don’t want to take part, you don’t have to give a reason. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you’d like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is 7 pages long, including the Consent Form. Please make sure you have read and understood all the pages.

**WHAT IS THE PURPOSE OF THE STUDY?**

The main aim of this study is to determine whether a person’s genes (the characteristics that are passed down to us from our parents), the structure of their brain and the way their brain responds to tasks influences whether they experience any benefits from taking the antipsychotic, clozapine. As a control participant, you will not receive clozapine as part of this study.

The study is partly funded by *NZPERF (New Zealand Pharmacy Education & Research)*
The study investigators are:
Dr Bruce Russell, School of Pharmacy, Ph 03 4797272
WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

You have been chosen to participate in this study because you meet the requirements for a control participant outlined in our study protocol.

The study will be carried out at the Clinical Research Centre at the Faculty of Medical and Health Sciences, University of Auckland.

If you choose to participate in the study, you will visit the study site for half a day; there we would like you to take part in the following:

- You will perform some tests on a touch screen computer to measure how good your memory and learning is.
- You will talk to one of our study psychiatrists or nurse about how you are feeling and any symptoms you may be experiencing.
- A person trained to take blood samples will withdraw a blood sample from a vein in your arm that we will use to look at your genes and antipsychotic levels.
- You will have an MRI scan that we will use to learn about the structure of your brain and how it works during certain tasks.
- We will collect information about the weak electrical signals generated by your brain, using a technique called electroencephalography (EEG). (We are collecting both EEG and MRI data because the EEG is able to give us information about brain activity milliseconds after it happens but will only provide information about activity on the surface of the head, whereas functional MRI tells us about the activity in the whole brain but is delayed by 6-10 seconds so is less sensitive.)
- You will be asked to provide a urine sample to test for the presence of illicit drugs. This will not exclude you from the study but we are required to check for scientific validity of our results.

After your visit, you will be free to go. We will provide a taxi if you need one.

More Information:
What are genes?

Each person has a DNA make-up (their genes) that is different from that of everybody else (except in the case of identical twins). This genetic make-up is a mixture of the genes of our parents. The precise way genes are mixed varies from child to child within the same family, so having the same parents does not mean that two children will have exactly the same genes. We already know that some health conditions and disorders are definitely inherited through the genes (hereditary conditions), but we do not know how many conditions are explained by genetic inheritance. Inherited genes may explain why some people are more resistant and some people more prone to disorders that have not yet been identified as hereditary. The research in which you are invited to participate will investigate genetic make-up to look for any link...
between antipsychotic responsive psychotic symptoms and clozapine-responsive or resistant schizophrenia and inherited genes.

Because the research investigates genetic make-up, this identifies you as a participant as well as your particular genetic characteristics. This information is confidential and will not be disclosed, stored or used in any way without your informed consent.

In particular the investigators of the research will not claim any right, ownership or property in your individual genetic information or that of your kinship group, hapū or iwi, without having first sought and obtained your informed consent to the transfer of any such right, ownership or property. Your consenting to participate in the DNA sampling for the proposed study will not be construed as creating any right or claim on the part of the researcher/sponsor to your genetic information.

Some iwi disagree with storage of tissue or blood samples citing whakapapa, and advise their people to consult prior to participation in research where this occurs. However, it is acknowledged that individuals have the right to choose to participate.

The blood sample we collect from you will be used to investigate whether differences in your genetic code make a difference to your susceptibility to experiencing a psychotic episode. Your blood sample will be used for this purpose alone; we are not permitted to test for any other medical disorders or abnormalities.

On completion of the study, we would like to keep your sample so we may use it in a future study looking at psychotic episodes and schizophrenia. If you do not wish for your sample to be kept, any remaining blood will be destroyed by incineration or returned to you at your request.

What will happen in the MRI scan?
In the final part of the study, we will use a method called magnetic resonance imaging (MRI) to learn about the structure of your brain and look at changes that occur during two tasks. Blood oxygen levels increase when a part of the brain is active and can tell us about which parts of your brain are working during certain tasks. For this part of the study, you will be asked to lie in the MRI scanner, up to your shoulders, and to be very still. You will be monitored continuously and will be able to communicate with the investigator during this procedure.

The MRI session will last for approximately 45 minutes. For the tasks, you will be asked to respond to some noise or picture by pushing a button. The picture will be shown on a large screen that is easy to see using the mirror that is mounted close to your face. This mirror will also give you a full view of the room. All the time, we will be checking on you to make sure you are comfortable and feel safe. Some people do not like small spaces and may wish to come out of the scanner. If this happens to you, there is a button you may press that will alert us and we can stop the scan immediately.

