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Investigation of Potential Biomarkers of Treatment-Resistant Schizophrenia

Meghan Elizabeth McIlwain

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy (Pharmacy)
The University of Auckland, 2013
Abstract

Approximately one third of all people with schizophrenia are diagnosed as having treatment-resistant schizophrenia (TRS) because they still suffer from persistent moderate-to-severe positive symptoms despite treatment with first line medications. Typically they also have poor-outcomes due to the length of time taken to reach this diagnosis which often takes many years. Clozapine remains the gold standard treatment for people with TRS where other antipsychotics have failed. Yet its use is limited by potentially fatal side effects and there is still a subset, approximately 50-70% of patients treated with clozapine, who do not experience clinically significant improvements in their symptoms, they are then said to be clozapine-resistant or have ultra-treatment-resistant schizophrenia (UTRS). Several lines of evidence suggest that TRS may have a neurobiological basis distinct from schizophrenia that responds to different pharmacological intervention. The neurobiological basis of UTRS is yet to be investigated.

Patients responsive to second generation antipsychotics (first-line responders) and those responsive to clozapine monotherapy (clozapine-responsive; TRS) were compared to each other, to those who do not respond to clozapine monotherapy (clozapine resistant; UTRS) and to a healthy control group. Two non-invasive magnetic resonance imaging (MRI) techniques were used to examine the structure and integrity of white matter pathways in the brain and potential alterations in the content of neurometabolites; diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (1H-MRS), respectively.

Patients with TRS had widespread reductions in white matter (WM) integrity compared to controls. Perhaps surprisingly, responders to first-line antipsychotics and those with UTRS were indistinguishable from controls. Those with TRS had lower fractional anisotropy (FA) in the right
superior longitudinal fasciculus than those with UTRS which could suggest that this region is “hyperconnected” in patients with UTRS. $^1$H-MRS revealed that people with TRS who are responsive to clozapine have increased glutamate + glutamine (Glx) content in the putamen compared to those with UTRS.

Glx levels in the putamen may represent an important biomarker of response to clozapine treatment where levels are higher in those who are responsive and lower in those with UTRS. Reduced white matter integrity may also be a potential biomarker for individuals with TRS. Patients with UTRS are the most treatment resistant and yet WM integrity and neurometabolite content were no different to control subjects. This suggests that UTRS might have a distinct underlying pathophysiology to other forms of schizophrenia.

Future research should be undertaken to investigate whether these changes are present before treatment with clozapine is initiated.
Dedication

*He aha te mea nui?*

*He tangata.*

*He tangata.*

*He tangata.*

*What is the most important thing?*

*It is people.*

*It is people.*

*It is people.*

This work is dedicated to the study participants and their families. Thank you for sharing your stories with me.
Acknowledgements

First and foremost, I want to thank my primary supervisor Dr Bruce Russell and my co-supervisor Professor Rob Kydd. It has been an honour to complete my PhD (and Masters before that) with your guidance. Bruce, you helped me to discover my passion for research and it’s changed the path of my life. I am grateful for the opportunities you gave me, the important work that you trusted me with and your unwavering faith in my abilities. Rob, I appreciate the insight and encouragement you provided over the years— you were always there with a cool head and a warm heart.

I’m thankful to our post-doc, Dr Valerie (Val) Anderson. Val, you’ve been a role model for me both professionally and personally. Often when faced with a difficult decision, I ask myself what you would do. Working with you was great fun – I think of the many moments of laughter we shared when things were going well and in challenging times, too.

I’m grateful to Professor John Shaw who was the Head of the School of Pharmacy when I started my PhD and to Professor Julia Kennedy who is the current Head of School for her support.

Special thanks to current and past members of the Psychopharmacology and Neural Dynamics group at The University of Auckland who I have been fortunate to complete this journey with: Dr Louise Curley, Dr Reem Jan, Dr Grace Wang, Dr Joanne Lin, Dr Mirjana Stojkovic, Carolyn McNabb, Jade Zhang and Alice Mason.

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Thank you to Dr Frederick Sundram for support with DTI analysis and to our wonderful statistician, Avinesh Pillai. Thank you to the team at the Centre for Advanced MRI: Dr Brett Cowan, Anna-Maria Lydon, Hilary Miller, Dr Beau Pontré, Rachel Heron, Shelley Park, Kate Handley, Carol Caunter, Angela Harrison and Marie Rooney. Thank you so much for being flexible and accommodating with our participants – including dog-sitting duties for the participant who thought it would be okay for their four-legged friend to lie on their lap during the MRI session.

Waitemata and Counties Manukau Mental Health Services area played a crucial role in supporting this research. Thank you to all the doctors, nurses and key workers throughout the wider Auckland region who helped us to find our participants. And thank you in particular to Associate Professor Wayne Miles for his invaluable advice on matters concerning ethical approval for the project.

The New Zealand Schizophrenia Research Group (NZSRG) supported the work presented in this thesis and I was honoured to be invited to join the NZSRG as a research representative. In particular I’d like to acknowledge the important insights offered by Dr Robert Miller and my friend Amanda Luckman.

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Last but certainly not least, thank you to my wonderful parents, Chris and Kathy McIlwain for all of your support and encouragement. I am blessed to have an amazing group of friends who have listened to and supported me along the way; thank you Abbie Devine, Michelle Cagney and Elaine Chen, Chris Kenedi and Firas Mudafar.
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<th>Description</th>
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<tbody>
<tr>
<td>$^{1}$H-MRS</td>
<td>Proton magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AF</td>
<td>Arcuate fasciculus</td>
</tr>
<tr>
<td>ALE</td>
<td>Activation likelihood estimation</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariates</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>$B_0$</td>
<td>The main magnetic field produced by the MRI scanner</td>
</tr>
<tr>
<td>CB</td>
<td>Cingulum bundle</td>
</tr>
<tr>
<td>CGI</td>
<td>Clinical Global Impression Scale</td>
</tr>
<tr>
<td>Cho</td>
<td>Choline</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CPZE</td>
<td>Chlorpromazine equivalents (mg)</td>
</tr>
<tr>
<td>Cr</td>
<td>Creatine</td>
</tr>
<tr>
<td>CRLB</td>
<td>Cramer-Rao lower bound</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>DAI-30</td>
<td>Drug attitude inventory (30 questions)</td>
</tr>
<tr>
<td>DLPFC</td>
<td>Dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>ECT</td>
<td>Electroconvulsive therapy</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
</tr>
<tr>
<td>ERP</td>
<td>Event related potential</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional anisotropy</td>
</tr>
<tr>
<td>FAST</td>
<td>FMRIB’s Automated Segmentation Tool</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>FDG</td>
<td>2-[18F]fluoro-2-deoxy-D-glucose</td>
</tr>
<tr>
<td>FEP</td>
<td>First episode psychosis</td>
</tr>
<tr>
<td>FES</td>
<td>First episode schizophrenia</td>
</tr>
<tr>
<td>FFR</td>
<td>Full functional recovery</td>
</tr>
<tr>
<td>FMRIB</td>
<td>Functional magnetic resonance imaging of the brain</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full-width half maximum</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>GCI</td>
<td>General Cognitive Index</td>
</tr>
<tr>
<td>Gln</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Glu</td>
<td>Glutamate</td>
</tr>
<tr>
<td>Glx</td>
<td>Glutamate, glutamine and GABA complex</td>
</tr>
<tr>
<td>GM</td>
<td>Grey matter</td>
</tr>
<tr>
<td>GMV</td>
<td>Grey matter volume</td>
</tr>
<tr>
<td>GPC</td>
<td>Glycerophosphorylcholine</td>
</tr>
<tr>
<td>GSMA</td>
<td>Genome scan meta-analysis</td>
</tr>
<tr>
<td>IFOF</td>
<td>Inferior fronto-occipital fasciculus</td>
</tr>
<tr>
<td>ILF</td>
<td>Inferior longitudinal fasciculus</td>
</tr>
<tr>
<td>MD</td>
<td>Mean diffusivity</td>
</tr>
<tr>
<td>MOAS</td>
<td>Modified Overt Aggression Scale</td>
</tr>
<tr>
<td>MPRAGE</td>
<td>Magnetisation prepared rapid gradient echo</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>ms</td>
<td>Milliseconds</td>
</tr>
<tr>
<td>MTR</td>
<td>Magnetic transfer ratio</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetyl aspartate</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl D-aspartate</td>
</tr>
<tr>
<td>NMDAR</td>
<td>N-methyl D-aspartate receptor</td>
</tr>
<tr>
<td>PANSS</td>
<td>Positive and Negative Syndrome Scale</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>PCh</td>
<td>Phosphorylcholine</td>
</tr>
<tr>
<td>PCP</td>
<td>Phencyclidine</td>
</tr>
<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>PD</td>
<td>Parallel diffusivity</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PRESS</td>
<td>Point resolved spin echo</td>
</tr>
<tr>
<td>RD</td>
<td>Radial diffusivity</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>RSWG</td>
<td>Remission in Schizophrenia Working Group</td>
</tr>
<tr>
<td>rTMS</td>
<td>Repetitive transcranial magnetic stimulation</td>
</tr>
<tr>
<td>SANS</td>
<td>Scale for Assessment of Negative Symptoms</td>
</tr>
<tr>
<td>SAPS</td>
<td>Scale for the assessment of positive symptoms</td>
</tr>
<tr>
<td>SLF</td>
<td>Superior longitudinal fasciculus</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>SVS</td>
<td>Single voxel spectroscopy</td>
</tr>
<tr>
<td>T</td>
<td>Tesla</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>THC</td>
<td>Tetrahydrocannabinol</td>
</tr>
<tr>
<td>TI</td>
<td>Inversion time</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>TRS</td>
<td>Treatment-resistant schizophrenia</td>
</tr>
<tr>
<td>UTRS</td>
<td>Ultra-treatment-resistant schizophrenia</td>
</tr>
<tr>
<td>VOI</td>
<td>Voxel of interest</td>
</tr>
<tr>
<td>WM</td>
<td>White matter</td>
</tr>
<tr>
<td>WMV</td>
<td>White matter volume</td>
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Overview

In 1952 the initiation of treatment with chlorpromazine for patients experiencing psychotic episodes marked a turning point in the treatment of schizophrenia and psychiatric conditions in general. Importantly, it was recognised that chlorpromazine was effective pharmacotherapy in patients with schizophrenia due to distinct antipsychotic effects rather than sedative properties. The antipsychotic action of chlorpromazine illuminated the pathophysiology of schizophrenia giving rise to the dopamine hypothesis and in the process the field of neuropsychopharmacology was born. Chlorpromazine reduced episodes of violence and days in seclusion by 90-95%; large state hospitals some with over 15,000 beds were downsized or closed. (Carpenter & Davis, 2012) The success of chlorpromazine led to the development of a number of agents with similar mechanisms of action, now termed first-generation antipsychotics and, over subsequent years, these were progressively introduced into clinical settings. A substantial number of patients improved with treatment using these medications. However, extrapyramidal side effects, including the potentially irreversible movement disorder tardive dyskinesia, were a problem. This led to the introduction of a number of newer antipsychotics with little or no effect on the extrapyramidal system, the second-generation antipsychotics. These developments have meant that there are now over 50 antipsychotics available with various receptor binding profiles. However, despite this proliferation there remain a number of patients whose psychotic symptoms are not alleviated by the first or second generation antipsychotics, forming a group designated treatment resistant schizophrenia (TRS).

Approximately a third of patients with schizophrenia suffer from TRS which represents a subset of patients with poor-outcome schizophrenia i.e. those with persistent moderate-to-severe positive symptoms despite standard antipsychotic treatment. The onset of schizophrenia is typically in early adulthood and the impact of TRS is profound for the individual, their families and ultimately the community as a whole. This may manifest as the decreased likelihood of living independently,
being in an intimate relationship, achieving formal education or undertaking paid employment.

The next major breakthrough in the treatment of schizophrenia was the introduction of clozapine during the early 1970s, which remains the gold standard for TRS where other antipsychotics have failed. Initially withdrawn from the market after cases of fatal agranulocytosis were reported, clozapine was reintroduced with compulsory white blood cell count monitoring following a study by Kane et al in 1988 who documented the superior efficacy of clozapine in treatment-resistant patients. Yet there is still a subset, approximately 50-70% of patients treated with clozapine, who do not experience clinically significant improvements in their symptoms and may or may not respond to augmentation with other antipsychotics – clozapine-resistant or ultra-treatment-resistant schizophrenia (UTRS). (Buckley et al., 2001; Chakos, Lieberman, Hoffman, Bradford, & Sheitman, 2001; Mouaffak et al., 2006)

This thesis reports on MRI findings in people with schizophrenia grouped according to their response to antipsychotic medication. This grouping was chosen because at present treatment selection is empirical and based upon the minimisation of side effects because there are no distinct advantages between antipsychotic agents, with the exception of clozapine. Further, this approach is consistent with the initiative launched by the National Institute of Mental Health (NIMH) ‘Research on Biomarkers for Mental Disorders’ to facilitate the accurate prediction of disease risk, course and therapeutic responses.

Several lines of evidence including diffusion tensor imaging (DTI) and proton magnetic resonance spectroscopy (¹H-MRS), discussed in the following chapters, suggest that TRS may have a neurobiological basis distinct from schizophrenia that responds to pharmacological intervention – this is yet to be investigated in UTRS. Previous reports have found that poor response to treatment with antipsychotics is associated with reduced WM integrity and alterations in glutamatergic neurometabolites, specifically increased glutamate in the anterior cingulate cortex and striatum. The identification of biological characteristics or biomarkers for TRS is valuable as this may allow the prescription of clozapine or other effective antipsychotic combinations early on in the
individual’s course of illness and therefore a faster return to normal function. Selecting the most appropriate treatment as soon as possible is critical since there is evidence to suggest that multiple relapses have biological consequences and is associated with a decline in response readiness across episodes.

In the work reported in this thesis, we aimed to discover potential biomarkers of TRS based on an individual’s response (or lack thereof) to treatment with clozapine which is consistent with a recently proposed classification of schizophrenia and in line with current treatment algorithms. Patients responsive to second generation antipsychotics (first-line responders) and clozapine monotherapy (clozapine-responsive; TRS) are compared to each other and to those who do not respond to clozapine monotherapy (clozapine resistant; UTRS), in addition to a healthy control group.

The identification of a biomarker requires significant resources and multiple experiments which are beyond the scope of the present study; however, it is hoped that this first analysis will provide leads for future longitudinal research that will identify useful biomarkers of TRS. Two non-invasive magnetic resonance imaging (MRI) techniques are used to examine: 1.) the structure and integrity of white matter pathways in the brain and 2.) alterations in the concentrations of neurometabolites; using DTI and $^1$H-MRS, respectively.

Outline of thesis

Chapter one introduces the concept of treatment resistant schizophrenia. The bulk of the chapter comprises a published review of the pharmacotherapy available for treatment resistant schizophrenia; the role of clozapine and options for those unresponsive to clozapine treatment.

Chapter two describes the study sample recruited and grouped according to treatment response. A publication that comprehensively describes the study sample examining the level of treatment response in three groups of people with schizophrenia according to remission criteria and validated symptom severity scales is introduced and commented upon.
Chapter three provides an introduction to proton magnetic resonance spectroscopy and findings relevant to treatment resistant schizophrenia while the results from this study are presented in chapter four in the form of a paper submitted for publication.

Chapter five is an introduction to diffusion tensor imaging and findings relevant to treatment resistant schizophrenia while the results from this study are presented in chapter six in the form of a paper submitted for publication.

Chapter seven presents a discussion of the findings of the present study in the context of the literature, the strengths and limitations of the current study, the implications for the treatment of schizophrenia and finally future directions for further research.
Chapter One: Treatment resistant schizophrenia

The following chapter is adapted from the following publication:

http://dx.doi.org/10.2147/NDT.S12769

1.1 Introduction

Schizophrenia is a disabling mental illness with a lifetime prevalence of ~0.7% worldwide. (Saha, Chant, Welham, & McGrath, 2005) Typically beginning in early adolescence outcomes for patients are variable but the course of illness is chronic often marked with periods of relapse despite treatment. Schizophrenia has a significant and often devastating impact on social and occupational functioning for patients, often due to residual negative symptoms and cognitive deficits. (Marder, 2006) This may manifest as the decreased likelihood of living independently, being in an intimate relationship, achieving formal education or being in paid employment. (Gureje, Herrman, Harvey, Morgan, & Jablensky, 2002; Jablensky et al., 2000; Thornicroft et al., 2004; Wheeler, 2007) There are a range of antipsychotic medications available, including first generation antipsychotics (FGAs) and second generation antipsychotics (SGAs). (Jones et al., 2006; Stroup et al., 2009) However, sub-optimal therapeutic response in terms of psychotic symptoms is common and affects up to one third of people. (Lehman A, 2004) Negative symptoms may be classified as primary (part of the disease process itself) or secondary (to factors such as depression, drug-induced akinesia or a suspicious withdrawal) (Carpenter, Heinrichs, & Wagman, 1988) and are generally less amenable to treatment. (Erhart, Marder, & Carpenter, 2006; Stahl & Buckley, 2007) Antipsychotic agents have no demonstrable efficacy for primary enduring or “deficit” negative symptoms. (Kirkpatrick, Fenton, Carpenter, & Marder, 2006) Improvements in this symptom domain are largely a consequence of a reduction in positive symptoms. (Breier, Schreiber, Dyer, & Pickar, 1991; Tandon et al., 1993) While antipsychotic agents improve attention in people with schizophrenia, (Harvey & Keefe, 2001; Mishara & Goldberg, 2004) the effects observed for other cognitive impairments are
inconsistent (Mortimer, 1997) and may include worsening (Bilder, 1997; Green & Braff, 2001). The net impact of an antipsychotic agent on cognitive function is determined by the beneficial effect on attention and adverse effects related to anticholinergic activity and extrapyramidal side effects (EPSE). (Tandon, Nasrallah, & Keshavan, 2010). Furthermore, it has been postulated that a practice effect may account for beneficial effects observed. (Goldberg et al., 2007). There are no apparent consistent differences between antipsychotic agents with respect to their effect on cognition. (Davidson et al., 2009; Hill, Bishop, Palumbo, & Sweeney, 2009; Keefe et al., 2007). Because of the consequences of inadequate symptom control, effective treatment strategies are required for people with treatment-resistant schizophrenia (TRS).

Several definitions of treatment-resistant schizophrenia exist and vary in their specificity. The criteria employed by Kane et al. (1988) to define treatment-resistant (or treatment-refractory) schizophrenia in the pivotal trial comparing clozapine to chlorpromazine is used frequently in clinical trials and audit settings. (Kane, Honigfeld, Singer, & Meltzer, 1988; Kerwin & Bolonna, 2005). Kane et al. classified participants as treatment-resistant if: improvement had not been demonstrated after three periods of treatment with antipsychotics (from two or more different chemical classes) in the previous five years equivalent to 1000 mg per day of chlorpromazine (CPZ) for 6 weeks and participants had had no episodes of good functioning in the previous 5 years Brief Psychiatric Rating Scale (BPRS) total score ≥45, Clinical Global Impressions (CGI) score ≥4 and score ≥4 on 2 or 4 positive symptoms items. (Kane et al., 1988). Conley et al. presented a modified version of these criteria to reflect clinical practice patterns and a better understanding of optimal dosing: two antipsychotic trials (400-600 mg CPZ equivalents per day) for 4 to 6 weeks with no clinical improvement, no period of good social or occupational functioning for >5 years, BPRS total score >45 and a score of >4 on 2 of 4 positive items. (Conley & Kelly, 2001).

Clozapine has been shown to be more effective than other antipsychotics in treatment-resistant populations in several studies (Kane et al., 1988; Lewis et al., 2006; Stroup et al., 2009) however, the occurrence of adverse effects, some of which are potentially life-threatening are important...
limitations. In addition to those who are intolerant to clozapine, only 30% to 50% experience clinically significant symptom improvement. (Buckley et al., 2001; Chakos et al., 2001) This has prompted unlicensed prescribing and antipsychotic combination strategies (with or without clozapine) for which there is the potential for increased side effects and little robust evidence to support this practice.

This review will summarise key studies and recent evidence for treatment strategies for people not responding to non-clozapine antipsychotic agents and people not responding or only partially responding to clozapine. The literature reviewed was identified by a systematic search of Ovid Medline & Medline In-Process, Embase (combined file 1947- present), Cochrane Central Register of Controlled Trials (CENTRAL/CCTR) and PsycINFO, supplemented by hand searches of reference lists. The evidence is presented in three sections: clozapine monotherapy versus other antipsychotics, clozapine augmentation strategies and options for clozapine-intolerant or clozapine-resistant people. The first section is divided into two parts comparing clozapine monotherapy to FGAs and SGAs; each part is stratified by the level of evidence presented. The clozapine augmentation section is first stratified by level of evidence (meta-analysis or randomised controlled trial) then by specific treatment strategy. The structure of this section reflects the relative availability of evidence regarding the treatment combinations considered. The third section, treatment options for those who are intolerant or resistant to clozapine, discusses alternative antipsychotic monotherapy and non-pharmacological treatments.

1.2 Clozapine monotherapy

The World Psychiatric Association Section on Pharmacopsychiatry utilized data from approximately 1,600 randomised controlled trials of 51 FGAs and 11 SGAs in the treatment of schizophrenia. (Tandon et al., 2008) Modest benefits were observed for the use of SGAs compared to FGAs for negative, cognitive and depressive symptoms, and with a lower risk of tardive dyskinesia. These benefits were mainly attributed to the ability of SGAs to provide improvement in positive symptoms, equivalent to that of FGAs, with a lower risk of extra pyramidal side effects.
There were no consistent differences between SGAs in terms of efficacy with the exception of clozapine which was found to be more efficacious than other antipsychotics in people who had not responded to one or more other antipsychotics. Adequate trials of adequate doses of FGAs and SGAs were found to be key variables in optimizing effectiveness of antipsychotic agents. In terms of treatment response and adverse effects, substantial individual variability was observed. SGAs offer the advantage of fewer acute extrapyramidal symptoms and less likelihood of tardive dyskinesia but produce greater metabolic side effects. Meta-analyses published subsequent to this summary statement and key trials regarding the use of clozapine are presented below.

1.2.1 Clozapine monotherapy versus FGA Agents

1.2.1.1 Meta-analyses

Leucht et al compared treatment outcomes between SGAs and FGAs in people with schizophrenia in general in a meta-analysis of 150 double-blind randomized studies including 21,533 participants. (Leucht, Corves, et al., 2009) The meta-analysis by Essali et al also compared treatment outcomes between those taking FGAs versus SGAs and was largely based on the same data. (Essali, Al-Haj Haasan, Li, & Rathbone, 2009) Four SGA agents emerged as superior to FGA agents: clozapine, amisulpride, olanzapine and risperidone. (Leucht, Corves, et al., 2009) The majority of studies (121) were of 12 weeks duration; 17 were of 6 months duration and 12 were longer than 12 months. It has been postulated that EPSE associated with FGAs may mimic the symptoms of schizophrenia and in early randomized controlled trials (RCTs) falsely suggest that SGAs are superior. (Geddes, Freemantle, Harrison, & Bebbington, 2000; Leucht, Wahlbeck, Hamann, & Kissling, 2003; Rosenheck, 2005) In order to avoid this potential problem, only participants taking ≤ 12 mg per day haloperidol (or ≤ 600 mg per day chlorpromazine equivalents for low-potency FGAs) were included in this meta-analysis. Positive and Negative Symptom Scale (PANSS) and BPRS scores were used to assess overall efficacy and specific symptoms domains all of which were found to be more amenable to treatment with clozapine, olanzapine, amisulpride or risperidone versus FGAs.
Treatment with clozapine produced medium effect sizes: overall symptoms -0.52 (95% confidence intervals [CI]: -0.75 to -0.29, p<0.0001), positive symptoms -0.36 (CI: -0.56 to -0.16, p<0.0001), negative symptoms -0.27 (CI: -0.42 to -0.13, p<0.0001), depression -0.51 (CI: -0.87 to -0.14, p=0.006). Amisulpride and olanzapine were found to be superior to FGAs with the following effect sizes: overall symptoms -0.31 (CI:-0.44 to -0.19, p<0.0001) and -0.28 (CI: -0.38 to -0.18, p<0.0001) respectively, positive symptoms -0.22 (CI: -0.37 to -0.06, p=0.005) and -0.15 (CI: -0.21 to -0.09, p<0.0001), negative symptoms -0.27 (CI: -0.40 to -0.14, p<0.0001) and -0.32 (CI: -0.47 to -0.16, p<0.0001), depression -0.37 (CI: -0.51 to -0.24, p<0.0001) and -0.27 (CI: -0.35 to -0.19, p<0.0001).

The effect sizes associated with risperidone were small and the improvement observed on the depression subscale was not significant: overall symptoms -0.13 (CI: -0.22 to 0.05, p=0.002), positive symptoms -0.13 (CI: -0.20 to -0.05, p=0.001), negative symptoms -0.13 (CI: -0.21 to -0.06, p<0.0001), depression -0.10 (CI: -0.23 to 0.03, p=0.145). Industry sponsorship, comparator dose and prophylactic EPSE medication were assessed as moderator variables but did not yield any consistent effects. Leucht et al concluded that this reflects the fact that FGAs and SGAs are heterogeneous classes of compounds and argued that such categorization can lead to improper generalization and confusion. (Leucht, Corves, et al., 2009)

1.2.2 Randomised controlled trials

Meltzer et al investigated the use of clozapine versus FGAs in treatment responsive participants during a 24 month study. (Meltzer, Bobo, Lee, Cola, & Jayathilake, 2010) Significant improvements in psychopathology, quality of life and global functioning were observed in both the clozapine (n=40) and FGA group (n=45) after taking a range of antipsychotic agents; most commonly haloperidol but also perphenazine, fluphenazine, loxapine, thioridazine, thiothixene, molindone and amoxapine. While a similar improvement in psychopathology was observed, significantly more relapse/rehospitalisation drop-outs occurred in those taking FGAs (19 relapse related hospitalizations in 10 participants versus 11 relapse related hospitalizations in 4 participants
treated with clozapine). There were no differences in the occurrence of EPSEs between clozapine and the FGA group however, clozapine was associated with more weight gain.

In a 12 week double blind trial, Krakowski et al randomly assigned participants with schizophrenia or schizoaffective disorder to receive clozapine (n=33), olanzapine (n=34) or haloperidol (n=33). (Krakowski, Czobor, & Nolan, 2008) People with a history of non-response or intolerance to any of the three study medications were excluded. Aggression was assessed using the Modified Overt Aggression Scale (MOAS) and a cognitive task battery tested general executive function, visuospatial ability, psychomotor function, and visual and verbal memory. In the general cognitive index (GCI) no significant improvement was observed in the haloperidol or clozapine group while clozapine was the most efficient medication in reducing aggression. An important limitation was the concomitant, prophylactic use of benztropine 4mg/day for EPSE in the group taking haloperidol, which may increase anticholinergic cognitive impairment. Participants taking haloperidol showed no increase in body weight, blood lipids or glucose. (Krakowski, Czobor, & Citrome, 2009)

1.3 Clozapine monotherapy versus other SGA Agents

1.3.1 Meta-analyses

The Cochrane Schizophrenia Group performed a meta-analysis in order to compare several commonly used SGA agents in terms of efficacy and tolerability in people with schizophrenia or schizophrenia-like psychoses. (Leucht, Komossa, et al., 2009) The primary outcome measure selected to assess this was change in total PANSS score, with positive and negative subscores as secondary outcomes. Outcomes were reported using weighted mean difference (WMD) in terms of PANSS scores and the dropout rate due to poor efficacy was included as a further outcome measure. Seventy-eight randomized, double-blind studies were included for analysis of which 28 included treatment with clozapine.

The results relating to clozapine were different to those anticipated based on previous reports. No significant differences were found when comparing the total PANSS scores between clozapine and
olanzapine (N=619), quetiapine (N=232), risperidone (N=466) or ziprasidone (N=146), however, clozapine was found to be significantly more efficacious than zotepine (N=59, WMD=-6.0, p=0.002).

The results for a decrease in positive symptoms reflected those found for overall symptoms while quetiapine was found to be more efficacious than clozapine on the negative symptom subscore (N=142, WMD=2.2, p<0.001). Clozapine was favoured over risperidone when comparing dropout rates due to poor efficacy (N=627, RR=0.40 95% CI 0.23-0.70, p=0.001). These unexpected results may be due to the low or very low doses of clozapine that were used in many of the studies included; several had an upper limit of 400 mg/day and five used dosages under 210 mg/day. In the pivotal studies that established clozapine’s effectiveness, the average daily dose of clozapine was 600 mg/day and 523 mg/day. (Kane et al., 1988; Rosenheck et al., 1997) Furthermore the participants included in these trials may not have been as treatment refractory as those in other studies demonstrating clozapine’s superiority over other SGA agents.

Substantial concerns about the side effects induced by SGA agents such as weight gain and metabolic syndrome may offset modest differences in their effectiveness. In a meta-analysis of head-to-head comparisons of the metabolic effects between SGA agents, Rummel-Kluge et al assessed weight gain and changes in cholesterol and glucose over 48 studies. (Rummel-Kluge et al., 2010) There were three main clusters in terms of these outcomes: olanzapine and clozapine produced the greatest elevation in weight, cholesterol and glucose (with no significant difference between the two agents) followed by quetiapine, risperidone and sertindole with intermediate elevations. Aripiprazole and amisulpride showed lower elevations and ziprasidone the lowest. The authors noted that the dose of antipsychotic influenced some of the results in metaregressions; for example a high dose of olanzapine tended to produce a greater difference in the outcome measure in favour of the comparator drug. Another important caveat is that data regarding prior antipsychotic treatment for the participants in the selected studies were not available for analysis.
1.3.2 Randomised controlled trials

Phase 2 of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) recruited 99 participants who discontinued treatment with olanzapine, quetiapine, risperidone or ziprasidone in phase 1 or 1B of the trial primarily due to inadequate efficacy. (McEvoy et al., 2006) Participants were randomized to blinded treatment with another newer SGA not previously received in the trial (olanzapine n=19, quetiapine n=15 or risperidone n=16) or open label treatment with clozapine (n=49). At 3-month assessments, participants treated with clozapine experienced a greater reduction in PANSS total score (mean=-11.7, SE=3.2) than participants treated with quetiapine (mean=2.5, SE=4.8) or risperidone (mean=4.1, SE=1.9) but not olanzapine (mean=-3.2, SE=2.3). Clozapine was only significantly better than quetiapine on the PANSS general psychopathology subscale (mean=-4.7, SE=1.5 versus mean=2.3, SE=2.5, p=0.006). Time to discontinuation for any reason was significantly longer for clozapine (median= 10.5 months) than for risperidone (2.8 months) or quetiapine (median=3.3 months) but not olanzapine (median=2.7 months). Time to discontinuation is subject to bias in this phase of the study since treatment allocation was known to both clinicians and participants there may have been reluctance to discontinue clozapine, it being widely considered the best option for treatment-resistant schizophrenia. The data from this study supports the conclusion that, for participants who prospectively failed to improve with an SGA, treatment with clozapine was more effective than switching to another SGA.

Phase 3 of CATIE allowed 270 participants who had discontinued antipsychotics in phases 1 and 2 to select treatment from nine antipsychotic regimens with the help of their study doctor. (Stroup et al., 2009) Approximately equal numbers of participants chose seven of the nine antipsychotics including clozapine (33 to 41 participants each agent). The study used a double-blind design with the exception of those treated with clozapine which was open label. The blinding of treatment with clozapine would have required additional monitoring of all treatment groups for clozapine specific safety issues and in doing so may have affected the ecological validity of the other agents. All of the commonly used treatments were associated with substantial symptom improvement at 3
months and 6 months; with the exception of aripiprazole at 3 months and ziprasidone and quetiapine at 6 months. A total of 106 participants discontinued treatment; there were no significant differences in the proportions of participants who discontinued the commonly selected medicines (range 33-46%). However, discontinuation due to lack of efficacy was lower for clozapine (5%), risperidone, quetiapine, and ziprasidone (0-5%) than olanzapine, aripiprazole and combination treatment (13-18%). Adverse effects were problematic in the group taking clozapine; the rates of adverse events classified as moderate or severe were highest for clozapine (35%), quetiapine (45%) and combination antipsychotic treatment (30%). Clinically significant weight gain of at least 7% was common with clozapine (32%), combination antipsychotic treatment (39%) and olanzapine (23%). All other SGA agents were associated with weight loss, in particular aripiprazole and ziprasidone which produced the greatest monthly weight loss of 0.64 kg and 0.59 kg respectively; clozapine produced a gain of 0.59 kg per month.

Krakowski et al reported that olanzapine outperformed clozapine in terms of neurocognitive function in a study of 100 physically aggressive inpatients with schizophrenia or schizoaffective disorder. (Krakowski et al., 2008) With respect to metabolic parameters, participants taking olanzapine gained the most weight compared to clozapine or haloperidol, but clozapine was associated with the greatest increases in serum cholesterol, triglycerides and glucose. (Krakowski et al., 2009) In the General Cognitive Index (GCI) olanzapine was found to be superior (improvement was approximately 0.5 standard deviations (SD)) to both clozapine and haloperidol; this was also associated with a decrease in aggression which was assessed using the MOAS. Rather than concluding that olanzapine has a procognitive effect it is perhaps more likely that olanzapine has less cognitive liability; clozapine has strong intrinsic anticholinergic activity compared to olanzapine. (Chengappa et al., 2000) Nonetheless, treatment with clozapine markedly reduced aggression suggesting that the antiaggressive effects of olanzapine may be mediated by different neuronal pathways.
It has been suggested that a decrease in serum cholesterol may result in aggression due to the subsequent decrease in brain serotonergic activity given that cholesterol determines the availability of serotonin receptors and transporters. (Engelberg, 1992) In a post-hoc analysis of the relationship between serum cholesterol levels and aggression in these groups, Krakowski et al found a negative correlation at baseline. (Krakowski & Czobor, 2010) Based on changes in total cholesterol (TC) over the 12 week study period the investigators used a Glimmix regression model to predict changes in aggression (Krakowski, pers comm). For those taking haloperidol it was predicted that a 141.9% increase in physical aggression was associated with a decrease of 1SD unit in TC levels. Participants whose cholesterol increased by 1SD in the clozapine group were predicted to be 67.6% (p<0.001) less physically aggressive than those whose cholesterol did not change. It was then postulated that the antiaggressive effects of clozapine may have been further enhanced by an increase in cholesterol.

The UK Cost Utility of the Latest Antipsychotic Drugs in Schizophrenia Study 2 (CUtLASS 2) included 136 people with schizophrenia and related disorders whose medication was being changed due to sub-optimal response to two or more previous antipsychotic agents. (Lewis et al., 2006) Participants were randomly allocated to receive clozapine or another SGA agent (risperidone, olanzapine, quetiapine or amisulpride) selected by the treating clinician. The trial was rater-blind and outcome assessments were carried out for 87% of the participants at 12, 26 and 52 weeks following randomization. No significant advantage was observed for those taking clozapine compared to other SGA agents in the Quality of Life score (3.36 points, 95% CI 0.46 to 7.71) however a significant improvement was seen in the PANSS total score (-4.93, 95% CI -8.82 to -1.05). At twelve weeks the group taking clozapine reported that their mental health was significantly better than those taking other SGA agents. There were no significant differences between the treatment groups in the rate of adverse effects including weight gain.

