4-23-2019 10:00 AM

Early Treatment Response in First Episode Psychosis: A 7-Tesla Magnetic Resonance Spectroscopic Study of Glutathione and Glutamate

Kara Dempster
The University of Western Ontario

Supervisor
Palaniyappan, Lena
The University of Western Ontario

Graduate Program in Neuroscience
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

Early Treatment Response in First Episode Psychosis: A 7-Tesla Magnetic Resonance Spectroscopic Study of Glutathione and Glutamate

Abstract

Approximately one third of patients with schizophrenia fail to respond to dopamine-blocking antipsychotic medications. While treatment resistant schizophrenia (TRS) is generally thought to be present from the onset of first episode psychosis (FEP), prospective identification of these patients remains clinically challenging. We investigated the association of glutamate and glutathione with time to response in the anterior cingulate cortex (ACC) of minimally-treated patients with FEP (n=26) and healthy controls (n= 27) using an ultra-high field 7T MRI protocol. Higher ACC glutathione at baseline was associated with decreased time to achieve 50% symptom improvement. There were no significant differences between patients and controls on measures of glutamate, or glutathione. For the first time, we have demonstrated an association between glutathione and longitudinal treatment response. Interventions that increase brain glutathione may provide new treatment options for individuals with FEP.

Keywords

Treatment resistant schizophrenia, first episode psychosis, antipsychotic response, magnetic resonance spectroscopy (MRS), glutamate, glutathione, anterior cingulate cortex

Acknowledgments

Firstly, I would like to acknowledge my supervisor, Dr. Lena Palaniyappan for his support and guidance throughout my completion of this project. Lena, you have mentored me both in a research capacity and clinically, and I will forever be grateful for the wealth of knowledge I have gained from you.

I would also like to express immense gratitude to Dr. Peter Williamson who provided me with mentorship early in my training and without who's influence I would likely never have applied to the Clinical Investigator Program, or pursued this Master's degree.

A special thanks goes out to the other members of my advisory committee, Dr. Jean Théberge and Dr. Ingrid Johnsrude. I appreciate the feedback and encouragement I received throughout the completion of this project immensely.

I will be forever grateful for the support and hardworking efforts of other individuals on my research team. First and foremost, patient recruitment would not have been as enjoyable and successful without Michael MacKinley, who has also helped me with innumerable (and sometimes random) tasks throughout the completion of this work. I would also like to acknowledge Peter Jeon who obtained the spectroscopic data, and otherwise made scans more enjoyable.

I also extend my gratitude to the PEPP team, including all the case managers who have been a pleasure to work with over the past couple of years. Particular thanks goes out to the psychiatrists who supported our work and helped with recruitment including Dr. Raj Harricharan, Dr. Julie Richard, Dr. Priya Subramanian, and Dr. Hooman Ganjavi.

For their expertise in scanning, I would like to thank Dr. Joe Gati, and Trevor.

Finally, I would like to extend my gratitude to my husband, Christopher Poss, for his unwavering support throughout my research journey. I would not have been able to complete this project without him. Lastly, I must acknowledge my dear son Caden, who accompanied me on this journey, both in utero, and then as a newborn; everything I did from the day you were born is to ensure you have everything you need in this life.

Contributions

I (Kara Dempster) performed all statistical analyses, recruited FEP patients for participation in the study, ensured completion of follow-up PANSS scales, participated in the 6 month diagnosis consensus conference, and wrote this document. MM helped with recruitment of FEP patients, recruited healthy control participants, and facilitated data collection. PJ collected and fitted all MRS data. LP was the primary supervisor for this entire project as well as participated in data collection, the consensus diagnosis, and helped with statistical analyses and editing. JT provided support in formalizing the MRS protocol and was a second reader for the final version of this thesis.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1 What is Schizophrenia?

Schizophrenia, a chronic psychotic disorder characterized by delusions, hallucinations, disorganized speech and behaviors, ranks among the top three most disabling medical conditions worldwide (Murray et al, 2012). Schizophrenia is relatively common, affecting approximately 1% of the population (McGrath et al, 2008), with an onset generally in late adolescence or early adulthood. Thought to be neurodevelopmental in origin, the illness is complex, and the underlying pathogenesis remains poorly understood. Many individuals who eventually develop schizophrenia demonstrate a progressive period of social decline, often referred to as a "prodrome", prior to developing overt symptoms of psychosis (Rossler and Rossler, 1998; Schultz-Lutter, 2009). Prodromal symptoms may present several years prior to the development of an acute psychotic episode, and may be characterized by attenuated symptoms of psychosis, and/or a general and non-specific decline in social and overall functioning. The advent of an acute psychotic episode is characterized by the onset of positive symptoms which may include hallucinations (most commonly auditory in nature, although visual, tactile, and olfactory are occasionally experienced), delusions (fixed false beliefs which go against cultural and social norms), and/or disorganized speech (thought form that is tangential and difficult to follow) and behaviors. Negative symptoms are experienced as a paucity of regular human affect and experience, and commonly endorsed negative symptoms include apathy, avolition, flattening of affective reactivity, and lack of social interest. Negative symptoms may be present for months to years prior to the onset of positive symptoms, and are generally less responsive to currently available treatment interventions. For a diagnosis of schizophrenia, the DSM-5 (American Psychiatric Association, 2013) requires the presence of two of: hallucinations, delusions,