What will happen in the EEG?
You will be fitted with headphones and an EEG (electroencephalography) cap, which we will use to measure changes in weak electrical signals generated by your brain during two tasks. This will involve having a cap of recording sensors, which we will fill with a conductive gel, placed on the surface of your head. The EEG will take approximately 20 minutes, plus about 20 minutes for fitting the cap.

How many people will be in the study?
This study will probably involve approximately 80 people, including approximately 20 control participants.

What is the time span for the study?
The study will start in January 2015 and finish in July 2016.
Will health information be collected?
We will not collect any health information from you, other than what you tell us.

WHAT ARE THE POSSIBLE BENEFITS AND RISKS OF THIS STUDY?

It is unlikely that you will obtain any personal benefit from taking part in this study; however, your participation may help to allow us to decide which people might benefit from clozapine in the future.

In the unlikely event that we detect anything medically relevant in your scan, we will inform you of this and refer you to an appropriate medical specialist. Because the images are not routinely reviewed by a radiologist, we are unable to perform diagnostic scans for medical purposes.

WHO PAYS FOR THE STUDY?

The study is partly funded by NZPERF (New Zealand Pharmacy Education & Research) and the University of Auckland.
You will be compensated for your participation in this study with a gift voucher.

WHAT IF SOMETHING GOES WRONG?

If you were injured in this study, which is unlikely, you would be eligible for compensation from ACC just as you would be if you were injured in an accident at work or at home. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won’t affect your cover.

WHAT ARE MY RIGHTS?

Your participation in the study is entirely voluntary. If you agree to take part, you are free to withdraw at any stage of the study (between now and the end of the study – 30 May 2016). You are not required to give a reason for your decision and there will be no penalty for withdrawing. If you choose to withdraw from the study, your data will be discarded.

You are entitled to see any data we collect from you, as well as copies of any final published works resulting from this study.

Any information we collect from you is confidential. No material that could personally identify you will be used in any reports on this study. Any illegal activities that you disclose or that are detected during the urine drug screen will remain confidential within the limits of the law.
Please feel free to contact the investigators if you have any questions about this study. Further information can be obtained from Carolyn McNabb, Dr Bruce Russell or Prof. Rob Kydd.

If you have any queries or concerns regarding your rights as a participant in this study, you can contact an independent Health and Disability Advocate. This is a free service provided under the Health and Disability Commissioner Act:
Telephone: 0800 555 050
Free Fax: 800 2787 7678 (0800 2 SUPPORT)
WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?

Upon completion of the study, your records will be stored for 10 years in a secure place at Faculty of Medical and Health Sciences, University of Auckland. All computer records will be password protected. All future use of the information collected will be strictly controlled in accordance with the Privacy Act. Taking part in this study will not change your regular treatment and you will continue to receive normal care after the study is completed. If you do not wish for your blood to be kept for use in a further study, any remaining blood will be destroyed or returned to you at your request.

We expect to have results from this study by 2016 and will keep you informed of any publications or results released to the community.

WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

If you have any questions, concerns or complaints about the study at any stage, you can contact:
Dr Bruce Russell, School of Pharmacy, Ph 09 3737599 Ext 86429
Carolyn McNabb, School of Pharmacy, Ph 09 3737599 Ext 88468
Prof. Rob Kydd, Department of Psychological Medicine, Ph 09 3737599 Ext 83774

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Email: advocacy@hdc.org.nz

If you require Māori cultural support, talk to your whānau in the first instance. Alternatively you may contact the administrator for He Kamaka Waiora (Māori Health Team) by telephoning 09 486 8324 ext 2324

If you have any questions or complaints about the study you may contact the Auckland and Waitematā District Health Boards Maori Research Committee or Maori Research Advisor by telephoning 09 4868920 ext 3204

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone: 0800 4 ETHICS
Email: hdecs@moh.govt.nz
Consent Form

Please tick to indicate you consent to the following

<table>
<thead>
<tr>
<th>Statement</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>I have read, or have had read to me in my first language, and I understand the Participant Information Sheet.</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
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<td>☐</td>
<td>☐</td>
</tr>
<tr>
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<td>☐</td>
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</tr>
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<td>I consent to my GP or current provider being informed of any significant abnormal results obtained during the study.</td>
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<tr>
<td>I consent to the research staff collecting my blood to test my antipsychotic levels</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>I consent to the research staff keeping my blood/DNA for use in a future study</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>I consent to providing a urine sample for drug/alcohol screening</td>
<td>☐</td>
<td>☐</td>
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<td>☐</td>
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<td>I understand my responsibilities as a study participant.</td>
<td>☐</td>
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</tr>
</tbody>
</table>
I wish to receive a summary of the results from the study. ☐ Yes ☐ No

Declaration by participant:

I hereby consent to take part in this study.