Suicide has been identified as the leading cause of premature death among people with schizophrenia. (Cohen, Test, & Brown, 1990) The International Suicide Prevention Trial (InterSePT)
assessed the risk for suicidal behaviour in 980 participants with schizophrenia or schizoaffective disorder treated with clozapine compared to olanzapine over a two year period. (Meltzer et al., 2003) Participants in this study, 26.8% of whom were refractory to previous treatment, were considered at high risk for suicide because of previous attempts or the presence of suicidal ideation. The study was conducted as an open-label trial with masked ratings. Suicidal behaviour, defined as suicide attempts and hospitalizations to prevent suicide, was observed less frequently in those taking clozapine versus olanzapine (hazard ratio 0.76, 95% CI 0.58 to 0.97). Worsening on the CGI-Suicide Severity or implicit worsening as demonstrated by occurrence of suicidal behaviour was also less frequent in those taking clozapine (hazard ratio 0.78, 95% CI 0.61 to 0.99). Fewer clozapine treated participants attempted suicide, required hospitalizations or rescue interventions to prevent suicide (34 versus 55, p=0.03, 82 versus 107, p=0.05 and 118 versus 155, p=0.01 respectively). The need for concomitant antidepressants or anxiolytics/soporifics was also less frequent in those taking clozapine compared to olanzapine (221 versus 258, p=0.01 and 301 versus 331, p=0.03). Although the number of completed suicides was greater in the clozapine group (5 clozapine-treated participants versus 3 olanzapine-treated participants, p=0.73) this was not significant and this study was not powered to evaluate this as an endpoint. It was recognised by the investigators at the outset that the study would need to include 20,000 participants to detect a decreased relative risk for suicide deaths with clozapine therapy by 20%.

In a randomized double-blind trial, Harvey et al compared the cognitive performance of 130 people with schizophrenia after 12 weeks of treatment with clozapine (n=69) or ziprasidone (n=61). (Harvey et al., 2008) All participants were either resistant or intolerant to previous antipsychotic treatment. Clozapine-treated participants showed improvement on the Rey Auditory Verbal Learning Test (RAVLT; episodic memory) and the Stroop interference test (executive function) but not the Trail-Making Test (TMT; parts A and B; processing speed) compared to those taking ziprasidone. None of the individual items were observed to improve at 12-weeks between the treatment groups; however the composite score improved significantly in those taking
ziprasidone compared to clozapine, (effect size $d=0.54$, $p=0.029$). One possible explanation for these results is that clozapine may interfere with the performance benefits of practice effects. Although it appears that ziprasidone is superior in reducing cognitive deficits in this short-term trial, clinical efficacy in terms of symptom control was not reported. Another explanation is that the findings of this phase four study are due to chance and it must be noted that this trial was funded by Pfizer, the makers of ziprasidone.

Davies et al compared clozapine to available SGA agents in a UK multi-centre, rater blind RCT in people with psychosis eligible for clozapine to assess cost-effectiveness. (Davies et al., 2008) Over a one year period, it was found that clozapine was associated with higher quality-adjusted life years (QALYs) than other SGA agents, but at an additional cost. The probability that clozapine is cost-effective reached 50% if in order to gain 1 QALY the decision-makers were willing to pay £33,000. In other words, if the decision-makers were willing to pay less than £33,000 to gain 1 QALY, other SGA agents may be more cost-effective than clozapine. However, this trial was conducted with a relatively small number of participants ($n=67$ clozapine; $n=69$ other SGA agents) and post-hoc calculations indicated that the power to detect significant differences in net money benefit was low (50% if important differences in costs and QALYs were defined as £1600 and one-twentieth of a QALY, respectively). Furthermore, it may not be possible to extrapolate the results to longer-term clozapine treatment or to a population of primarily treatment-resistant people. The authors also noted that clozapine may be more cost-effective if fewer participants had clozapine initiated as an inpatient than in this RCT.

The present review found two RCTs comparing clozapine monotherapy to treatment with high dose olanzapine (Kumra, Kranzler, Gerbino-Rosen, Kester, De Thomas, et al., 2008; Meltzer et al., 2008) and a further study examining treatment with ziprasidone with treatment-resistant participants. (Sacchetti et al., 2009) These studies will be discussed in detail below.
1.4 Clozapine augmentation

Despite proven efficacy in people with schizophrenia showing sub-optimal response to other antipsychotics, only 30% to 50% of people will experience clinically significant symptom improvement with clozapine treatment. (Buckley et al., 2001; Chakos et al., 2001) One to two-thirds of people will continue to experience positive symptoms with adequate doses of clozapine or will be unable to reach adequate levels due to side effects that prevent further dose increases. (Chakos et al., 2001) Antipsychotic monotherapy is preferred over augmentation according to schizophrenia treatment algorithms; for people who do not respond to first-line antipsychotics clozapine is recommended. Therefore clozapine augmentation strategies should be implemented only for those who experience insufficient response to clozapine monotherapy. An operational definition of non-response to clozapine or ‘ultra resistant’ schizophrenia is: BPRS improvement of <20% despite a trial with clozapine for ≥ 8 weeks and plasma levels > 350 μg/L, no stable period of good social and/or occupational functioning for ≥ 5 years, GAF ≤ 40, BPRS total score ≥ 45, CGI score ≥ 4 and a score of ≥ 4 on 2 of 4 positive symptom items. (Mouaffak et al., 2006)

1.4.1 Meta-analyses

1.4.1.1 Augmentation with other antipsychotics

The present review found four meta-analyses concerning the augmentation of clozapine treatment with another antipsychotic for people with an inadequate response to clozapine monotherapy. (Barbui, Signoretti, Mule, Boso, & Cipriani, 2009; Cipriani, Boso, & Barbui, 2009; Correll, Rummel-Kluge, Corves, Kane, & Leucht, 2009; Taylor & Smith, 2009) These meta-analyses were based on essentially the same data, the largest of which was conducted by Barbui et al and arrived at similar conclusions with the exception of Correll et al. (Barbui et al., 2009; Correll et al., 2009)

Barbui et al selected 21 studies to determine the efficacy of a second antipsychotic in combination with clozapine. (Barbui et al., 2009) The number of trials evaluating each augmentation agent was
chlorpromazine n=1, pipothiazine n=2, amisulpride n=1, sulpiride n=7 and the remainder used risperidone (n=10). The mean length of follow up was 13.8 weeks (SD=19.6) and the trials were divided into either short-term studies of less than 10 weeks duration or long-term studies. Clozapine combination strategies were favored in fourteen open (non-blind), randomized studies in terms of effect size or standardized mean difference (SMD) from various outcome scales (SMD=-0.80, 95% CI -1.14 to -0.46). However, this trend was not apparent in six of the RCTs (SMD=-0.12, 95% CI -0.57 to 0.32). Subgroup analysis by trial duration revealed a similar trend: the open studies favored clozapine combinations in both long and short-term trials, the blinded studies showed no advantage for clozapine combinations of either duration.

Correll et al found antipsychotic combinations in general to be advantageous over monotherapy in a meta-analysis of 19 studies (1229 participants) in terms of all cause discontinuation (n=1052, RR 0.65, 95% CI 0.54 to 0.78) and less study-specific inefficacy (n=1202, RR 0.76, 95% CI 0.63 to 0.90). (Correll et al., 2009) The mean trial duration was 12.1 weeks (range 4 to 52 weeks). The most commonly used antipsychotic was clozapine, though a variety of antipsychotic combinations were used. In terms of lack of efficacy as defined by each study, co-treatment including clozapine was superior to antipsychotic monotherapy (n=764, RR 0.75, 95% CI 0.61 to 0.93), however the specific augmenting agents were not presented separately within the results. Meaningful results regarding specific psychopathology and adverse events could not be calculated due to insufficient data. Sensitivity analyses identified 5 efficacy moderators: clozapine combinations, concurrent polypharmacy initiation, Chinese trials, trial duration >10 weeks and SGA + FGA combinations. Meta-regression of variables from sensitivity analyses identified three significant moderators associated with superior efficacy of antipsychotic combinations: similar doses in the mono- and poly-therapy arm (p=0.006, coeff=0.48), SGA + FGA combinations (p=0.027, coeff=0.39) and concurrent polypharmacy initiation (p=0.050, coeff=0.35). The findings of this study differ from other meta-analyses of antipsychotic combination treatment and it is important to note that the positive results for antipsychotic combinations observed were primarily from Chinese studies not
included in the other meta-analyses. A high degree of heterogeneity within the database and possible publication bias further obscured the significance of these findings.

Overall, it appears that the evidence considered for clozapine augmentation with another antipsychotic in these meta-analyses is weak and observed benefits are moderate at best. One consideration to take into account is that these reviews combined results of all antipsychotic augmentation irrespective of mechanism of action.

1.4.1.2 Augmentation with anticonvulsants

Dysfunctional glutamatergic neurotransmission is postulated to be an important component underlying the pathophysiology of schizophrenia. (Goff & Coyle, 2001) Lamotrigine is an anticonvulsant drug that inhibits excessive glutamate release in the brain by antagonism of sodium channels and increases gamma-aminobutyric acid (GABA) release. It has been used as an augmenting agent on this basis. (Cunningham & Jones, 2000; Leach, Baxter, & Critchley, 1991)

Tiihonen et al examined the advantages of combining clozapine with lamotrigine in five randomized placebo-controlled trials (161 participants) of 10 to 24 weeks duration. (Tiihonen, Wahlbeck, & Kiviniemi, 2009) On the primary outcome measure the total score for symptoms of psychosis, clozapine-lamotrigine combination was superior to clozapine-placebo combination (SMD 0.57, 95% CI 0.25 to 0.89; NNT 4, 95% CI 3 to 6). The secondary outcome measures also favored this combination (SMD 0.34, 95% CI 0.02 to 0.65 for decreasing positive symptoms and SMD 0.43, 95% CI 0.11 to 0.75 for improving negative symptoms). The incidence of severe adverse effects or drop-out rate did not differ between the treatment groups. No significant heterogeneity was observed in the meta-analysis. Importantly, this is the first evidence to date of efficacy for any pharmacological treatment in clozapine-resistant schizophrenia and it is noted by the authors that similar benefits may not be observed with lamotrigine and other antipsychotic agents apart from clozapine. The effect size for total score for symptoms of psychosis was 0.57 suggesting beneficial effects for general symptoms which are known to be robust predictors of functional outcomes; however scores were not available for all studies. (Green, 1996)
1.4.1.3 Augmentation with NMDA Agonists

Like anticonvulsants, the use of N-methyl-D-aspartate (NMDA) enhancing agents is predicated on the glutamate hypothesis of schizophrenia, specifically NMDA receptor hypofunction. Antagonists of NMDA receptors such as phencyclidine and ketamine produce psychotic symptoms and neurocognitive deficits in human subjects and exacerbate psychotic symptoms in people with schizophrenia. (Krystal et al., 1994; Lahti, Holcomb, Medoff, & Tamminga, 1995; Malhotra et al., 1997) Agonists at the obligatory NMDA-glycine binding site are glycine, D-serine and D-alanine and the partial agonist D-cycloserine, as opposed to agonists at the NMDA recognition site which are excitotoxic. These agents in addition to sarcosine which increases the availability of glycine in the synapse by inhibiting the glycine transporter-1 (GlyT-1) have been investigated as potential therapeutic agents for schizophrenia. Tsai et al performed a meta-analysis of 26 double-blind, placebo controlled trials in approximately 800 people taking an NMDA agonist in addition to stable doses of antipsychotic medication for at least four weeks. (Tsai & Lin, 2010) Almost all studies used the PANSS to assess symptom severity. The pooled effect size (ES) of clinical efficacy of NMDA agonist augmentation compared to placebo for total psychopathology was 0.40 (95% CI 0.22-0.58) and significant improvement was noted for depressive, negative, cognitive, positive and general symptoms. Treatment with glycine, D-serine and sarcosine was associated with improvement in multiple symptom domains while D-cycloserine was not. The concomitant antipsychotic used appeared to affect the efficacy of the NMDA-enhancing agent; those treated with risperidone or olanzapine improved, but those treated with clozapine did not. Gastrointestinal (GI) upset and nausea were noted more often in some glycine trials while other side effects were equivalent for NMDA-enhancing agents and placebo. Despite a moderate effect size, the efficacy of these agents may have been overstated due to limitations within the study. For instance, studies were only included if they provided “enough data to calculate and effects size” and a test for homogeneity revealed that there may have been systematic differences among the included studies. Another
important caveat is that D-cycloserine, D-serine, D-alanine and sarcosine are protected by US patents for which the study author is a patent holder.

1.4.2 Randomized controlled trials

1.4.2.1 Augmentation with anticonvulsants

Topiramate is a GABAergic anticonvulsant drug indicated as add-on pharmacotherapy for adults and children with primary generalized tonic-clonic and partial-onset seizures. It has been used for people with schizophrenia to correct a postulated glutamate deregulation due to NMDA receptor hypofunction. Topiramate is thought to potentiate inhibitory GABAergic transmission (probably through a non-benzodiazepine mechanism) and inhibit the activity of kainite on the AMPA/kainate receptor subtype. (Ängehagen, Shank, Hansson, Rönnbäck, & Ben-Menachem, 2001; Arnone, 2005; Gibbs, Sombati, DeLorenzo, & Coulter, 2000; Shank, Gardocki, Streeter, & Maryanoff, 2000; White, Brown, Woodhead, Skeen, & Wolf, 1997)

Two studies have examined the use of topiramate as an adjunct to treatment to clozapine with contrasting results. Afshar et al conducted a double-blind trial over eight weeks with 32 people receiving clozapine treatment for at least two months. (Afshar et al., 2009) Participants were randomized to receive up to 300 mg per day of topiramate (n=16) or placebo in addition to clozapine (n=16). Total PANSS scores at baseline were similar between the groups indicating a suboptimal response to clozapine monotherapy (topiramate group 96.87 ±21.98; placebo group 101.87 ± 23.05 p=0.53). Clinically significant improvement was defined as a >20% decrease in total PANSS score and was observed in eight participants (50%) in the topiramate group and two in the placebo group (12.5%; p<0.05). The differences in the groups’ total PANSS mean scores were reported at both three and eight weeks and favoured topiramate augmentation; -11.18 ± 8.72 versus -1.56 ± 9.23, p= 0.005 and -20.00 ± 11.96 versus -1.31 ± 11.13, p<0.001 respectively. At eight weeks a number of side effects were more prevalent in the topiramate group such as hypersalivation (75.0% versus 34.7%, p≤0.05) but this was reported to be present in some participants prior to the study, psychomotor retardation (50.0% versus 6.2%, p≤0.01), paresthesia
(37.5% versus 6.2%, p≤0.05). Weight loss was also reported more commonly in the topiramate group (37.5% versus 6.2%, p≤0.05). However, the authors reported that there were no differences observed in body mass index (BMI) between the groups or within each group over the trial period. None of the participants dropped out of the trial due to drug-induced adverse effects. While the results of this small trial appear to favour topiramate augmentation, the follow-up period is relatively short. Furthermore, the investigators did not assess cognitive impairment, a well-documented, dose-dependent adverse effect of topiramate that is particularly relevant to people with schizophrenia. (Arif et al., 2009; Gilliam et al., 2003; Lee et al., 2003; Thompson, Baxendale, Duncan, & Sander, 2000)

The double blind RCT by Muscatello et al was a methodologically robust 24 week study that failed to replicate the benefits of topiramate add-on pharmacotherapy reported by Afshar et al. (Muscatello et al., 2010) People receiving clozapine for at least one year, at a stable dose for at least one month with a BPRS score of ≥25 were eligible to participate. The clozapine dose remained unchanged throughout the study and participants non-compliant with all 10 study visits were excluded. No last observations were carried forward (LOCF) since this introduces assumptions which can under- or overestimate the effects of treatment. (Streiner, 2002) Participants did not receive any antidepressants or anticonvulsants for a period of 2 months prior to the study. A maximum dose of 200 mg/day topiramate was added to clozapine treatment (n=19; placebo n=24). No significant improvement in positive, negative, affective or overall symptomatology from baseline to week 24 was observed. In the topiramate group a significant reduction was observed using the scale for the assessment of positive symptoms (SAPS) subscale for bizarre behaviour (including clothing and appearance, aggressive behaviour, stereotyped behaviour and social and sexual behaviour). (Andreasen, 1984

http://www.movementdisorders.org/UserFiles/file/Long_SAPS_2000_publish(1).pdf.) No significant effects on cognitive functioning were observed as measured by the Stroop test, verbal fluency and the Wisconsin Card Sorting Test (WCST). No serious adverse events were reported;
however adjunctive topiramate was more frequently associated with asthenia, sedation and paresthesia while constipation and hypersalivation were reported in the placebo group. There was no significant change in body weight from baseline to the end of the trial for the topiramate group.

It is possible that this trial did not prove topiramate to be as useful for clinical symptomatology as the previous study because a lower dose of topiramate was used (200 mg/day versus 300 mg/day). Yet this dose was chosen based on findings by Deutsch et al in order to avoid cognitive impairment which was not assessed by Afshar et al. (Deutsch et al., 2003) Furthermore, the very small topiramate group (n=19) means that only a large change in SAPS or WCST would produce a statistically significant difference. From these studies it appears that at doses low enough to preserve cognitive function, topiramate is of little benefit for clinical symptoms.

1.4.2.2 Augmentation with cognitive enhancing agents

Memantine is a weak non-selective NMDA receptor antagonist approved for use in the treatment of moderate to severe Alzheimer’s disease. De Lucena et al studied the effects of 20 mg per day memantine combined with clozapine treatment for negative symptoms over 12 weeks. (De Lucena et al., 2009) This double-blind trial was small (memantine n=10 and placebo n=11) and consisted of those taking clozapine for at least ten years for TRS. Significant improvements were seen at week 12 in the active treatment group for the total BPRS score (19.00 versus 43.18, p=0.001) and on the positive and negative symptom subscales (4.10 versus 9.18, p=0.007 and 6.10 versus 13.55, p=0.001). Those taking memantine also showed an 6.12-point (95% CI 4.45 to 7.79) increase in mean score on the Mini-Mental State Examination (MMSE) although this is not the most sensitive measure of cognitive functioning. (Lancu & Olmer, 2006) SAS score and body weight were not significantly different between the groups. Based on results from animal studies, it has been postulated that memantine may improve cognitive function by up-regulating the expression of brain derived neurotrophic factor (BDNF) in humans. (Meisner et al., 2008) In this study however, de Lucena did not detect an association between memantine treatment and increased serum BDNF levels which have been highly correlated with cerebrospinal fluid BDNF levels. (Pan, Banks, Fasold,
Bluth, & Kastin, 1998) This may be due to the small sample size or clozapine treatment prior to randomisation that may also have increased serum BDNF levels。(Gama et al., 2007)

From this small trial, it appears that memantine may have beneficial effects in treatment-resistant people taking clozapine in particular; previous studies have not reported this effect in people taking atypical antipsychotics apart from clozapine。(Lieberman et al., 2009) Other cognitive enhancing agents such as CX516 (an ampakine) and modafinil (a wakefulness promoting agent) have shown less promising results in recent randomized controlled trials。(Freudenreich et al., 2009; Goff et al., 2008) CX516 did not improve PANSS scores after 4 weeks of co-administration with clozapine (n=24), olanzapine (n=18) or risperidone (n=9) and was associated with fatigue, insomnia and epigastric upset compared to placebo。(Goff et al., 2008) In an eight week trial, modafinil did not worsen psychosis in 35 people taking clozapine concurrently but also failed to reduce fatigue, negative symptoms or cognitive deficits。(Freudenreich et al., 2009)

1.4.2.3 Augmentation with aripiprazole

As a partial D<sub>2</sub> agonist, aripiprazole’s mechanism of action is distinct from other antipsychotics. It is a partial agonist at 5-HT<sub>1A</sub> receptors, an agonist at 5-HT<sub>2</sub> receptors and has been described as the prototype of a new generation of antipsychotic agents; the dopamine-serotonin system stabilisers。(Tamminga & Carlsson, 2002) Partial agonism may be a beneficial property by allowing optimal neurotransmission, for instance, by acting as an antagonist in areas where there is an abundance of dopamine causing psychosis while acting as an agonist at receptor sites where low dopaminergic tone would produce adverse effects such as EPSE or hyperprolactinaemia。(Rivas-Vasquez, 2003) Adverse effects associated with this drug such as somnolence, headache, light-headedness and GI upset may be explained by its affinity for several other receptors including D<sub>4</sub>, D<sub>4</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>7</sub>, α<sub>1</sub> and H<sub>1</sub>.

Millar et al studied aripiprazole or placebo in combination with clozapine in suboptimally controlled outpatients over a period of 16 weeks。(Millar, Felter, & Landsberg, 2008) Participants in
this double-blind randomized study were on a stable dose of clozapine for at least three months and had gained at least 2.5 kg since starting clozapine. At week 16, co-treatment with aripiprazole was associated with a significant decrease in mean weight compared to placebo (aripiprazole 2.53 kg, placebo 0.018 kg; p<0.001) and waist circumference (aripiprazole -2.00 cm, placebo 0 cm; p<0.001). Both treatment groups showed similar improvement in the Global Assessment of Functioning (GAF). Improvements on the Epworth Sleepiness Scale (ESS) and Fatigue Syndrome Inventory (FSI) were observed in both groups; a significant difference in favour of aripiprazole was only seen in week 1.

In an open-label extension of a 16 week double-blind placebo controlled trial (reviewed in the meta-analysis by Taylor 2009), Fleischhacker et al administered aripiprazole (5 to 15 mg/day) in combination with clozapine to all participants. (Fleischhacker et al., 2010) For participants previously randomized to adjunctive placebo then treated with adjunctive aripiprazole for 12 weeks, the weight loss from the end of the double-blind phase was greater (1.74 kg versus adjunctive aripiprazole: 0.47 kg). This finding suggests that while the weight loss was maintained in the initial aripiprazole group, this effect may plateau after a period of time. Clinically relevant weight loss from baseline was seen in 13% of those previously in the placebo group and in 21% of those taking aripiprazole for 28 weeks. Differences in PANSS scores were not significant between treatment groups in either phase of the study. The authors reported that symptom improvements were maintained, however only the week 16 PANSS results were reported. Similarly, it was reported that participants who switched from placebo to aripiprazole at week 12 had reduced total cholesterol (TC), LDL cholesterol and triglycerides but data illustrating this were not supplied.

Phase 3 of the CATIE study included only 2 participants receiving this combination of antipsychotics and therefore it was not meaningful to report these separately. However, the positive outcomes regarding weight loss in these randomized controlled trials correspond with findings in CATIE III; treatment with aripiprazole was associated with the most monthly weight loss (0.64 kg) (Stroup et al., 2009). It appears from these trials that the addition of aripiprazole counteracts or at least
decelerates the weight gain as a result of clozapine treatment without causing clinical deterioration or improvement.

1.5 Options for clozapine-intolerant or clozapine-resistant people

1.5.1 Alternative antipsychotics

Two RCTs focused specifically on clozapine versus high dose olanzapine in TRS. (Kumra, Kranzler, Gerbino-Rosen, Kester, DeThomas, et al., 2008; Meltzer et al., 2008) Olanzapine is structurally similar to clozapine but has a different receptor affinity profile being a weaker agonist for α₁ and α₂ receptors relative to its D₂, D₄ and 5HT₂A antagonism. In a 6-month double-blind RCT Meltzer et al. examined the efficacy and tolerability of high-dose olanzapine (target dose 25 mg to 45mg/day; mean dose = 34 mg/day; n= 19) versus clozapine (target dose 300 mg to 900 mg/day; mean dose = 564 mg/day; n = 21) in treatment-resistant participants with schizophrenia or schizoaffective disorder. (Meltzer et al., 2008) Between six weeks and six months of treatment, significant and robust improvements were observed in both groups using multiple measures of psychopathology. The GAF significantly favored clozapine (p<0.01) however, there were no other significant differences between each group. While it appears in this small trial that high-dose olanzapine was as effective as clozapine, significantly more weight gain in the olanzapine group may limit its use. At six months, the mean increase in body mass index (BMI) for those taking olanzapine was 2.2 versus 0.3 for those taking clozapine (p=0.006).

Kumra et al. (Kumra, Kranzler, Gerbino-Rosen, Kester, De Thomas, et al., 2008) concluded in a 12-week controlled comparison of 39 adolescents with TRS that clozapine was superior to high-dose olanzapine (included in meta-analysis by Rummel-Kluge et al.). (Rummel-Kluge et al., 2010) In an open-label extension of this study, the authors investigated the metabolic side effects of these treatments at 24 weeks and the clinical response at 12 weeks of 10 of the 19 olanzapine-treated participants who were switched to clozapine due to non-response. (Kumra, Kranzler, Gerbino-Rosen, Kester, DeThomas, et al., 2008) Clinical response was defined as a decrease of at least 30% on the BPRS and a CGI-Improvement rating of 1 (very much improved) or 2 (much improved).
this basis, seven of the 10 participants switched to clozapine were found to respond to clozapine. Metabolic side effects were similarly problematic in both treatment groups but direct comparisons between the groups were difficult to make due to the large proportion of participants switched to clozapine. It should also be noted that the mean weight of the participants at the beginning of this trial corresponded to an age-adjusted, population-based mean BMI percentile of 91.3 (SD = 10.0), which may be accounted for by exposure to SGA agents prior to study entry.

With a much higher affinity for 5-HT₂ receptors than D₂ receptors, ziprasidone has one of the highest serotonin/dopamine binding ratios of the SGA group and a low affinity for H₁ and α₁ receptors. Sacchetti et al investigated the use of ziprasidone compared to clozapine over an 18 week period in acutely unwell people (mean PANSS total score ~107) with a history of multiple refractoriness to antipsychotics using a double-blind design. (Sacchetti et al., 2009) Decreases in the PANSS score were similar in each group; clozapine -24.5 (95% CI -29.7 to -19.2) and ziprasidone -25.0 (95% CI -30.2 to -19.8). Discontinuation rates due to adverse effects were similar however, ziprasidone offered the advantage of a more favourable metabolic profile (in terms of weight, fasting glucose, total cholesterol, LDL cholesterol and triglycerides). Reductions in movement disorders assessed by the Simpson-Angus Scale (SAS) and Abnormal Involuntary Movement Scale (AIMS) scores were also observed with ziprasidone but not clozapine. Clozapine-intolerant and clozapine-resistant participants were not distinguished from one another in this study which may have affected the results. The investigators also acknowledge that the mean dosage of clozapine (346 mg/day) was within the therapeutic range but lower than may be used in clinical practice.

1.5.2 Non-pharmacological treatment
A thorough appraisal of the value of non-pharmacological treatment options is beyond the scope of this review, however in the context of treatment resistance it is important to acknowledge the potential role of cognitive behavioural therapy (CBT) and electroconvulsive therapy (ECT).
1.5.2.1  Cognitive Behavioural Therapy

Recent studies have shown that CBT may be beneficial for those resistant to clozapine. Barretto et al compared CBT for psychosis (n=12) to non-specific social support also termed “befriending” (BF, n=9) over 20 individualized therapy sessions over 3 weeks and at 6 months. (Barretto et al., 2009) The clozapine dose remained the same for all participants throughout the trial and the rater was blinded for the participants’ intervention. At six months modest improvements were observed in the BPRS total score (CBT mean=25.00, SD 6.85 versus BF mean=19.00, SD 8.38, p=0.0092), PANSS total score (CBT mean=74.11, SD 8.76 versus BF mean=66.54 SD 13.95, p=0.0447) and PANSS general symptom subscale (CBT mean=38.44, SD 6.63 versus BF mean=33.45 SD 7.27, p=0.0147). Participants with residual negative symptoms such as conceptual disorganisation, emotional withdrawal and blunted affect were excluded from the study. Although this approach is rational, since such people may not be able to engage in and benefit from CBT, this limits the generalizability of the findings; many people with TRS have residual negative symptoms.

Turkington et al compared CBT (n=31) and BF (n=28) over a five year period in individuals with schizophrenia and persistent positive symptoms despite adequate trials of antipsychotic medication. (Turkington et al., 2008) Improvements were observed with CBT in overall symptoms severity (NNT=10.36, 95% CI -10.21 to -10.51) and level of negative symptoms (NNT=5.22, 95% CI 5.06 to 5.37). While these results suggest that CBT may improve outcomes for participants, there was a significant break between the intervention which was completed at nine months and follow-up at 18 months and 5 years. Intermediate follow-up assessments and booster sessions may have revealed greater benefits for CBT.

1.5.2.2  Electroconvulsive Therapy

Matheson et al performed a systematic meta-review to determine the benefits and adverse outcomes associated with ECT and repetitive transcranial magnetic stimulation (rTMS) for people with schizophrenia. (Matheson, Green, Loo, & Carr, 2010) In contrast to ECT which produces global
central nervous system excitation and generalized seizures, rTMS allows for targeted stimulation of superficial layers of the brain which may be effective for specific symptoms of schizophrenia. (Burt, Lisanby, & Sackeim, 2002) Furthermore, rTMS is subconvulsive and does not require an anaesthetic or muscle relaxant. Five systematic reviews with meta-analysis were included in this meta-review (2 ECT, 3 rTMS) and graded in terms of the quality of evidence. High quality evidence suggested a short-term, small effect with ECT for the improvement of global symptoms in participants with or without concurrent antipsychotics (RR=0.76, 95% CI 0.63 to 0.92). (Matheson et al., 2010; Tharyan & Adams, 2005) For rTMS, high quality evidence suggests a moderate to large decrease in auditory hallucinations (d=0.88, 95% CI 0.52 to 1.23). (Aleman, Sommer, & Kahn, 2007; Matheson et al., 2010) No evidence was found for long-term therapeutic or adverse effects of either treatment.

Lévy-Rueff et al conducted a retrospective chart review of 19 participants with schizophrenia or schizoaffective disorder non-responsive or only partially responsive to pharmacological agents. (Lévy-Rueff, Gourevitch, Lôo, Olié, & Amado, 2010) In addition to antipsychotic medication, participants received maintenance ECT (M-ECT) beyond acute episodes of psychosis. Participants received an average of 47 sessions of bilateral M-ECT at one to eight week intervals for a mean period of 43 months. Improvements in mood, delusions, anorexia, suicidal ideation and anxiety were observed but symptom scores were not reported. With M-ECT the mean duration of yearly hospitalizations decreased by 80% within this cohort from 10.5 months (SD 17 months) in the year preceding M-ECT to 2.1 months (SD 2.04 months). The mean duration of each hospitalization decreased by 40%, from 4.13 months (SD 4.0 months) prior to M-ECT to 2.53 months (SD 3.47). An improvement in daily functioning was also reported for most participants; two participants were discharged from full-time hospitalization and one returned to employment.

1.6 Conclusion

The results of the large trials CATIE and CUTLASS challenged the widely held belief that SGAs are superior to FGAs in treatment responsive schizophrenia. One concept that remains unchanged is
Clozapine’s superiority over both SGAs and FGAs in treatment-resistant schizophrenia; a finding reinforced by the second phase of each of these studies (and in the case of CATIE the third phase also) and the recent meta-analyses and RCTs presented in this review. In addition to people with treatment-resistant schizophrenia, studies suggest that clozapine may be useful for those at high risk of suicide or aggression. The adverse effects of clozapine are significant ranging from acute events such as agranulocytosis to insidious weight gain and the onset of the metabolic syndrome. Many studies reported that clozapine treatment produced the greatest increase in BMI and/or body weight, closely followed by olanzapine.

The evidence supporting clozapine augmentation is weak and the benefits observed were moderate at best with the exception of lamotrigine. In the meta-analysis by Tiihonen et al lamotrigine produced significant improvements in the total PANSS or BPRS score and positive and negative symptom subscales. The use of the NMDA receptor antagonist memantine was supported by one recent RCT which reported improvements in the MMSE, total BPRS and positive and negative symptom subscales. Limited evidence suggests that NMDA agonists may produce clinical improvements in participants taking olanzapine or risperidone, but not clozapine, while the addition of topiramate to clozapine was of little benefit at doses low enough to preserve cognitive function. Clozapine augmentation with aripiprazole resulted in weight loss or at least halted further weight gain without causing clinical deterioration or improvement.

Recent RCTs suggest that high dose olanzapine may be an important alternative for people intolerant or resistant to clozapine; evidence for the use of ziprasidone in these conditions is limited. CBT in addition to a non-clozapine antipsychotic for people not responding or intolerant to clozapine is supported by small trials. ECT (with or without concurrent antipsychotic medication) was found to produce small, short-term improvements in global functioning while significant improvements specifically in auditory hallucinations were observed with rTMS. However, more studies are required to determine the long-term and adverse effects of these treatments.
In terms of clinical practice recommendations where there is a lack of evidence from RCTs to guide treatment, clinicians should review single case reports or case series which are beyond the scope of this review. When implementing a treatment strategy for which there is limited evidence ensure that the treatment trial is adequate with objective outcome measures, for example the PANSS. Larger trials with prospective data using clinically important outcomes measured by well-validated, approved instruments are needed to accurately compare the agents available for the treatment of schizophrenia. Future trials concerning clozapine augmentation strategies should aim to make a distinction between augmenting agents rather than comparing the results of all antipsychotic augmentation irrespective of mechanism of action.

1.7 Advances since publication
Since the publication of this review in March 2011 a number of reviews have been published on the pharmacotherapy of treatment-resistant schizophrenia arriving at the same conclusions as above. Clozapine remains the most effective antipsychotic for TRS. (Miyamoto, Miyake, Jarskog, Fleischhacker, & Lieberman, 2012). The present review did not find sufficient data to suggest that high dose olanzapine was as effective as clozapine treatment and a recent meta-analysis confirmed this finding. (Souza, Kayo, Tassell, Martins, & Elks, 2013) The evidence for antipsychotic polypharmacy is weak and the evidence for augmentation of clozapine treatment in particular remains marginal at best. (Maiocchi & Bernardi, 2013; Taylor, Smith, Gee, & Nielsen, 2012) The addition of aripiprazole to clozapine treatment provided no benefit in terms of psychopathology. (Cipriani et al., 2013) A meta-analysis of rTMS studies confirmed that this treatment strategy may be particularly helpful for patients experiencing treatment-resistant auditory verbal hallucinations. (Slotema, Blom, van Lutterveld, Hoek, & Sommer) ECT was also identified as a potentially beneficial adjunct to pharmacotherapy in patients with TRS; however, no effect size was reported and the extent to which this option is beneficial remains unknown. (Pompili et al., 2013) The review by Miyamoto provides a comprehensive summary of future drug development strategies and studies investigating agents other that antipsychotics as adjuncts to
treatment including celecoxib, minocycline, omega-3 fatty acid, estrogen and erythropoietin; however, the efficacy of novel agents and the novel strategies using alternatives to antipsychotics have not been proven and require further exploration. (Miyamoto et al., 2012)
Chapter Two: Study sample

The following chapter is adapted from the following article submitted for publication in late 2013 and describes the demographics of the patients who took part in the research presented in the following chapters. In order to investigate treatment response using proton magnetic resonance spectroscopy (1H-MRS) and diffusion tensor imaging (DTI) it was necessary to first establish the participants’ symptom severity at the time of the study. Positive and Negative Syndrome Scale (PANSS) interviews were performed and the scores are presented in this chapter in addition to other validated symptom scores such as the Clinical Global Impression (CGI) scale and the Remission in Schizophrenia Working Groups’ (RSWG) criteria for symptomatic remission.


2.1 Abstract

Objective: Clinical outcomes for people with schizophrenia such as the ability to function socially, live independently, maintain employment and be relatively symptom-free are variable. With the increasing availability of effective pharmacotherapy and psychotherapeutic options, a way to facilitate standardised comparisons of outcomes was required. The Remission in Schizophrenia Working Group developed a consensus definition and operational criteria of remission as applied to schizophrenia. To the best of our knowledge, this remission for schizophrenia criteria has been not applied to a New Zealand sample and the aim of this study was to compare remission rates to those reported internationally.

Method: The present study recruited people with schizophrenia from the wider Auckland region ranging in their response to antipsychotic treatment in order to assess rates of remission and demographic variables associated with fulfilling the criteria.
Results: Remission rates were low; in this sample only 9 out of 62 (14.5%) met the remission criteria. The PANSS items that most frequently prevented participants from achieving remission were lack of spontaneity and flow of conversation, followed by blunted affect (32.3%) and conceptual disorganisation (30.6%). Factors associated with achieving remission criteria were female gender (p=0.015) and a short duration of untreated psychosis (p=0.037). No significant associations were detected for duration of illness, age at onset of schizophrenia or ethnicity.