disorganized speech, grossly disorganized or catatonic behavior, or negative symptoms, lasting for at least one month, with an overall 6 months of general functional decline. Though not currently reflected in formal diagnostic criteria, cognitive impairments are a well-established phenomenon experienced by the majority of patients with schizophrenia, are detectable even prior to the development of acute psychosis (Mesholam-Gately et al, 2009), and are associated with impaired functioning (Fujii and Wylie, 2003; Cervellione et al, 2007; Smith et al, 2002).

The course of schizophrenia is heterogeneous (Modestin et al, 2003; Thara, 2004), and response to treatment is variable. While some individuals return to their previous functional level following the onset of psychosis, for many, the illness is characterized by periodic relapses, and some experience chronic psychotic symptoms with a deteriorating functional course (Bleuler, 1972; Harding et al, 1987). However, in more recent years, compelling evidence has demonstrated that intervening earlier in the course of schizophrenia is associated with better outcomes longitudinally (Birchwood, Todd, and Jackson, 1998; Bertolote and McGorry, 2005; Malla et al, 2002). The first episode of psychosis (FEP) is of considerable clinical and research interest. Because the onset of psychosis occurs at a pivotal time in development (Hafner et al, 1998) in terms of educational, occupational, and relational attainment, the development of a FEP has the potential to completely derail a young person's life trajectory. It has increasingly become recognized that multiple interventions, including pharmacological, psychological, and social and educational supports, are warranted in order to optimize functioning and long-term prognosis in this group of young people.

While there are several different antipsychotic medications available, many individuals only respond partially, or do not respond at all, to dopamine-blocking agents, which characterize the majority of agents to date with antipsychotic efficacy. Although some studies have

demonstrated superiority of certain medications over others (Leucht et al, 2003), given the extensive variation in presentation and course, it is difficult to apply clinical findings on an individualized basis. Though somewhat controversial, there is some evidence that untreated psychosis is associated with worse long-term outcomes, and may even be neurotoxic (Black et al, 2001; McGlashan, 2006). In one of the most comprehensive longitudinal studies of first-episode psychosis patients to date, the overall cumulative duration of active psychosis predicted brain volume loss over the course of 7 years (Andreasen at al, 2013). It has been hypothesized that early psychopharmacological intervention improves the outcome and prognosis of schizophrenia (Wyatt, 1991), and therefore, a priority in the management of patients with FEP, should be to obtain sufficient response to an antipsychotic agent in a timely manner.

1.2 Treatment Resistant Schizophrenia

Since the introduction of chlorpromazine in the 1950's, antipsychotic medications have been the primary biological treatment modality for patients with schizophrenia. These medications facilitate remission for many patients, and the discovery of this class of medications has allowed countless patients to live independently in the community, as opposed to being chronically institutionalized. Unfortunately, however, antipsychotic medications are not helpful for all patients with schizophrenia. Despite ongoing advances in neuroscience, and new drug development, approximately 20-33% of patients are considered to be treatment resistant (TR) (Meltzer, 1997; Lindenmayer, 2000) and continue to experience symptoms despite receiving evidenced-based antipsychotic therapy. Although some individuals who fail to respond to an initial agent, may respond to an alternative antipsychotic trial (while objective definitions of what constitutes a trial have varied, more recent guidelines (Howes et al, 2018) suggest that a trial should entail 4-6 weeks on an antipsychotic medication of adequate dose), response rates

decrease with subsequent medication trials, with response rates as low as 7% in patients who have failed two antipsychotic trials (Kinon et al, 1993). Currently, there are no objective methods to predict who will develop TR prospectively, leading to a period of medication trial and error, which is both frustrating for the patient who continues to suffer, and the clinician. Research that may facilitate prognostication of eventual treatment response may help guide early pharmacological intervention, and improve illness course, and recovery, for these particular patients.