Participant’s name: ____________________________
Signature: ____________________________ Date: ____________________________

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant’s questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher’s name: ____________________________
Signature: ____________________________ Date: ____________________________
Participant Information Sheet

Study title: Predicting whether clozapine will be an effective treatment: The Understanding Clozapine Response (CloRes) Study
Locality: WDHB, ADHB and CMDHB
Ethics committee ref.: NZ/1/88C5013

Lead investigator: Dr Bruce Russell
Contact phone number: (03) 4797272

You are invited to take part in a study to see if you are a person who will respond to clozapine. Whether or not you take part is your choice. If you don’t want to take part, you don’t have to give a reason, and it won’t affect the care you receive. If you do want to take part now but change your mind later, you can pull out of the study at any time.

This Participant Information Sheet will help you decide if you’d like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide, you may want to talk about the study with other people, such as family, whānau, friends or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is 8 pages long, including the Consent Form. Please make sure you have read and understood all the pages.

What is the purpose of the study?

The main aim of this study is to determine whether a person’s genes (the characteristics that are passed down to us from our parents), the structure of their brain and the way their brain responds to tasks influence whether they experience any benefits from taking clozapine. Everyone participating in the study will receive clozapine at a dose that has been decided by their doctor.

The study is partly funded by NZPERF (New Zealand Pharmacy Education & Research)

The study investigators are:
Dr Bruce Russell, School of Pharmacy, Ph 03 4797272
WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

You have been chosen to participate in this study because your doctor has decided that you might benefit from treatment with clozapine.

The study will be carried out at the Clinical Research Centre at the Faculty of Medical and Health Sciences, University of Auckland.

Firstly, a doctor or nurse will ask you questions about how you have been feeling recently. This is so that we can measure any improvements that might be due to clozapine once you start taking your medicine.

If you choose to participate in the study, you will visit the study site for half a day; there we would like you to take part in the following:

- You will perform some tests on a touch screen computer to measure how good your memory and learning is.
- A person trained to take blood samples will withdraw a blood sample from a vein in your arm that we will use to look at your genes and medication levels.
- You will have an MRI scan that we will use to learn about the structure of your brain and how it works during certain tasks.
- We will collect information about the weak electrical signals generated by your brain, using a technique called electroencephalography (EEG). (We are collecting both EEG and MRI data because the EEG is able to give us information about brain activity milliseconds after it happens but will only provide information about activity on the surface of the head, whereas functional MRI tells us about the activity in the whole brain but is delayed by 6-10 seconds so is less sensitive.)
- You will be asked to provide a urine sample to test for the presence of illicit drugs. This will not exclude you from the study but we are required to check for scientific validity of our results.

After your visit, we will keep in touch and invite you for a second visit after 3 months. At this second visit, we would like you to speak to the same doctor or nurse about how you have been feeling and then have a second MRI scan (which will be shorter than the first time), EEG and computer test.

More Information:
What are genes?
Each person has a DNA make-up (their genes) that is different from that of everybody else (except in the case of identical twins). This genetic make-up is a mixture of the genes of our parents. The precise way
genes are mixed varies from child to child within the same family, so having the same parents does not mean that two children will have exactly the same genes. We already know that some health conditions and disorders are definitely inherited through the genes (hereditary conditions), but we do not know how many conditions are explained by genetic inheritance. Inherited genes may explain why some people are more resistant and some people more prone to disorders that have not yet been identified as hereditary. The research in which you are invited to participate will investigate genetic make-up to look for any link between the response to treatment (in this case clozapine) and inherited genes.

Because the research investigates genetic make-up, this identifies you as a participant as well as your particular genetic characteristics. This information is confidential and will not be disclosed, stored or used in any way without your informed consent.

In particular the investigators of the research will not claim any right, ownership or property to your individual genetic information or that of your kinship group, hapū or iwi, without having first sought and obtained your informed consent to the transfer of any such right, ownership or property. Your consenting to participate in the DNA sampling for the proposed study will not be construed as creating any right or claim on the part of the researcher/sponsor to your genetic information.

Some iwi disagree with storage of tissue or blood samples citing whakapapa, and advise their people to consult prior to participation in research where this occurs. However, it is acknowledged that individuals have the right to choose to participate.