Conclusions: Despite low rates of remission, patients in this sample were classed as minimally to mildly ill using other validated symptom scales, suggesting that failure to meet the remission criteria does not equate to severe illness. Future research should focus on functional recovery in addition to appropriate assessment of symptom severity.

2.2 Introduction

Clinical outcomes for people with schizophrenia such as the ability to function socially, live independently, maintain employment and be relatively symptom-free are variable (Bellack, 2006; Schennach et al., 2012). In order to achieve these outcomes, patients would first need to experience an improvement in core signs and symptoms to the extent that any residual psychopathology was below the diagnostic threshold and behaviour was largely unaffected. Remission of the core symptoms of schizophrenia can therefore be considered a prerequisite for positive clinical outcomes or a return to optimal social functioning. Traditional prognostic predictions of poor outcome meant that schizophrenia was viewed as a lifelong, chronic illness with little hope of recovery (Rund, 1990). However, with better knowledge of the course of illness and improved therapeutic options this viewpoint began to shift (Carr, 1983). With the increasing availability of effective pharmacotherapy and psychotherapeutic options, a way to facilitate standardised comparisons of outcomes was required.

The Remission in Schizophrenia Working Group (RSWG) developed a consensus definition and operational criteria of remission as applied to schizophrenia (Andreasen et al., 2005). Remission
was defined as a low-mild symptom intensity level, where such absent, borderline, or mild symptoms do not influence an individual’s behaviour (Andreasen et al., 2005). Core items from various symptom rating scales were selected to assess the three dimensions of psychopathology. The relevant criteria from the Positive and Negative Symptom Scale (PANSS) (Kay, Fiszbein, & Opler, 1987) were delusions (item P1), unusual thought content (G9), hallucinatory behaviour (P3), conceptual disorganisation (P2), mannerisms/posturing (G5), blunted affect (N1), social withdrawal (N4) and lack of spontaneity (N6). Simultaneous ratings of mild or less (scores ≤ 3) on all items is required for a period of at least six months for symptomatic remission (Andreasen et al., 2005).

Although cognitive function has been found to be the best predictor of social and functional outcome for people with schizophrenia, it is intentionally not incorporated into the RSWG remission criteria due to the continuous nature of impairments (Green, 1996). The time course of cognitive impairment in schizophrenia is a rapid decline at the beginning of the illness and then an apparent plateau where deficits do not correlate with chronicity measures (Bilder et al., 2000; Censits, Ragland, Gur, & Gur, 1997; Goldberg, Hyde, Kleinman, & Weinberger, 1993). However, the purpose of the RSWG remission criteria is to capture the episodic intensity of psychotic symptoms. Since the relationship between specific symptoms and specific cognitive deficits is not well understood it was not possible to include psychosocial or cognitive dysfunction into definitions of remission in schizophrenia (Andreasen et al., 2005). The applicability of the RSWG severity criteria has been confirmed in clinical trials of people with schizophrenia (Beitinger, Lin, Kissling, & Leucht, 2008). The ability to predict functional outcomes for people with schizophrenia is particularly relevant in New Zealand following a shift to community-based care for the majority of patients (Joseph & Kearns, 1996).

The aim of the current study was to apply the remission for schizophrenia criteria to a cross-sectional New Zealand sample to examine the proportion of people who achieved remission, in comparison with international figures. To the best of our knowledge, this is the first time that the remission for schizophrenia criteria has been applied to a New Zealand sample. People with
schizophrenia were recruited from the wider Auckland region belonging to three treatment groups; those taking a second generation non-clozapine antipsychotic, clozapine monotherapy (clozapine-responsive, treatment-resistant schizophrenia, TRS) and a group who were taking a combination of two antipsychotics having failed to respond to antipsychotic monotherapy (antipsychotic augmentation). It was anticipated that the rate of symptomatic remission in a New Zealand sample of patients that could provide informed consent would be similar to international reports which range from 25% (Austin et al., 2013; Beitinger et al., 2008; Mosolov, Potapov, & Ushakov, 2012) to 55% (Lambert, Karow, Leucht, Schimmelmann, & Naber, 2010; Schennach et al., 2012). Furthermore, it was expected that those taking either a second generation antipsychotic or clozapine monotherapy would be more likely to experience symptomatic remission than those taking antipsychotic augmentation.

2.3 Method

The recruitment process was conducted as part of a larger cross-sectional study seeking biomarkers of treatment resistant schizophrenia by using magnetic resonance imaging (MRI), electroencephalography (EEG) combined with event-related potentials (ERPs), cognitive testing and genetic screening. The study was conducted in the School of Pharmacy, University of Auckland between March 2011 and July 2013 with approval from the Northern X Regional Ethics Committee. Patients who met the DSM-IV criteria for schizophrenia were identified by their treating clinician either from a community mental health centre or a forensic psychiatric inpatient unit; the diagnosis was then checked against patient notes. Subsequent to obtaining informed consent, data regarding duration of illness and a medication history was collected from clinical notes and patient interviews. Medication histories were initially recorded by a registered pharmacist (M. E. M.) and then verified by chart review or community pharmacy records. All participants were stabilised on antipsychotic medication for at least six weeks and categorised according to their treatment history and response to antipsychotic medication. Diagnostic criteria for treatment resistant schizophrenia were taken from published treatment algorithms such as those implemented by the Royal
The Australian New Zealand College of Psychiatrists (RANZCP), the UK's National Institute for Clinical Excellence (NICE) and the American Psychiatric Association (APA) (Association, 2004; NICE, 2002; Royal Australian New Zealand College of Psychiatrists, 2005). These algorithms define treatment-resistant schizophrenia in patients who do not experience significant symptom improvement after trials with at least two antipsychotic agents at therapeutic doses for a minimum of six weeks each.

The three treatment groups in this study comprised of those taking i) a second generation (atypical) non-clozapine antipsychotic, ii) clozapine monotherapy (treatment resistant; TRS), or iii) antipsychotic augmentation. All participants were between the ages of 18 and 45. The exclusion criteria were a history of traumatic brain injury, other neurologic illness, significant physiological comorbidity, non-compliance with antipsychotic medication and contraindications to MRI scanning (for the subsequent MRI study).

Symptom severity was assessed using the PANSS and scores were also converted to Clinical Global Impression for Schizophrenia scores based on severity but not improvement (Haro et al., 2003; Kay et al., 1987). The investigator was trained and assessed by an expert psychiatrist; ratings in the test environment were acceptable if 80% of the PANSS items were within a range of the expert standard ±1. Each PANSS interview (one interview per participant, approximately one hour in duration) was conducted by the same validated investigator (M. E. M.) to avoid introducing interviewing bias. The findings of the clinical interviews were corroborated with the patients’ family, caregiver or mental health professional (treating psychiatrist or key worker). Attitude towards antipsychotic treatment was assessed using the Drug Attitude Inventory 30 (DAI-30) (Hogan, Awad, & Eastwood, 1983). In order to address potential bias resulting from differences in the duration of illness, participants were matched for age on a group basis. Similar numbers of female participants were included in each treatment group. The number of participants was determined by the requirements of the larger study to identify genetic, EEG/ERP and MRI biomarkers of treatment resistant schizophrenia.
Patients with a score of 3 or less simultaneously on the following PANSS items are considered to meet the symptomatic severity remission criteria: delusions (item P1), unusual thought content (G9), hallucinatory behaviour (P3), conceptual disorganisation (P2), mannerisms/posturing (G5), blunted affect (N1), social withdrawal (N4) and lack of spontaneity (N6) (Andreasen et al., 2005). Chlorpromazine equivalents (CPZEs) were calculated in order to compare antipsychotic daily doses using formulae with power transformation (Andreasen, Pressler, Nopoulos, Miller, & Ho, 2010) except for amisulpride, which in the absence of a power formula was calculated using expert consensus regarding antipsychotic dosing (Gardner, Murphy, O’Donnell, Centorrino, & Baldessarini, 2010). Associations between remission status and demographic variables of the sample were examined, including sex, age, ethnicity, age of onset of schizophrenia, duration of illness, duration of untreated psychosis, forensic history, PANSS scores, accommodation status, medication prescribed and chlorpromazine equivalents.

Statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) version 19.0 software. The treatment group mean and standard deviation was calculated for scalar data such as age and duration of illness. Analyses of Variance (ANOVAs) were conducted in order to compare the means of the three treatment groups with Bonferroni correction for multiple comparisons. After the participants were classified according to the remission criteria, independent t-tests and chi squared (exact) tests were performed to explore the relationship between remission status and demographic variables.

2.4 Results
2.4.1 Participants
A total of 156 people with schizophrenia were approached to participate in this study. Twenty-eight people declined to participate and 64 did not meet the inclusion criteria (Figure 1). A total of 64 people with schizophrenia were included. One participant dropped out and one was excluded from analysis due to the detection of a brain abnormality at the time of the MRI scan, leaving a total of 62 for the final analysis.
Figure 1 Recruitment summary

The demographic and clinical data of the study participants (n=62) are summarized below (Table 1). Antipsychotic medication at the time of the study is shown in Table 2. There were no significant differences in sex, age, duration of illness, duration of untreated psychosis, DAI-30 scores, PANSS total scores or any of the PANSS or CGI subscales. There was a significant difference in terms of ethnicity between the groups; Maori were over-represented in the clozapine monotherapy group (n=14 versus n=4 and n=7 in the first-line and augmentation groups, respectively, p=0.007). There were significantly more people with a forensic history in the clozapine monotherapy and antipsychotic augmentation groups than the first-line responder group (p=0.008). The augmented
group had significantly higher antipsychotic dosing equivalents than the other treatment groups (806 mg ±365 mg versus 436 mg ±190 mg and 452 mg ±215 mg respectively).

Table 1: Demographic data of study participants by treatment group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>First-line responders (n=20)</th>
<th>Clozapine monotherapy (n=21)</th>
<th>Antipsychotic augmentation (n=21)</th>
<th>Overall sample (%) (n=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>16</td>
<td>17</td>
<td>49 (79.0%)</td>
</tr>
<tr>
<td>Female</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>13 (21.0%)</td>
</tr>
<tr>
<td><strong>Age (mean years, SD)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>First-line responders (n=20)</td>
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</tr>
<tr>
<td>Male</td>
<td>31.2 (8.2)</td>
<td>33.6 (7.9)</td>
<td>35.3 (6.4)</td>
<td>33.4 (7.6)</td>
</tr>
<tr>
<td>Female</td>
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<td></td>
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</tr>
<tr>
<td>Ethnicity</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Maori</td>
<td>4</td>
<td>14</td>
<td>7</td>
<td>25 (40.3%)a</td>
</tr>
<tr>
<td>Pacific Island</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>13 (21.0%)</td>
</tr>
<tr>
<td>NZ European</td>
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<td>7</td>
<td>14 (22.6%)</td>
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<tr>
<td>Other European</td>
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<td>Asian</td>
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<tr>
<td>African</td>
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<td>3 (4.8%)</td>
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<tr>
<td>Middle Eastern</td>
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<td>0</td>
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<td>1 (1.6%)</td>
</tr>
<tr>
<td><strong>Clinical features</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at onset of schizophrenia (mean years, SD)</td>
<td>21.8 (5.4)</td>
<td>20.8 (4.1)</td>
<td>24.4 (6.9)</td>
<td>22.3 (5.7)</td>
</tr>
<tr>
<td>Duration of illness (mean years, SD)</td>
<td>9.4 (7.7)</td>
<td>12.8 (6.6)</td>
<td>10.9 (5.2)</td>
<td>11.0 (6.6)</td>
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<tr>
<td>Duration of untreated psychosis (mean months, SD)</td>
<td>13.0 (15.2)</td>
<td>9.5 (17.1)</td>
<td>21.0 (23.0)</td>
<td>14.5 (19.1)</td>
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<td>Forensic history</td>
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<td>15</td>
<td>36b</td>
</tr>
<tr>
<td><strong>Psychiatric ratings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANSS total score (mean, SD)</td>
<td>61.5 (13.2)</td>
<td>59.6 (14.5)</td>
<td>64.0 (12.1)</td>
<td>61.7 (13.2)</td>
</tr>
<tr>
<td>PANSS positive score (mean, SD)</td>
<td>14.4 (5.8)</td>
<td>12.0 (5.3)</td>
<td>13.5 (5.6)</td>
<td>13.3 (5.6)</td>
</tr>
<tr>
<td>PANSS negative score (mean, SD)</td>
<td>17.4 (6.2)</td>
<td>19.0 (7.2)</td>
<td>20.4 (6.9)</td>
<td>19.0 (6.8)</td>
</tr>
<tr>
<td>PANSS general score (mean, SD)</td>
<td>29.7 (6.5)</td>
<td>28.7 (6.1)</td>
<td>30.1 (4.9)</td>
<td>29.5 (5.8)</td>
</tr>
<tr>
<td>CGI severity positive score (mean, SD)</td>
<td>2.40 (1.10)</td>
<td>2.02 (1.09)</td>
<td>2.36 (1.02)</td>
<td>2.26 (1.07)</td>
</tr>
<tr>
<td>CGI severity negative score (mean, SD)</td>
<td>2.15 (1.00)</td>
<td>2.24 (1.06)</td>
<td>2.41 (0.94)</td>
<td>2.27 (0.99)</td>
</tr>
<tr>
<td>CGI severity depressive score (mean, SD)</td>
<td>2.05 (0.71)</td>
<td>2.03 (0.93)</td>
<td>1.78 (0.92)</td>
<td>1.95 (0.85)</td>
</tr>
<tr>
<td>CGI severity cognitive score (mean, SD)</td>
<td>2.46 (0.90)</td>
<td>2.43 (1.00)</td>
<td>2.76 (0.98)</td>
<td>2.55 (0.96)</td>
</tr>
<tr>
<td>DAI-30 score</td>
<td>13.1 (7.8)</td>
<td>12.6 (12.7)</td>
<td>11.8 (14.0)</td>
<td>12.5 (11.7)</td>
</tr>
<tr>
<td><strong>Accommodation Status</strong></td>
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<td>Inpatient</td>
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<tr>
<td>Assisted living</td>
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<td>13</td>
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<tr>
<td>Community (independently)</td>
<td>5</td>
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<td>Community (with everyday support from family or caregiver)</td>
<td>8</td>
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</table>

Note: a p =0.007, b p=0.008

Table 2: Antipsychotic medication by treatment group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>First-line responders (n=20)</th>
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<tr>
<td>Community (with everyday support from family or caregiver)</td>
<td>8</td>
<td>2</td>
<td>3</td>
<td>13</td>
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</tbody>
</table>
Antipsychotic medication | First-line responders (n=20) | Clozapine monotherapy (n=21) | Antipsychotic augmentation (n=21) |
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<td>Olanzapine</td>
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</tr>
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<td>Risperidone</td>
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<td>Clozapine Monotherapy</td>
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<td>Clozapine + amisulpride</td>
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<td>Clozapine + aripiprazole</td>
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<td>Clozapine + risperidone</td>
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<td>Aripiprazole + olanzapine</td>
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<td>Olanzapine + quetiapine</td>
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<tr>
<td>Risperidone + quetiapine</td>
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</tr>
<tr>
<td>Risperidone + ziprasidone</td>
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<tr>
<td>Daily dose of antipsychotic medication (CPZE, mg)</td>
<td>436* (±190)</td>
<td>452* (±215)</td>
<td>806* (±365)</td>
</tr>
</tbody>
</table>

Note: *p<0.001

2.4.2 Rates of remission

Nine patients (14.5% of the sample) met the remission criteria at the time of the study Table 3.

The clozapine monotherapy group had the highest proportion of people in remission (n = 5/21; 23.8%), followed by the first-line responder group (15%) and antipsychotic augmentation group (4.8%). However, a chi square test did not reveal a significant association between clozapine treatment and remission (p=0.135).

**Table 3 Number of participants that met the remission criteria by treatment group**

<table>
<thead>
<tr>
<th>Number of participants in remission (% of treatment group)</th>
<th>First-line responders (n=20)</th>
<th>Clozapine monotherapy (n=21)</th>
<th>Antipsychotic augmentation (n=21)</th>
<th>Overall sample (n=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/20 (15%)</td>
<td>5/21 (23.8%)</td>
<td>1/21 (4.8%)</td>
<td>9/62 (14.5%)</td>
<td></td>
</tr>
<tr>
<td>8/41 (20%)</td>
<td></td>
<td>1/21 (4.8%)</td>
<td>9/62 (14.5%)</td>
<td></td>
</tr>
</tbody>
</table>

2.4.3 Demographics of remitters versus non-remitters

A higher proportion of female participants met the remission criteria than male participants; 38.5% versus 8.1%, respectively. Chi squared (exact) tests revealed that female gender was significantly associated with achieving remission status ($\chi^2 = 7.601, p=0.015, \text{Cohen’s } d = 0.748$). There was no
significant difference between the two groups in terms of age at the time of the study and no significant associations were detected for Maori (p=0.47), Pacific Island (p=0.082) or New Zealand European ethnicity (p=0.673). Age at onset of schizophrenia and duration of illness did not differ between the groups; however, duration of untreated psychosis was significantly shorter in the remission group (6.9 months versus 15.8 months, p=0.037, Cohen’s d = 0.575). PANSS total scores (p<0.001) and positive (p<0.001) and negative (p<0.001) and general (p=0.007) subscale scores were higher in those that did not meet the remission criteria. CGI positive, negative and cognitive scores but not depressive scores were higher in the non-remitter group (all p<0.001). People in the remitter group were taking significantly lower antipsychotic daily doses (378 ±170 CPZEs versus 598 ±325 CPZEs; p=0.007). There were no significant associations between remission and DAI-30 scores, accommodation status or forensic history.

Table 4 Comparison of participants that did and did not meet the remission criteria

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>In remission</th>
<th>Not in remission</th>
<th>Significant p value (&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4 (8.1%)</td>
<td>45 (91.9%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>5 (38.5%)</td>
<td>8 (61.5%)</td>
<td>p=0.015</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>35.1 (6.6)</td>
<td>33.1 (7.8)</td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maori</td>
<td>3</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Pacific Island</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Other European</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>African</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Middle Eastern</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical features (mean, SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at onset of schizophrenia (years)</td>
<td>20.6 (3.3)</td>
<td>22.6 (6.0)</td>
<td></td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td>14.5 (7.3)</td>
<td>10.4 (6.4)</td>
<td></td>
</tr>
<tr>
<td>Duration of untreated psychosis (months)</td>
<td>6.9 (8.9)</td>
<td>15.8 (20.1)</td>
<td>p=0.037</td>
</tr>
<tr>
<td>Positive for forensic history</td>
<td>5 (13.9%)</td>
<td>31 (86.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Psychiatric ratings (mean, SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANSS total score (mean, SD)</td>
<td>42.9 (7.1)</td>
<td>64.9 (11.2)(^\dagger)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>PANSS positive score (mean, SD)</td>
<td>7.9 (1.5)</td>
<td>14.2 (5.9)(^\dagger)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>PANSS negative score (mean, SD)</td>
<td>11.0 (3.1)</td>
<td>20.3 (6.3)(^\dagger)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>PANSS general score (mean, SD)</td>
<td>24.0 (5.3)</td>
<td>30.4 (5.4)(^\dagger)</td>
<td>p=0.007</td>
</tr>
<tr>
<td>CGI severity positive score (mean, SD)</td>
<td>1.17 (0.19)</td>
<td>2.44 (1.04)(^\dagger)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Characteristic</td>
<td>In remission n=9</td>
<td>Not in remission n=53</td>
<td>Significant p value (&lt;0.05)</td>
</tr>
<tr>
<td>---------------</td>
<td>-----------------</td>
<td>-----------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>CGI severity negative score (mean, SD)</td>
<td>1.21 (0.21)</td>
<td>2.45 (0.96)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>CGI severity depressive score (mean, SD)</td>
<td>2.41 (1.02)</td>
<td>1.87 (0.81)</td>
<td></td>
</tr>
<tr>
<td>CGI severity cognitive score (mean, SD)</td>
<td>1.72 (0.49)</td>
<td>2.69 (0.95)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>DAI-30 score</td>
<td>15.6 (10.0)</td>
<td>12.0 (12.0)</td>
<td></td>
</tr>
<tr>
<td>Antipsychotic medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily dose in CPZE</td>
<td>380 (±170)</td>
<td>598 (±325)</td>
<td></td>
</tr>
<tr>
<td>Accommodation Status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inpatient</td>
<td>3 (16.7%)</td>
<td>15 (83.3%)</td>
<td></td>
</tr>
<tr>
<td>Assisted living</td>
<td>1 (7.7%)</td>
<td>12 (92.3%)</td>
<td></td>
</tr>
<tr>
<td>Community (independently)</td>
<td>5 (27.8%)</td>
<td>13 (72.2%)</td>
<td></td>
</tr>
<tr>
<td>Community (with everyday support from family or caregiver)</td>
<td>0 (0%)</td>
<td>13 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

2.4.4 Remission criteria not fulfilled

In order to assess which PANSS items prevented a participant from meeting the remission criteria, a single PANSS-remission-item analysis was performed (Figure 2). 40.3% of participants scored >3 on lack of spontaneity and flow of conversation, followed by blunted affect (32.3%) and conceptual disorganisation (30.6%).

![Remission criteria not fulfilled](image)

*Figure 2 Percentage of participants not achieving the PANSS remission criteria.*
2.5 Discussion

The rate of remission in the overall sample was 14.5% and excluding the antipsychotic augmentation group the rate was 20%; both of which are lower than anticipated because the majority of participants were outpatients (44/62 or 71%) and the CGI scores corresponded to minimally to mildly ill in all treatment groups. The clozapine treatment group had the highest rate of remission (n=5/21; 23.8%); however, a chi square test did not reveal a significant relationship between clozapine treatment and remission. Factors associated with a medium effect size for achieving remission criteria were female gender (38.5% for female participants, 8.1% for males, $\chi^2 = 7.601, p=0.015$, Cohen’s $d=0.748$) and a short duration of untreated psychosis (6.9 months versus 15.8 months, $p=0.037$, Cohen’s $d=0.575$). People who achieved remission were also taking lower doses of antipsychotic medication (380 mg ±170 mg CPZEs versus 598 mg ±325 mg CPZEs; $p=0.007$). No significant associations were detected for duration of illness, age at onset of schizophrenia or ethnicity. Lack of spontaneity and flow of conversation (40.3%), followed by blunted affect (32.3%) and conceptual disorganisation (30.6%) were the core PANSS items that most frequently prevented patients from fulfilling the remission criteria.

The rate of remission in this New Zealand sample appears to be lower than international studies, which range from around 25% (Austin et al., 2013; Beitinger et al., 2008; Mosolov et al., 2012) to 55% (Lambert et al., 2010; Schennach et al., 2012). At the lower end of the range, a meta-analysis of six antipsychotic drug trials in patients with schizophrenia assessed rates of remission at three time points: 4 weeks (27.2%; symptom severity criteria only), 28 weeks (20.3%; severity and time criteria; three studies) and 52 weeks (32.4%; severity and time criteria; one study) (Beitinger et al., 2008). While a 10 year follow up study of 304 patients in Denmark observed the remission rates to be 22%, 29% and 25% at 2 years, 5 years and 10 years, respectively (Austin et al., 2013). At the higher end of the range, a recent study of 186 patients in Germany reported a remission rate of 54% at one year (Schennach et al., 2012) and a review of thirty studies found that when the time criteria was omitted, 56% of patients met the remission criteria (Lambert et al., 2010).
longitudinal study of 249 people with chronic schizophrenia reported that 50.2% met remission criteria at the seven year follow-up time point (Henry et al., 2010). While the treatment groups were well-matched with respect to demographic and clinical features, the numbers are smaller than international studies. Furthermore, because so few patients met the remission criteria, this makes it difficult to determine factors associated with remission. Since data were collected cross-sectionally, it was possible to analyse only the symptomatic criteria; we cannot comment on the stability of remission amongst this population. However, other groups have found that the time criterion is rarely achieved, which may be due to high drop-out rates and for this reason several studies have chosen to focus on the symptom severity criteria instead (Beitinger et al., 2008; Buckley, Harvey, Bowie, & Loebel, 2007; Helldin, Kane, Karilampi, Norlander, & Archer, 2006; Jager et al., 2009; Kane et al., 2007; McIntyre, Fallu, & Konarski, 2006; Novick et al., 2007; Peuskens, Kaufman, & Van Vleymen, 2007).

One possible explanation for the lower rate of symptomatic remission in our study is the inclusion of inpatients, which was identified as a source of variability in review studies (Beitinger et al., 2008; Lambert et al., 2010) (Table 5). The inpatients included in this study were stabilised on their antipsychotic treatment and despite residual symptoms were not experiencing an acute episode of psychosis as many participants are at baseline in international studies (Austin et al., 2013). Furthermore, because the inpatient unit is a forensic psychiatric unit, there may be legal as opposed to medical reasons for a participant’s inpatient status. However, a cross-sectional study of 288 patients with chronic schizophrenia reported that 55% achieved remission during inpatient treatment (Jager et al., 2009). Schennach et al recruited inpatients at baseline and while remission rates were not reported at this time point, 54% achieved remission at one year in the community (Schennach et al., 2012). Furthermore, outpatient status does not necessarily confer symptomatic remission as indicated by Mosolov et al in a cross-sectional study of outpatients with chronic schizophrenia (n=203), which reported 31.5% of patients met the criteria at baseline and 26.1% maintained remission at 6 months (Mosolov et al., 2012). Therefore, it is unlikely that the inclusion
of inpatients (18/62; 29% of the sample) in the present study can account for the low symptomatic remission. Whether patients with a forensic history differ from the general schizophrenia population in terms of psychopharmacologic needs or response to medication has not been conclusively demonstrated (Stone & Niz, 2004). There is some evidence to suggest that forensic patients have more severe illness with persistent auditory hallucinations that do not respond to first-line antipsychotics (Maier, 1992; Tardiff, 1992). On the other hand, studies have shown that forensic patients respond well to clozapine treatment (Ebrahim, Gibler, Gacono, & Hayes, 1994; Maier, 1992). A significant proportion of participants in our study had a forensic history and although we found this did not preclude fulfilling the remission criteria it may affect the generalizability of the findings.

**Table 5** Summary of recent studies reporting remission rates and the inclusion of inpatients versus outpatients.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Patient sample</th>
<th>Remission rate reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austin <em>et al</em>, 2013</td>
<td>Longitudinal (ten year follow-up)</td>
<td>Patients with a chronic schizophrenia spectrum disorder (n=304). 7% were inpatients or living in supported accommodation.</td>
<td>39% sustained symptom remission at 10-year follow-up.</td>
</tr>
<tr>
<td>Mosolov <em>et al</em>, 2012</td>
<td>Cross-sectional assessment of remission at baseline; follow-up assessment at 6 months.</td>
<td>Chronic schizophrenia (n=203). Outpatients at both time points.</td>
<td>31.5% met remission criteria at baseline and 26.1% maintained remission at follow-up.</td>
</tr>
<tr>
<td>Schennach <em>et al</em>, 2012</td>
<td>Longitudinal (one year follow-up)</td>
<td>Chronic schizophrenia (n=186). Inpatients at baseline and all discharged at follow-up.</td>
<td>54% achieved remission at one year in the community.</td>
</tr>
<tr>
<td>Henry <em>et al</em>, 2010</td>
<td>Longitudinal (seven year follow-up)</td>
<td>Chronic schizophrenia spectrum disorder (n=249). Average time spent living independently in the 2 years preceding assessment was 22.1 months (but number living independently not reported separately).</td>
<td>50.2% met symptomatic remission criteria.</td>
</tr>
</tbody>
</table>

Duration of untreated psychosis has been identified as a significant predictor of achieving the remission criteria (Lambert *et al*., 2010). In our sample, those who met the remission criteria had a shorter duration of untreated psychosis (6.9 months versus 15.8 months, p=0.037), which is in line
with several studies (Chang et al., 2013; Jeppesen et al., 2008; Marshall et al., 2005; Verma, Subramaniam, Abdin, Poon, & Chong, 2012; Wunderink, Sytema, Nienhuis, & Wiersma, 2009).

Medication adherence and a positive attitude towards treatment have also been identified as significant predictors of achieving remission (Lambert et al., 2010; Schennach et al., 2012). While it was not possible to obtain a formal measure of adherence to treatment from the clinical notes, DAI-30 scores were collected to assess attitudes towards treatment with higher scores indicating more positive attitudes towards treatment and therefore an increased likelihood of adherence. DAI-30 scores were higher in the remitter group but this was not significant (15.6±10.0 versus 12.0 ±12.0, p=0.356); however, there was a large range in scores for both groups and a larger sample size may have clarified the importance of this measure. Furthermore, the DAI-30 is a self-reported measure of adherence.

A relationship between duration of illness and achieving remission has been reported such that those with a longer duration of illness are less likely to achieve remission (Brousse et al., 2010; Haro, Novick, Suarez, Ochoa, & Roca, 2008; Jager et al., 2007). While our study did not detect this association, our participants had a similar duration of illness (mean = 11.0 years, SD=6.6) to other studies and the remission rates were still lower. Patients who met the remission criteria were receiving lower doses of antipsychotic medication, which is also in agreement with previous work (Schennach et al., 2012). One explanation is that those who met the criteria are less treatment resistant and therefore require lower doses; however, the inclusion of the antipsychotic augmentation group greatly increases the CPZEs in the non-remitter group. Another issue when calculating CPZEs for the combination antipsychotic treatment group is that CPZEs for different antipsychotics may not be simply additive; however at this time there is no alternative approach to represent this data (Andreasen et al., 2010). We found that a greater proportion of female participants met the remission criteria which is in line with other studies (Haro et al., 2008; Lambert et al., 2010; Schennach et al., 2012), although this association is not consistently replicated (Jaaskelainen et al., 2012). Better outcomes for female patients with schizophrenia are
thought to be due to neurodevelopmental and psychosocial factors and the neuroprotective role of oestrogen (Ochoa, Usall, Cobo, Labad, & Kulkarni, 2012; Salem & Kring, 1998). The putative protective and anti-dopaminergic effects of oestrogen are thought to account for the delayed onset of illness in females and a second peak in onset around the time of menopause with decreasing levels of oestrogen (Hafner et al., 1993; Riecher-Rossler et al., 1994).

The PANSS items that most frequently prevented participants from achieving remission in this sample were lack of spontaneity and flow of conversation (40.3%), followed by blunted affect (32.3%) and conceptual disorganisation (30.6%). Some studies have reported that persistent negative symptoms more frequently prevent patients from meeting the remission criteria (Austin et al., 2013; Schennach et al., 2012); however, in this sample positive and negative symptoms were equally important. This is in agreement with the meta-analysis by Beitinger et al and linear regression analyses, which demonstrated only definitions of remission containing both positive and negative symptoms independently predicted functional outcome (Beitinger et al., 2008; Cassidy, Norman, Manchanda, Schmitz, & Malla, 2010). It should also be noted that the most problematic items, i.e. lack of spontaneity and flow of conversation, blunted affect and to a lesser extent conceptual disorganisation, have been found to be particularly subjective with poor inter-rater reliability (0.55, 0.39 and 0.68 respectively) (Norman, Malla, Cortese, & Diaz, 1996).

While most patients did not fulfil the remission criteria and there was a significant difference in CGI scores between the remitters and non-remitters (with the exception of depressive symptoms), symptom severity using the CGI could still be classified as minimal to mild. This suggests that not achieving the remission criteria does not represent severe illness nor does it preclude a high level of functioning. A follow-up study of the cohort described by Henry et al found that 31% of people who attained full functional recovery (FFR) at 14 months and 7.5 years failed to meet the remission criteria at 14 months (Alvarez-Jimenez et al., 2011). On the other hand, of the people who attained remission at 8 months, only 14% went on to achieve FFR. The authors found that functional and vocational recovery was more important to attaining and maintaining FFR than symptomatic
remission. Lambert et al came to similar conclusions, noting that patients in remission do not “automatically” have an adequate level of functioning or quality of life due to, for instance, enduring cognitive or affective symptoms (Lambert et al., 2010). With this in mind, future work should aim to include functional and cognitive assessments in addition to symptom severity measurements.

2.6 Conclusions
To our knowledge, this study is the first to apply the RSWG symptomatic remission criteria to a New Zealand sample. The rate of remission in the sample was lower than international reports, even when the antipsychotic augmentation group was excluded. Despite low rates of remission, patients in this sample were classed as minimally to mildly ill using other validated symptom scales, suggesting that failure to meet the remission criteria does not equate to severe illness. Future research should include assessments of medication adherence which has been identified as a significant predictor of remission and focus on functional recovery by incorporating functional and cognitive assessments in addition to symptom severity.
Chapter Three: Proton Magnetic Resonance Spectroscopy

3.1 Introduction

This chapter provides an introduction to proton magnetic resonance spectroscopy ($^1$H-MRS) as background to the experimental study reported later in this thesis. Neurometabolites of interest are discussed individually and findings in relationship to schizophrenia are briefly presented. Neural circuitry abnormalities relevant to the neurometabolites of interest are summarised in addition to $^1$H-MRS studies investigating treatment response and the effects of antipsychotics in patients with schizophrenia.

3.2 Principles of Proton Magnetic Resonance Spectroscopy

$^1$H-MRS is a non-invasive magnetic resonance imaging (MRI) technique that allows the measurement of certain metabolically important chemical constituents (neurometabolites) of the human brain in vivo (Martin, Capone, Schneider, Hennig, & Thiel, 2001). In order for a neurometabolite to be reliably measured it must be present at concentrations in the millimolar range and it must be able to rotate rapidly in solution (i.e. not be bound to a membrane or organelle). This is because $^1$H-MRS measures the resonance or the nuclear spin of molecules in a magnetic field following excitation with a radiofrequency pulse (Maddock & Buonocore, 2012b). As the protons relax back into alignment with the main magnetic field of the MRI scanner ($B_0$) energy is emitted. A spectrogram is a plot of the intensity of emission on the vertical axis and the frequency at which the compound resonates on the horizontal axis (Figure 3). In $^1$H-MRS, the frequency is indicated in parts per million (ppm) to enable comparison between spectra acquired at different field strengths. This is because the resonant frequency of a compound varies according to the strength of the magnetic field; doubling the strength of the magnetic field doubles the frequency at which a compound resonates.
3.3 Metabolites of interest

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetylaspartate</td>
<td><img src="image1.png" alt="Structure" /></td>
</tr>
<tr>
<td>Creatine</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
<tr>
<td>Glutamate</td>
<td><img src="image3.png" alt="Structure" /></td>
</tr>
<tr>
<td>Choline</td>
<td><img src="image4.png" alt="Structure" /></td>
</tr>
<tr>
<td>Glutamine</td>
<td><img src="image5.png" alt="Structure" /></td>
</tr>
<tr>
<td>Gamma-aminobutyric acid</td>
<td><img src="image6.png" alt="Structure" /></td>
</tr>
</tbody>
</table>

Figure 3 Sample $^1$H-MRS spectra (TE=30ms) from the dorsolateral prefrontal cortex in a patient with schizophrenia.