Treatment resistant schizophrenia (TRS) is associated with a tenfold higher cost of health care, and various other associated conditions that contribute to individual and societal burden (Kennedy et al, 2014). In a systematic analysis of studies between 1996-2012, TRS patients were found to have more comorbidities (including cigarette smoking and alcohol and other drug abuse), were more likely to experience adverse medication events, had worse quality of life, were more likely to be unemployed, and were more likely to express suicidal ideation. TRS patients appear to have more severe cognitive deficits (De Bartolomeis et al, 2013; Frydecka et al, 2016) and are significantly more impaired on tasks of daily functioning (Iasevoli et al, 2016). Alarmingly, patients with TRS have also been shown to have a decreased life expectancy (Tandon et al, 2009) and higher rates of completed suicide (Green et al, 2007). In addition to the clear catastrophic personal costs associated with being afflicted by refractory illness, this condition is associated with considerable societal morbidity, including decreased employment (Marwaha and Johnson 2004) and significantly higher direct and indirect costs of healthcare (Zeidler et al. 2012). Despite the significant personal and societal costs associated with TRS, there is a paucity of evidence-based treatment options for this subgroup of patients, and the lack of available options may relate to a generally poor understanding of both the presentation, and

underlying pathophysiology of TRS.

TRS may represent a distinct subtype of illness, with a varying course and pathophysiology from treatment-responsive schizophrenia. More recently, it appears possible to differentiate TRS from treatment responsive schizophrenia early on in the course of illness (Lally et al, 2016). While there are no objective measures to predict treatment resistance prospectively, several demographic and illness-related factors have been associated with poor treatment response including negative symptoms (Lindemayer et al, 2000), younger age at onset of illness (Jomli et al, 2012; Mohamed, 2013), poor premorbid adjustment and more severe cognitive impairment (Meltzer, 1997; Lindemayer, 2000), the presence of neurological soft signs (Smith et al, 1999), male gender, longer illness duration, and longer duration of untreated psychosis (Carbon and Correll, 2014). In a prospective study of 375 patients with first-episode, non-affective psychosis investigating baseline sociodemographic, premorbid, and clinical predictors of antipsychotic response at 6 weeks, family psychiatric history, previous hospitalization, longer duration of untreated illness (DUI) and duration of untreated psychosis (DUP), and poor premorbid adjustment during adolescence were associated with poor antipsychotic response (Crespo-Facorro et al, 2013). It certainly appears that those patients, who are not functioning as well at initial presentation, have a higher likelihood of responding poorly to treatment. In a South-African study of 133 patients with first-episode psychosis, non-responders after 3-months of depot antipsychotic medication (to enhance compliance) were characterized by significantly poorer functional outcome, poorer quality of life, a greater burden of cognitive impairment, and had more neurological soft signs at initial presentation of illness (Bonginkosi et al, 2015). In a large prospective study of antipsychotic response in first-episode psychosis, shorter duration of untreated psychosis, longer duration of treatment, and higher baseline scores of general and

negative symptoms were associated with response (Zhang et al, 2014). While there are several clinical factors associated with eventual treatment resistance, these factors are non-specific, and it is difficult to extrapolate these findings to make individual clinical decisions.

A major barrier to advancing the understanding of TRS has been the lack of an objective, universally-accepted definition. While overtime, a few different criteria have been proposed, various research studies have defined the phenomenon slightly differently, making it difficult to compare results. Criteria for TRS generally necessitate first, a lack of response to antipsychotic treatment, although, what stipulates "lack of response" has been inconsistently defined. Most definitions additionally require an individual to demonstrate treatment failure on more than one medication, taken over variable periods of time. One of the most well cited definitions of treatment resistance by Kane et al (1998), required a failure to respond to at least 3 antipsychotic treatments in the preceding 5 years, with medications being from two different classes, and of at least 1000 mg chlorpromazine equivalents, with no significant period of good functioning during that time, and a symptom severity of >45 on the Brief Psychiatric Rating Scale (BPRS). While this definition is comprehensive, the time period required to meet criteria is substantial, and would contribute to significant delays in recognizing TRS early on in the illness course. More recent definitions have attempted to reconcile the clinical need for earlier recognition, and hence, intervention, of individuals with TRS.