The blood sample we collect from you will be used to investigate whether differences in your genetic code make a difference to whether you benefit from taking clozapine. Your blood sample will be used for this purpose alone; we are not permitted to test for any other medical disorders or abnormalities. On completion of the study, we would like to keep your sample so we may use it in a future study looking at treatment response. If you do not wish for your sample to be kept, any remaining blood will be destroyed by incineration or returned to you at your request.

**What will happen in the MRI scan?**

In the final part of the study, we will use a method called magnetic resonance imaging (MRI) to learn about the structure of your brain and look at changes that occur during two tasks. Blood oxygen levels increase when a part of the brain is active and can tell us about which parts of your brain are working during certain tasks. For this part of the study, you will be asked to lie in the MRI scanner, up to your shoulders, and to be very still. You will be monitored continuously and will be able to communicate with the investigator during this procedure.

The MRI session will last for approximately 45 minutes. For the tasks, you will be asked to respond to some noise or picture by pushing a button. The picture will be shown on a large screen that is easy to see using the mirror that is mounted close to your face. This mirror will also give you a full view of the room. All the time, we will be checking on you to make sure you are comfortable and feel safe. Some people do not like small spaces and may wish to come out of the scanner. If this happens to you, there is a button you may press that will alert us and we can stop the scan immediately.

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How many people will be in the study?  
This study will involve approximately 80 people.

What is the time span for the study?  
The study will start in January 2015 and finish in May 2016.

Will health information be collected?  
Yes. We will collect information about how you are feeling at the beginning of the study and at three months. We will also collect information about your past medications. This will be done by Dr Bruce Russell, who is a pharmacist.

What are the possible benefits and risks of this study?  
It is unlikely that you will obtain any personal benefit from taking part in this study; however, your participation may help allow us to decide which people might benefit from clozapine in the future.

In the unlikely event that we detect anything medically relevant in your scan, we will inform you of this and refer you to an appropriate medical specialist. Because the images are not routinely reviewed by a radiologist, we are unable to perform diagnostic scans for medical purposes.

Who pays for the study?  
The study is partly funded by NZPERF (New Zealand Pharmacy Education & Research) and the University of Auckland.
You will be compensated for your participation in this study with a gift voucher.

What if something goes wrong?  
If you are injured in this study, which is unlikely, you will be eligible for compensation from ACC, just as you would be if you were injured in an accident at work or at home. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

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**WHAT HAPPENS AFTER THE STUDY OR IF I CHANGE MY MIND?**

Upon completion of the study, your records will be stored for 10 years in a secure place at the Faculty of Medical and Health Sciences, University of Auckland. All computer records will be password protected. All future use of the information collected will be strictly controlled in accordance with the Privacy Act.

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If you do not wish for your blood to be kept for use in a further study, any remaining blood will be destroyed or returned to you at your request.

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- Prof. Rob Kydd, Department of Psychological Medicine, Ph 09 3737599 Ext 83774

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### Consent Form

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Declaration by participant:

I hereby consent to take part in this study.

Participant’s name:

Signature: ___________________________ Date: ___________________________

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant’s questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher’s name:

Signature: ___________________________ Date: ___________________________
Magnetic Resonance Imaging involves the use of an extremely powerful magnet. For your **safety** please answer the following questions

Have you had a previous MRI scan?  
☐ yes ☐ no

Do you have **or** have you **ever** had a cardiac pacemaker?  
☐ yes ☐ no

Do you have a brain aneurysm clip?  
☐ yes ☐ no

Have you **ever** had an injury to the **eye** with a metallic object or fragment?  
☐ yes ☐ no

Have you had any previous surgery?  
☐ yes ☐ no

**Please list**  
__________________________________________________________

Do you have any allergies to medications?  
☐ yes ☐ no

**Please list**  
__________________________________________________________

Do you have any of the following:

Anaemia, blood disorders, kidney disease or seizures?  
☐ yes ☐ no

**FEMALE PATIENTS**

Is there any chance that you could be pregnant?  
☐ yes ☐ no

Are you currently breastfeeding?  
☐ yes ☐ no

**PLEASE ANSWER THE QUESTIONS ON THE BACK OF THIS SHEET**
DO YOU HAVE ANY OF THE FOLLOWING?

- Implanted cardiac defibrillator
- Implanted electronic or magnetic device
- Metallic stent, filter or coil
- Cochlear implant or other ear implant
- Heart valve prosthesis
- Any type of prosthesis (eye, limb etc)
- Joint replacement
- Screws, plates or wires in bones or joints
- Shunt (spinal, intraventricular, or heart)
- Vascular or drug access port or catheter
- Radiation seeds or implants
- Medication patches (Nicotine or hormone)
- Tattoo or permanent makeup
- Dentures or partial plate
- Hearing aid
- Shrapnel, bullets or other metal

BEFORE ENTERING THE MR SCAN ROOM

You must remove all metallic objects, including jewellery, watches, keys, coins, credit cards, pens, cell phones, hearing aids, clothing with metallic zips and fasteners, metallic threads, or glitter finishes. You may be asked to change into a gown.