Figure 4 The molecular structures of six commonly studied neurometabolites.
3.3.1 N-Acetyl Aspartate (NAA)

N-acetylaspartate (NAA) is produced exclusively in neurons and is considered to be a marker of density of viable neuronal tissue (Meyerhoff et al., 1993; Moffett, Ross, Arun, Madhavarao, & Namboodiri, 2007). It is present at high concentrations (up to 10mM or greater) in the brain (Bluml, 1999; Miyake, Kakimoto, & Sorimachi, 1981; Pan & Takahashi, 2005). Due to its abundance and prominent proton signal in magnetic resonance spectroscopy (MRS) spectrograms, NAA is one of the most reliable markers for brain MRS studies (Barany et al., 1987; Fan, Higashi, Lane, & Jardetzky, 1986; Luyten & den Hollander, 1986). The three approximately equivalent hydrogen atoms in the acetate moiety resonate in $^1$H-MRS to produce a single sharp peak at 2.02ppm (Figure 3, Figure 4). Other acetylated compounds resonate at this frequency and make small contributions to the NAA peak such as N-acetylaspartylglutamate (NAAG); the most concentrated peptide in the brain which may contribute up to 15-25% to the acetate signal of this peak. N-acetylneuraminic acid and the underlying coupled resonances of glutamate and glutamine also contribute, however the peak at 2.02ppm is still attributable predominantly to NAA (Govindaraju, Young, & Maudsley, 2000). NAA is synthesised from aspartate (the carboxylate anion of aspartic acid which is an amino acid) and acetyl coenzyme A in neuronal mitochondria (Moffett et al., 2007; Szulc et al., 2013) (Figure 1, Figure 2). NAA is hydrolysed in oligodendrocytes to form aspartate and acetate. NAAG is also produced in neurons from NAA and glutamate and is thought to serve a cell-signalling function. (Neale, Bzdega, & Wroblewska, 2000) NAAG is released into the extracellular space where it is hydrolysed to produce NAA and glutamate.

Following release into the synapse, NAAG (but not NAA) is able to activate presynaptic group II metabotropic glutamate receptors (mGluR2/3) (Wroblewska et al., 1997) which inhibits neurotransmitter release including glutamate (Xi, Baker, Shen, Carson, & Kalivas, 2002; Zhong et al., 2006). In this way NAAG is thought to reduce glutamate excitotoxicity (Slusher et al., 1999); however, this also depends on the activity of the NAAG hydrolysing enzyme, glutamatedecarboxypeptidase II (GCP II). High levels of GCP II relieve the mGluR2/3 inhibition of
synaptic glutamate release and increase the level of extracellular glutamate resulting in enhanced glutamatergic neurotransmission (Arun, Madhavarao, Moffett, & Namboodiri, 2008). In vitro experiments have shown that clozapine and haloperidol increase NAA in dopaminergic-like human neuroblastoma cells and suggest that NAA and NAAG expression may be modulated by D₂ receptor blockade (Arun et al., 2008).

Figure 5 NAA synthesis and metabolism

A reduction in NAA is thought to reflect neuronal or axonal loss or mitochondrial dysfunction (Meyerhoff et al., 1993; Sager et al., 2001). There is evidence that NAA levels reflect reversible changes in neuronal viability, therefore NAA is more accurately a measure of either permanent loss or reversible dysfunction of neuronal tissue (Gasparovic, Arfai, Smid, & Feeney, 2001; Moffett et al., 2007). Given the well-documented reductions of both grey and white matter in patients with schizophrenia and its ease of measurement, NAA is the most extensively studied neurometabolite in these patients including three meta-analyses (Haijma et al., 2012; Steen, Hamer, & Lieberman, 2005). In a meta-analysis of 64 studies reporting NAA in patients with schizophrenia compared to healthy control subjects Steen et al found consistent evidence of NAA reductions in patients with
schizophrenia in specific brain regions. Compared to controls NAA was reduced in patients with schizophrenia by >5% in temporal grey and white matter, frontal grey and white matter and by >10% in the cerebellum and hippocampus. Smaller reductions were also observed in the anterior cingulate cortex (ACC) and thalamus. The basal ganglia, occipital cortex and posterior cingulate cortex appeared to be unaffected. No significant elevations in NAA were found for patients with schizophrenia (Steen et al., 2005). Brugger et al. evaluated 97 1H-MRS studies and confirmed the findings of the first meta-analysis; NAA reductions were present in the frontal lobe, temporal lobe and thalamus of patients with schizophrenia while in individuals considered to be at high risk of developing schizophrenia, significant reductions were only found in the thalamus (Brugger, Davis, Leucht, & Stone, 2011). The degree of NAA reduction was not correlated with duration of illness suggesting a lack of progression (Brugger et al., 2011). Kraguljac et al reviewed 103 studies and reported that NAA was globally decreased in patients with schizophrenia compared to controls — this finding was most consistent in the frontal lobe and basal ganglia (Kraguljac, Reid, White, Jones, et al., 2012). Consistent NAA reductions in the basal ganglia are not in agreement the earlier meta-analysis by Steen et al; however, the latter meta-analysis included 103 studies of patients with schizophrenia as opposed to 64 studies and 1H-MRS studies are being conducted at higher field strengths which may account for this divergent finding.

3.3.2 Creatine
Creatine (Cr) levels are generally interpreted as a measure of the global health of brain parenchyma and reductions are attributed to trauma and impaired cellular function (Tofts & Waldman, 2004) (Figure 4). Present in both grey and white matter in all the major cell types, Cr has an essential role in central nervous system (CNS) energy homeostasis (Maddock & Buonocore, 2012b). Cr can be phosphorylated (to form phosphocreatine) by creatine kinase (CK) in the presence of adenosine triphosphate (ATP). This reaction is reversible so that in the presence of adenosine diphosphate (ADP), ATP can be regenerated (Maddock & Buonocore, 2012b). Cr and phosphocreatine (PCr) cannot be reliably distinguished using 1H-MRS; the term “creatine” refers to
the combined signal (Cr+PCr) from both compounds. Together, these compounds give rise to two
singlet peaks; one at approximately 3.03ppm and another at 3.91ppm (Govindaraju et al., 2000)
(Figure 3). However, the concentration of Cr is relatively stable and homogenous throughout the
brain hence Cr is commonly used as an internal standard and other metabolites are expressed as a
ratio to creatine (Maddock & Buonocore, 2012b; Ross & Sachdev, 2004); however, the Cr signal
may increase or decrease in association with pathological conditions for instance stroke and brain
trauma. (Lei, Berthet, Hirt, & Gruetter, 2009; Signoretti et al., 2010) Studies of Cr in patients with
schizophrenia have produced conflicting results reporting no differences (Galinska et al., 2009;
Ohrmann et al., 2008), elevations (Wood et al., 2008) or reductions (Ohrmann et al., 2007)
compared to control subjects. A recent meta-analysis of 103 studies found no significant
alterations in Cr levels in patients with schizophrenia regardless of whether data were reported as
absolute metabolite concentrations or ratios (Kraguljac, Reid, White, Jones, et al., 2012).

3.3.3 Choline
Choline (Cho) containing compounds, phosphorylcholine (PCh) and glycerophosphorlcholine (GPC)
give rise to a prominent singlet peak at 3.21ppm (Figure 3, Figure 4) (Govindaraju et al., 2000).
While choline-containing phospholipids are found throughout the brain in myelin and cell
membranes (mainly phosphotidylcholine), only freely mobile Cho compounds are measurable by
$^1$H-MRS. Therefore the Cho signal reflects free choline compounds and is influenced by the density
of cell membranes and myelin turnover. Increased synthesis (for instance in tumours) or
degradation of membrane phospholipids is associated with an increase in Cho concentrations
(Boulanger, Labelle, & Khiat, 2000; Geddes, Panchalingam, Keller, & Pettegrew, 1997). For this
reason the Cho signal is interpreted as a reflection of membrane turnover and/or overall cell
density. Some studies have reported increases in Cho, for example in the caudate nucleus of
antipsychotic-naïve patients with schizophrenia (Bustillo et al., 2002) and in the parietal white
matter of acutely ill patients (Auer et al., 2001). These findings were not supported by the meta-
analysis by Kraguljac et al that found no significant alterations in Cho levels in patients with schizophrenia relative to controls (Kraguljac, Reid, White, Jones, et al., 2012).

3.3.4 Glutamate, Glutamine and Glx

After NAA, glutamate (Glu) is the second most concentrated mobile metabolite in the brain, yet due to its chemical structure it is significantly more difficult to quantify using standard MRI sequences (Govindaraju et al., 2000). Instead of producing distinct singlet peaks that arise from methyl groups (like those present in NAA, Cho and Cr), the methylene and methine groups give rise to broad, complex peaks at 2.34ppm, 2.08ppm (which is obscured by NAA) and 3.74ppm (Figure 3, Figure 4). Glutamine (Gln) produces peaks which are difficult to distinguish from those of glutamate at approximately 2.44, 2.12 (also obscured by NAA) and 3.75ppm (Figure 3). Gln is present in the brain at concentrations which are about 40% to 60% of Glu and as a result the Gln signal confounds the Glu signal (Govindaraju et al., 2000; Neale et al., 2000). It is possible to resolve the Glu and Gln peaks at 3T using a short echo time (TE) but the accuracy is greatly increased with the use of a dedicated sequence such as a J-resolved PRESS sequence (Lei et al., 2009). Without using an optimised sequence, measurements of these overlapping peaks are interpreted as the combined signal from Glu and Gln plus minor contributions from gamma-aminobutyric acid (GABA) and glutathione which is termed “Glx” (Maddock & Buonocore, 2012b). Glx represents a good approximation of the total Glu and Gln pool available for glutamatergic neurotransmission (Chen & Swanson, 2003; Maddock & Buonocore, 2012b; Signoretti et al., 2010).

Glutamate is the main excitatory neurotransmitter in the brain while Gln is as a non-neuroactive intermediate required for the recycling of amino acid neurotransmitters, mainly Glu and GABA (Figure 6). Gln is also involved in regulating brain ammonia a by-product of amino acid catabolism (Waagepetersen, Sonnewald, & Schousboe, 2007). Glu released into the synapse is taken up into astrocytes and converted into Gln via glutamine synthetase. N-methyl D-aspartate (NMDA) receptor hypofunction (a type of Glu receptor) is thought to increase the activity of glutamine synthetase to produce increased Gln levels (Rodrigo & Felipo, 2007). Importantly it is not possible
to differentiate between intra- and extracellular Glu or Gln using $^1$H-MRS even with specialised MRI sequences (Glodzik-Sobanska et al., 2006).

![Glutamate and glutamine metabolic cycle](image)

**Figure 6** Glutamate and glutamine metabolic cycle

Glutamatergic abnormalities are thought to be a central feature of the underlying pathophysiology of schizophrenia (see section 3.4). A recent meta-analysis of 28 studies found that frontal Glu is lower while frontal Gln is higher in patients with schizophrenia compared to healthy controls (Marsman et al., 2013). Both Glu and Gln levels in the frontal region decreased progressively with age in patients with schizophrenia; however, this meta-analysis included only cross-sectional data therefore this conclusion is preliminary. There is some evidence that Glu levels decrease during healthy aging (Kaiser, Schuff, Cashdollar, & Weiner, 2005); nonetheless a difference was still detected between patients and controls in this meta-analysis. Marsman *et al* also reported decreasing NAA levels in patients but, this did not account for the progressive decrease in Glu levels.
3.4 Neural circuitry abnormalities in schizophrenia

The current recent model of glutamate function in patients with schizophrenia with the most supporting evidence places N-methyl D-aspartate (NMDA) receptor hypofunction at the centre of circuitry abnormalities underlying schizophrenia (Lisman et al., 2008). Dissociative anaesthetics and potent N-methyl D-aspartate receptor (NMDAR) blockers such as ketamine and phencyclidine (PCP) are capable of producing schizophrenia-like psychotic symptoms in healthy individuals (Linn, Negi, Gerum, & Javitt, 2003; Luby, Cohen, Rosenbaum, Gottlieb, & Kelley, 1959; Rowland et al., 2005) while drugs that reduce or inhibit Glu release, such as lamotrigine or topiramate, have been found to lessen the psychotropic effects of ketamine in healthy individuals (Anand et al., 2000; Krystal et al., 2005) and improve positive symptoms in patients with schizophrenia (Patil et al., 2007; Tiihonen et al., 2005). These findings suggest that NMDA receptor hypofunctioning causes increased and desynchronised glutamate release. Although NMDA receptors are found throughout the brain, the present model suggests that neurodevelopmental formation abnormalities in the glutamate synapses at particular sites cause schizophrenia: specifically synapses on the γ-aminobutyric acid (GABA) interneurons in the cerebral cortex and the hippocampus (a region which warrants further study but is beyond the scope of this study). In healthy individuals, GABA interneurons provide negative feedback to the glutamatergic pyramidal cells; however, in people with schizophrenia GABA levels in the chandelier and basket interneurons are decreased (as assessed by GAD67 mRNA in studies of post-mortem tissue) and as a result NMDARs are relatively insensitive to glutamate (Guidotti et al., 2000; Volk, Austin, Pierri, Sampson, & Lewis, 2000). To compensate for “low” Glu levels, GABA synthesis and release is down-regulated and Glu output is increased (Lisman et al., 2008).

An important consequence of the failure of this negative feedback loop is increased dopamine (DA) levels in the mesolimbic and mesocortical dopaminergic pathways. Hypofunctional NMDA synapses in the prefrontal cortex cause overactivation of the cortico-brainstem Glu pathway leading to increased Glu in the ventral tegmental area (VTA) which in turn directly stimulates the
mesolimbic DA pathway and excessive DA release in the nucleus accumbens which results in the positive symptoms of psychosis. A separate population of cortico-brainstem glutamate neurons indirectly regulate the mesocortical DA pathway (which projects from the VTA to the prefrontal cortex) via inhibitory GABA interneurons. Excessive Glu in the VTA activates the GABA interneurons which then inhibit the mesocortical DA neurons and produce negative and cognitive symptoms of schizophrenia (Laruelle, Kegeles, & Abi-Dargham, 2003; Sesack, Carr, Omelchenko, & Pinto, 2003).

3.5 $^{1}$H-MRS studies of treatment response in schizophrenia

Recent work, utilising positron emission tomography (PET) has suggested an excess of presynaptic DA synthesis capacity in the striatum of patients with schizophrenia is associated with the response to first line antipsychotics while treatment resistant individuals have normal presynaptic DA synthesis capacity (Demjaha, Murray, McGuire, Kapur, & Howes, 2012). $^{1}$H-MRS data in the anterior cingulate cortex (ACC) were also collected for a subset of patients; responders to antipsychotics (n=8), those with treatment resistant schizophrenia taking a non-clozapine antipsychotic (TRS; n=6) and healthy controls (n=10). Relative to healthy controls, Glu was elevated in the group with TRS but not the responder group, although this may have been confounded by Gln signal contamination. No group differences were seen in Glx levels; however, responders to antipsychotic treatment had lower NAA in the ACC than both controls and those with TRS. These preliminary findings (summarised in Table 6) suggest that neurochemical imaging may be used to stratify patients according to treatment response; however, this sample did not include patients taking clozapine despite a diagnosis of TRS. Another important consideration is that the treatment resistant group had significantly higher PANSS scores, therefore these differences may be due to the presence of active symptoms (Demjaha et al., 2013).
Table 6  Positron Emission Tomography and proton magnetic resonance spectroscopy findings in patients with schizophrenia responsive and resistant to antipsychotic treatment (Demjaha et al, 2012 & 2013)

<table>
<thead>
<tr>
<th>Measurement (method)</th>
<th>Treatment responders</th>
<th>Treatment resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striatal dopamine synthesis capacity (PET)</td>
<td>Elevated relative to healthy volunteers</td>
<td>Normal</td>
</tr>
<tr>
<td>ACC Glu (^1H-MRS)</td>
<td>Normal</td>
<td>Elevated relative to healthy volunteers</td>
</tr>
<tr>
<td>ACC NAA (^1H-MRS)</td>
<td>Decreased relative to healthy volunteers and treatment resistant patients</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Egerton et al compared first episode schizophrenia (FES) patients in symptomatic remission (n=15; eleven receiving a SGA and four no longer receiving medication) and a symptomatic, non-remission group (n=17; sixteen receiving a SGA and one no longer receiving medication) (Egerton et al., 2012). Elevated Glu/Cr was detected in the anterior cingulate cortex (ACC), but not the thalamus, in patients who remained symptomatic following at least one course of antipsychotic treatment compared to those in symptomatic remission. After the removal of outliers, significantly higher Glx/Cr was observed in the non-responder group. A healthy control group was not included and because the study was cross-sectional, it was not possible to determine the effects of medication on Glu/Cr. The authors also acknowledged Gln contamination of the Glu peak was a limitation.

De la Fuente-Sandoval et al studied glutamate levels in the right associative striatum in a sample of first episode of psychosis (FEP) patients (n=24) compared to healthy controls (n=18); a treatment resistant group was not included (de la Fuente-Sandoval et al., 2013). Following four weeks of clinically effective treatment with oral risperidone, increased Glu and Glx at baseline normalised and were similar to healthy controls. An important limitation of this study is that the authors reported uncorrected ‘absolute concentration’ values which do not account for patient and MRI system factors such as head size and position within the head coil or drift and variability of intrinsic gain in the amplifiers of the (radiofrequency) RF receiver system (Maddock & Buonocore, 2012a).

3.6  Effects of antipsychotics on neurometabolites

A recent systematic review of ^1H-MRS changes after antipsychotic treatment included fourteen longitudinal and eight cross-sectional studies of patients with schizophrenia (Szulc et al., 2013).
Most of the longitudinal studies included patients taking a variety of antipsychotics rather than specific medication groups; however there were some exceptions (Ertugrul et al., 2009; Goff et al., 2002; Szulc et al., 2005). Increased NAA levels within the frontal lobe and thalamus were noted in the short-term (≤ 12 weeks) longitudinal studies but this effect disappeared with longer observation. Cross-sectional studies reported that patients treated with second generation antipsychotics (SGAs) had higher NAA in the frontal lobe and the anterior cingulate cortex (ACC) than those treated with first generation antipsychotics (FGAs). Four out of six longitudinal studies revealed a significant decrease in Glx in the frontal and temporal lobes, ACC and thalamus following antipsychotic treatment (Aoyama et al., 2011; Choe et al., 1996; Szulc et al., 2011; Theberge et al., 2007). On the contrary one study reported no change in Glx after 1, 6 and 12 months of treatment (in a group treated with quetiapine, risperidone, clozapine or haloperidol) (Bustillo et al., 2010) and another reported increased Glx in the ACC after olanzapine treatment in 10 patients (Goff et al., 2002). These findings are consistent with a recent meta-analysis that reported that Glu and Gln levels decrease progressively with age in patients with schizophrenia compared to healthy controls; however it is not known whether this is a medication effect or related to disease progression (Marsman et al., 2013).

The effects of clozapine specifically have been investigated in one small longitudinal trial and one cross-sectional study (Bustillo et al., 2001; Ertugrul et al., 2009). Ertugrul et al performed 1H-MRS evaluation in the dorsolateral prefrontal cortex (DLPFC) of ten patients at baseline and after eight weeks of clozapine treatment. A significant improvement in symptom severity was observed in the PANSS total scores and PANSS positive, negative and general subscores. At follow-up, there was a significant increase in NAA/Cr ratios compared to baseline measures (1.73±0.23 versus 1.91±0.26, t=2.20, P=0.05). Limitations of this study are the small sample size with no control group, no adjustments for the proportion of CSF within the voxel, the inclusion of patients who were intolerant to other antipsychotics as opposed to treatment-resistant and a relatively short washout period of one week prior to clozapine initiation (Ertugrul et al., 2009). Bustillo et al investigated the
effects of clozapine (n=19) and haloperidol (n=19) in the caudate nuclei and frontal lobe compared to healthy controls (n=21). There were no group differences in the caudate nuclei when the data were corrected for the proportion of cerebrospinal fluid (CSF) within the voxel. The haloperidol group had significantly lower NAA than the controls while the clozapine group had intermediate NAA levels though not significantly different to either the haloperidol group or controls. While this study included a control group and performed CSF-correction, there were important differences between the patient groups, for instance the clozapine group had a longer lifetime exposure to antipsychotics and an earlier age of onset than those who took haloperidol (Bustillo et al., 2001).

3.7 Regions of interest

3.7.1 Frontal lobe regions
The ACC has been investigated in ¹H-MRS studies of treatment response described above since structural abnormalities within this region may be predictive of conversion to psychosis in those considered to be at high risk. In addition functional MRI studies show that antipsychotic treatment may normalise functional activity in this region (Pantelis et al., 2003; Snitz et al., 2005). Dopaminergic dysregulation in the DLPFC has been associated with schizophrenia (Bertolino et al., 2000; Deutch, 1992; Weinberger, Berman, & Zec, 1986). Patients with high baseline DLPFC volumes and elevated metabolic activity (determined by PET) were more likely to experience an improvement in negative symptoms following treatment with clozapine (Molina et al., 2003).

3.7.2 Putamen
The pharmacological feature common to all antipsychotic medication is dopamine D₂ receptor blockade in the striatum which consists of two structures, the putamen and caudate nucleus, separated by a white matter tract called the internal capsule. Several imaging studies using positron emission tomography (PET) have confirmed that >60% striatal D₂ receptor blockade is required to achieve an antipsychotic response while occupancies higher than 80% are associated with extrapyramidal side effects (EPSEs) (Farde et al., 1992; Kapur, Zipursky, Jones, Remington, & Houle, 2000; Nordström et al., 1993). The relationship between response to antipsychotics and D₂
receptor occupancy in other regions of the brain has also been investigated; however, striatal D₂ occupancy predicted treatment response better than D₂ occupancy in the frontal, thalamic and temporal regions (Agid et al., 2006).

Putamen volumes have been proposed as a longitudinal marker of treatment responsiveness in patients with schizophrenia and post-mortem studies have revealed microstructural correlates of treatment responsiveness such as fewer mitochondria per synapses in this region (Buchsbaum et al., 2003; Mitelman, Canfield, Chu, et al., 2009; Roberts, 2013). ¹H-MRS studies of the putamen have reported no differences in NAA, Cr or Cho in either chronically ill, medicated patients (Bertolino et al., 1996; Heimberg, Komoroski, Lawson, Cardwell, & Karson, 1998; Ohara et al., 2000) or unmedicated (Bertolino et al., 1998) patients with schizophrenia. Most of these studies used small sample sizes (Ohara et al, n= 10, Bertolino 1998 n=10 and 1996 n=12), none measured Glu or Glx and all were composed of either unmedicated patients with schizophrenia or a group of medicated patients receiving a range of medications (e.g. FGAs, SGAs and clozapine). De la Fuente-Sandoval et al reported increased striatal Glu in first episode patients and ultra-high risk subjects (de la Fuente-Sandoval et al., 2011). Furthermore, this group reported increased Glu levels in the right associative striatum at baseline subsequently normalised and were similar to healthy controls after four weeks of effective treatment with risperidone (de la Fuente-Sandoval et al., 2013). These findings suggest that glutamatergic abnormalities in striatal structures may be a marker of treatment response; however the authors were criticised for reporting uncorrected ‘absolute concentrations’ which do not account for patient and MRI system factors (as mentioned above in 3.5) (Maddock & Buonocore, 2012a).

3.8 Conclusion

This chapter has described the basic theory underlying ¹H-MRS and reviewed findings using this technology in patients with schizophrenia showing differing responses to pharmacological treatment. In patients with schizophrenia NAA appears to be decreased compared to healthy control subjects and there does not appear to be significant differences in Cr or Cho levels between
patients and control subjects. Glu and Gln concentrations are lower in patients with schizophrenia and decrease with age at a faster rate than control subjects. $^1$H-MRS of treatment response suggest that elevated Glu or Glx is associated with poor response to antipsychotic medication in the ACC and striatum – this may be related to or a consequence of NMDA receptor hypofunction but the mechanism has yet to be determined. The following chapter presents the aims and hypotheses for the $^1$H-MRS study presented in this thesis based on the review above. In order to investigate neurometabolite alterations based on treatment response three groups of patients with schizophrenia were included and stratified based on the treatment prescribed. These findings are presented in the form of a journal article prepared for publication.
Chapter Four: Glutamate and glutamine in treatment-resistant schizophrenia and ultra-treatment-resistant schizophrenia

4.1 Abstract

Background: This study tested the hypothesis that patients with treatment-resistant schizophrenia (TRS) could be differentiated from responders to first-line medication, those with ultra-treatment-resistant schizophrenia (UTRS) and healthy controls based on alterations in glutamate and glutamine levels.

Methods: In this cross-sectional study, proton magnetic resonance spectroscopy ($^1$H-MRS) data were obtained using a point resolved spin echo (PRESS) sequence, echo time (TE) 30 ms on a 3T Siemens Magnetom Skyra from sixteen healthy controls and forty-two patients with schizophrenia; those who were antipsychotic-responsive (taking a second generation non-clozapine antipsychotic; n=15), those with TRS (clozapine monotherapy, clozapine-responsive; n=16) and those with ultra-treatment-resistant schizophrenia (UTRS; clozapine-resistant, antipsychotic augmentation; n=11). Voxels were placed in the dorsolateral prefrontal cortex (DLPFC; 2cm$^3$), the ACC (2cm$^3$) and the putamen (1.5 x 1.5 x 3.5cm). The following metabolites were estimated in ratio to creatine (Cr): N-acetyl-aspartate (NAA), glutamate (Glu), glutamate + glutamine (Glx) and choline (Cho).

Results: Successful treatment with clozapine monotherapy in patients with TRS was associated with higher Glx levels in the putamen compared to those with clozapine resistant UTRS (mean difference (MD) =0.360 (95% CI), p=0.05, Cohen’s $d$ = 1.221) and this result remained significant when participants who tested positive for tetrahydrocannabinol (THC) were removed from the analysis. There were no significant correlations between Glx/Cr ratios and positive and Negative Syndrome Scale (PANSS) total scores, PANSS subscale scores or antipsychotic dose. There were no significant group differences in the concentrations of other neurometabolites (NAA/Cr, Glu/Cr or GPC+PCh/Cr) in the DLPFC, ACC or putamen.
Conclusions: These results require replication in future, prospective studies examining response to specific antipsychotic medications but it may be that Glx in striatal structures such as the putamen represents a marker of response to clozapine treatment.

4.2 Introduction

Treatment-resistant schizophrenia (TRS) represents a subset of people with schizophrenia with poor outcomes who experience moderate-to-severe positive symptoms despite adequate treatment with first-line antipsychotic medication (Elkis & Meltzer, 2010). It is estimated that treatment resistance affects 30-60% of patients with schizophrenia and while clozapine is the gold-standard treatment for this group, individual responses vary (Association, 2004). Approximately 50-70% of clozapine-treated patients do not experience clinically significant improvements in their symptoms (termed clozapine-resistant or ultra-treatment-resistant schizophrenia; UTRS) (Buckley et al., 2001; Chakos et al., 2001; Mouaffak et al., 2006). The residual symptoms experienced by patients with TRS have devastating effects on quality of life for the individuals and their families in addition to the financial burden for wider society which is estimated to cost more than $34 billion annually within the USA alone (Kennedy, Altar, Taylor, Degtiar, & Hornberger, 2013).

The glutamate hypothesis of schizophrenia proposes that the disorder arises from neurodevelopmental abnormalities that result in disrupted glutamatergic neurotransmission and in particular N-methyl-d-aspartate (NMDA)-receptor mediated signalling (Belforte et al., 2010). Glutamatergic excitotoxicity may underlie the deteriorating course of the illness, ultimately leading to dopaminergic dysregulation and the symptoms of schizophrenia (Olney & Farber, 1995; Sharp, Tomitaka, Bernaudin, & Tomitaka, 2001). Changes in Glu have been examined in relationship to treatment resistance with two recent proton magnetic resonance spectroscopic (\(^1\)H-MRS) studies of patients with first episode psychosis (FEP) reporting that Glu levels are associated with the response to antipsychotic medication (de la Fuente-Sandoval et al., 2013; Egerton et al., 2012). De la Fuente-Sandoval et al, reported that after four weeks of effective treatment with risperidone, increased Glu levels in the right associative striatum at baseline normalised and were similar to
healthy controls (de la Fuente-Sandoval et al., 2013). Elevated Glu has also been reported in the anterior cingulate cortex (ACC) of patients with FEP who remained symptomatic following at least one course of antipsychotic treatment compared to those in symptomatic remission (Egerton et al., 2012). Other work has examined the role of dopamine in treatment resistance. A positron emission tomography (PET) study has found an excess of presynaptic dopamine synthesis capacity in the striatum is associated with response to first line antipsychotics; while treatment-resistant groups have normal pre-synaptic dopamine synthesis capacity (Demjaha et al., 2012).

It is as yet unknown whether the association between Glu and treatment response persists beyond the early (medication naïve) phase of schizophrenia. A meta-analysis reported that elevated frontal glutamatergic activity is seen in un-medicated patients during the early phase of schizophrenia with subsequent progressive reductions in frontal Glu and Gln levels with age (Marsman et al., 2013). Glutamate levels similar to or lower than control subjects have been found in studies of medicated patients with schizophrenia (Bustillo et al., 2011; Reid et al., 2010; Rowland et al., 2012; Theberge et al., 2003). In contrast a recent study of patients with schizophrenia reported that Glu levels in the ACC were linked to treatment response (Demjaha et al., 2013). Relative to healthy controls, patients with persistent psychotic symptoms despite treatment with a non-clozapine antipsychotic displayed elevated Glu levels in the ACC whereas those who responded to treatment had the same levels as healthy control participants.

To further explore the relationship between glutamatergic compounds and TRS we recruited three groups of patients with established schizophrenia; those taking a second generation non-clozapine antipsychotic (antipsychotic-responsive; first-line responders), those on clozapine monotherapy (clozapine-responsive; treatment-resistant) and a group taking a combination of two antipsychotics (clozapine-resistant; ultra-treatment-resistant schizophrenia; UTRS). These groupings are consistent with the recently proposed classification of schizophrenia subtypes based on treatment response and in are in line with current treatment algorithms (Association, 2004; Farooq, Agid, Foussias, & Remington, 2013; NICE, 2002; Royal Australian New Zealand College of Psychiatrists,
We chose to investigate the levels of glutamate and glutamine (Glx) in the ACC, dorsolateral prefrontal cortex (DLPFC) and the putamen. The ACC has been investigated in \(^1\)H-MRS studies described above, because structural abnormalities within this region may be predictive of conversion to psychosis in those at high risk, in addition functional MRI studies show that antipsychotic treatment may normalise functional activity in this region (Pantelis et al., 2003; Snitz et al., 2005). In addition dopaminergic dysregulation in the DLPFC has been associated with schizophrenia (Bertolino et al., 2000; Deutch, 1992; Weinberger et al., 1986). Patients with high baseline DLPFC volumes and elevated metabolic activity (determined by PET) were more likely to experience an improvement in negative symptoms following treatment with clozapine (Molina et al., 2003). Finally, putamen volumes have been proposed as a longitudinal marker of treatment responsiveness in patients with schizophrenia and post-mortem studies have revealed microstructural correlates of treatment responsiveness such as fewer mitochondria per synapses in this region (Mitelman, Canfield, Chu, et al., 2009; Roberts, 2013).

The model of glutamate function in patients with schizophrenia with the most supporting evidence places N-methyl D-aspartate (NMDA) receptor hypofunction at the centre of circuitry abnormalities in schizophrenia (Lisman et al., 2008). This model suggests that NMDA receptor hypofunctioning causes increased and desynchronised glutamate release – to compensate for “low” glutamate levels, GABA synthesis and release is down-regulated and glutamate output is increased (Lisman et al., 2008). An important consequence of the failure of this negative feedback loop is increased dopamine (DA) levels in the mesolimbic and mesocortical dopamine pathways which is thought to cause the positive and the negative/cognitive symptoms of schizophrenia, respectively (Laruelle et al., 2003; Sesack et al., 2003).

On the basis of earlier studies examining neurometabolite levels and treatment response (de la Fuente-Sandoval et al., 2013; Demjaha et al., 2013; Egerton et al., 2012; Molina et al., 2003), we hypothesized that in the three areas examined 1.) Glutamate (Glu) and glutamate + glutamine (Glx) would be similar to healthy controls in responders to first-line treatment. 2.) Glu and Glx would be
elevated in those with TRS compared to first-line responders and controls. 3.) Glu and Glx would be highest in those with UTRS.

4.3 Method
4.3.1 Participants
This study was approved by the Regional Ethics Committee. Patients who met the DSM-IV criteria for schizophrenia were identified by their treating clinician either from a community mental health centre or a forensic psychiatric inpatient unit; the diagnosis was checked against patient notes. Subsequent to obtaining written informed consent, data regarding duration of illness and medication history were collected and then verified from clinical notes and patient interviews. All participants were stabilised on antipsychotic medication for at least six weeks and categorised according to their treatment history and response to antipsychotic medication. Patient classification (first-line responder, TRS or UTRS) was based on the treatment at the time of the study and prescribed in accordance with criteria for TRS using published algorithms (Association, 2004; NICE, 2002; Royal Australian New Zealand College of Psychiatrists, 2005). These algorithms define TRS in patients who do not experience significant symptom improvement after trials with at least two antipsychotic agents using therapeutic doses for at least six weeks. The three treatment groups in this study comprised those taking i) a second generation (atypical) non-clozapine antipsychotic (first-line responders), ii) clozapine monotherapy (clozapine-responsive, TRS), or iii) a combination of antipsychotics (clozapine-resistant; UTRS) having failed a trial of clozapine monotherapy. Clozapine could be one of the antipsychotics used in combination or the combination could be two alternative antipsychotics as long as past failure with clozapine monotherapy could be confirmed from clinical notes as opposed to clozapine cessation or down-titration secondary to side-effects. A healthy control group with no history of mental or neurological illness was recruited through advertising in the community. Age, sex, years of education and ethnicity were matched on a group basis. All participants were between the ages of 18 and 45 years. Exclusion criteria included a history of traumatic brain injury (loss of
consciousness for more than three minutes following brain trauma), neurological illness, significant physiological comorbidity or contraindication to MRI.

Symptom severity was assessed using the PANSS and scores were also converted to Clinical Global Impression (CGI) for Schizophrenia scores based on severity but not improvement (Kay et al., 1987). All PANSS interviews were conducted by the same trained investigator. The findings from clinical interviews were corroborated with the patients’ family, caregiver or mental health professional (treating psychiatrist or key worker).

The World Health Organisation’s Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) was used to collect recreational drug use histories from all participants (Newcombe, Humeniuk, & Ali, 2005). ProScreen Cups© (US Diagnostics Inc., PSCupA-6MBAU) were used to screen urine samples collected at each study session for amphetamines, cocaine, benzodiazepines, tetrahydrocannabinol (THC) or opiates. For detection limits please refer to Appendix 1: Detection limits for ProScreen Cups© recreational drug screen kit. Standardised premorbid IQ scores were determined using a computerised version of the “spot the real word” test within the IntegNeuro test battery (Brain Resource Company, Sydney, Australia) (Baddeley, Emslie, & Nimmo-Smith, 1993; Gordon, 2003; Gordon, Cooper, Rennie, Hermens, & Williams, 2005). Antipsychotic chlorpromazine equivalents (CPZEs) were calculated to compare daily doses using formulae with power transformation (Andreasen et al., 2010), except for amisulpride which in the absence of a power formula was calculated using expert consensus regarding antipsychotic dosing (Gardner et al., 2010). The sample size was based on previous studies that have examined the relationship between antipsychotic treatment and spectroscopic measures in schizophrenia (Bustillo et al., 2008; Ertugrul et al., 2009).

4.3.2 Image acquisition

Imaging was performed using a 3T Siemens Magnetom Skyra (Siemens, Germany). A 32-channel head coil was used for the majority of imaging sessions; where the participant could not fit comfortably into this coil due to head size limitations, a 20-channel head coil was used (n=4).
standard T1-weighted MR scan was acquired (magnetisation prepared rapid gradient echo; MPRAGE; Repetition Time (TR), 1900 ms; Echo Time (TE), 2.39 ms; inversion time (TI), 900 ms; flip angle, 9 deg; voxel size 0.9x0.9x0.8mm).