Many definitions have emphasized the requisite of several antipsychotic trials prior to making a determination of TRS status. For example, the UK National Institute for Clinical Excellence (NICE) defines treatment resistance as "unsatisfactory clinical improvement despite consecutive use of two antipsychotics, one of which should be an atypical agent for 6 to 8 weeks duration" (NICE, 2002). Defining resistance based on the prerequisite of having switched

medications without the use of objective symptom scales however, may be problematic. The decision to switch medications may be influenced by extraneous factors unrelated to lack of response. For example, an individual may refuse to switch medications for a variety of reasons (side effects, tolerability or lack of drug coverage). In addition, patients vocalizing higher levels of distress related to symptoms, may prompt more frequent switching relative to patients who continue to be symptomatic but are not as vocal, perhaps due to lack of insight. Therefore, the use of standardized symptom scales in defining resistance, is crucial, and unfortunately, has not been reflected in most traditional TRS definitions. However, defining resistance as failure to reach some predetermined, arbitrary symptom score may be additionally problematic. Application of an absolute symptom threshold in characterizing TRS risks over-categorizing resistance in individuals with high initial symptom burdens, who may never reach a defined threshold despite having improved considerably from baseline. Similarly, a patient with a milder presentation of illness in terms of symptom severity, may meet threshold criteria, without having experienced much improvement from their own baseline.

The use of criteria that employ relative symptom improvement from baseline may control for some of the inherent variability in illness severity which may confound determination of whether a person has responded sufficiently. Until recently, there has been no consensus in the field regarding the extent of relative improvement necessary for defining adequate response. While Leucht et al (2007) have suggested that sufficient response be operationalized as a 50% reduction in scores on the Brief Psychiatric Rating Scale (BPRS), or Positive and Negative Syndrome Scale (PANSS), the Treatment Response and Resistance in Psychosis (TRRIP) working group has defined resistance as having a symptomatic reduction of less than 20% from baseline (Howes et al, 2017). However, given that most patients with FEP respond robustly to

antipsychotics (Robinson et al, 1999), a <20% improvement threshold may not be sufficiently sensitive to identify poor response at this stage of illness.

In reality, response to treatment may be best conceptualized in a continuous manner, with some individuals being highly treatment resistant, others being highly treatment responsive, and the majority showing an intermediate response. Indeed, using growth mixture modelling (GMM), Marques et al (2011) found that positive symptom antipsychotic response over 6 weeks was best represented by four trajectories. 48% were partial responders and showed approximately a 20% improvement in symptoms. 22% were responders and showed a 50% improvement, while 10% were dramatic responders and showed a 75% improvement. Finally, approximately 20% were categorized as non-responders. Although there have been numerous studies investigating treatment resistance, including recent efforts at developing globally-applicable criteria (Howes et al, 2017), the optimal symptomatic improvement required for response is debatable, and has not been well-defined, particularly for patients with FEP. In addition to the lack of objective criteria in defining TRS, there are multiple factors, including medication non-adherence, substance use, and differences in individual pharmacokinetics, that lead to challenges in determining whether an individual is truly medication resistant.

One of the biggest challenges in accurately identifying TRS relates to the issue of medication adherence. In general, non adherence to antipsychotic medications is associated with poorer outcomes including lower rates of remission, more time required in hospital (Caseiro et al, 2012), and non-adherent individuals may be mistakenly identified as being treatment resistant (Correll et al, 2011). The large CATIE study found rates of medication non adherence to be approximately 74% within 18 months (Lieberman et al, 2005). With non-adherence being the norm rather than the exception, it is exceedingly difficult to both estimate rates of true TRS, and

accurately identify it clinically. Given that partial adherence has been associated with an increased risk of relapse, a partially-adherent individual may appear to be treatment resistant if it is assumed they are taking their medication as prescribed. Many studies investigating TRS have failed to accurately separate poor adherence from true treatment resistance. More recently, the more frequent use of long acting injectable (LAI) antipsychotic medications may help address this concern. Another potential method of separating poor adherence from true resistance involves monitoring of antipsychotic serum levels; however, this is not available at many centres for all agents used, and may be undesirable from a patient perspective.

In addition to poor medication adherence, ongoing use of illicit drugs may complicate an apparently treatment resistant presentation. In investigating treatment resistance, it is important to thoroughly screen for substance use, and continue to inquire regarding use patterns, particularly in patients who remain symptomatic. Urine toxicology screens may be helpful in determining drug use however, in many cases, frequent urine sampling is not practical or possible, and many newer drugs of abuse do not screen positive on current traditional drug panels.

Although many definitions of treatment resistance stipulate a lowest acceptable antipsychotic dosage threshold, several other factors may influence how a particular individual responds to a given dose of an antipsychotic medication. Factors such as gender, body habitus, general health status, gastrointestinal absorption, and other drug interactions may influence the effective dosage that an individual receives. Therefore, while a person may be taking a seemingly effective dose, due to pharmacokinetics and pharmacodynamics, they may not be absorbing a truly effective dose for them. Of particular relevance, is the fact that CYP 1A2 is induced by cigarette smoking (Hukkanen et al, 2011), which decreases the effective dosage of

many antipsychotic medications. Therefore, while outlining minimal dose thresholds in defining treatment resistance is helpful, it is important to keep in mind that several extraneous factors may influence the absolute dosage a particular individual will receive.