Owing to the loud noises emitted by the MR system, you will be given

If you answer YES or are uncertain regarding any of the above, please contact us on (09) 303 5966 prior to your appointment.

USE OF YOUR IMAGES

As a University it may be useful to use your images (without your name or other identifying details) for all or some of the following purposes -

- education and training by Centre for Advanced MRI staff
- scientific publications, reports and presentations
- University teaching
- publicity material for the Centre for Advanced MRI
- the Centre for Advanced MRI website and websites of organisations we collaborate with (e.g. Siemens the manufacturer of the machine)
- publicity materials for non-profit organisations
- television documentaries or other public interest media
- databases that may be published on the internet

I give consent for my images to be used for the above purposes provided that all details that could allow me to be identified have been removed

[ ] yes [ ] no
I confirm that the above information is correct to the best of my knowledge.

Signature ___________________________________ Date ___/___/____

Screening form checked by ______________________
### 8.9. Positive and Negative Syndrome Scale (PANSS)

Circle the degree of severity for all the PANSS items

<table>
<thead>
<tr>
<th>PANSS ITEMS</th>
<th>Absent</th>
<th>Minimal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Mod/severe</th>
<th>Severe</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Delusions</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
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<td>2. Conceptual organisation</td>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>3. Hallucinatory behaviour</td>
<td>1</td>
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<td>5. Grandiosity</td>
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<td>8. Blunted Affect</td>
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<td>9. Emotional Withdrawal</td>
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<td>10. Poor Rapport</td>
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<td>11. Passive/Apathetic Social Withdrawal</td>
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<td>12. Difficulty in Abstract Thinking</td>
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<td>13. Lack of Spontaneity and Flow of Conversation</td>
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<td>17. Guilty Feelings</td>
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<td>19. Mannerism &amp; Posturing</td>
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<td>21. Motor Retardiation</td>
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<td>26. Lack of Judgement and Insight</td>
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<td>27. Disturbance of Volition</td>
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<td>28. Poor Impulse Control</td>
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<td>29. Preoccupation</td>
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<td>30. Active Social Avoidance</td>
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8.10. SLICE TIMING CORRECTION FOR MULTIBAND SEQUENCES

Multislice mode = interleaved

Slices = 64

Multiband acceleration factor = 8

Number of shots = 8 (64/8=8)

Increment slice (requires increment-n-slice interleave pattern)

\[
\text{if } n_{\text{shots}} \text{ is even} \\
\quad n_{\text{increment}} = \left(\frac{n_{\text{shots}}}{2}\right) - 1; \\
\text{if } n_{\text{increment}} \text{ is even} \\
\quad n_{\text{increment}} = n_{\text{increment}} - 1; \\
\text{end} \\
\text{end}
\]

So

\[n(\text{increment})=(8/2)-1 = 3\]

Increment slice = 3

Slice excitation order =

0, 8, 16, 24, 32, 40, 48, 56
3, 11, 19, 27, 35, 43, 51, 59
6, 14, 22, 30, 38, 46, 54, 62
1, 9, 17, 25, 33, 41, 49, 57
4, 12, 20, 28, 36, 44, 52, 60
7, 15, 23, 31, 39, 47, 55, 63
2, 10, 18, 26, 34, 42, 50, 58
5, 13, 21, 29, 37, 45, 53, 61
8.11. MODULARITY AS A FUNCTION OF THE GAMMA THRESHOLD

FIGURE 70. THE VALUE OF GAMMA DIRECTLY INFLUENCES THE DIFFERENCE IN MODULARITY BETWEEN HEALTHY CONTROLS AND INDIVIDUALS WITH SCHIZOPHRENIA. FLR = FIRST-LINE RESPONDERS; TRS = TREATMENT-RESISTANT SCHIZOPHRENIA; UTRS = ULTRA-TREATMENT-RESISTANT SCHIZOPHRENIA.
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