\(^1\)H-MRS data were acquired by single voxel spectroscopy (SVS) using a point resolved spin echo (PRESS) sequence (80 averages; TR, 2000 ms; TE, 30 ms). Using this method three mutually orthogonal slices are stimulated successively and the voxel of interest (VOI) is the result of the intersection of these slices. Only the spins in this region are measured. Three \(^1\)H-MRS voxels of interest were acquired: (1) a 2cm\(^3\) voxel placed in the left dorsolateral prefrontal cortex; (2) a 2cm\(^3\) voxel placed in the anterior cingulate cortex; (3) a 1.5 x 1.5 x 3.5cm voxel centred on the left putamen (Figure 7).

4.3.3 \(^1\)H-MRS data processing
Spectra were analysed using LCModel (Provencher, 2001). To ensure high-quality data, data sets that met one or more of the following were excluded: (a) obvious movement artifact on imaging or spectroscopy; (b) metabolite concentration uncertainties that exceeded a Cramer-Rao Lower Bound (CRLB), as provided by LCModel, of 20%; (c) spectra with an LCModel full-width half maximum (FWHM) that exceeded 0.1 ppm. We determined the amount of grey matter, white matter and cerebrospinal fluid (CSF) in the \(^1\)H-MRS voxels by segmenting participants’ structural scan using FMRIB’s Automated Segmentation Tool (FAST). This tool determines the tissue type of each voxel whilst also correcting for variations in spatial intensity. Neurometabolite ratios expressed as a ratio to creatine containing compounds (creatine and phosphocreatine; Cr +PCR) were corrected for CSF fraction within the voxel based on the assumption that CSF has NAA, Cre, Cho and Glu levels of zero. For example, if the voxel CSF component was 10% and tissue component (GM+WM) was 90%, the LC model yielded ratio was divided by 0.9. This technique has been employed in other studies with good test-retest reliability (Brooks, Friedman, & Stidley, 1999; Mullins et al., 2003). Differences in metabolite concentrations in grey versus white matter have been reported (Hetherington et al., 1994; Schuff et al., 2001), therefore any region in which voxel
tissue component composition varied significantly, the metabolite ratios were covaried for the proportion of GM in the voxel. Metabolite ratios in the putamen were covaried for GM because the voxel was larger than the putamen, which is a grey matter structure, and captured surrounding WM which was not relevant.

Figure 7 Three orthogonal reference views for single-voxel $^1$H-MRS acquisition; column A= left DLPFC, B= ACC, C= left putamen.
treatment groups using an ANOVA in SPSS. The effect of variables on NAA levels were assessed using one-tailed Pearson’s correlations. NAA/Cr ratios were compared with current symptom severity collected on the day of imaging. Potential confounders were concomitant medications in the patient groups and recreational drug use across all groups.

4.5 Results

4.5.1 Participants

A total of 156 people with schizophrenia and 30 healthy controls were approached to participate in this study. Due to the strict exclusion criteria only 58 people (16 controls and 42 patients) were included in the final analysis. The demographic and clinical data of the study participants are summarized in Table 7. There was a difference in years of education between the groups (F(3,39)=2.9, p=0.044); post hoc tests showed that the group with TRS had fewer years of education than the controls (p=0.025). There were no significant differences between the treatment groups in sex, age, premorbid IQ scores, duration of untreated psychosis, PANSS total scores or any of the subscales or any of the CGI scores. There was a difference in duration of illness between the treatment groups (F(2,39)=3.4, p=0.042) and in the dose of antipsychotic treatment at the time of the study (F(2,39)=9.8, p<0.001). Post hoc tests showed that the first-line responders had a shorter duration of illness than the group with TRS (p=0.036) and the group with UTRS was receiving higher doses of antipsychotic medication than both the first-line responders and the group with TRS (both p=0.001).

Antipsychotic medication prescribed in the first-line responder group included olanzapine (n=6), risperidone (n=5), aripiprazole (n=3) and amisulpride (n=1). The combinations of antipsychotics in the group with UTRS were clozapine and amisulpride (n=3), clozapine and aripiprazole (n=4), clozapine and risperidone (n=1), clozapine and quetiapine (n=1). There were two individuals who failed a trial of clozapine monotherapy due to inadequate response whose current antipsychotic medications did not include clozapine: aripiprazole and quetiapine (n=2). Five participants tested positive for THC but no other recreational drugs at the time of scanning; first-line responders (n=3),
TRS (n=1), UTRS (n=1). Patients were taking concomitant psychotropic medications as follows: benztrapine (n=5), citalopram (n=2), clomipramine (n=1), fluoxetine (n=1), lamotrigine (n=1), lithium (n=1), lorazepam (n=1), nicotine replacement therapy patches (n=3), nortriptyline (n=1), paroxetine (n=2), sodium valproate (n=7) and zopiclone (n=1) (Table A).

Table 7 Demographic data of study participants by treatment group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>First-line responders (n=15)</th>
<th>TRS (n=16)</th>
<th>UTRS (n=11)</th>
<th>Controls (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>12</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Age (mean years, SD)</td>
<td>30.7 (7.2)</td>
<td>33.7 (8.6)</td>
<td>35.0 (7.3)</td>
<td>34.1 (7.9)</td>
</tr>
<tr>
<td>Education (mean years, SD)</td>
<td>12.4 (3.0)</td>
<td>11.1 (2.8)*</td>
<td>12.4 (2.2)</td>
<td>13.8 (2.1)*</td>
</tr>
<tr>
<td>Standardised premorbid IQ (mean, SD)</td>
<td>-1.059 (1.137)</td>
<td>-0.673 (1.039)</td>
<td>-1.118 (1.138)</td>
<td>-0.326 (0.823)</td>
</tr>
<tr>
<td>Duration of illness (mean years, SD)</td>
<td>7.4 (5.3)*</td>
<td>13.0 (7.0)*</td>
<td>11.3 (5.4)</td>
<td>-</td>
</tr>
<tr>
<td>Duration of untreated psychosis (mean months, SD)</td>
<td>13.3 (15.9)</td>
<td>7.4 (7.2)</td>
<td>21.1 (21.5)</td>
<td>-</td>
</tr>
</tbody>
</table>

Psychiatric ratings

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>First-line responders (n=15)</th>
<th>TRS (n=16)</th>
<th>UTRS (n=11)</th>
<th>Controls (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANSS total score (mean, SD)</td>
<td>59.9 (11.1)</td>
<td>59.7 (15.0)</td>
<td>62.4 (12.5)</td>
<td>-</td>
</tr>
<tr>
<td>PANSS positive subscale score (mean, SD)</td>
<td>13.4 (5.3)</td>
<td>11.4 (5.5)</td>
<td>14.0 (5.8)</td>
<td>-</td>
</tr>
<tr>
<td>PANSS negative subscale score (mean, SD)</td>
<td>17.1 (5.8)</td>
<td>19.7 (6.7)</td>
<td>20.0 (6.9)</td>
<td>-</td>
</tr>
<tr>
<td>PANSS general subscale score (mean, SD)</td>
<td>29.5 (5.9)</td>
<td>28.6 (6.6)</td>
<td>28.4 (4.2)</td>
<td>-</td>
</tr>
<tr>
<td>CGI severity positive score (mean, SD)</td>
<td>2.27 (1.00)</td>
<td>1.84 (1.07)</td>
<td>2.33 (1.18)</td>
<td>-</td>
</tr>
<tr>
<td>CGI severity negative score (mean, SD)</td>
<td>2.05 (0.86)</td>
<td>2.37 (1.02)</td>
<td>2.32 (0.96)</td>
<td>-</td>
</tr>
<tr>
<td>CGI severity depressive score (mean, SD)</td>
<td>2.13 (0.68)</td>
<td>2.15 (0.99)</td>
<td>1.70 (0.69)</td>
<td>-</td>
</tr>
<tr>
<td>CGI severity cognitive score (mean, SD)</td>
<td>2.43 (0.85)</td>
<td>2.44 (1.08)</td>
<td>2.80 (0.99)</td>
<td>-</td>
</tr>
<tr>
<td>Dose at time of scan (chlorpromazine equivalents)</td>
<td>426.5 (205.8)</td>
<td>446.0 (242.3)</td>
<td>855.4 (369.6)**</td>
<td>-</td>
</tr>
<tr>
<td>Positive test for THC</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Smoking status (% smokers)</td>
<td>87%</td>
<td>56%</td>
<td>73%</td>
<td>31%</td>
</tr>
<tr>
<td>Head coil used (32 channel/20 channel)</td>
<td>13/2</td>
<td>15/1</td>
<td>10/1</td>
<td>16/0</td>
</tr>
</tbody>
</table>

Note: *p<0.05, FWE-corrected, **p<0.001, FWE-corrected

4.5.2 Quality of spectra

A total of 58 spectra were acquired in the left DLPFC, 50 in the ACC and 42 in the left putamen (Table 8). Fewer voxels were acquired in the ACC and putamen than the DLPFC due to time constraints; these were the last sequences performed during a one hour and fifteen minute scanning session. In the DLPFC and the ACC, there were no significant differences between the
groups in terms of the scanner FWHM, or the FWHM and signal to noise ratio (SNR) calculated by LC Model. For the putamen voxel, there was a significant difference in the scanner FWHM (F=2.911, p=0.047) but not the LC Model FWHM or SNR. Post hoc tests revealed that the scanner FWHM was higher for the group with TRS versus controls (24.9±3.2 versus 21.3±2.4, p=0.047, FWE-corrected).

Table 8 FWHM and SNR for each region by treatment group

<table>
<thead>
<tr>
<th>Variable</th>
<th>First-line responders</th>
<th>TRS</th>
<th>UTRS</th>
<th>Controls</th>
<th>F (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DLPFC (n=58)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scanner FWHM (ppm)</td>
<td>21.3 (2.3)</td>
<td>21.7 (1.9)</td>
<td>20.8 (3.0)</td>
<td>21.0 (2.7)</td>
<td>0.390 (0.761)</td>
</tr>
<tr>
<td>LC Model FWHM (ppm)</td>
<td>0.07 (0.01)</td>
<td>0.07 (0.01)</td>
<td>0.07 (0.01)</td>
<td>0.06 (0.02)</td>
<td>0.654 (0.584)</td>
</tr>
<tr>
<td>LC Model SNR</td>
<td>30.9 (8.2)</td>
<td>29.9 (6.6)</td>
<td>30.1 (6.4)</td>
<td>34.8 (7.8)</td>
<td>1.479 (0.231)</td>
</tr>
<tr>
<td><strong>ACC (n=50)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scanner FWHM (ppm)</td>
<td>21.5 (1.9)</td>
<td>20.1 (2.2)</td>
<td>20.8 (3.0)</td>
<td>20.1 (3.2)</td>
<td>0.918 (0.440)</td>
</tr>
<tr>
<td>LC Model FWHM (ppm)</td>
<td>0.08 (0.01)</td>
<td>0.07 (0.01)</td>
<td>0.08 (0.01)</td>
<td>0.07 (0.01)</td>
<td>1.303 (0.285)</td>
</tr>
<tr>
<td>LC Model SNR</td>
<td>31.4 (6.7)</td>
<td>31.9 (8.4)</td>
<td>25.9 (4.5)</td>
<td>32.2 (8.1)</td>
<td>1.669 (0.187)</td>
</tr>
<tr>
<td><strong>Putamen (n=42)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scanner FWHM (ppm)</td>
<td>21.7 (3.1)</td>
<td>24.9 (3.2)*</td>
<td>21.5 (3.2)</td>
<td>21.3 (2.4)*</td>
<td>2.911 (0.047)*</td>
</tr>
<tr>
<td>LC Model FWHM (ppm)</td>
<td>0.08 (0.01)</td>
<td>0.09 (0.01)</td>
<td>0.08 (0.01)</td>
<td>0.08 (0.01)</td>
<td>0.896 (0.452)</td>
</tr>
<tr>
<td>LC Model SNR</td>
<td>17.3 (3.6)</td>
<td>16.3 (2.1)</td>
<td>17.8 (3.8)</td>
<td>19.7 (3.1)</td>
<td>2.122 (0.114)</td>
</tr>
</tbody>
</table>

Note: * p<0.05, FWE-corrected

4.5.3 Voxel tissue components

In the DLPFC and putamen there were no significant group differences in percentage of GM, WM or CSF (Table 9).

In the ACC there were differences in the percentage of GM and CSF but not WM; F=4.678 (p=0.006) and F=7.206 (p<0.001), respectively. Post-hoc analysis showed that the group with TRS had a lower percentage of GM in the voxel placed in the ACC than control subjects (58.4±6.2% versus 63.9±4.8%, p=0.050) and first-line responders (58.4±6.2% versus 64.2±5.1%, p=0.032). Glu levels are higher in GM than in WM (Pan, Mason, Pohost, & Hetherington, 1996; Pouwels & Frahm, 1998). Accordingly, the raw metabolite ratios (not corrected for CSF content) were covaried for the proportion of GM in the voxel.
Tissue segmentation revealed that the voxel placed over the putamen, which is a subcortical GM structure, captured a large percentage of surrounding white matter; therefore, the raw metabolite ratios (not corrected for CSF content) in the putamen were covaried for the proportion of GM in the voxel.

Table 9 Voxel tissue segmentation

<table>
<thead>
<tr>
<th></th>
<th>First-line responders</th>
<th>TRS</th>
<th>UTRS</th>
<th>Controls</th>
<th>F (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DLPFC (n=58)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM (%, SD)</td>
<td>n = 15</td>
<td></td>
<td>n = 16</td>
<td>n = 11</td>
<td>n = 16</td>
</tr>
<tr>
<td></td>
<td>17.7 (5.8)</td>
<td>21.1 (7.6)</td>
<td>18.6 (5.3)</td>
<td>16.3 (5.6)</td>
<td>1.675 (0.183)</td>
</tr>
<tr>
<td>WM (%, SD)</td>
<td>80.0 (6.9)</td>
<td>75.2 (10.4)</td>
<td>78.7 (6.2)</td>
<td>81.6 (6.8)</td>
<td>1.908 (0.139)</td>
</tr>
<tr>
<td>CSF (%, SD)</td>
<td>2.3 (1.3)</td>
<td>3.6 (2.8)</td>
<td>2.7 (1.1)</td>
<td>2.0 (1.5)</td>
<td>2.210 (0.097)</td>
</tr>
<tr>
<td><strong>ACC (n=50)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM (%, SD)</td>
<td>n = 14</td>
<td></td>
<td>n = 14</td>
<td>n = 9</td>
<td>n = 13</td>
</tr>
<tr>
<td></td>
<td>64.2 (5.1)</td>
<td>58.4 (6.2)</td>
<td>58.3 (4.7)</td>
<td>63.9 (4.8)</td>
<td>4.678 (0.006)*</td>
</tr>
<tr>
<td>WM (%, SD)</td>
<td>13.0 (2.2)</td>
<td>15.2 (4.4)</td>
<td>11.7 (3.5)</td>
<td>15.1 (4.3)</td>
<td>2.390 (0.081)</td>
</tr>
<tr>
<td>CSF (%, SD)</td>
<td>22.4 (4.3)</td>
<td>26.3 (6.7)</td>
<td>29.6 (3.9)</td>
<td>20.4 (4.3)</td>
<td>7.206 (&lt;0.001)*</td>
</tr>
<tr>
<td><strong>Putamen (n=42)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GM (%, SD)</td>
<td>n = 12</td>
<td></td>
<td>n = 8</td>
<td>n = 9</td>
<td>n = 13</td>
</tr>
<tr>
<td></td>
<td>12.6 (5.4)</td>
<td>14.8 (7.5)</td>
<td>11.1 (5.2)</td>
<td>12.9 (10.5)</td>
<td>0.337 (0.799)</td>
</tr>
<tr>
<td>WM (%, SD)</td>
<td>87.4 (5.4)</td>
<td>85.0 (7.7)</td>
<td>88.9 (5.2)</td>
<td>86.7 (11.5)</td>
<td>0.336 (0.799)</td>
</tr>
<tr>
<td>CSF (%, SD)</td>
<td>0.1 (0.1)</td>
<td>0.2 (0.4)</td>
<td>0.0 (0)</td>
<td>0.4 (1.2)</td>
<td>0.732 (0.540)</td>
</tr>
</tbody>
</table>

4.5.4 1H-MRS findings

4.5.4.1 DLPFC

There were no significant differences in CSF-corrected NAA/Cr, Glu/Cr or GPC+PCh/Cr metabolite ratios in the DLPFC (Table 10). There were significant group differences in CSF-corrected Glx/Cr ratios (F=2.814, p=0.048; Table 10; column F); post hoc analysis revealed that the first-line responder group had significantly higher Glx/Cr than the group with UTRS (mean difference (MD) =0.25 (95% CI), p=0.041, Cohen’s d= 1.204). The group differences were maintained after covarying for duration of illness in the three patient groups (F=3.886, p=0.029, Table 10, column F); the first-line responder group had significantly higher Glx/Cr than the group with UTRS (mean difference (MD) =0.28 (95% CI), p=0.031).

A linear regression was carried out with Glx/Cr as a dependent variable and PANSS scores independent variables adjusted for GM and duration of illness. There was no evidence that there was a significant relationship between Glx/Cr and total PANSS scores (R²=0.046, p=0.813), PANSS positive scores (R²=0.049, p=0.672), PANSS negative scores (R²=0.046, p=0.785) or PANSS general...
scores ($R^2=0.049$, $p=0.658$). When the antipsychotic daily dose in CPZE$s$ was investigated as an independent variable adjusted for GM and duration of illness, there was also no significant correlation with Glx/Cr ($R^2=0.064$, $p=0.378$).

### Table 10 Metabolite ratios in the left dorsolateral prefrontal cortex (mean, SD)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>First-line responders</th>
<th>TRS</th>
<th>UTRS</th>
<th>Controls</th>
<th>$F^\text{a}$ (p-value)</th>
<th>$F^\text{b}$ (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>16</td>
<td>11</td>
<td>16</td>
<td>58 (patients and controls)</td>
<td>42 (patients only)</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.53 (0.25)</td>
<td>1.46 (0.25)</td>
<td>1.60 (0.31)</td>
<td>1.59 (0.23)</td>
<td>0.860 (0.467)</td>
<td>0.910 (0.411)</td>
</tr>
<tr>
<td>Glu/Cr</td>
<td>1.21 (0.17)</td>
<td>1.19 (0.24)</td>
<td>1.06 (0.15)</td>
<td>1.14 (0.19)</td>
<td>1.435 (0.243)</td>
<td>2.625 (0.086)</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>1.47 (0.25)</td>
<td>1.39 (0.27)</td>
<td>1.22 (0.15)</td>
<td>1.31 (0.19)</td>
<td>2.814 (0.048)</td>
<td>3.886 (0.029)</td>
</tr>
<tr>
<td>GPC+PCh/Cr</td>
<td>0.33 (0.05)</td>
<td>0.35 (0.03)</td>
<td>0.36 (0.04)</td>
<td>0.33 (0.04)</td>
<td>1.815 (0.155)</td>
<td>1.287 (0.288)</td>
</tr>
</tbody>
</table>

Note: 

- $^\text{a}$ Analysis of variance (ANOVA), within-group factor metabolite level, between group factor treatment group
- $^\text{b}$ Univariate analysis of covariate (ANCOVA), within-group factor metabolite level, between-group factor treatment group
- $^\text{c}$ One spectrum was rejected because of a Cramer-Rao lower bound exceeding 20%.
- $^\text{d}$ One spectrum was removed; outlier value.

#### 4.5.4.2 ACC

There were no significant differences in the raw NAA/Cr, Glu/Cr, Glx/Cr or GPC+PCh/Cr metabolite ratios (i.e. not corrected for CSF) in the ACC (Table 11); there were also no significant group differences for the CSF corrected values (data not shown). After covarying for the proportion of GM in the voxel for all four groups (Table 11, column F_a) and the proportion of GM plus the duration of illness for the three patient groups (Table 11, column F_b), there were still no significant group differences for NAA/Cr, Glu/Cr, Glx/Cr or GPC+PCh/Cr in the ACC.

### Table 11 Metabolite ratios in the anterior cingulate cortex (mean, SD)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>First-line responders</th>
<th>TRS</th>
<th>UTRS</th>
<th>Controls</th>
<th>$F^\text{a}$ (p-value)</th>
<th>$F^\text{b}$ (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>14</td>
<td>9</td>
<td>13</td>
<td>50 (patients and controls)</td>
<td>37 (patients only)</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.22 (0.26)</td>
<td>1.19 (0.17)</td>
<td>1.25 (0.23)</td>
<td>1.33 (0.17)</td>
<td>1.342 (0.273)</td>
<td>0.806 (0.456)</td>
</tr>
<tr>
<td>Glu/Cr</td>
<td>1.47 (0.24)</td>
<td>1.33 (0.22)</td>
<td>1.35 (0.18)</td>
<td>1.52 (0.31)</td>
<td>1.715 (0.177)</td>
<td>0.735 (0.487)</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>1.76 (0.36)</td>
<td>1.59 (0.33)</td>
<td>1.64 (0.20)</td>
<td>1.83 (0.44)</td>
<td>1.280 (0.293)</td>
<td>0.193 (0.826)</td>
</tr>
<tr>
<td>GPC+PCh/Cr</td>
<td>0.263 (0.034)</td>
<td>0.280 (0.035)</td>
<td>0.267d (0.021)</td>
<td>0.269 (0.021)</td>
<td>0.862 (0.468)</td>
<td>0.251 (0.779)</td>
</tr>
</tbody>
</table>

Note: 

- $^\text{a}$ Univariate analysis of covariate (ANCOVA), within-group factor metabolite level (not CSF-corrected), between-group factor treatment group, and proportion of grey matter as a covariate.
- $^\text{b}$ Univariate analysis of covariate (ANCOVA), within-group factor metabolite level (not CSF-corrected), between-group factor treatment group, and proportion of grey matter and duration of illness as covariates.
- $^\text{c}$ One spectrum was removed; outlier value.
4.5.4.3 Putamen

There were no significant differences in the raw NAA/Cr, Glu/Cr or GPC+PCh/Cr metabolite ratios (i.e. not corrected for CSF) in the putamen (Table 12); there were also no significant group differences for the CSF corrected values (data not shown). After covarying for the proportion of GM in the voxel for all four groups (Table 12, column F\(^3\)) and the proportion of GM plus the duration of illness for the three patient groups (Table 12, column F\(^b\)), there were still no significant group differences for NAA/Cr, Glu/Cr, or GPC+PCh/Cr in the putamen.

There were significant group differences in CSF-corrected Glx/Cr ratios (F=3.797, p=0.018) and the raw (i.e. not corrected for CSF; Figure 8) Glx/Cr ratios covaried for the proportion of GM within the voxel (F=3.386, p=0.028; Table 12, column F\(^a\)). Post hoc tests revealed that the group with TRS had significantly higher Glx/Cr than the group with UTRS (mean difference (MD) =0.378 (95% CI), p=0.034, FWE-corrected, Cohen’s d =1.221). The group differences were maintained after covarying for duration of illness in the three patient groups (F=3.653, p=0.041, Table 12, column F\(^b\)); the group with TRS had significantly higher Glx/Cr than the group with UTRS (mean difference (MD) =0.360 (95% CI), p=0.054).

A linear regression was carried out with Glx/Cr as a dependent variable and PANSS independent variables adjusted for GM and duration of illness. There was no evidence of a significant relationship between Glx/Cr and total PANSS scores (R\(^2\)=0.112, p=0.322), PANSS positive scores (R\(^2\)=0.100, p=0.414), PANSS negative scores (R\(^2\)=0.083, p=0.664) or PANSS general scores (R\(^2\)=0.105, p=0.370). When antipsychotic daily dose in CPZE was used as an independent variable adjusted for GM and duration of illness, there was also no significant correlation with Glx/Cr (R\(^2\)=0.196, p=0.065).
Table 12 Metabolite ratios in the left putamen (mean, SD)

<table>
<thead>
<tr>
<th></th>
<th>First-line responders</th>
<th>TRS</th>
<th>UTRS</th>
<th>Controls</th>
<th>F (p-value)a</th>
<th>F (p-value)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>8</td>
<td>9</td>
<td>13</td>
<td></td>
<td></td>
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<tr>
<td>n (patients and controls)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.06 (0.19)</td>
<td>1.01 (0.24)</td>
<td>1.22 (0.16)</td>
<td>1.12 (0.16)</td>
<td>2.173 (0.108)</td>
<td>2.180 (0.135)</td>
</tr>
<tr>
<td>Glu/Cr</td>
<td>1.12 (0.13)</td>
<td>1.23 (0.19)</td>
<td>1.08 (0.17)</td>
<td>1.16 (0.13)*</td>
<td>1.243 (0.308)</td>
<td>1.630 (0.217)</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>1.50 (0.20)</td>
<td>1.81 (0.36)</td>
<td>1.42 (0.27)</td>
<td>1.51 (0.21)*</td>
<td>3.386 (0.028)*</td>
<td>3.653 (0.041)*</td>
</tr>
<tr>
<td>GPC+PCh/Cr</td>
<td>0.258 (0.018)</td>
<td>0.254 (0.033)</td>
<td>0.271 (0.038)</td>
<td>0.267 (0.033)</td>
<td>0.798 (0.503)</td>
<td>0.722 (0.496)</td>
</tr>
</tbody>
</table>

a Univariate analysis of covariate (ANCOVA), within-group factor metabolite level (not CSF-corrected), between-group factor treatment group, and proportion of grey matter as a covariate.

b Univariate analysis of covariate (ANCOVA), within-group factor metabolite level (not CSF-corrected), between-group factor treatment group, and proportion of grey matter and duration of illness as a covariates.

c One spectrum was removed; outlier value.

Figure 8 Mean glutamate + glutamine levels scaled to creatine (Glx/Cr) for the putamen in first-line treatment responders, patients with treatment-resistant schizophrenia (TRS), patients with ultra-treatment-resistant schizophrenia (UTRS) and healthy controls. There were significant group differences in Glx ratios (F=3.653, p=0.041) covaried for proportion of grey matter in the voxel and duration of illness; the group with TRS had significantly higher Glx/Cr than the group with UTRS (mean difference (MD) =0.360 (95% CI), p=0.054).
4.6 Discussion

To our knowledge, this is the first study to compare neurometabolite levels in patients with TRS taking clozapine with first-line responders and the first report to report levels in those with UTRS. The patients were similarly responsive to their respective antipsychotic treatment which can be shown by their PANSS scores (Table 7). Evaluation with $^1$H-MRS revealed significant group differences in Glx/Cr in the DLPFC and putamen. In the DLPFC, responders to first-line treatment were found to have elevated Glx/Cr compared to those with UTRS; however, this difference disappeared when five patients who tested positive for THC were removed from the analysis (Table A 2). In the putamen, the group with TRS had significantly higher Glx/Cr than the group with UTRS and this result remained significant when participants who tested positive for THC were removed from the analysis (Table A 4). In DLPFC and putamen, there were no significant correlations between Glx/Cr ratios and PANSS total scores, PANSS subscale scores or antipsychotic dose (CPZE). There were no significant group differences in the other neurometabolites (NAA/Cr, Glu/Cr or GPC+PCh/Cr) in the DLPFC, ACC or putamen.

Overall, the groups were well-matched for demographic and clinical variables; however, there was a significant difference in the duration of illness: the first-line responders had a shorter duration of illness than the group with TRS (7.4 ±5.3 years versus 13.0 ±7.0 years, p=0.036). Progressive decreases in Glu and Gln concentrations in patients with schizophrenia were reported in a recent meta-analysis; in order to account for the group difference in the present study the duration of illness was included as a covariate (Marsman et al., 2013). Another potential confounder is smoking status; there was a higher percentage of smokers in the patient groups (87%, 56% and 73%) compared to controls (31%); which is in line with epidemiological studies of smoking rates in patients with schizophrenia (Dervaux & Laqueille, 2008). However, the effect of smoking on neurometabolite content has been reported for NAA and choline containing compounds rather than glutamatergic compounds (Durazzo, Gazdzinski, Banys, & Meyerhoff, 2004; Gallinat et al., 2007; Gallinat & Schubert, 2007; Kraguljac, Reid, White, den Hollander, & Lahti, 2012). Other
considerations are concomitant medications which were either not allowed (de la Fuente-Sandoval et al., 2013) or not reported in previous studies (Demjaha et al., 2013; Demjaha et al., 2012; Egerton et al., 2012). Adherence to treatment was based on pharmacy records and self-report while recreational drug use at the time of the study was determined using a urine screen and data analysed separately by excluding those with a positive test for recreational drugs. During the recruitment process we used stringent exclusion criteria to exclude those with a history of TBI. The main spectroscopic limitation for data acquisition was that we did not collect water unsuppressed spectra as a reference for metabolite peaks; the levels are reported as a ratio to Cr. This approach is associated with high levels of accuracy and resilience to variations in SNR but is predicated upon the assumption that there is no group difference in Cr level (Kanowski, Kaufmann, Braun, Bernarding, & Tempelmann, 2004). In support of this approach, a recent meta-analysis reported that absolute levels and ratio data were consistent in schizophrenia and that Cr did not appear to be significantly affected in the areas studied which included the DLPFC, ACC and basal ganglia (Kraguljac, Reid, White, Jones, et al., 2012). Spectroscopic data was the last sequence during a one hour fifteen minute scanning session and due to time constraints, difficulty with manual shimming and participants’ restlessness towards then end of the session, fewer voxels were acquired in the ACC and putamen than the DLPFC. However, we applied strict criteria regarding the quality of spectra included for analysis but this meant that fewer spectra for the group with UTRS were included in analysis. Despite a significant group difference in MRI FWHM in the putamen, there was no difference in LCModel FWHM or SNR in this region. There was also no group difference in any spectral quality measure within the DLPFC or ACC. We only studied the left DLPFC and left putamen to reduce imaging time. While other groups have failed to demonstrate glutamatergic laterality in patients with schizophrenia (Bustillo et al., 2011; Chang et al., 2007), we acknowledge that bilateral $^1$H-MRS examination of these regions is required to generalise these findings.
We did not find a difference in Glx/Cr or Glu/Cr between the first-line responders compared to controls. This is finding is in line with previous studies showing that response to antipsychotics is associated with normal Glu and Glx levels; de la Fuente-Sandoval et al reported that after four weeks of successful treatment with oral risperidone FEP patients (n=24) demonstrated no difference in Glx or Glu levels in the associative striatum compared to controls (n=18). In a cross-sectional study, Demjaha et al found that eight responders to non-clozapine antipsychotics had similar Glu and Glx levels to healthy volunteers (Demjaha et al., 2013). Egerton et al reported that in 15 patients taking a SGA and in symptomatic remission had lower Glx/Cr and Glu/Cr in the ACC than non-remitted patients (n=17) although this study did not include a control group of psychiatrically healthy subjects (Egerton et al., 2012).

We expected to find elevated Glx in the group with TRS based on previous reports of elevated Glu and/or Glx in non-responders (de la Fuente-Sandoval et al., 2013; Demjaha et al., 2013; Egerton et al., 2012). However, we only observed elevated Glx in the putamen of the group with TRS compared to the group with UTRS. There are several methodological differences that could account for this such as duration of illness (FEP versus schizophrenia), symptom severity and antipsychotic medication prescribed (earlier reports did not include patients taking clozapine).

Demjaha et al refer to their group as being “treatment-resistant”; however, their patients were not necessarily treated with clozapine despite a long duration of illness. In our study, the group with TRS were all stabilised on clozapine monotherapy and there were no significant differences in symptom severity between our treatment groups; PANSS total scores were 59.9 (±11.1), 59.7 (±15.0) and 62.4 (±12.5) for first-line responders, those with TRS and those with UTRS respectively. Whereas the total PANSS scores between treatment groups reported by Demjaha et al differed significantly (104.3 ±10.6 versus 50.7 ±5.8) which may have uncovered differences due in part to “state” rather than “trait” differences. Other methodological differences such as reporting metabolite ratios versus concentrations and implementing CSF-correction of metabolite peaks may also account for group differences between the present study and treatment-resistant patients in
previous reports. In the regions where we found group differences in Glx, the DLPFC and putamen, we did not observe any correlations with antipsychotic dose in CPZEs which this is in line with other studies and suggests that antipsychotics have no appreciable effect on glutamate metabolites (Aoyama et al., 2011; Bustillo et al., 2010; de la Fuente-Sandoval et al., 2009; Szulc et al., 2005; Theberge et al., 2007). In contrast an animal study found that treatment with clozapine or olanzapine, but not risperidone or aripiprazole, significantly reduced frontal cortical glutamate and glutamine but the effects in the hippocampus and striatum were less pronounced (McLoughlin et al., 2009). A further animal study suggested that this effect may be due to receptors other than D2 since six months of haloperidol treatment had no significant effect on Glu or Gln in several areas of the rat brain (Bustillo et al., 2006).

We anticipated that the group with UTRS, who were not responsive to clozapine monotherapy, would have the highest levels of glutamatergic compound of all the groups. Interestingly, this group had significantly lower Glx/Cr than responders to clozapine (group with TRS) in the putamen and were not significantly different to control subjects or other treatment groups in the DLPFC and ACC. To our knowledge, this is the first study to examine neurometabolites in patients who are clozapine-resistant (UTRS) and despite the small numbers in this group, our finding may support a proposed mechanism of action for clozapine’s unique efficacy in TRS – increased glutamatergic neurotransmission via inhibition of glycine transporters or by interacting with the glycine site of the NMDA receptor (Javitt, Duncan, Balla, & Sershen, 2004; Schwieler, Linderholm, Nilsson-Todd, Erhardt, & Engberg, 2008). NMDA receptors require both Glu and a coagonist at the glycine site to be fully active (Currá & Pallotta, 1996). Glycine coagonists are promising agents in patients stabilized on non-clozapine antipsychotics (Heresco-Levy 2005, Tsai 1998, Tsai 2006) but glycine and D-serine, do not improve symptoms for patients taking clozapine (Evins 2000, Tsai 1999) and d-cycloserine may even worsen negative symptoms in clozapine treated patients (Goff 1996 & 1999). This may be due to full occupation of the glycine site with clozapine treatment, reflected in high Glx in the group with TRS versus the group with UTRS in the present study; however, there was no
significant difference in clozapine dose between these groups (TRS n=8, mean daily clozapine dose = 369 ± 173mg/day versus UTRS n=7 (out of 9) taking clozapine as part of augmentation, mean daily clozapine dose = 386 ±157mg/day; p=0.846). In the present study, the group with TRS did not have higher Glx levels in the DLPFC or ACC; this was restricted to the putamen. It has been suggested that Glu anomalies may be restricted to DA rich regions of the brain such as the striatum (de la Fuente-Sandoval et al., 2011). Goff et al, reported a similar finding in patients switched from conventional antipsychotics to olanzapine, i.e. increased Glx/Cr in the cingulate cortex was found in patients whose negative symptoms improved following the switch (Goff et al., 2002). Therefore, while antipsychotic treatment may not directly influence glutamatergic abnormalities as discussed above, changes in glutamatergic levels may only occur in those who respond to antipsychotic treatment; i.e. an increase in Glx or Glu may be associated with response to clozapine treatment. These results are limited by the cross-sectional design of the study and require replication in prospective studies examining the response to specific antipsychotics but it may be that Glx in striatal structures such as the putamen represents a marker of response to clozapine treatment. Future studies should investigate glutamatergic anomalies in patients prior to clozapine treatment then following successful treatment and in those with UTRS taking additional glutamatergic therapies. ¹H-MRS sequences optimised to detect glycine/Cr in addition to Glx or Glu/Cr in the brain may aid the identification of those who would benefit from augmentation with a glutamatergic agent.
Chapter Five: Diffusion tensor imaging

5.1 Introduction

Schizophrenia is thought to arise from dysconnectivity which can be described as the abnormal or pathological integration of brain processes. Schizophrenia has a heterogeneous presentation including positive symptoms such as psychosis, negative symptoms such as social withdrawal and cognitive impairment. Focal brain abnormalities that can account for the range of symptoms observed in schizophrenia have not been identified; instead widespread dysconnectivity or the dysconnection hypothesis is thought to explain the heterogeneous presentation of the disorder (Stephan, Friston, & Frith, 2009). In the brain, the white matter (WM) is the anatomical infrastructure connecting grey matter (GM) regions. DTI is a non-invasive imaging technique that allows the characterization of major WM fibre bundles in vivo and has been used extensively since the late 1990s to investigate dysconnectivity in schizophrenia and WM abnormalities in several other conditions such as multiple sclerosis, traumatic brain injury and bipolar affective disorder. However, few studies have used this technique to investigate treatment resistance or responsiveness to antipsychotics in schizophrenia.