Overall, while some objective definitions of TRS have been proposed, the use of these definitions is variable, and there continue to be problems related to lack of objectivity in utilizing these criteria.

1.3 Time Course of Treatment Resistance

Historically, chronicity has frequently been assumed as being a prerequisite for resistance, and it was previously speculated that TRS developed overtime following a longer duration of psychosis, and successive relapses. While there is a subset of patients who seem to develop resistance following a period of initial response, in general, evidence has shown that a treatment-resistant course often declares itself from the onset of illness (Agid et al, 2011). In a retrospective study of treatment response 2-20 years after an initial diagnosis of schizophrenia, the majority of TRS patients developed resistance early and remained resistant throughout the course of their illness (Kolakowska et al, 1985). Two studies have specifically investigated the prevalence of TR in FEP. Lally et al (2016) used retrospective chart analyses to investigate the course of treatment resistance following a FEP. They found that 34% of patients met criteria for treatment resistance at 5-year follow-up, and that of these, 70% did not respond to antipsychotic medications from illness onset. In another 10-year retrospective study of treatment resistance after FEP, 23% of patients met criteria for treatment resistance, and of these, 84% were characterized as being resistant from treatment initiation (Demjaha et al, 2017). Differences between these studies may relate to the application of variable criteria for TRS. Lally et al (2016) considered patients to be treatment resistant if they were on clozapine at any point, or showed

"little symptomatic improvement" after being on two different antipsychotic medications. Demajaha et al (2017) classified those who continued to have positive symptoms (a rating of at least one or more positive symptoms of at least "moderate" severity) after two antipsychotic trials as treatment resistant. In addition, neither of these studies applied stringent measurements of medication compliance. Overall, it seems that for the majority, treatment resistance may represent a stable, neurobiological phenomena, present from the first episode of illness, though recognizing it at that time, remains clinically challenging.

While treatment resistance, in many ways, is poorly understood, there appears to be a better understanding of the time course of antipsychotic treatment response, which is closely related to the concept of resistance. As the bulk of antipsychotic response occurs within the first few weeks (Agid et al, 2003) following drug initiation (a meta-analysis by Suzuki et al (2011) of patients with chronic schizophrenia found that two-thirds of the response occurred within the first three weeks), longer drug trials may be unproductive and result in negative clinical outcomes. Applying a diagnostic test review model of studies investigating early treatment response in patients with primarily chronic schizophrenia, Samra et al (2015) found that failing to achieve at least a minimal response (characterized by improving by 20% on the PANSS or BPRS) at week 2, predicted failing to achieve a 50% improvement at 4-12 weeks. A similar pattern of treatment response being evident within the first few weeks following antipsychotic initiation has been demonstrated in FEP samples. Logistic regression analyses were applied to a large sample of patients with FEP registered in the European First Episode Schizophrenia Trial (EUFEST) to test whether response at 2, 4, or 6 weeks predicted remission at one-year. 2-week treatment response predicted remission status at one-year at a rate of 61%. Including response at 4 and 6 weeks improved correct classification of remission to 63% and 68% respectively (Derks

et al, 2010). In another group of antipsychotic-naïve patients with first-episode psychosis, treatment response at week 4 was associated with categorical treatment response at one-year (Ucok et al, 2011). Based on analyses of response patterns, Schennach-Wolff et al, (2010) concluded that patients with FEP should improve by at least 30% in total symptoms by week 2 in order to achieve response and remission. However, Gallego et al, (2011) found that in FEP, the estimated cumulative response to antipsychotic medications was 39.58% by week 8, and 65.16% by week 16, but did not find that earlier response patterns (at weeks 2, 4, or 8) were predictive of response at week 16. Therefore, while treatment resistance can be recognized early on in FEP, and treatment response in the initial weeks following antipsychotic initiation may be highly predictive of overall eventual responsiveness, it is unclear exactly when response should be noted in terms of predicting longer term illness trajectory.