The idea that schizophrenia is the result of abnormal connections between regions is longstanding. The term “schizophrenia” coined by Bleuler refers to the “splitting” of different mental domains (Bleuler, 1911). However, Wernicke was the first to propose this idea by suggesting that psychosis was the result of anatomical disruptions of association fibre tracts which he referred to as “sejunction” or the state of being disjoined (Wernicke, 1906). Cellular and synaptic abnormalities for dysconnectivity have also been proposed, both of which are thought to have structural correlates. At the cellular level, abnormal inter-regional functional coupling might be due to structural impairments in connectivity, such as the aberrant wiring of association fibres during neurodevelopment (Bullmore, Frangou, & Murray, 1997). Disturbances in synaptic plasticity is an alternative explanation that has structural consequences which effect the distribution and
morphology of dendritic spines in addition to alterations in receptor density, phosphorylation status and sub-unit composition (Friston, 1998). These explanations are not exclusive and may coexist; however, neurophysiological, neuropharmacological, post-mortem evidence and genetic studies all support altered synaptic plasticity.

This chapter will review the basic theoretical underpinnings of DTI, the interpretation of DTI measures and the key findings in patients with schizophrenia compared to psychiatrically-healthy control subjects. Symptom sub-types that are correlated with altered diffusion measures in specific regions will be summarised. The few studies that have specifically investigated the impact of antipsychotic medication on DTI measures or used these to examine treatment resistance will also be discussed.

5.2 Principles of diffusion tensor imaging

DTI uses the movement of water molecules to estimate the directionality of WM tracts (Mori, 2007). Water molecules diffuse freely in a random manner known as Brownian motion when in unstructured space. This type of movement or motion is known as isotropic and reflects the equal probability of the water molecules moving in any given direction. When motion or diffusion is constrained by a structure such as a cell membrane then the diffusion has an apparent direction and is said to be anisotropic. For example, water molecules diffuse more freely along an axon which is a rigid, tubular structure, than across the walls of the axon; the diffusion has an apparent directionality and is anisotropic. Using DTI, magnetic field gradient pulses encode the diffusion direction in the MRI signal. Diffusion causes randomisation of the nuclear magnetic resonance (NMR) spin phase which results in signal reduction. The amount of reduction detected in a diffusion direction (or gradient) provides a quantitative measure of the diffusion that occurs in that direction. Accordingly, only diffusion occurring in the direction of a particular pulse can be detected. In order to characterise diffusion, which is a three-dimensional process, several diffusion gradients must be applied to calculate the mean diffusion in each voxel (Basser, Mattiello, & LeBihan, 1994; Pierpaoli, Jezzard, Basser, Barnett, & Di Chiro, 1996).
Fractional anisotropy (FA) values quantify the magnitude of the directional preference of diffusion in WM and range from zero (isotropic diffusion) to one (anisotropic, diffusion principally in one direction, Figure 9). The orientation of axonal bundles can be estimated from the FA values for a particular brain region; however, there are several factors to consider when interpreting these values including voxel size and whether fibres cross each other. It is desirable to collect diffusion data at as high a resolution as possible (i.e. with the smallest voxel size possible such as $1 \times 1 \times 1 \text{ mm}^3$) in order to avoid the problem of partial voluming. This occurs when the WM structure does not align perfectly with the voxel grid (Metzler-Baddeley, O’Sullivan, Bells, Pasternak, & Jones, 2012). The result is that voxels at the edges of the WM structure partially contain the WM of interest but also GM and CSF. Partial voluming can affect diffusion measures globally though it is most relevant to tracts that border the ventricles such as the fornix or the genu of the corpus callosum (Alexander, Hasan, Lazar, Tsuruda, & Parker, 2001; Metzler-Baddeley et al., 2012; Pfefferbaum & Sullivan, 2003). Furthermore, approximately 90% of WM voxels contain crossing fibres which has important implications for DTI measurements which approximate WM tracts at the macroscopic level (Jeurissen, Leemans, Tournier, Jones, & Sijbers, 2013). For example a single voxel may contain fibre populations with different spatial orientations resulting in an apparent decrease in FA (Pierpaoli et al., 2001).
Figure 9 Regions on the FA map (left) that appear white have high FA values close to one such as the white matter tracts. In areas where isotropic diffusion occurs, the tissue appears darker such as the CSF in the ventricles and the FA values are close to zero. FA values reflect the fibre orientation; in areas where there is a coherent axonal bundle the diffusion ellipsoid has one prominent direction. In areas with crossing fibres or without any boundaries to diffusion such as in the CSF, the FA values are low. The eigenvalues of the diffusion tensor, x, y and z are used to calculate diffusion measures.

In the central nervous system, oligodendrocytes produce myelin sheaths which coat the axons of neurons forming a lipid layer of insulation. The axon itself is a rigid, tubular structure sufficient to generate anisotropy in white matter. In order to test the extent to which myelination contributes to FA, Song et al compared the FA of normal and demyelinated mice (Song et al., 2002). FA was reduced by 20% in the latter group suggesting that while myelination contributes to this measure of diffusion, the axon provides the greatest FA. It is important to take these uneven contributions to FA into account since changes in the axon versus the myelin cannot be distinguished from one another on the basis of results from DTI. Boretius et al illustrated this with a mouse model of multiple sclerosis using the drug cuprizone, which causes demyelination without damaging axons, and myelin oligodendrocyte glycoprotein peptide (MOG\textsubscript{35-55}), which causes demyelination in additional to axonal damage. It was not possible to distinguish between the demyelinated group and the demyelinated plus axonal damage group using FA values (Boretius et al., 2012). However, combining DTI data with histological techniques or \textsuperscript{1}H-MRS may help to clarify the underlying pathology in WM.
Other measures obtained from DTI such as parallel diffusivity (PD) and radial diffusivity (RD) are important for delineating axonal damage versus demyelination in schizophrenia (Scheel, Prokscha, Bayerl, Gallinat, & Montag, 2013) (Figure 10). PD, also known as axial diffusivity, is defined as the first and largest eigenvalue ($\lambda_1$) and reflects water diffusion parallel to the WM fibres. Eigenvalues are coefficients of the diffusion tensor which give the magnitude of diffusion along each of three mutually orthogonal directions (which are given by corresponding eigenvectors). According to animal studies a decrease in this measure is indicative of axonal damage (Budde, Xie, Cross, & Song, 2009; Loy et al., 2007; Song et al., 2003). RD is the mean of the second and third eigenvalue and increases in RD indicate demyelination (Song et al., 2005). Mean diffusivity (MD) is the average of all three eigenvalues and reflects the amount of overall diffusion. In the case of axonal damage or demyelination FA values would be reduced (Scheel et al., 2013).

Figure 10 The diffusion tensor ellipsoid and equations for calculating fractional anisotropy (FA), mean diffusivity (MD), parallel diffusivity (PD) and radial diffusivity (RD); adapted from Scheel et al. 2013.

5.3 Findings in schizophrenia

Schizophrenia is thought to be the result dysconnectivity i.e. abnormal connections between brain regions (Andreasen et al., 1999; Friston, 2005; Friston, 1998; Friston & Frith, 1995; Kubicki, Westin,
McCarley, & Shenton, 2005). Because WM is the anatomical infrastructure connecting GM regions, the integrity of this tissue is important, it has also been suggested to play an integral part in psychotic disorders (McGuire & Frith, 1996). While structural changes such as enlarged ventricles are well documented in schizophrenia, changes that occur in the WM are not well understood.

Two main theories regarding WM alterations, the global theory and the macro-circuit theory, have been proposed. The former states that WM reductions occur uniformly throughout the brain and may be due to genetic abnormalities in the protein expression that controls myelination (Konrad & Winterer, 2008). The latter argues that specific WM tracts are disrupted either as a cause or consequence of a disorder in the GM regions they connect (Konrad & Winterer, 2008). Whole brain WM volume in patients with schizophrenia were only decreased by 1% relative to controls in a meta-analysis of 58 volumetric studies (Wright et al., 2000). A further meta-analysis of 17 voxel-based morphometry (VBM) studies revealed consistent reductions in frontal WM regions and the bilateral internal capsule in line with the macro-circuit theory (Di, Chan, & Gong, 2009).

In 1998 Buchsbaum et al. conducted the first DTI study in patients with schizophrenia to investigate the disturbances in frontal-striatal-thalamic circuitry that had been reported in psychopharmacology and animal studies (Buchsbaum et al., 1998). The authors reported that patients with schizophrenia had decreased FA and in conjunction with co-registered PET (positron emission tomography) showing decreased cellular metabolism using 2-[18F]fluoro-2-deoxy-D-glucose (FDG) in these structures, subsequently concluded that there is diminished frontal-striatal connectivity. There is now a growing body of literature about WM abnormalities in schizophrenia with more than 100 DTI studies investigating patients, high-risk populations and unaffected family members. Three meta-analyses DTI studies of patients with schizophrenia have been performed to date (Bora et al., 2011; Ellison-Wright & Bullmore, 2009; Patel et al., 2011) plus a meta-analysis of WM development in healthy adolescents (discussed further in section 5.3.1) which highlights areas that may be key targets in the neurodevelopmental abnormalities that have been associated with schizophrenia (Peters et al., 2012).
In order to identify consistent WM changes, Ellison-Wright and Bullmore carried out a meta-analysis of fifteen studies reporting the whole-brain, voxel-based analysis of FA (Ellison-Wright & Bullmore, 2009). Together the studies included 407 patients with schizophrenia and 383 controls. Using the Genome Scan Meta-analysis (GSMA) modification of activation likelihood estimation (ALE) the investigators analysed 112 coordinates of FA reductions and found no increases in FA. Two areas emerged with significantly lower FA than control subjects; thirteen out of the fifteen studies reported lower FA in the participants with schizophrenia that were within 20mm of one or other of these regions’ maxima. The first region was in the left frontal lobe deep WM; the second was located in the left temporal lobes deep WM. Several WM tracts transverse these regions; the first interconnects the frontal lobe, thalamus and cingulate gyrus and the second interconnects the frontal lobe, insula, hippocampus-amygdala and occipital lobe. The results of this meta-analysis suggest that these two networks are abnormal in schizophrenia and may contribute to the associated cognitive deficits and symptoms. Decreased FA in the fornix is associated with memory impairment and decreased FA in the left cingulum bundle with deficits in performance monitoring (Nestor et al., 2004; Nestor et al., 2007). Disruption in the inferior longitudinal fasciculus (ILF) is thought to contribute to the impaired social cognition observed in schizophrenia (Ashtari, Cottone, et al., 2007).

Building on the first meta-analysis of findings from DTI, Bora et al conducted a meta-analysis of findings from VBM using signed differential mapping (SDM) (Bora et al., 2011). Reported peak coordinates were used to create a meta-analytical maps weighted by the size of the contributing studies. The main advantage of the SDM approach over ALE is that the meta-analytical maps show both positive and negative differences between controls and the patients in the analysis. Schizophrenia was associated with decreased FA and/or WM in several regions; the interhemispheric fibres (corpus callosum), anterior thalamic radiation, inferior longitudinal fasciculi, inferior frontal occipital fasciculi, cingulum and fornix. Compared to studies of WM volume, DTI was a more sensitive measure of WM abnormalities in schizophrenia. Based on 24
DTI studies comparing 699 patients with schizophrenia and 681 healthy controls FA was significantly reduced in three clusters; the largest was in the bilateral genu of the CC extending to the anterior cingulate cortex (ACC)/medial frontal WM and the right anterior limb of the internal capsule (ALIC) and the right external capsule/corona radiata. The second largest cluster identified was in the left temporal WM, retroventricular internal capsule, external capsule including fibres from the left inferior frontal occipital fasciculi, ILF and fornix. The third cluster was detected in the right temporal WM and included fibres from the right inferior frontal occipital fasciculi and ILF. There were no clusters in which FA was higher in patients with schizophrenia than controls. Illness chronicity was associated with more severe WM deficits suggesting that the findings are biased towards the characteristics of patients with schizophrenia who have a poor prognosis.

Abnormal interhemispheric dysconnectivity is a consistent finding in patients with schizophrenia (Crow, Paez, & Chance, 2007; Hulshoff Pol et al., 2004; Mohr, Pulvermuller, Cohen, & Rockstroh, 2000). As the largest WM structure the CC connects the left and right hemispheres and has been an area of intense interest in DTI studies of patients with schizophrenia. A relatively small meta-analysis of two has shown that patients with schizophrenia have significantly lower FA in the splenium than control subjects but not in the genu (Patel et al., 2011). Data from seven studies including 202 patients with schizophrenia and 213 healthy controls yielded a modest effect size of 0.527 (p=0.001) for FA reductions in the splenium and 0.233 for the genu which was not significant. The analysis included a mix of first episode and chronic patients which may have influenced the findings which was also acknowledged. It was also noted that studies with fewer male participants reported a larger effect size for the splenium suggesting potential sex differences (Patel et al., 2011).

FA is the most commonly reported diffusion measure in patients with schizophrenia; relatively few report MD, RD and PD (Scheel et al., 2013). However, Clark et al assessed MD in addition to FA in patients with schizophrenia (n=35) and controls (n=29) (Clark et al., 2011). Compared to controls, the patient group had significantly higher MD attributed to tissue atrophy or differences in tissue
density, in several tracts including the right anterior thalamic radiations, the forceps minor, the bilateral inferior fronto-occipital fasciculus (IFO), the temporal component of the left SLF, and the bilateral uncinate. An important limitation of this study was that diffusion data were only collected in six directions. Scheel et al conducted a detailed analysis of FA reduction including PD, RD and MD in patients with schizophrenia (n=17) compared to controls (n=15) using twelve diffusion directions (Scheel et al., 2013). In patients significant FA reductions were detected throughout the WM with a frontal predominance. Corresponding increases in RD, and therefore MD, accounted for the FA reductions while no significant differences in PD were detected; however, this study was underpowered to enable the detection of subtle differences in PD. Overall, these results need to be replicated in larger samples but it is suggested that the driving force behind FA reduction in patients with schizophrenia is an increase in RD which suggests decreased myelination (Scheel et al., 2013).

5.3.1 Illness stage and DTI measures
DTI studies of healthy individuals report significant increases in FA from early childhood through to adolescence and early adulthood (Ashtari, Cervellione, et al., 2007; Snook, Plewes, & Beaulieu, 2007). This is thought to reflect several processes including increasing myelination, fibre packing density and fibre coherence (Beaulieu, 2002). Schizophrenia is thought to have neurodevelopmental origins and abnormal trajectories of development are associated with the typical onset of psychosis in adolescence (Insel, 2010; Shaw, Gogtay, & Rapoport, 2010). For these reasons, adolescence is a crucial period in development and highly relevant to the pathophysiology of schizophrenia. Peters et al conducted a DTI study of 78 healthy adolescents which they compared to a small meta-analysis of seven studies also assessing FA in healthy adolescents (Peters et al., 2012). In the study sample, bilateral increases in FA with age were observed which were most prominent in the left SLF, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, and anterior thalamic radiation. Results from the meta-analysis supported these findings; the most consistent was an increase in FA in the bilateral SLF. Furthermore, the role of the SLF in language
development was investigated in the study sample. A positive association was found between FA in the bilateral SLF and verbal working memory performance and partially mediated increases in verbal fluency as a function of increasing age.

Due to confounders such as medication and the potentially progressive nature of DTI changes attributable to the disease process in schizophrenia, first-episode schizophrenia (FES) has been the focus of several studies. Widespread reductions in FA have been reported at the time of first episode psychosis in several studies (Luck, Malla, Joober, & Lepage, 2010; Perez-Iglesias et al., 2010; Ruef et al., 2012), though some did not replicate these findings and reported no differences to controls (Lee et al., 2012; Mulert et al., 2012). Furthermore, FA reductions have been detected in patients with schizophrenia prior to antipsychotic treatment (Cheung et al., 2008; Guo et al., 2012) and in people at high risk of psychosis (Carletti et al., 2012; Clemm von Hohenberg et al., 2013). A recent systematic review of FES studies found that although the pattern of disruptions in WM is not totally consistent, the main areas affected are the frontal, fronto-temporal and fronto-limbic connections and tracts including the SLF, cingulum bundle, uncinate fasciculus and CC (Samartzis, Dima, Fusar-Poli, & Kyriakopoulos, 2013). Overall, findings in FES are less consistent than those reported in schizophrenia which may be because FA reductions are more subtle in the early stages of schizophrenia or related to the effects of medication.

For healthy adults in whom volume declines are not necessarily detectable, a review of DTI studies found there are age-related declines in FA (Sullivan & Pfefferbaum, 2006). This decline occurs in a linear fashion from about the age of 20 years onwards and it has a frontal distribution equivalent in men and women. In patients with schizophrenia, the exact progression of these changes is less clear (Fitzsimmons, Kubicki, & Shenton, 2013). While several studies (Friedman et al., 2008; Kong et al., 2011; Mitelman, Nikiforova, et al., 2009) and the meta-analysis by Bora et al (Bora et al., 2011) suggest that more severe FA reductions were associated with illness chronicity, a review of 32 studies published since this meta-analysis presented conflicting results (Fitzsimmons et al., 2013). The majority of studies presented positive findings i.e. patients with schizophrenia had
lower FA than control participants in several regions (Cui et al., 2011; Kitis et al., 2011; Kubicki et al., 2011; Miyata et al., 2012; Nakamura et al., 2012). On the other hand, a study specifically investigating the effect of age on FA in patients with schizophrenia recruited two patient groups (≤55 years and ≥56 years) and two matched control groups (Voineskos et al., 2010). Voineskos et al reported that although the young patients had lower FA than the young controls, there were no significant differences between the older patient group and the older control group; patients did not demonstrate accelerated age-related decline compared with healthy controls in any WM tract. This contradicts earlier studies and the meta-analysis conducted by Bora et al which reported that more severe FA reductions were associated with illness chronicity (Bora et al., 2011). One possible explanation for this finding is that Voineskos et al included only community-dwelling patients with cognitive scores in the non-demented range which may suggest that this highly functioning group may possess resilience to WM disruptions (Voineskos et al., 2010). In which case, the findings of this study may support those of treatment response that report better WM integrity is associated with achieving remission rather than detecting the effects of illness chronicity (Garver, Holcomb, & Christensen, 2008; Reis Marques et al., 2013). Overall, most studies indicate that WM abnormalities such as reduced FA do exist in chronic schizophrenia though their distribution and progression remain unclear and medication effects might be an important confound (discussed further in section 5.3.3).

5.3.2 Treatment response and white matter integrity
The relationship between WM alterations and treatment response was first investigated by examining WM volumes. A reduction in WM volume was observed in a structural MRI study of patients who responded to haloperidol, risperidone or ziprasidone (n=13) as determined by a reduction in the Schedule for the Assessment of Positive Symptoms (SAPS) scores (Christensen, Holcomb, & Garver, 2004). A non-significant increase in WM was reported in the non-responder group, though, this was in only three patients (Christensen et al., 2004). Only a handful of studies to date have used DTI to assess WM integrity in relation to treatment response; two cross-sectional
(Luck et al., 2011; Mitelman et al., 2006) and three longitudinal (Garver et al., 2008; Mitelman, Canfield, Newmark, et al., 2009; Reis Marques et al., 2013). These studies are discussed in detail in Chapter Six and the longitudinal studies summarised in Table 13. Overall, it appears that lower FA is associated with a poor response to treatment and a tentative hypothesis is that successful antipsychotic treatment may restore WM integrity as part of the therapeutic response.
Table 13 DTI studies examining WM and response to antipsychotic treatment

<table>
<thead>
<tr>
<th>Study</th>
<th>Follow-up period, antipsychotic medication,</th>
<th>Scanner, diffusion directions</th>
<th>Participant group</th>
<th>Findings at baseline</th>
<th>Findings at follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garver et al, 2008(Garver et al., 2008)</td>
<td>28 days Risperidone (n=5), haloperidol (n=4), ziprasidone (n=4)</td>
<td>1.5T, 6 directions</td>
<td>Responders (n=8)</td>
<td>At baseline, the patients who would go on to show a clinically significant response to antipsychotic treatment had higher MD than controls suggesting impaired WM integrity.</td>
<td>Significant decrease in MD compared to baseline measurements.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Poor responders (n=5)</td>
<td>No differences compared to controls.</td>
<td>No significant difference compared to baseline; a trend towards increased MD was detected.</td>
</tr>
<tr>
<td>Mitelman et al, 2009(Mitelman, Canfield, Newmark, et al., 2009)</td>
<td>4 years Antipsychotic medication not specified</td>
<td>1.5T, 6 directions</td>
<td>Responders (n=23)</td>
<td>Patient groups were not differentiated at baseline. Compared to controls, patients had lower FA in the fronto-parietal WM and larger posterior frontal WM volumes</td>
<td>Patient groups were not separately compared to controls at follow-up. Healthy subjects had a more pronounced decline in anisotropy compared to all patients.</td>
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<td></td>
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<td></td>
<td>Poor responders (n=26)</td>
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<td></td>
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<tr>
<td>Reis Marques et al, 2013 (Reis Marques et al., 2013)</td>
<td>12 weeks First-episode psychosis SGAs (n=58), FGAs (n=5)</td>
<td>3T, 32 directions</td>
<td>Responders (n=20)</td>
<td>No differences compared to controls.</td>
<td>Increase in FA positively correlated with exposure to antipsychotics.</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Poor responders (n=22)</td>
<td>Lower FA than responders and controls, mainly in the uncinate, cingulum and corpus callosum.</td>
<td>Increase in FA positively correlated with exposure to antipsychotics; however, FA was still lower than the responder group.</td>
</tr>
</tbody>
</table>
5.3.3 Effects of antipsychotics
Volumetric studies of the effects of antipsychotics have produced conflicting findings. Hulshoff Pol et al. reported no association between higher cumulative antipsychotic exposure and volume changes and that treatment may even be associated with less prominent volumetric changes (Hulshoff Pol & Kahn, 2008). In a longitudinal study of FES patients, Ho et al. found increased antipsychotic exposure was associated with brain volume reduction and that this association was stronger than illness related features (Ho, Andreasen, Ziebell, Pierson, & Magnotta, 2011). Differential effects of antipsychotics on brain volume have also been associated with second generation antipsychotics that are reported to have a neuroprotective effect versus first generation antipsychotics (Miyamoto, Duncan, Marx, & Lieberman, 2004). A recent meta-analysis of progressive volumetric changes and the relationship with antipsychotic medication included eight studies which investigated WM volume changes over an average of 72.4 weeks. While reductions in whole brain volume and ventricular enlargement were observed in patients with schizophrenia at baseline compared to controls, no significant differences in WM were detected at either baseline or follow-up. Progressive decreases in GM volume and ventricular enlargement were observed in patients, the GMV decreases were inversely correlated with antipsychotic exposure (Fusar-Poli et al., 2013).

Since DTI studies are able to detect WM abnormalities not apparent in volumetric studies, antipsychotic medication is frequently identified as a confounder in studies using DTI patients with schizophrenia. To reduce the contribution of this confound studies have focused on first-episode, drug naïve patients but relatively few have investigated the effects of these agents on WM integrity. A small study of twelve patients and eleven controls found that high FA in the left frontal WM correlated significantly with higher exposure to antipsychotic medication (Minami et al., 2003). Similarly Okugawa et al reported that in a sample of 25 patients with schizophrenia (plus a control group, n=21), FA in the left middle cerebellar peduncles correlated significantly with antipsychotic dose (Okugawa et al., 2004). In studies of treatment response, Reis Marques et al reported a
positive correlation between cumulative antipsychotic exposure and FA (Reis Marques et al., 2013) while Luck et al reported no significant association with antipsychotic dose (Luck et al., 2011).

Moreover, preclinical studies suggest that antipsychotics such as quetiapine can attenuate cuprizone induced demyelination (Chandran et al., 2012) and may even enhance oligodendrocyte regeneration and myelin repair (Zhang et al., 2012).

5.3.4 Symptom types
DTI has been extensively used to investigate the relationship between WM abnormalities and specific symptom types in schizophrenia either by comparing patient groups with and without a particular symptom or correlating symptom scores with DTI measures. These studies are summarized below and grouped according to the positive and negative syndrome scale (PANSS) by positive (Table 14), negative (Table 15), general (Table 16) and multiple symptom types where more than one domain is affected (Table 17).

Most studies report FA values and the most often studied symptom type is auditory verbal hallucinations (AVH). Several groups report increased FA in the SLF or arcuate fasciculus (AF) in patients experiencing AVH (Hubl et al., 2004; Rotarska-Jagiela et al., 2009; Seok et al., 2007; Shergill et al., 2007), though decreases in FA in the AF were also reported (Catani et al., 2011). Reductions in FA associated with the deficit syndrome were reported in the uncinate fasciculus, right SLF and right inferior fronto-occipital fasciculus (IFOF) (Kitis et al., 2012; Rowland et al., 2009; Voineskos et al., 2013). A variety of general symptoms and total symptom scores have also been investigated but no clear pattern has emerged. There is a considerable degree of heterogeneity in the results which may be due to several sources; voxel-based versus a region of interest (ROI) fibre tracking methods and different magnetic field strengths. A limitation of several studies is the small sample size and in particular early studies used minimal diffusion directions to acquire DTI data. Another important consideration is the impact of publication bias since studies with significant correlations are more likely to be published than those with negative findings, although there have been recent exceptions (Spalletta, Piras, Alex Rubino, Caltagirone, & Fagioli, 2013).
### 5.3.4.1 Positive symptoms

**Table 14** DTI studies in which diffusion measures are correlated with the presence of positive symptoms in patients with schizophrenia

<table>
<thead>
<tr>
<th>Authors</th>
<th>Symptom type</th>
<th>Sample</th>
<th>Scanner, diffusion directions</th>
<th>Area affected</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdul-Rahman et al, 2012 (Abdul-Rahman et al., 2012)</td>
<td>Positive; auditory hallucinations, Delusions</td>
<td>32 males with schizophrenia, 44 male controls</td>
<td>3T 15 directions</td>
<td>Arcuate fasciculus (AF), frontal aspects,</td>
<td>Patients with schizophrenia had lower FA in the frontal aspects of the left AF than healthy controls. Greater left lateralization in terms of FA and PD in the temporal lobe was associated with delusions and hallucinations (only PD was significant, not FA).</td>
</tr>
<tr>
<td>Catani et al, 2011 (Catani et al., 2011)</td>
<td>Positive; auditory hallucinations</td>
<td>28 patients with schizophrenia (17 with a history of auditory hallucinations, 11 without), 59 controls</td>
<td>1.5T 64 directions</td>
<td>Arcuate fasciculus, perisylvian fissure language pathways between posterior temporal and anterior regions in the inferior frontal and parietal lobe</td>
<td>Reduced FA observed in patients with schizophrenia. Greatest and bilateral reductions in FA were seen in patients with auditory verbal hallucinations. Patients without AVH had decreased FA only in the left AF.</td>
</tr>
<tr>
<td>Cheung et al, 2011(Cheung et al., 2011)</td>
<td>Positive subscale of PANSS</td>
<td>34 patients with first-episode schizophrenia (never medicated), 32 controls</td>
<td>1.5T 25 directions</td>
<td>R frontal lobe, L anterior cingulate gyrus, L superior temporal gyrus, R middle temporal gyrus, R middle cingulate gyrus, L cuneus</td>
<td>Reduced FA was identified in the patient group. A positive correlation was seen between positive symptom scores and FA values in the left fronto-occipital fasciculus and left inferior longitudinal fasciculus. Patients with FA values approaching control levels had the highest positive symptom scores.</td>
</tr>
<tr>
<td>Cui et al, 2011(Cui et al., 2011)</td>
<td>Positive subscale of PANSS</td>
<td>25 patients with paranoid schizophrenia (16 male, 9 female), 18 psychotic bipolar mania (10 male, 8 female), 30 controls (18 male, 12 female)</td>
<td>3T 15 directions</td>
<td>L posterior corona radiata</td>
<td>Reduced FA was associated with positive symptoms in the schizophrenia group. Reduced FA was also seen in the bipolar group however the deficits were bilateral suggesting overlapping WM pathology.</td>
</tr>
<tr>
<td>de Weijer, 2011(de Weijer et al., 2011)</td>
<td>Auditory verbal hallucinations</td>
<td>44 patients (25 male, 19 female), 42 controls (23 male, 19 female)</td>
<td>3T 30 directions</td>
<td>Arcuate fasciculus</td>
<td>FA values were significantly lower in the schizophrenia group. More severe positive symptoms were correlated with higher magnetic transfer ratio (MTR) bilaterally suggesting degraded AF integrity.</td>
</tr>
<tr>
<td>Authors</td>
<td>Symptom type</td>
<td>Sample</td>
<td>Scanner, diffusion directions</td>
<td>Area affected</td>
<td>Findings</td>
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<tr>
<td>De Weijer, 2013 (de Weijer et al., 2013)</td>
<td>Auditory verbal hallucinations</td>
<td>35 patients with schizophrenia with AVH, 35 non-psychotic individuals with AVH, 36 controls</td>
<td>3T 30 directions</td>
<td>Arcuate fasciculus</td>
<td>FA was decreased in the AF in patients with schizophrenia only suggesting decreased FA is not specifically related to hallucinations but is a feature of the disease process or schizophrenia in general. Increased MTR values were seen in both groups with AVH and could indicate increased myelination in language pathways.</td>
</tr>
<tr>
<td>Fujiwara, 2007 (Fujiwara et al., 2007)</td>
<td>Positive subscale of PANSS</td>
<td>42 patients (21 male, 21 female), 24 controls (12 male, 12 female)</td>
<td>3T 12 directions</td>
<td>Anterior and posterior cingulum</td>
<td>Reduced FA was found bilaterally in patients and the reduction was left accentuated in the anterior CBs. FA negatively correlated with positive symptom scores bilaterally in the posterior cingulum bundle.</td>
</tr>
<tr>
<td>Hubl et al, 2004 (Hubl et al., 2004)</td>
<td>Auditory verbal hallucinations</td>
<td>13 patients with AVH 13 without AVH, 13 controls (8 male, 5 female in each group)</td>
<td>1.5T 6 directions</td>
<td>Arcuate fasciculus (temporo-parietal section), anterior corpus callosum</td>
<td>Higher FA in patients with AVH than in patients without AVH or controls. Comparing the two patient groups, this difference was most pronounced in the L hemispheric fibre tracts including the cingulate bundle.</td>
</tr>
<tr>
<td>Authors</td>
<td>Symptom type</td>
<td>Sample</td>
<td>Scanner, diffusion directions</td>
<td>Area affected</td>
<td>Findings</td>
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<tr>
<td>Knöchel et al, 2012 (Knochel et al., 2012)</td>
<td>Hallucinations, positive subscale of PANSS</td>
<td>28 patients (13 male, 15 female), 18 first-degree relatives (9 male, 9 female), 22 controls (12 male, 10 female)</td>
<td>3T 6 directions</td>
<td>Arcuate fasciculus</td>
<td>FA was positively correlated with hallucination scores in patients and relatives. In patients, positive PANSS scores and psychosis-related symptoms and subjective cognitive function (assessed by the Eppendorf Schizophrenia Inventory) were also positively correlated with FA.</td>
</tr>
<tr>
<td>Mulert et al, 2012 (Mulert et al., 2012)</td>
<td>Auditory verbal hallucinations</td>
<td>10 patients with paranoid schizophrenia: 5 with AVH (4 male, 1 female), 5 without AVH (4 male, 1 female), 10 controls (8 male, 2 female)</td>
<td>1.5T 6 directions</td>
<td>Interhemispheric auditory pathway</td>
<td>Patients with AVH had higher FA than those without AVH.</td>
</tr>
<tr>
<td>Rotarska-Jagiela et al, 2009 (Rotarska- Jagiela et al., 2009)</td>
<td>Auditory verbal hallucinations</td>
<td>24 patients with AVH, 24 controls (12 male, 12 female in each group)</td>
<td>3T 6 directions</td>
<td>Arcuate Fasciculus</td>
<td>Increased FA values positively correlated with severity of AVH but also with illness duration.</td>
</tr>
<tr>
<td>Seok et al, 2007 (Seok et al., 2007)</td>
<td>Auditory hallucinations</td>
<td>15 patients with AH (8 male, 7 female), 15 patients without AH (7 male, 8 female), 22 controls (11 male, 11 female)</td>
<td>1.5T 32 directions</td>
<td>L frontal superior longitudinal fasciculus</td>
<td>While both patient groups showed decreased FA in this region, the mean FA in the hallucinating group correlated positively with severity of auditory hallucinations.</td>
</tr>
<tr>
<td>Shergill et al, 2007 (Shergill et al., 2007)</td>
<td>Auditory hallucinations</td>
<td>33 patients (30 male, 3 female), 40 controls (35 male, 5 female)</td>
<td>1.5T 64 directions</td>
<td>Superior longitudinal fasciculus, anterior cingulum</td>
<td>Increased FA was correlated with the propensity to experience AH.</td>
</tr>
<tr>
<td>Authors</td>
<td>Symptom type</td>
<td>Sample</td>
<td>Scanner, diffusion directions</td>
<td>Area affected</td>
<td>Findings</td>
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<tr>
<td>Spalletta et al, 2013 (Spalletta et al., 2013)</td>
<td>Somatic hallucinations and delusions</td>
<td>75 patients (50 male, 25 female): 22 with somatic hallucinations, 28 with somatic delusions, 75 controls (50 male, 25 female)</td>
<td>3T 30 directions</td>
<td>None</td>
<td>While increased grey matter (GM) volume was detected in the bilateral thalamic nuclei of controls, GM MD and WM FA did not differ between the patient groups with and without psychotic somatic phenomena.</td>
</tr>
<tr>
<td>Whitford et al, 2010 (Whitford et al., 2010)</td>
<td>Psychotic symptoms</td>
<td>19 male patients with chronic schizophrenia, 19 male controls</td>
<td>3T 51 directions</td>
<td>Corpus callosum</td>
<td>FA was reduced and RD was increased in the frontal fibres of the patient group suggesting myelin abnormalities. Mode increases were observed in parietal fibres in the patient group suggesting a relative absence of crossing fibres. FA was positively correlated with reality distortion scores in the frontal fibres while RD was negatively correlated with the scores.</td>
</tr>
</tbody>
</table>
### 5.3.4.2 Negative symptoms

**Table 15 DTI studies in which diffusion measures are correlated with the presence of negative symptoms in patients with schizophrenia**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Symptom type</th>
<th>Sample</th>
<th>Scanner, diffusion directions</th>
<th>Area affected</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kitis et al, 2012(Kitis et al., 2012)</td>
<td>Deficit syndrome</td>
<td>29 patients with schizophrenia (11 deficit; 7 male, 4 female, 18 non-deficit; 9 male, 9 female), 17 controls (9 male, 8 female)</td>
<td>1.5T 60 directions</td>
<td>Uncinate fasciculus</td>
<td>Reduced FA was found in the patients with deficit schizophrenia compared to non-deficit patients and controls.</td>
</tr>
<tr>
<td>Nakamura et al, 2012(Nakamura et al., 2012)</td>
<td>Avolition</td>
<td>58 patients, 58 controls (38 male, 20 female in each group)</td>
<td>1.5T 6 directions</td>
<td>Anterior corpus callosum</td>
<td>FA negatively correlated with the avolition score on the Scale for the Assessment of Negative Symptoms (SANS).</td>
</tr>
<tr>
<td>Rowland et al, 2009(Rowland et al., 2009)</td>
<td>Deficit syndrome</td>
<td>20 patients with schizophrenia (10 deficit; 10 non-deficit; 8 male, 2 female in each group), 11 controls (8 male, 3 female)</td>
<td>3T 32 directions</td>
<td>R superior longitudinal fasciculus, frontal WM</td>
<td>Reduced FA was found in the deficit group compared to controls and the non-deficit group (approaching statistical significance, p=0.07).</td>
</tr>
<tr>
<td>Voineskos et al, 2013(Voineskos et al., 2013)</td>
<td>Deficit syndrome</td>
<td>36 patients with schizophrenia (18 deficit, 18 non-deficit), 18 individually matched controls (14 male, 4 female in each group)</td>
<td>1.5T 23 directions</td>
<td>R inferior longitudinal fasciculus, R arcuate fasciculus, L uncinate fasciculus</td>
<td>Patients with deficit schizophrenia decreased FA and increased MD compared to non-deficit patients and controls. These findings were replicated by the investigators in an unmatched population based approach (same deficit group versus 59 patients with non-deficit schizophrenia, and 79 health controls).</td>
</tr>
<tr>
<td>Wolkin et al, 2003(Wolkin et al., 2003)</td>
<td>Negative symptoms</td>
<td>10 male patients</td>
<td>Magnet strength not reported 6 directions</td>
<td>Inferior frontal WM</td>
<td>FA was negatively correlated with SANS total score.</td>
</tr>
</tbody>
</table>
### General and other symptoms

#### Table 16 DTI studies in which diffusion measures are correlated with the presence of general and other symptoms in patients with schizophrenia

<table>
<thead>
<tr>
<th>Authors</th>
<th>Symptom type</th>
<th>Sample</th>
<th>Scanner, diffusion directions</th>
<th>Area affected</th>
<th>Findings</th>
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</thead>
<tbody>
<tr>
<td>Antonius et al, 2011</td>
<td>General; poor insight into illness</td>
<td>36 schizophrenia/schizoaffective patients (9 female, 27 male), no control group</td>
<td>1.5T 8 directions</td>
<td>Fronto-temporal and parietal regions</td>
<td>Reduced FA was related to misattribution of symptoms in parietal and temporal brain regions. High FA in frontal and parietal regions was related to reduced insight.</td>
</tr>
<tr>
<td>Bracht et al, 2013</td>
<td>General; volitional motor activity</td>
<td>21 patients with schizophrenia (13 male, 8 female), 21 controls (13 male, 8 female)</td>
<td>3T 42 directions</td>
<td>Cortico-basal ganglia motor pathways: right anterior cingulate cortex (rACC), pre-supplementary motor area (pre-SMA) and SMA-proper, primary motor cortex (M1), caudate nucleus, putamen, pallidum</td>
<td>Higher probability indices forming part of a bundle of interest (PIBI) values were found in patients in motor control pathways. Positive correlations between PIBI values and motor activity were found in different pathways of the motor system for each group. It was suggested that patients with schizophrenia use cortical pathways involving the supplementary motor area to compensate for basal ganglia dysfunction.</td>
</tr>
<tr>
<td>Hoptman et al, 2004 (Hoptman et al., 2004)</td>
<td>Motor Impulsivity</td>
<td>25 male schizophrenia/schizo-affective patients</td>
<td>1.5T 6 directions</td>
<td>Negative correlations: R inferior frontal WM, dorsal anterior cingulate, the insula, putamen, caudate, R middle temporal gyrus, L inferior parietal lobule Positive correlations: L postcentral gyrus, L supplementary motor area, L superior frontal gyrus, R middle and superior temporal gyrus, and L fusiform gyrus.</td>
<td>The negative correlations occurred in regions involved in response inhibition functions and suggest that dysfunction in circuitry of emotional regulation plays a role in aggressive/impulsive behaviour. The positive correlations may indicate great WM integrity in areas involved with response preparation or execution.</td>
</tr>
<tr>
<td>Authors</td>
<td>Symptom type</td>
<td>Sample</td>
<td>Scanner, diffusion directions</td>
<td>Area affected</td>
<td>Findings</td>
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<tr>
<td>Kubota et al, 2012 (Kubota et al., 2012)</td>
<td>Alexithymia (deficits in emotional self-awareness)</td>
<td>44 patients, 44 controls (26 male, 18 female in each group)</td>
<td>3T, 81 directions</td>
<td>Corpus callosum, L superior longitudinal fasciculus, L inferior longitudinal fasciculus, inferior occipito-frontal fasciculus, anterior and posterior thalamic radiation, precuneus white matter</td>
<td>FA was negatively correlated with Toronto Alexithymia Scores (TAS-20).</td>
</tr>
<tr>
<td>Leitman et al, 2007 (Leitman et al., 2007)</td>
<td>Affective prosody</td>
<td>19 schizophrenia/schizo-affective patients (17,2; 18 male, 1 female), 19 controls</td>
<td>1.5T 6 directions</td>
<td>VOICEID task correlations: Medial geniculate nucleus, primary auditory radiations, dorsal and ventral stream auditory projections, corpus callosum, orbitofrontal cortex, cingulum Distorted tunes task correlations: Primary auditory radiations, dorsal and ventral stream auditory projections, amygdala.</td>
<td>FA was negatively correlated with voice emotion identification task (VOICEID) scores which measures the ability to decode emotions based on tone of voice. FA was also negatively correlated with distorted tunes task scores which measure the ability to detect incorrect notes within common melodies. These results suggest that deficits in lower-level auditory processing contribute to higher order failures of neurocognition.</td>
</tr>
<tr>
<td>Sim et al, 2009</td>
<td>Passivity phenomenon (feelings that one’s experiences are controlled by an external agency)</td>
<td>11 patients with passivity (6 male, 5 female), 25 without passivity (21 male, 4 female), 32 controls (19 male, 13 female)</td>
<td>3T 15 directions</td>
<td>Frontal cortex WM, cingulate gyrus, basal ganglia, thalamus</td>
<td>Increased FA was observed in patients with passivity phenomenon in the regions identified except for the thalamus which showed decreased FA.</td>
</tr>
<tr>
<td>Walther et al, 2011(Walther et al., 2011)</td>
<td>Motor activity</td>
<td>19 patients (12 male, 7 female), 24 controls (11 male, 13 female)</td>
<td>3T 42 directions</td>
<td>R supplemental motor area (SMA), R precentral gyrus, posterior cingulum</td>
<td>FA was negatively correlated with activity levels in the patient group.</td>
</tr>
</tbody>
</table>
### Multiple symptom types

<table>
<thead>
<tr>
<th>Authors</th>
<th>Symptom type</th>
<th>Sample</th>
<th>Scanner, diffusion directions</th>
<th>Area affected</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asami et al, 2013 (Asami et al., 2013)</td>
<td>Positive, negative and general; disorganised thoughts and attention deficit</td>
<td>26 male patients with schizophrenia, 25 male controls</td>
<td>3T 51 directions</td>
<td>Middle longitudinal fasciculus (MdLF)</td>
<td>Patients with schizophrenia had lower FA in the bilateral MdLF. FA of the left MdLF was negatively correlated with the Disorganized Thoughts Factor while FA of the right MdLF showed a negative correlation with Poor Attention scores.</td>
</tr>
<tr>
<td>Boos et al, 2013 (Boos et al., 2013)</td>
<td>Total PANSS and positive, negative and general subscales</td>
<td>126 patients with schizophrenia (101 male, 25 female), 123 of their non-psychotic siblings (56 male, 67 female), 109 controls (54 male, 55 female)</td>
<td>1.5T 32 directions</td>
<td>Arcuate fasciculus</td>
<td>Patients with higher PANSS scores showed lower FA bilaterally in the AF.</td>
</tr>
<tr>
<td>Herbsman et al, 2010 (Herbsman &amp; Nahas, 2010)</td>
<td>Total PANSS scores</td>
<td>13 unmedicated patients (but were previously medicated, no medication history was obtained), 16 controls</td>
<td>3T 6 directions</td>
<td>Anterior portion of the occipital lobe bordering on the parietal lobe</td>
<td>FA correlated bilaterally with total PANSS scores however, only the cluster details for the L forceps major were reported. There was a positive correlation with total PANSS scores in this region.</td>
</tr>
<tr>
<td>Michael et al, 2008</td>
<td>Total PANSS and positive, negative and general subscales</td>
<td>45 patients (33 male, 12 female), no control group (since the study aimed to explicitly correlate PANSS scores with DTI measures and controls would all score the minimum values on the PANSS)</td>
<td>3T 12 directions</td>
<td>Corpus callosum, R superior longitudinal fasciculus, L inferior longitudinal fasciculus</td>
<td>FA negatively correlated with all three PANSS scores. Mean diffusivity and axial diffusivity positively correlated with PANSS scores, suggesting disruption of WM integrity. Radial diffusivity (which is thought to reflect dysmyelination or demyelination) did not correlate with the scores.</td>
</tr>
<tr>
<td>Authors</td>
<td>Symptom type</td>
<td>Sample</td>
<td>Scanner, diffusion directions</td>
<td>Area affected</td>
<td>Findings</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>-------------------------------</td>
<td>------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Skelly et al, 2008 (Skelly et al., 2008)</td>
<td>Positive and negative PANSS subscales</td>
<td>25 patients with chronic schizophrenia (18 male, 7 female), 25 controls (16 male, 9 female)</td>
<td>3T 12 directions</td>
<td>uncinate fasciculus, R sagittal stratum, L superior longitudinal fasciculus,</td>
<td>FA values were negatively correlated with positive PANSS scores. FA values were positively correlated with negative PANSS scores in one region only: R insula.</td>
</tr>
<tr>
<td>Szeszko et al, 2008 (Szeszko et al., 2008)</td>
<td>Positive and negative symptoms</td>
<td>33 patients with recent onset schizophrenia (21 male, 12 female), 30 controls (18 male, 12 female)</td>
<td>1.5T 25 directions</td>
<td>Bilateral uncinate fasciculus, inferior fronto-occipital fasciculus</td>
<td>Low FA was correlated with greater severity of negative symptoms (alogia, affective flattening, and worse verbal learning/memory functioning) in the uncinate fasciculus. High FA in the inferior fronto-occipital fasciculus correlated with more severe delusions and hallucinations.</td>
</tr>
</tbody>
</table>
5.4 Conclusion

This chapter has described the basic theory of DTI and reviewed findings in patients with schizophrenia including the limited number of studies regarding treatment response. In patients with schizophrenia widespread reductions in FA and increases in MD have been observed which suggest decreased WM integrity relative to control subjects. Decreased FA is present at the FES and prior to treatment with antipsychotic medication. Most studies report reduced FA in patients with chronic schizophrenia though their distribution and progression remain unclear and may be influenced by medication effects. Poor response to antipsychotics was associated with low FA in several regions relative to controls and patients with schizophrenia who respond well to treatment in DTI studies investigating treatment response. Several studies have investigated the relationship between WM abnormalities and specific symptom types in schizophrenia; the most often studied symptom type is auditory verbal hallucinations (AVH). A variety of general symptoms and total symptom scores have also been investigated but no clear pattern has emerged. The aims and hypotheses of the study presented in this thesis are based on the findings reviewed above. The next chapter presents the DTI findings three groups of people with schizophrenia, stratified by treatment response. These findings are in the form of a journal article submitted for publication.
Chapter Six: White matter integrity is reduced in treatment-resistant but not ultra-treatment-resistant schizophrenia

Meghan E. Mcilwain, Valerie M. Anderson, Frederick Sundram, Rob R. Kydd, Bruce R. Russell

6.1 Abstract

Background: Compromised white matter (WM) integrity is a well-replicated finding in people with schizophrenia and there is some evidence to suggest WM changes are related to response to antipsychotic treatment. This study tested the hypothesis that individuals with treatment-resistant schizophrenia (TRS) could be differentiated from responders to first-line medication, those with ultra-treatment-resistant schizophrenia (UTRS) and healthy controls based on WM integrity.

Methods: Diffusion tensor imaging (DTI) data were obtained from first line responders taking a second generation non-clozapine antipsychotic (n=18), clozapine responsive monotherapy (TRS; n=18), a group who did not respond well to clozapine monotherapy and were taking a combination of two antipsychotics i.e. clozapine-resistant or UTRS, (n=14) and healthy controls (n=20). Tract-based spatial statistics (TBSS) was used to compare fractional anisotropy (FA), mean diffusivity, parallel diffusivity and radial diffusivity across the groups.

Results: Symptom severity was similarly low in all treatment groups. The group with TRS had significantly lower FA compared to healthy controls in the superior longitudinal fasciculi (SLF; bilateral), the corpus callosum including the posterior corona radiata (bilateral) and the corticospinal tract (bilateral). Lower FA in the group with TRS was also detected in the right SLF compared to the group with UTRS. The first-line responders and the group with UTRS were indistinguishable from the healthy controls.
Conclusions: This study suggests that DTI measures may be useful biomarkers of TRS. However, this did not apply to the group with UTRS which may represent a subtype of schizophrenia that has a distinct underlying pathophysiology.

6.2 Introduction

Treatment-resistant schizophrenia (TRS) is a relatively common subtype of schizophrenia, which affects between 30% to 60% of patients and is a costly condition both socially and economically (Kennedy et al., 2013; Lehman A, 2004). Clozapine is the most effective antipsychotic for those with TRS; however, omitting those who are intolerant to clozapine, 50-70% do not experience clinically significant improvements in their symptoms (clozapine-resistant or ultra-treatment-resistant schizophrenia; UTRS) (Buckley et al., 2001; Chakos et al., 2001; Mouaffak et al., 2006). Treatment-resistant patients experience a significantly lower quality of life than those in remission. Conservative estimates of the financial burden estimate that more than $34 billion annually is spent in direct medical costs within the USA alone (Kennedy et al., 2013).

It has been suggested that TRS may have a neurobiological basis. Structural MRI studies of the brain have revealed that treatment resistance is associated with morphological features such as lower volumes in most brain structures (Lawrie et al., 1995) and reduced grey matter volume (Bodnar et al., 2012; Szoszko et al., 2012). White matter (WM) is the anatomical infrastructure connecting grey matter regions with disruptions also reported in schizophrenia (Andreasen et al., 1999; Friston, 2005; Friston, 1998; Friston & Frith, 1995; Kubicki et al., 2005). However, the relationship between white matter changes and treatment-resistance is unclear. This may be due to differences between patient groups (First episode psychosis (FEP) versus schizophrenia), the medications prescribed, the definition of treatment response employed or DTI acquisition parameters and statistical analysis (Reis Marques et al., 2013).

There have been several DTI studies investigating chronic schizophrenia and some have included patients receiving clozapine or a combination of antipsychotics however the participants were not
analysed as separate groups (Camchong, MacDonald, Bell, Mueller, & Lim, 2011; Kubicki et al., 2008; Whitford et al., 2010). Recently Holleran et al compared patients with schizophrenia prior to commencing clozapine with age and gender matched healthy controls (Holleran et al., 2013). While this study has the advantage of assessing TRS before the potential confound of clozapine treatment there was no comparison made with those who respond well to first-line antipsychotic treatment.

A handful of studies utilising DTI have investigated WM integrity in response to treatment with antipsychotics and shown conflicting results. A small longitudinal study assessing whole-brain mean diffusivity (MD) found that responders to antipsychotics (n=8) had increased MD (impaired WM integrity) compared with healthy controls at baseline. Response to treatment with haloperidol, risperidone or ziprasidone over a 28 day period was defined as a >60% reduction of baseline Schedule for Assessment of Positive Symptoms (SAPS) scores or a reduction of the total SAPS psychosis score to <10. However, at follow up the responders showed a significant decrease in MD compared to baseline (interpreted as a restorative effect) while the non-responders (n=5) showed a trend towards increased MD (Garver et al., 2008). A cross-sectional study using the Keefe criteria to define poor outcome, averaged FA values across Brodmann’s areas (Mitelman et al., 2006). The good outcome group (n=51) had less extensive FA reductions in the whole brain than the poor outcome group (n=53), higher FA in frontal and cingulate regions and increases in regional WM volumes particularly in the left hemisphere (Mitelman et al., 2006). A follow-up study of this cohort where patients were rescanned after four years found that the poor outcome group (n=26) had lower FA in frontal and parietal regions than the good outcome group (n=23) (Mitelman, Canfield, Newmark, et al., 2009).

Another cross-sectional study using DTI-tractography examining the response to antipsychotics in first episode psychosis (FEP) produced similar findings (Luck et al., 2011). Response to atypical antipsychotics was assessed using the Remission in Schizophrenia Working Group (RSWG) criteria (Andreasen et al., 2005). Poor response to treatment (n=24) was associated with lower FA along
the uncinate and superior longitudinal fasciculus (SLF) (n=20). The largest and most recent study of WM integrity and treatment response evaluated FEP patients after 12 weeks of antipsychotic treatment and the RSWG criteria to define response (Reis Marques et al., 2013). Post-hoc analyses revealed that at baseline non-responders (n=22) had lower FA than either the healthy controls (n=52) or responders (n=20) in the uncinate, corpus callosum and the cingulum. In contrast at baseline the responders were indistinguishable from controls. After 12-weeks, there was an increase in FA in regions that differentiated responders from non-responders (the uncinate, corpus callosum and cingulum) which positively correlated with cumulative antipsychotic exposure (Reis Marques et al., 2013).

In summary, while there are studies investigating schizophrenia and structural determinants of resistance to antipsychotics, there have been no reports of DTI measures associated with clozapine response or resistance. In this study, we recruited three groups of patients with established schizophrenia; first line responders taking a second generation non-clozapine antipsychotic, clozapine responsive monotherapy (treatment-resistant schizophrenia (TRS) and a group who did not respond well to clozapine monotherapy and were taking a combination of two antipsychotics i.e. clozapine-resistant or ultra-treatment-resistant schizophrenia (UTRS). These groupings are consistent with a recently proposed and new classification of schizophrenia subtypes, based on treatment responses which are in line with current treatment algorithms (Association, 2004; Farooq et al., 2013; NICE, 2002; Royal Australian New Zealand College of Psychiatrists, 2005). A voxel-based approach was chosen to assess all brain regions without a priori hypotheses since this is the first DTI study to approach treatment classification in this way. Furthermore, we aimed to control for differences in illness stage by matching for duration of illness across the groups, which may have led to inconsistent findings in earlier studies. We predicted that a reduction in WM integrity would be more pronounced in those less responsive to antipsychotic medication. On the basis of the earlier studies reported above, we hypothesised that: 1) the patients with schizophrenia would have lower FA than control subjects; 2) the first-line responders would have
higher FA than the groups with TRS and UTRS; 3) the group with UTRS would have the lowest FA of the treatment groups.

6.3 Method
6.3.1 Participants
This study was approved by the Regional Ethics Committee. Patients who met the DSM-IV criteria for schizophrenia were identified by their treating clinician either from a community mental health centre or a forensic psychiatric inpatient unit; the diagnosis was checked against patient notes. Subsequent to obtaining written informed consent, data regarding duration of illness and medication history were collected and then verified from clinical notes and patient interviews. All participants were stabilised on antipsychotic medication for at least six weeks and categorised according to their treatment history and response to antipsychotic medication. Patient classification (first-line responder, TRS or UTRS) was based on the treatment type at the time of the study and prescribed in accordance with criteria for TRS using published algorithms (Association, 2004; NICE, 2002; Royal Australian New Zealand College of Psychiatrists, 2005). These algorithms define TRS in patients who do not experience significant symptom improvement after trials with at least two antipsychotic agents using therapeutic doses for at least six weeks. The three treatment groups in this study comprised those taking i) a second generation (atypical) non-clozapine antipsychotic (first-line responders), ii) clozapine monotherapy (clozapine-responsive, TRS), or iii) a combination of antipsychotics (clozapine-resistant; UTRS) having failed a trial of clozapine monotherapy. Clozapine could be one of the antipsychotics used in combination or the combination could be two alternative antipsychotics as long as failure with clozapine monotherapy could be confirmed from clinical notes as opposed to clozapine cessation or down-titration secondary to side-effects. A healthy control group with no history of mental or neurological illness was recruited through advertising in the community. Age, sex, years of education and ethnicity were matched on a group basis. All participants were between the ages of 18 and 45. Exclusion
criteria included a history of traumatic brain injury (loss of consciousness for more than three minutes), neurological illness, significant physiological comorbidity or contraindication to MRI.

Symptom severity was assessed using the Positive and Negative Symptom Scale (PANSS) and scores were also converted to Clinical Global Impression for Schizophrenia (CGI) scores based on severity but not improvement (Kay et al., 1987). All PANSS interviews were conducted by the same trained investigator. The findings from clinical interviews were corroborated with the patients’ family, caregiver or mental health professional (treating psychiatrist or key worker).

The World Health Organisation’s Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) was used to collect recreational drug use histories from all participants (Newcombe et al., 2005). ProScreen Cups© (US Diagnostics Inc., PSCupA-6MBAU) were used to screen urine samples collected at each study session for amphetamines, cocaine, benzodiazepines, tetrahydrocannabinol (THC) or opiates. For detection limits, please refer to Appendix 1: Detection limits for ProScreen Cups© recreational drug screen kit. Standardised premorbid IQ scores were determined using a computerised version of the “spot the real word” test within the IntegNeuro test battery (Brain Resource Company, Sydney, Australia) (Baddeley et al., 1993; Gordon, 2003; Gordon et al., 2005). Antipsychotic chlorpromazine equivalents (CPZE) were calculated to compare daily doses using formulae with power transformation (Andreasen et al., 2010), except for amisulpride which in the absence of a power formula was calculated using expert consensus regarding antipsychotic dosing (Gardner et al., 2010). The sample size was based on previous studies examining the relationship between treatment outcomes and DTI measures in schizophrenia (Garver et al., 2008; Luck et al., 2011; Mitelman, Nikiforova, et al., 2009). WM integrity was measured by FA, mean diffusivity (MD), parallel diffusivity (PD) and radial diffusivity (RD) data using TBSS (Tract-Based Spatial Statistics) (Smith et al., 2006), part of FSL (Smith et al., 2004) on a Grid Engine Linux cluster.
6.4 Image acquisition

Imaging was performed using a 3T Siemens Magnetom Skyra (Siemens, Germany). A 32-channel head coil was used for the majority of scans; where the participant could not fit comfortably into this coil, a 20-channel head coil was used (n=5). Diffusion-weighted images were acquired using an echo planar imaging (EPI) sequence with the following parameters: repetition time (TR) 8900 ms, echo time (TE) 95 ms, field of view (FOV) 240 mm, 122 x 122 matrix, 2.0 mm slice thickness, isotropic voxel size 2.0 x 2.0 x 2.0 mm$^3$. An acceleration factor (GRAPPA) of two was used. Sixty-seven slices were acquired parallel to the anterior commissure-posterior commissure (AC-PC) line with diffusion-weighting factor b=1000s/mm$^2$ in 64 gradient directions. Eight scans without diffusion weighting (b=0) scans were acquired. Prior to assessing acquired scans via the image processing pipeline, scans were examined for artefacts including subject motion, excess signal-to-noise and incomplete acquisitions. First, FA images were created by fitting a tensor model to the raw diffusion data using FMRIBs Diffusion Toolbox (FDT), and then brain-extracted using the Brain Extraction Tool (BET) (Smith, 2002). All subjects' FA data were then aligned to the FMRIB58_FA target image using the nonlinear registration tool FNIRT (Andersson, Jenkinson, & Smith, 2007a, 2007b) which uses a b-spline representation of the registration warp field (Rueckert et al., 1999) and transformed into standard space (Montreal Neurologic Institute (MNI) Image 152 standard). Next, the FA image across subjects was created and thinned at a threshold of 0.3 to exclude low anisotropic regions and create a mean FA skeleton which represents the centres of all WM tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton. Anatomical localisation of significant WM clusters was determined using the probabilistic cortical, subcortical and WM tractography atlases provided in FSL.

6.5 Statistical analysis

Whole-brain statistical analyses were performed using Randomise (v2.9). Threshold-free cluster enhancement was used for statistical comparisons, using a nonparametric permutation test, in which group membership was permuted 5000 times to generate a null distribution for each
contrast. Unpaired t-tests were performed to explore group differences and a single regression
correlation (Pearson) using Randomise (v2.9) was performed to investigate the association
between FA and daily antipsychotic dose in CPZE. Significant clusters were identified where voxel-
wise p-values were corrected for multiple comparisons (p<0.05, Family-Wise Error corrected). Age
and sex were demeaned prior to analysis and included as covariates of no interest (Bose et al.,
2009). Analyses of socio-demographic and clinical characteristics were conducted using the
Statistical Package for Social Sciences (SPSS) version 19.0 software package. Analyses of Variance
(ANOVAs) were used to compare continuous variables with Tukey correction for multiple
comparisons.

6.6 Results

6.6.1 Participants

A total of 70 people (20 controls and 50 patients) were included in the final analysis. The
demographic and clinical data of the study participants are summarized in Table 18. There were no
significant group differences in sex, age, duration of illness, duration of untreated psychosis,
 premorbid IQ, PANSS total scores, or any of the subscales or CGI scores. There was a significant
group difference in years of education (F(3,66)=4.1, p=0.01); post hoc tests showed that the group
with TRS had fewer years of education than controls (p<0.01). Antipsychotic medication used by
first-line responders included olanzapine (n=8), risperidone (n=6), aripiprazole (n=3) and
 amisulpride (n=1). The combinations of antipsychotics in the group with UTRS were clozapine and
 amisulpride (n=4), clozapine and aripiprazole (n=4), clozapine and risperidone (n=2), clozapine and
 quetiapine (n=1). There were three individuals who failed a trial of clozapine monotherapy due to
 inadequate response whose current antipsychotic medications did not include clozapine:
 aripiprazole and quetiapine (n=2) and risperidone and quetiapine (n=1). Seven participants tested
 positive for THC but no other recreational drugs at the time of study (first-line responders n=3, TRS
 n=1, UTRS n=3).
Table 18 Demographic data (mean (SD) unless otherwise stated) of study participants by treatment group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>First-line responders (n=18)</th>
<th>TRS (n=18)</th>
<th>UTRS (n=14)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n)</td>
<td>14</td>
<td>14</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Female (n)</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>32 (8)</td>
<td>33 (8)</td>
<td>35 (7)</td>
<td>33 (8)</td>
</tr>
<tr>
<td><strong>Education (years)</strong></td>
<td>12 (2.8)</td>
<td>11 (2.7)*</td>
<td>13 (2.1)</td>
<td>14 (2.0)*</td>
</tr>
<tr>
<td><strong>Premorbid IQ (standardized scores)</strong></td>
<td>-0.781 (0.982)*</td>
<td>-0.955 (1.170)</td>
<td>-0.885 (0.883)</td>
<td>-0.838 (1.286)*</td>
</tr>
<tr>
<td><strong>Duration of illness (years)</strong></td>
<td>10 (7.9)</td>
<td>13 (6.7)</td>
<td>12 (4.8)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Duration of untreated psychosis (months)</strong></td>
<td>12 (14.7)</td>
<td>11 (18.1)</td>
<td>23 (27.5)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Psychiatric ratings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PANSS total score</td>
<td>59.5 (11.0)</td>
<td>59.6 (14.1)</td>
<td>61.2 (10.9)</td>
<td>-</td>
</tr>
<tr>
<td>PANSS positive subscale score</td>
<td>13.4 (5.4)</td>
<td>11.9 (5.5)</td>
<td>12.6 (5.1)</td>
<td>-</td>
</tr>
<tr>
<td>PANSS negative subscale score</td>
<td>17.1 (5.7)</td>
<td>18.8 (6.8)</td>
<td>19.5 (7.6)</td>
<td>-</td>
</tr>
<tr>
<td>PANSS general subscale score</td>
<td>28.9 (6.0)</td>
<td>28.8 (6.2)</td>
<td>29.1 (4.4)</td>
<td>-</td>
</tr>
<tr>
<td>CGI severity positive score</td>
<td>2.21 (0.95)</td>
<td>1.98 (1.14)</td>
<td>2.24 (0.95)</td>
<td>-</td>
</tr>
<tr>
<td>CGI severity negative score</td>
<td>2.09 (0.92)</td>
<td>2.22 (1.05)</td>
<td>2.36 (1.03)</td>
<td>-</td>
</tr>
<tr>
<td>CGI severity depressive score</td>
<td>2.04 (0.73)</td>
<td>2.02 (1.00)</td>
<td>1.62 (0.81)</td>
<td>-</td>
</tr>
<tr>
<td>CGI severity cognitive score</td>
<td>2.43 (0.91)</td>
<td>2.51 (1.04)</td>
<td>2.50 (0.88)</td>
<td>-</td>
</tr>
<tr>
<td>Dose at time of scan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(chlorpromazine equivalents)</td>
<td>421.6 (191.6)</td>
<td>438.2 (229.3)</td>
<td>843.2 (377.4)*</td>
<td>-</td>
</tr>
<tr>
<td>Positive test for THC (n)</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: *p<0.05, FWE-corrected

No premorbid IQ score for one participant

*TRS = treatment-resistant schizophrenia, UTRS = ultra-treatment-resistant schizophrenia, PANSS = Positive and Negative Symptom Scale, CGI = Clinical Global Impression, THC = tetrahydrocannabinol

6.7 TBSS results

6.7.1 Patients versus healthy controls

No statistically significant differences in FA, MD, PD or RD were found between controls or people with schizophrenia. Similarly pair-wise comparisons showed no significant differences in any diffusion measures between controls and either the first-line responders or the group with UTRS.
Five significant clusters were found in which the group with TRS had lower FA and PD than controls (p<0.05, FWE-corrected; Table 19, Figure 11, Figure 12).

Table 19 White matter regions of lower FA and PD in the group with TRS compared to healthy controls

<table>
<thead>
<tr>
<th>FA clusters</th>
<th>MNI152 Co-ordinates of peak voxel (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Matter Area/Tract</td>
<td>Significant cluster size (number of voxels)</td>
</tr>
<tr>
<td>Corpus callosum (CC) forceps major (R), body of CC (R), superior longitudinal fasciculus (SLF; R), corticospinal tract (CST) (R)</td>
<td>2335</td>
</tr>
<tr>
<td>CC forceps major (L), SLF (L), inferior longitudinal fasciculus (ILF; L), cingulum (L), inferior fronto-occipital fasciculus (IFOF; L), anterior thalamic radiation (L)</td>
<td>1982</td>
</tr>
<tr>
<td>Anterior thalamic radiation (L), CST (R), CC Anterior corona radiata (R), uncinate fasciculus (R), CC body Forceps minor (R)</td>
<td>1577</td>
</tr>
<tr>
<td>CC; body, splenium and forceps major (L), CST (L), SLF (L), SLF temporal part (L)</td>
<td>1557</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PD clusters</th>
<th>MNI152 Co-ordinates of peak voxel (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Matter Area/Tract</td>
<td>Significant cluster size (number of voxels)</td>
</tr>
<tr>
<td>Inferior fronto-occipital fasciculus (R), Uncinate fasciculus (R), CC body Forceps minor (R)</td>
<td>153</td>
</tr>
<tr>
<td>CC splenium (L), Forceps major (L)</td>
<td>80</td>
</tr>
<tr>
<td>CC Anterior corona radiata (R), uncinate fasciculus (R)</td>
<td>39</td>
</tr>
<tr>
<td>Inferior longitudinal fasciculus (L), CC Forceps major (L)</td>
<td>38</td>
</tr>
<tr>
<td>CC Forceps major (L)</td>
<td>8</td>
</tr>
</tbody>
</table>

Note: FA = Fractional anisotropy, PD = parallel diffusivity, MNI = Montreal Neurological Institute
Figure 11 White matter maps showing lower FA in the group with TRS compared to the healthy controls (p<0.05, FWE- corrected). Red-yellow voxels represent regions in which FA is significantly lower in the group with TRS relative to controls. The FA white matter skeleton is represented by green voxels and the images are overlaid on to the mean FA image in MNI 152 brain space (radiologic view with Z co-ordinates). FA= fractional anisotropy; TRS = treatment-resistant schizophrenia; FWE = family-wise error; MNI = Montreal Neurological Institute

Figure 12 White matter maps showing significantly lower PD in the group with TRS compared to the healthy controls (p<0.05, FWE- corrected). Red-yellow voxels represent regions in which PD is significantly lower in the group with TRS relative to controls. The FA white matter skeleton is represented by green voxels and the images are overlaid on to the mean FA image in MNI 152 brain space (radiologic view with Z co-ordinates). PD = parallel diffusivity, FA= fractional anisotropy; TRS = treatment-resistant schizophrenia; FWE = family-wise error; MNI = Montreal Neurological Institute
6.7.2 Comparison of treatment groups

No statistically significant differences in diffusion measures were detected between first-line responders and either the group with TRS or the group with UTRS. The group with TRS had significantly lower FA than the group with UTRS in the right SLF (p<0.05, FWE-corrected; MNI peak coordinates x=50, y=104, z=103; 366 voxels; t= 4.94; Figure 13). No significant differences in any other diffusion measures were observed between the group with TRS and the group with UTRS.

![Figure 13](image)

**Figure 13** White matter maps showing significantly lower FA in the group with TRS than the group with UTRS (p<0.05, FWE-corrected). Red-yellow voxels represent regions in which FA is significantly lower in the group with TRS relative to the group with UTRS. The FA white matter skeleton is represented by green voxels and the images are overlaid on to the mean FA image in MNI 152 brain space (radiologic view, co-ordinates: X=50, Y=104, Z=103). FA= fractional anisotropy; TRS = treatment-resistant schizophrenia; UTRS = ultra-treatment-resistant schizophrenia; FWE = family-wise error; MNI = Montreal Neurological Institute

6.7.3 Correlation with antipsychotic dose

Using TBSS and Randomise there was no area identified where FA, MD, PD or RD correlated with antipsychotic daily dose in CPZEs.

6.8 Discussion

To our knowledge, this is the first study to investigate white matter integrity and the response to treatment in patients with TRS taking clozapine and patients with UTRS (Farooq et al., 2013). While there was variation in patient response to medication type, there were no differences in symptom severity scores using the PANSS. Using DTI, we found that first-line responders and those with UTRS were not significantly different to healthy controls in any diffusion measures; however the group with TRS had lower FA and PD in several regions relative to controls. There were no
significant differences between the treatment groups except for those with UTRS which had significantly higher FA in the SLF compared to those with TRS.

Our results for the group of first-line responders and those with TRS support our prediction i.e. that more disrupted WM integrity would be associated with treatment resistance. These findings are in agreement with a study of patients with first-episode psychosis which found responders to antipsychotics were not different to controls (Reis Marques et al., 2013) and a study of patients with TRS prior to initiation of clozapine treatment that reported widespread reductions in FA (Holleran et al., 2013). The latter study by Holleran et al reported reduced FA in several regions replicated by our study; throughout the CC, bilaterally in the SLF, and the ILF. Holleran et al also detected increased RD but no differences in PD in patients with TRS, in contrast we report the opposite. We found no differences in RD and five clusters mostly in the CC of lower PD in patients with TRS compared to controls which suggests axonal damage in these regions as opposed to myelin abnormalities. While the studies contained similar numbers of participants and both used 64 diffusion gradients, our data was collected at a higher field strength (3T versus 1.5T). These contrasting findings may represent a medication effect since the main difference between patients with TRS in these studies is exposure to clozapine.