Chapter 2: Defining Treatment Resistance in a First Episode Psychosis Sample

2.1 Rationale for Study

The course of schizophrenia is heterogeneous, with variable response to treatment. Approximately one third of patients (Meltzer, 1997; Lindenmayer, 2000) continue to experience symptoms despite treatment with dopamine-blocking antipsychotic agents, with response rates as low as 7% in patients who have failed two antipsychotic trials (Kinon et al, 1993). It appears that a treatment-resistant course may declare itself early in the course of illness (Kolakowska et al, 1985; Agid et al, 2011) with approximately 23-34% of patients being treatment resistant (TR) from their first episode of schizophrenia (FES) (Lally et al, 2016; Demjaha et al, 2017). Recently, the Treatment Response and Resistance in Psychosis (TRRIP) working group

published consensus guidelines (Howes et al, 2017) for defining treatment resistance. According to these guidelines, insufficient response is defined as <20% improvement on symptom domains (with positive, negative, and cognitive domains measured separately), with TR being defined as insufficient response achieved with two trials of antipsychotic medications of adequate duration and sufficient dosage. However, it is unclear whether the threshold of 20% symptom improvement will be appropriate for a FES sample where response rates are generally high (Derks et al, 2010). Alternative criteria for defining TR specific to FES are warranted in order to promote earlier recognition of TR. Furthermore, the role of negative symptoms in defining TR at FES has not yet been characterized. In this report, we study the utility of applying a "time-based" response cut-off, irrespective of the number of antipsychotic trials, that considers negative symptom improvement in predicting "probable TR" in a prospective FES sample. We studied the utility of a 20% response threshold (as defined by TRIPP), as well as a more stringent 50% response threshold (identified as a "good response" cut-off for clinical trials by Aboraya et al, 2017) in the domains of positive, negative, and total symptoms 6 months following FES. In keeping with previous literature (Meltzer et al, 1997; Lindenmayer et al, 2000) we hypothesized that the most valid criteria would categorize approximately 33% of the sample as probable TR. We hypothesized that approximately one third of individuals with FES would meet probable TR criteria at as early as 6 months after commencing antipsychotic treatment, and that negative symptoms would be important in characterizing probable TR in a FES sample.

2.2 Methods

2.2.1 Sample

Data were analysed retrospectively using a longitudinal, naturalistic sample of patients treated at the Prevention and Early Intervention Program for Psychosis (PEPP) in London,

Ontario between February 1997 and February 2002. This program provides assessment and treatment to individuals presenting with first-episode, non-affective psychoses using an assertive case-management model. Criteria for acceptance to the program include age between 16 and 50, symptoms meeting criteria for a Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition) (DSM-IV) psychotic disorder, and having never received prior antipsychotic treatment for greater than one month. Approval for the study was obtained from the University Human Ethics Committee for Health Sciences at the University of Western Ontario. Patients treated by the program during that time were invited to participate in a study involving symptoms and functional outcomes.

Diagnoses were established using the Structured Clinical Interview for DSM-IV (First and Gibbon, 1997) by trained research assistants, and confirmed by two senior psychiatrists and a clinical research psychologist, with consensus diagnosis conferences occurring at one-year follow-up. Individuals that ended up meeting criteria for a mood disorder with psychotic features, or any substance-induced psychosis, were excluded from the analysis. Positive and negative symptoms of psychosis were assessed using the SAPS (Andreasen, 1984) and SANS (Andreasen, 1983) at baseline, and at months 1, 2, 3, and 6. Interrater reliability on the SAPS and SANS demonstrated agreement within one point 93% of the time (Malla et al, 2005). Duration of untreated illness (DUI) was calculated as the period between the onset of any psychiatric symptoms and the time to antipsychotic treatment. Duration of untreated psychosis (DUP) was defined as the time between the onset of psychotic symptoms and the time to adequate (at a minimally effective dose) antipsychotic treatment. Premorbid adjustment was measured using the Premorbid Adjustment Scale (PAS) (Cannon-Spoor et al, 1982), with higher values indicating worse premorbid functioning. Symptom change overtime was calculated as the

difference between a particular symptom domain from baseline divided by the baseline symptom score (*e.g.* positive symptom improvement over month 1= (($SAPS_{baseline}$ - $SAPS_{month\ 1}$)/$SAPS_{baseline}$)x100%).

Adherence monitoring to antipsychotic medication treatment was accomplished via a weekly adherence log (as described in Malla et al, 2006). Adherence was scored on a scale of 0-4 (0=not adherent, 1=0-25%, 2=25-50%, 3=50-75%, 4=75-100% of prescribed doses taken). Scores were obtained through reports of case managers (who have frequent contact with patients and their families), in discussion with the primary psychiatrist. Patient and family reports were considered in making adherence assessments, as well as reviews of prescriptions and pill counts. Individuals were considered to be adherent if they scored a 4, meaning their compliance was estimated to be between 75-100%. Individuals that were not medication adherent (scores of 0, 1, 2, or 3) based on 6-month adherence measures were not included in the sample.