We predicted the group with UTRS would have the lowest FA of the groups but intriguingly, this group was no different to healthy controls. This may mean that UTRS represents a subtype of schizophrenia in which WM integrity plays a minor role and the lack of response to treatments should be further investigated, for example for differences in GM abnormalities (Palaniyappan et al., 2013). Those with UTRS were taking significantly higher doses of antipsychotics at the time of imaging than the first-line responders or those with TRS; 421.6 (±191.6) and 438.2 (±229.3) versus 843.2 (±377.4) CPZEs, respectively. The higher exposure to antipsychotic doses in this group may have had pro-myelinating or protective effects on WM integrity, which has been suggested by animal studies (Chandran et al., 2012; Zhang et al., 2012) but unlike Reis Marques et al we did not detect a correlation between antipsychotic exposure and FA or other diffusion measures (Reis
Marques et al., 2013). Our calculations were based on antipsychotic dose at the time of the study because the longstanding duration of illness in our sample meant that we were unable to accurately calculate lifetime exposure to antipsychotics.

In our sample, those with UTRS had higher FA in the right SLF than those with TRS. Our findings are similar to Catani et al who reported a relative preservation of microstructural integrity in the right arcuate fasciculus, the fourth component of the SLF, in patients without auditory verbal hallucinations (Catani et al., 2011; Makris et al., 2005). In contrast, other groups have reported higher FA in the SLF is associated with auditory hallucinations (Hubl et al., 2004; Knochel et al., 2012; Rotarska-Jagiela et al., 2009; Shergill et al., 2007) although this has not been consistently replicated (Catani et al., 2011; de Weijer et al., 2013). Research reporting a positive correlation between symptom severity and FA in the SLF has been interpreted as pathological “hyperconnectivity” and suggested to facilitate abnormal fronto-temporal communication, specifically, a functional imbalance between language production and perception areas (Rotarska-Jagiela et al., 2009). In our sample we did not find a significant difference on PANSS item 3 “auditory hallucinations” between those with TRS and UTRS using a non-parametric ANOVA suggesting that the regional difference may not be related to the presence of auditory hallucinations. This post hoc analysis was based on only one PANSS item which assesses the preceding seven days. The implementation of a rating scale specific to auditory hallucinations such as the Revised Hallucination Scale (RHS) would be more appropriate for exploring these associations (Morrison, Wells, & Nothard, 2002). Alternative explanations for increased FA values in the SLF include regionally defective axonal pruning during neurodevelopment (Lewis & Levitt, 2002; Rapoport, Addington, Frangou, & Psych, 2005) or, DTI imaging artefacts resulting from atrophy and shrinkage of crossing fibres, which might artificially enhance the FA values of the longitudinal fibres (Bae et al., 2006).

One of the strengths of the present study is that the groups had well-matched clinical variables, despite the chronic nature of treatment-resistance; for example there were no significant
differences in age or duration of illness between the groups. During the recruitment process we used stringent exclusion criteria for TBI to rule out WM alterations due to axonal shearing. We were also able to enhance the accuracy of diffusion tensor estimation by using a higher field strength (3T versus 1.5T) and with more diffusion directions (64) than several previous studies (Garver et al., 2008; Luck et al., 2011; Mitelman et al., 2006; Reis Marques et al., 2013).

The main limitation of the present study is that it is cross-sectional and therefore not possible to separate potential medication effects from abnormalities due to pathological processes. It was also not possible to apply longitudinal criteria to establish treatment response. However, CGI-severity scores ranged from approximately two to three across all groups which corresponds to minimally ill and mildly ill respectively. The advantage of including patients with mild symptoms is that the WM alterations are less likely to be due to the presence of active symptoms; “state” versus “trait” differences. The influence of recreational drugs and concomitant medications on WM integrity is not well understood. In our sample, seven participants tested positive for cannabis on the day of scanning. When we performed the analyses excluding these participants, the overall pattern of the results remained unchanged; however the distribution and size of clusters identified in those with TRS versus controls altered (Appendix 3: Supplementary information (Chapter 6).

Other DTI studies of chronic schizophrenia included patients taking concomitant medications such as benzodiazepines and antidepressants, the impact of which remains uncertain (Luck et al., 2011). There is as yet no consensus about how to define UTRS; we defined it as people treated with adequate doses of clozapine for a sufficient length of time who subsequently required a further antipsychotic or combination to achieve symptom control (and excluded those who could not continue treatment with clozapine due to its side effects e.g. excessive sedation etc.) . Future definitions may need to take into account the nature of residual symptoms poorly controlled by clozapine, e.g. clozapine-resistant or persistent positive versus negative symptoms as the two may have different underlying pathophysiologies.
The current study suggests that DTI measures may be useful biomarkers of TRS. The group with UTRS was indistinguishable from healthy controls or first line responders which suggests that UTRS represents a subtype with a distinct underlying pathophysiology in which WM integrity may only play a minor role or could be due to PK-PD factors. Further investigation is required to delineate the role of WM integrity in the underlying pathological processes of TRS and UTRS.
Chapter Seven: Discussion

7.1 Introduction

This chapter presents a general discussion of the work presented in this thesis. The aim of this research is outlined in the context of previous studies using DTI and $^1$H-MRS to investigate characteristics associated with response to antipsychotics or medication effects. The findings of the research presented in this thesis are summarised then followed by a detailed examination of study limitations and a discussion of its strengths. The chapter finishes with suggestions for future research and some general conclusions from this work.

7.2 Aim and context

The overall aim of this thesis was to determine potential biomarkers of treatment-resistant schizophrenia (TRS) by comparing patients responsive to second generation antipsychotics (first-line responders), clozapine monotherapy (clozapine-responsive; TRS) and those who do not respond to clozapine monotherapy (clozapine resistant; UTRS), in addition to a healthy control group. Research by others has suggested that poor response to treatment with antipsychotics is associated with reduced WM integrity and alterations in glutamatergic neurometabolites, specifically increased glutamate in the anterior cingulate cortex and striatum (de la Fuente-Sandoval et al., 2013; Demjaha et al., 2013; Egerton et al., 2012; Luck et al., 2011; Reis Marques et al., 2013). Two non-invasive magnetic resonance imaging (MRI) techniques would be useful: diffusion tensor imaging (DTI) to examine the structure and integrity of white matter pathways in the brain and proton magnetic resonance spectroscopy ($^1$H-MRS) to investigate alterations in neurometabolites.

The work presented in this thesis provides a starting point for addressing the important question of whether or not there is biological evidence to support the classification of schizophrenia based on treatment response to first-line antipsychotics or clozapine. While this research has been underway for several years it is also in line with a recently proposed classification system which
reflects rates of treatment response or resistance to first-line antipsychotics and clozapine (Farooq et al., 2013). The DTI and $^1$H-MRS experiments presented are unique and address an important issue in the literature i.e. the investigation of potential biomarkers of treatment response. While a small number of studies have used these techniques to investigate response to antipsychotic medication, there are important points of difference which preclude them from addressing the question of biomarkers based on the above treatment categories (Farooq et al., 2013).

Studies using $^1$H-MRS to investigate neurometabolite alterations following treatment with antipsychotics were performed in first episode psychosis (FEP) (de la Fuente-Sandoval et al., 2013) or first episode schizophrenia (FES) patients limiting the generalizability to patients with longer standing or treatment resistant schizophrenia (Egerton et al., 2012). Since patients were recruited at the early stages of illness, neither of these studies included patients taking clozapine. Furthermore, de la Fuente-Sandoval et al also received methodological criticism for presenting absolute metabolite concentrations that were not scaled to the unsuppressed water signal or another reference measurement (Maddock & Buonocore, 2012a). Demjaha et al included patients with schizophrenia; however, none of the patients with TRS were receiving clozapine treatment and unfortunately this study is also limited by the minimal sample size (responders n=8, TRS n=6) (Demjaha et al., 2013). Two studies specifically investigated the effects of clozapine on neurometabolite measures in patients with schizophrenia (Bustillo et al., 2001; Ertugrul et al., 2009). One small study (n=10) included only responders to clozapine which did not allow for comparisons to patients with a good response to first-line medications or those with UTRS (Ertugrul et al., 2009). Bustillo et al included 19 patients taking clozapine but also did not compare responders versus non-responders (Bustillo et al., 2001). Furthermore, neither of these studies used $^1$H-MRS to measure the neurometabolites Glu and Glx (Bustillo et al., 2001; Ertugrul et al., 2009).

DTI has been used to assess changes in white matter (WM) integrity following a good or poor response to antipsychotic treatment. Similar to $^1$H-MRS studies, this has been performed in FEP
patients (Luck et al., 2011; Reis Marques et al., 2013) or when studies included patients with schizophrenia, the patients were not stratified based on medication type (Garver et al., 2008; Mitelman, Canfield, Newmark, et al., 2009; Mitelman et al., 2006). Garver et al included only those taking haloperidol or SGAs and was limited by sample size (responders n=8, non-responders n=5) (Garver et al., 2008) while Mitelman et al group patients based on outcomes but did not specify medications (Mitelman, Canfield, Newmark, et al., 2009; Mitelman et al., 2006). Holleran et al compared patients with TRS prior to commencing clozapine treatment with healthy controls and no comparison made with those who respond well to first-line antipsychotic treatment (Holleran et al., 2013).

The patients recruited for the studies presented in this thesis had an established diagnosis of schizophrenia, were stabilised on antipsychotic treatment and a medication review was performed to assess past response to antipsychotics and ensure appropriate group allocation. Positive and Negative Syndrome Scale (PANSS) interviews were performed to assess symptom severity and it was determined that the treatment groups all displayed similarly low levels of residual symptoms. Accordingly, any group differences detected reflect trait differences rather than state differences and therefore active symptoms. Other strengths of this study are discussed below.

7.3 Summary of findings

A review of the literature surrounding the pharmacotherapy for schizophrenia presented in chapter one revealed that there is little difference in efficacy between first generation antipsychotics (FGAs) and second generation antipsychotics (SGAs) and that the extrapyramidal side effects (EPSEs) associated with the former are counter-balanced by equally important metabolic side effects of the latter. In meta-analyses and large randomised controlled trials, amisulpride, risperidone and olanzapine emerged as somewhat more efficacious than most other agents but did not surpass clozapine (Jones et al., 2006; Leucht, Corves, et al., 2009; Lieberman et al., 2005). The
review confirmed that despite insidious metabolic side effects and the potential for acute events such as agranulocytosis, clozapine is still the gold standard for patients with TRS. For those with TRS who do not experience symptomatic improvements with clozapine (UTRS) there are limited options; evidence for clozapine augmentation with a second antipsychotic is weak; the addition of aripiprazole was helpful for weight management without affecting symptom severity. The most promising agent for use in combination with clozapine is the anticonvulsant agent lamotrigine, which inhibits excessive glutamate release in the brain by antagonism of sodium channels and increases gamma-aminobutyric acid (GABA) release. Lamotrigine produced significant improvements in both positive and negative subscales in an RCT (Tiihonen et al., 2005). The evidence for other agents which act on glutamatergic neurotransmission such as topiramate is not as robust. N-methyl D-aspartate receptor (NMDAR) glycine-site agonists do not improve symptoms for patients treated with clozapine which was thought to relate to clozapine’s unique mechanism of action – inhibition of glycine transport which increases glutamatergic neurotransmission and enhances NMDA hypofunctioning. Alternatives to pharmacotherapy for UTRS have also been investigated with limited success.

Chapter two describes the recruitment process and the patients included in the study sample and their response to antipsychotic treatment using the Positive and Negative Symptom Scale (PANSS) scores. According to the Remission in Schizophrenia Working Group (RSWG) criteria, which requires a score of three or less simultaneously on eight key items from the PANSS scales, the rate of symptomatic remission in our sample was low (14.5% or 9/62). Despite this result, patients in this sample were classed as minimally to mildly ill using the Clinical Global Impression (CGI) scale which is a validated symptom scale, suggesting that failure to meet the remission criteria does not equate to severe illness. It is important to make the distinction between remission and response to treatment; remission refers to a state in which the patient is free of clinically significant symptoms whereas response can be defined as a significant improvement in the patient’s psychopathology irrespective of whether or not he/she is still symptomatic (Keith & Kane, 2003). This study grouped
patients based on antipsychotic treatment at the time of the study, prescribed in accordance with criteria for TRS using published algorithms which require symptom assessments before switching patients to another antipsychotic medication (Association, 2004; NICE, 2002; Royal Australian New Zealand College of Psychiatrists, 2005). Therefore, while the majority (53 out of 62) of our participants did not meet the remission criteria, they could still be classified as responders to the antipsychotic treatment that they were receiving. Furthermore, there were no significant differences between the three treatment groups in terms of PANSS scores (total, positive, negative or general) or CGI scores. Similarly, in the subset of patients included in the proton magnetic resonance spectroscopic (1H-MRS) analysis described in chapter four and diffusion tensor imaging (DTI) in chapter six, there were no differences between the treatment groups in their PANSS total or subscale scores or CGI scores. While we anticipated that the group with UTRS may have more severe residual symptoms, this was not the case and may reflect the fact that we were only able to recruit patients who were well enough to give informed consent to participate.

Evaluation with 1H-MRS (chapter four) found that patients with TRS receiving clozapine had higher Glx levels than those with clozapine resistant or UTRS in the putamen suggesting that Glx in striatal structures such as the putamen may represent a marker of response to clozapine treatment. This result remained significant when participants who tested positive for THC were removed from the analysis. Group differences in Glx ratios were only detected in the putamen, not the dorsolateral prefrontal cortex (DLPFC) or the anterior cingulate cortex (ACC) and it has been suggested that glutamatergic anomalies may be restricted to DA rich regions of the brain such as the striatum (de la Fuente-Sandoval et al., 2011). No significant group differences were found in the DLPFC, ACC or putamen for the other neurometabolites measured (expressed as a ratio to creatine): N-acetylaspartate (NAA/Cr), or choline containing compounds (GPC+PCh/Cr). This is consistent with recent 1H-MRS studies which found no differences associated with treatment response for NAA or choline containing compounds (Demjaha et al., 2013; Egerton et al., 2012) or differences only at the trend level for NAA (a significant increase in GPC+PCh/Cr was detected in the associative
striatum after four weeks of antipsychotic treatment but in this first episode of psychosis (FEP) study) (de la Fuente-Sandoval et al., 2013).

Results from the DTI study which was conducted to assess WM integrity in each group are described in chapter six. The group with TRS had significantly lower fractional anisotropy (FA; i.e. disrupted WM integrity) and lower parallel diffusivity (PD; interpreted as axonal damage) compared to healthy controls in several regions including the superior longitudinal fasciculi (SLF; bilateral), the corpus callosum including the posterior corona radiata (bilateral) and the corticospinal tract (bilateral). The first-line responders and the group with UTRS were indistinguishable from the healthy controls. These findings are similar to other studies in which reductions in FA were associated with poor outcome schizophrenia (Mitelman, Canfield, Newmark, et al., 2009; Mitelman et al., 2006) or non-response to treatment in FEP patients (Luck et al., 2011; Reis Marques et al., 2013) and one small study of treatment response over 4 weeks in patients with schizophrenia (Garver et al., 2008). Overall, DTI measures may be useful biomarkers for predicting clozapine response.

Interestingly the group with UTRS were also indistinguishable from healthy controls which suggests that UTRS might be a subtype of schizophrenia with a distinct underlying pathophysiology. Another possibility is that ‘normal’ WM integrity in the group with UTRS could be due to higher exposure to antipsychotic medication; however no region was identified in which diffusion measures (FA, MD, RD or PD) correlated with the daily antipsychotic dose in chlorpromazine equivalents (CPZEs). Another intriguing finding from the DTI study was that the group with TRS had lower FA in the right SLF compared to the group with UTRS which suggests that this region might be “hyperconnected” in patients with UTRS. Studies have reported that higher FA in the SLF is associated with auditory hallucinations (Hubl et al., 2004; Knochel et al., 2012; Rotarska-Jagiela et al., 2009; Shergill et al., 2007) although this has not been consistently replicated (Catani et al., 2011; de Weijer et al., 2013).
7.4 Potential mechanisms

Clozapine may enhance glutamatergic neurotransmission by inhibition of glycine transport or by interacting with the glycine site on the NMDA receptor (Javitt et al., 2004; Schwieler et al., 2008). Increased Glx in the putamen of the group with TRS compared to those with UTRS may be due to full occupation of the glycine site with clozapine treatment. Seven out of nine people with UTRS included in the spectroscopic analysis of the putamen were also taking clozapine and there was no difference in their daily clozapine dose compared to the group with TRS. This suggests that clozapine might not enhance glutamatergic neurotransmission in patients with UTRS which could be due to differences at the obligatory NMDA-glycine binding site in patients with UTRS.

In previous DTI studies investigating treatment response to antipsychotics, poor response was associated with low FA in several regions relative to controls and patients with schizophrenia who respond well to treatment. (Luck et al., 2011; Reis Marques et al., 2013) Furthermore Reis Marques et al reported increased FA and Garver et al reported decreased MD in responders relative to baseline after twelve and weeks of antipsychotic treatment respectively suggesting restoration of WM integrity. (Garver et al., 2008; Reis Marques et al., 2013) Interestingly, in the present DTI study the group with TRS had lower FA than controls despite responding well to clozapine treatment which suggests that while disrupted WM integrity may be associated with TRS, it does not preclude response to antipsychotics. Holleran et al reported widespread FA reductions in patients with TRS prior to commencing treatment with clozapine which suggests that FA reductions in the group with TRS are not a consequence of clozapine treatment. Furthermore, 11 out of 14 patients with UTRS included in the DTI analysis were taking clozapine in combination with another antipsychotic.

Possible explanations for widespread FA reductions in patients who develop TRS are i) long-standing neurodevelopmental abnormalities in WM pathways or ii) damage to WM pathways as a result of glutamate-induced excitotoxicity which may also be related to a long duration of untreated psychosis. It may be that patients with TRS have reduced WM integrity while those with
UTRS have normal WM integrity but the organisation of the WM fibres is abnormal and in some regions “hyperconnected”, for instance in the right SLF, i.e. damage versus disorganisation. While we did not find any regions in which diffusion measures correlated with antipsychotic dose (in CPZE), it is possible patients with UTRS had similar FA to control subjects due to medication effects of antipsychotics. We were not able to quantify lifetime exposure to antipsychotics for the groups but the UTRS was receiving the highest daily dose of antipsychotics in CPZEs and had the longest duration of illness. It could be that the group with UTRS at one point had lower FA similar to the group with TRS but higher exposure to antipsychotic medication produced an increase in FA via promyelinating effects suggested in preclinical studies (Chandran et al., 2012).

7.5 Limitations

The main limitation of the study is that it is cross-sectional; therefore it cannot be said with certainty that the differences found between the treatment groups are “state” differences related to the participants’ capacity for response to certain antipsychotic medications or effects of the medication itself. A recent meta-analysis reported an inverse relationship between cumulative antipsychotic exposure and GM but not WM volume. That is to say the higher the exposure to antipsychotics, the greater the GMV decreases; while no correlation was detected between duration of illness or symptom severity (Fusar-Poli et al., 2013). In order to investigate this potential confounder, we performed correlation analyses with antipsychotic daily dose converted to CPZEs for each imaging technique. No correlations were found between antipsychotic daily dose and diffusion measures or Glx/Cr which indicates that group differences in these measures may not be explained by exposure to antipsychotic medication. A more thorough way of investigating this potential confounder is to use lifetime exposure to antipsychotic medication (i.e. cumulative CPZEs) but the longstanding duration of illness in our sample meant that we were unable to accurately calculate lifetime exposure. Another consideration when calculating CPZEs for the group with UTRS is that the CPZE for each antipsychotic was summed and while at present there is no
alternative approach to this problem it may be that CPZEs are not simply additive (Andreasen et al., 2010). Furthermore, we did not use clozapine levels for such correlations since they were not collected at the time of the study for four participants and while plasma levels of >250ng/mL are recommended, the therapeutic range is not well-defined (Remington et al., 2013).

Patient selection relied on clinician reported notes; we did not employ the OPerational CRITeria (OPCRIT) diagnostic system (Craddock et al., 1996). However, the duration of illness in each of the treatment groups was sufficiently long to demonstrate diagnostic consistency; overall sample mean duration of illness = 11.0 ± 6.6 years; first-line responders = 9.4 ±7.7 years, TRS =12.8 ±6.6 years and UTRS =10.9 ±5.2 years. Assessment of adherence is important when establishing a diagnosis of TRS and UTRS. In our sample, adherence to antipsychotic medication was self-reported. We collected Drug Attitude Inventory (DAI-30) scores in order to assess attitudes towards treatment as an indirect measure of adherence and all three groups had similar scores as discussed in chapter two. Furthermore, most of our sample was supportive of taking prescribed medication. A significant proportion were inpatients (18/62), living in assisted accommodation (13/62) or in the community with everyday support from family or a caregiver (13/62). Furthermore, adherence and non-adherence to treatment is a dynamically changing behaviour and non-adherence is a common occurrence; more than 50% of patients with psychotic disorders become partially adherent or non-adherent within one year and 75% within two years (Keith & Kane, 2003). Given the cross-sectional design of our study and the long-standing duration of illness in our patient sample it may not have been possible to accurately represent lifetime adherence in this study.

There are important limitations inherent in the interpretation of fractional anisotropy (FA) cited in chapters five and six. FA values cannot distinguish between demyelination and axonal damage (Boretius et al., 2012). Furthermore, it is estimated that approximately 90% of WM voxels contain crossing fibres which has important implications for DTI measurements that approximate WM tracts at the macroscopic level (Jeurissen et al., 2013). A single voxel may contain fibre populations with different spatial orientations resulting in an apparent decrease in FA. It is therefore
recommended that a combination of techniques is used to assess WM integrity (Alba-Ferrara & de Erausquin, 2013). In order to clarify group differences in FA, mean diffusivity (MD), parallel diffusivity (PD) and radial diffusivity (RD) were assessed. We observed decreases in parallel diffusivity (PD) which overlapped with areas of decreased FA in the group with TRS and no changes in RD suggesting that reduced FA was related to axonal damage rather than demyelination.

We reported increased Glx but not Glu in the group with TRS compared to the group with UTRS. A limitation of this study was the difference in group numbers with only 11 people forming the group with UTRS. Nevertheless, high Glx levels in the putamen in those with TRS compared to the group with UTRS was detected with a large effect size (Cohen’s d = 1.221) which strongly suggests that this result that justifies further exploration. Other studies examining potential relationships between glutamatergic neurometabolites and treatment response have reported either alterations in both (de la Fuente-Sandoval et al., 2013; Egerton et al., 2012) or only Glu (Demjaha et al., 2013) acknowledging the limitations of measuring Glu. As discussed in chapters three and four, without the use of a J-PRESS sequence the Glu peak is contaminated by Gln (Hancu, 2009), therefore our finding of increased Glx in the group with TRS may be a more accurate measure of altered levels of glutamatergic compounds.

7.6 Strengths

In order to ensure that patients were placed in the correct treatment group, clinical notes were reviewed. A medication history was collected based on notes, medication charts for inpatients and community pharmacy records for outpatients. Strict criteria (loss of consciousness (LOC) for more than two minutes after head injury) were applied to exclude participants with traumatic brain injuries. When the duration of LOC could not be confirmed, the participant was not recruited. If a participant had a history of head injury with no or only momentary LOC medical notes were consulted and the participant was only included if a normal head CT or MRI after the injury took place was documented.
Other important confounders include concomitant medications and recreational drug abuse. Recreational drugs were tested for and the subsequent analyses conducted excluded those who tested positive for THC, which the urine screen showed was the only illicit substance used close to the time of study visits. The recreational use of cannabis is prevalent in patients with schizophrenia; compared to the general population patients with schizophrenia have a two-fold increase in rates of cannabis use disorders (Arseneault, Cannon, Witton, & Murray, 2004; Buckley, Miller, Lehrer, & Castle, 2009). Since cannabis use is associated with grey and white matter changes (Dekker et al., 2010; James et al., 2011; Peters et al., 2009), analyses were repeated excluding seven patients from the DTI data set and five from the spectroscopic data set who tested positive for THC on the study day. Group differences in the studies using DTI and 1H-MRS remained significant after excluding these participants. While factors such as cannabis use, smoking tobacco and concomitant medication are all potential confounders to be considered in the interpretation of these findings, it could be argued that the inclusion of patients with these confounders reflects clinical reality and makes our results more generalizable.

We also reported concomitant psychotropic medications in our sample (chapter four) but other covariates took precedence over accounting for this confounder. Other DTI studies of patients with schizophrenia included those also taking benzodiazepines and antidepressants, the impact of which remains uncertain (Luck et al., 2011). Antidepressants are associated with increased NAA (Gonul et al., 2006) while benzodiazepines have not been shown to significantly alter neurometabolite content (Yildiz et al., 2010). Preclinical studies suggest that mood stabilisers such as lithium and sodium valproate enhance glutamate reuptake (Dixon & Hokin, 1997, 1998).

As discussed above and in chapter two, our treatment groups had similar symptom scores on all PANSS and CGI ratings. The active symptoms of psychosis have been associated with alterations in DTI measures in several studies (Bertolino et al., 1998; Catani et al., 2011; Hoptman J, 2010). Because our groups can be deemed to have only minimal to mild symptoms it is anticipated that the group differences detected using MRI techniques were “trait” rather than “state” specific. We
also matched participants on a group basis for age, sex and duration of illness, although in the spectroscopic subset, the first-line responders had a shorter duration of illness than those with TRS or UTRS.

7.7 Future directions

Future work should aim to disentangle medication effects from underlying pathology with large, longitudinal studies capturing the transition from first-line antipsychotics to clozapine in those with TRS and augmentation for those who develop UTRS. In order to improve the accuracy of $^1$H-MRS measurements, water unsuppressed spectra should be acquired to allow neurometabolite quantification (as opposed to Cr ratios), shimming software such as FASTESTMAP greatly improve shimming and a MEGA-point resolved spectroscopy (MEGA-PRESS) sequence would allow the resolution of Glu, Gln and GABA peaks. Our demonstration of glutamatergic abnormalities in the putamen is in need of replication and whether this occurs in other areas relevant to the effects of antipsychotic action such as the ventral tegmental area (VTA), hippocampus and the caudate nucleus should also be investigated.

FA values cannot distinguish between myelin and axonal damage so the interpretation is further limited by the effects of partial voluming and crossing fibres. Since histological techniques applied in animal studies and post-mortem tissue are clearly not applicable in clinical studies, other non-invasive MRI techniques such as have been suggested to assess tissue integrity. Complete Fourier Direct MRI (CFD-MRI) models the effect of spin motion on the MRI signal which overcomes assumptions in other models such as the diffusion ellipsoid and represents the motion properties of spins in an asymmetric, unconstrained fashion (Özcan, 2013). In order to better describe the group differences in FA observed in the present study, high angular resolution diffusion-weighted imaging (HARDI) could be used to calculate fibre orientation distribution (FOD) which enables group comparisons or correlations of ‘apparent fibre density’ (Tournier, Calamante, Gadian, & Connelly, 2004).
Since NAA is a source of acetyl groups which are incorporated into myelin, \(^1\)H-MRS may be used to inform DTI findings (Chakraborty, Mekala, Yahya, Wu, & Ledeen, 2001; Mehta & Namboodiri, 1995; Moffett et al., 2007). Other groups have investigated the feasibility of this approach with promising results (Irwan, Sijens, Potze, & Oudkerk, 2005; Sato, Maruyama, Hoshida, & Minato, 2013; Steel et al., 2001). Our \(^1\)H-MRS data was collected at the same time as our DTI data, therefore it was not possible to use DTI results to guide \(^1\)H-MRS voxel placement; however, based on our DTI findings, future studies should do so.

Structural and functional MRI (fMRI), EEG combined with ERPs, cognitive testing data and samples for genetic screening were all collected from the participants in the present study. It is hoped that machine learning techniques which are able to combine inputs from various methods to identify key components for classification will help determine clinical measures for predicting treatment response. Another technique recently developed by Professor Nikola Kasabov from Auckland University of Technology (AUT) is the NeuCube, a three dimensional evolving neurogenetic brain cube of spiking neurons mimicking structural and functional areas of the human brain. The NeuCube is able to successfully model spatio-temporal data such as that obtained from EEG and fMRI as well as purely spatial information acquired from structural MRI and DTI (Kasabov, 2012).

7.8 Overall conclusion

Glutamate + glutamine (Glx) levels in the putamen may represent an important biomarker of response to clozapine treatment where levels are higher in those who are responsive and lower in those with ultra-treatment-resistant schizophrenia. Reduced white matter integrity measured in terms of fractional anisotropy may be a potential biomarker for individuals with treatment-resistant schizophrenia who do not respond to first-line antipsychotics. Using both DTI and \(^1\)H-MRS, significant differences were found between the groups with TRS and UTRS. Although the group with UTRS is the most treatment resistant, white matter integrity and neurometabolites were no different to controls. This may suggest that UTRS has a distinct underlying pathophysiology to other forms of schizophrenia. This data lays a solid foundation for future
research which should aim to determine whether these results are present before and after the
initiation of clozapine treatment in a prospective trial.
Appendix 1: Detection limits for ProScreen Cups© recreational drug screen kit

**Table A 1** The AS/NZS 4308:2008 (Australian New Zealand Standards) Immunoassay initial test cut off levels.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Initial cut-off level (for detection in urine)</th>
<th>Detection time frame limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>300 µg/L</td>
<td>2-6 days</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>200 µg/L</td>
<td>2-14 days</td>
</tr>
<tr>
<td>Cannabis</td>
<td>50 µg/L</td>
<td>2-30* days</td>
</tr>
<tr>
<td>Cocaine</td>
<td>300 µg/L</td>
<td>2-5 days</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>300 µg/L</td>
<td>2-6 days</td>
</tr>
<tr>
<td>Opiates</td>
<td>300 µg/L</td>
<td>2-5 days**</td>
</tr>
</tbody>
</table>

Note: * This extended time frame only applies to regular or heavy users of cannabis. **Methadone is detectable from 2-8 days.
### Appendix 2: Supplementary information (Chapter 4)

#### Table A1 Concomitant psychotropic medications and nicotine replacement therapy by treatment group

<table>
<thead>
<tr>
<th>Medication</th>
<th>First-line responders (n=15)</th>
<th>TRS (n=16)</th>
<th>UTRS (n=11)</th>
<th>Total (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benztropine</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Citalopram</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Lithium</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Nicotine replacement therapy (patches)*</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sodium Valproate</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Zopiclone</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

*None of the control subjects were taking nicotine replacement therapy

#### Table A2 Metabolite levels in the DLPFC excluding those participants who tested positive for THC at the study visit

<table>
<thead>
<tr>
<th></th>
<th>First-line responders</th>
<th>TRS</th>
<th>UTRS</th>
<th>Controls</th>
<th>$F^2$ (p-value)</th>
<th>$F^2$ (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>12</td>
<td>15</td>
<td>10</td>
<td>16</td>
<td>53 (patients and controls)</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.44 (0.24)</td>
<td></td>
<td>1.41 (0.24)</td>
<td>1.50 (0.25)</td>
<td>1.56 (0.23)</td>
<td>1.094 (0.361)</td>
</tr>
<tr>
<td>Glu/Cr</td>
<td>1.14 (0.16)</td>
<td></td>
<td>1.14 (0.23)</td>
<td>1.02 (0.15)</td>
<td>1.11 (0.18)*</td>
<td>1.085 (0.365)</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>1.38 (0.24)*</td>
<td></td>
<td>1.35 (0.25)</td>
<td>1.18 (0.15)</td>
<td>1.28 (0.19)$^d$</td>
<td>1.868 (0.148)</td>
</tr>
<tr>
<td>GPC+PCh/Cr</td>
<td>0.325 (0.060)</td>
<td></td>
<td>0.339 (0.030)</td>
<td>0.357 (0.044)</td>
<td>0.325 (0.045)</td>
<td>1.276 (0.293)</td>
</tr>
</tbody>
</table>

*Analysis of variance (ANOVA), within-group factor metabolite level, between group factor treatment group.

$^a$Univariate analysis of covariate (ANCOVA), within-group factor metabolite level, between-group factor treatment group, and duration of illness as a covariate.

$^b$One spectrum was rejected because of a Cramer-Rao lower bound exceeding 20%.

$^c$One spectrum was removed; outlier value.
<table>
<thead>
<tr>
<th>Table A 3</th>
<th>Metabolite levels in the ACC excluding those participants who tested positive for THC at the study visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>First-line responders</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.17 (0.28)</td>
</tr>
<tr>
<td>Glu/Cr</td>
<td>1.44 (0.26)</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>1.75 (0.41)</td>
</tr>
<tr>
<td>GPC+PCh/Cr</td>
<td>0.263 (0.037)</td>
</tr>
</tbody>
</table>

^a Univariate analysis of covariate (ANCOVA), within-group factor metabolite level, between-group factor treatment group, and proportion of grey matter as a covariate.

^b ANCOVA, within-group factor metabolite level (not CSF-corrected), between-group factor treatment group, and proportion of grey matter and duration of illness as a covariates.

^c One spectrum was removed; outlier value, n=7.

<table>
<thead>
<tr>
<th>Table A 4</th>
<th>Metabolite levels in the putamen excluding those participants who tested positive for THC at the study visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First-line responders</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
</tr>
<tr>
<td>NAA/Cr</td>
<td>1.06 (0.19)</td>
</tr>
<tr>
<td>Glu/Cr</td>
<td>1.14 (0.13)</td>
</tr>
<tr>
<td>Glx/Cr</td>
<td>1.57 (0.15)</td>
</tr>
<tr>
<td>GPC+PCh/Cr</td>
<td>0.259 (0.019)</td>
</tr>
</tbody>
</table>

^a Univariate analysis of covariate (ANCOVA), within-group factor metabolite level (not CSF-corrected), between-group factor treatment group, and proportion of grey matter as a covariate.

^b Univariate analysis of covariate (ANCOVA), within-group factor metabolite level (not CSF-corrected), between-group factor treatment group, and proportion of grey matter and duration of illness as a covariates.

^c One spectrum was removed; outlier value.
Appendix 3: Supplementary information (Chapter 6)

**Table A 1** White matter regions of lower FA and PD in the group with TRS compared to healthy controls excluding seven participants who tested positive for THC at the time of testing (n=63)

<table>
<thead>
<tr>
<th>FA clusters</th>
<th>White Matter Area/Tract</th>
<th>Significant cluster size (number of voxels)</th>
<th>t-statistic</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC Posterior Corona Radiata (Right)</td>
<td>797</td>
<td>3.76</td>
<td>70</td>
<td>97</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>CC Posterior Corona Radiata (Left)</td>
<td>258</td>
<td>3.68</td>
<td>111</td>
<td>95</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>CC Superior Corona Radiata (Left)</td>
<td>90</td>
<td>2.99</td>
<td>109</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>SLF (Right)</td>
<td>62</td>
<td>3.84</td>
<td>52</td>
<td>101</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>SLF (Right)</td>
<td>44</td>
<td>2.77</td>
<td>71</td>
<td>125</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>Corticospinal tract (Left)</td>
<td>19</td>
<td>2.79</td>
<td>106</td>
<td>97</td>
<td>128</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PD clusters</th>
<th>White Matter Area/Tract</th>
<th>Significant cluster size (number of voxels)</th>
<th>t-statistic</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inferior fronto-occipital fasciculus (R), Uncinate fasciculus (R)</td>
<td>409</td>
<td>3.58</td>
<td>73</td>
<td>165</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>CC splenium (L), Forceps major (L)</td>
<td>55</td>
<td>4.26</td>
<td>115</td>
<td>69</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>CC Anterior corona radiata (R), uncinate fasciculus (R)</td>
<td>16</td>
<td>3.71</td>
<td>66</td>
<td>154</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Inferior longitudinal fasciculus (L), CC Forceps major (L)</td>
<td>14</td>
<td>3.70</td>
<td>116</td>
<td>55</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>CC posterior corona radiata (L)</td>
<td>5</td>
<td>2.65</td>
<td>118</td>
<td>66</td>
<td>91</td>
</tr>
</tbody>
</table>
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