2.2.2 Defining Treatment Resistance

Probable TR status was investigated at 6 months after entry into the first episode psychosis program based on meeting defined thresholds for symptom change from baseline, in those who were medication adherent. We identified all individuals who were not responding to antipsychotic treatment based on 2 different response thresholds, regardless of whether or not alternative treatment options were tried. For positive, negative, and total symptom domains, we used 20%, and less than 50% improvement as cut-offs to identify subjects satisfying “probable TR” criteria. Following identifying “probable TR” individuals, individual item scores on the SAPS and SANS (as applicable) were examined at baseline to ensure that individuals meeting criteria met the threshold of at least moderate severity (more than one individual item >2) in terms of symptomology, as suggested by TRIPP (Howes et al, 2017).

2.2.3 Statistical Analysis

Goodness-of-fit tests based on chi-square statistics were performed on the 6 definitions of probable TRS. We tested the observed proportions against the expected proportion of 30% subjects being treatment resistant (Meltzer, 1997; Lindenmayer, 2000). Chi-square analyses were then performed to determine the univariate association between variables (ie, substance abuse, family history, gender) and probable TR status at 6 months, and t-tests were used to assess the association between continuous (ie, duration of untreated psychosis, age at onset) variables and probable TR status. For all t-tests, Levine's test for homogeneity of variances was conducted. For variables showing significant heterogeneity of variance, the corrected p-value was used. Logistic regression analyses were then applied to create a model predicting membership within the probable TR group using only the definitions that corresponded closely to estimated rates of TR of about 1/3 (Meltzer, 1997; Lindenmayer, 2000). All demographic and clinical factors found to be significantly associated with probable TR status using chi-square analyses or independent t-tests were included in the prediction models. Mann-Whitney U Tests were then used to compare the number of antipsychotic medication trials in individuals with probable and non-TR for each symptom improvement threshold.

2.3 Results

2.3.1 Final Sample

129 patients met criteria for a first episode schizophrenia spectrum disorder and were considered for inclusion in the analysis. Using only individuals that were medication adherent, resulted in a sample size of 93 FES patients (74 male and 19 female), while 36 (30.23%) were

not included due to being categorized as non-adherent. One male was missing SANS scores and therefore the total sample size was 92 for analyses assessing negative symptoms.

2.3.2 Rates of TR

We first investigated the prevalence of probable TR at 6 months using 20% and 50% symptom improvement thresholds for positive, negative and total symptoms (see table 2-1). Total symptom probable TR<50% and negative TR<20% at 6 months resulted in rates closest to those previously described in the literature (Meltzer, 1997; Lindenmayer, 2000) (rates of 37% and 33% respectively). We further tested the goodness of fit of these models using one-sample chi-square tests with the null hypothesis being that the proportion of TR individuals would be 33% for each criterion. The null hypothesis was rejected for all definitions, with the exception of TR negative <20% and TR total <50%, meaning the expected frequency of TR for these criteria was approximately 33%. Of those meeting criteria for TR negative <20%, 77% also met criteria for total symptom TR<50%, suggesting there was a high degree of overlap between these two categorizations of TR. Next, we investigated the predictive ability of demographic factors, and early symptom improvement, in determining membership to these classifications of probable TR (total symptoms <50%, and negative symptoms <20% criterion).

Table 2-1. Rates of Probable TR and mean number of antipsychotic trials using various criteria (n=92).

Domain	Criteria	# Prob TR	# AP trials M (SD)	# non TR	# AP trials M (SD)
Positive symptoms	<20%	2 (2%)	2.00 (1.41)	91 (98%)	1.30 (0.50)
	<50%	13 (14%)	1.38 (0.65)	80 (86%)	1.30 (0.05)
Negative symptoms	<20%	30 (33%)	1.23 (0.50)	62 (67%)	1.34 (0.54)
	<50%	56 (61%)	1.30 (0.54)	36 (39%)	1.31 (0.52)
Total symptoms	<20%	11 (12%)	1.36 (0.67)	81 (88%)	1.29 (0.51)
	<50%	34 (37%)	1.32 (0.59)	58 (63%)	1.29 (0.49)

M = mean; SD = standard deviation.

Table 2-2. Chi-Square Analyses for Goodness of Fit of Various TR Definitions.

	TR Pos<50	TR Pos<20	TR Neg<50	TR Neg<20	TR Total<50	TR Total<20
Chi-square	15.22	40.03	32.31	.01	.65	18.43
Significance	.000	.000	.000	.936	.420	.000

Df= 1 for all tests

2.3.3 Factors Associated with Total Probable TR <50% at 6 months

Patients meeting criteria for probable TR based on having a less than 50% total symptom improvement had worse premorbid functioning (*M*= .37, *SD*=.13) than patients not meeting probable TR criteria (*M*=.28, *SD*=.17) (*t*(80)= -2.526, *p*=.014), a longer duration of untreated illness (probable TR: *M*= 386.21, *SD*= 340.78; non TR: *M*=236.31, *SD*= 211.28), (*t*(48.37)= -2.31, *p*= 0.025), a lower baseline SAPS score (probable TR: *M*= 9.35, *SD*= 3.52; non TR: *M*=11.21, *SD*= 3.47), (*t*(90)= 2.463, *p*= .016) and less total symptom percentage improvement over 1 month (probable TR: *M*=16.39%, *SD*= 26.37; non TR: *M*=37.38%, *SD*= 23.46), (*t*(57)= 3.048, *p*=0.003), and 2 months (probable TR: *M*=20.87%, *SD*= 27.05; non TR: *M*= 48.70%, *SD*= 29.83), (*t*(60)=3.59, *p*=0.001).

Table 2-3. Factors Associated with Probable TR (<50% improvement on total symptoms).

Characteristic	Prob TR	Non TR	*t* or χ2	df	*p*-value
PAS	0.37/.17	0.28	-2.526	80	0.014*
DUP (Weeks)	104.04/134.98	68.10/105.38	-1.421	90	0.159
DUI (Weeks)	386.21/340.78	236.31/211.28	-2.313	48	0.025*
Age of Onset	23.96/8.66	24.45/7.80	0.275	90	0.784
SAPS baseline	9.35/3.52	11.21/3.47	2.463	90	0.062
SANS baseline	11.38/4.64	13.47/5.35	1.890	90	0.062
Total ΔSx_{1month} (%)	16.39	37.38	2.048	57	0.003*
Total ΔSx_{2month} (%)	20.87	48.71	3.585	60	0.001*
Substance abuse/dep. Y/N	9/25	14/44	0.062	1	0.803
Mode of onset I/A	23/9	44/14	0.172	1	0.678

Gender M/F	28/6	45/13	0.297	1	0.586
Family History Y/N	12/16	20/33	0.201	1	0.654
Number of AP switches	0.2941	0.2759	-0.186	90	0.853
Cpz eq over 6 months	249.51	213.92	-0.884	56	0.381

PAS= premorbid adjustment scale. DUP= duration untreated psychosis. DUI= duration untreated illness. Total symp change ratio is difference between baseline symptoms and 1, and 2 month symptoms/Baseline symptoms. I= insidious. A= acute. Y=yes. N= no. M= male. F= female. AP= antipsychotic. Cpz eq= chlorpromazine equivalence over 6 months. Note that for all t-tests, Levine's test for homogeneity of variances was conducted. For variables showing significant heterogeneity of variance, the corrected p-value was used.

Next, a logistic regression analysis was conducted to predict TR total <50% criteria using all predictors found to be associated with TR in univariate analyses (premorbid functioning, duration of untreated illness, baseline SAPS score, and total symptom change over months 1 and 2). A test of the full model was significant, indicating the model as a whole could reliably identify probable TR (total<50%) ($\chi 2$= 15.90, p= .007, df= 5). Prediction success overall was 82.2% (96.9% for non probable TR and 46.2% for probable TR), indicating the model was much stronger at ruling out TR. In analyzing the independent predictors in the model (see table 2-4), none of the variables independently predicted TR status, although total symptom change over 2 months demonstrated trend-level significance (p=.064). We tested for multicolinearity using the variance inflation factor (VIF) among the predictors using a threshold of 2, (with a tolerance of less than 0.9 for all predictors) with no evidence of multicolinearity detected.

Table 2-4. Binary logistic regression analysis of Probable TR total symptom <50% improvement criteria.

Predictor	Odds Ratio (95% CI)	P-value
Premorbid adjustment	35.56 (.17-7520.22)	.168
Duration of untreated illness	1.00 (.999-1.01)	.249
SAPS baseline	1.04 (.83-1.31)	.733
Total ΔSx_{1month} (%)	.98 (.94-1.02)	.227
Total ΔSx_{2month} (%)	.96 (.93-1.00)	.059

Total symp change ratio = (Baseline total symptoms – N month total symptoms)/Baseline total symptoms. χ^2 = 1590; P= 0.007, Nagelkerke r^2=0.426; B=-.901; SE= .329; Wald= 7.501; P= 0.006; Exp(B)= .406.

2.3.4 Factors Associated with TR Negative <20% at 6 months

Patients meeting criteria for TR based on having a less than 20% improvement of negative symptoms had worse premorbid functioning (probable TR: *M*=.38, *SD*= .18, non TR: *M*=.28, *SD*= .14), (*t*(80)=-2.79, *p*= .007), a longer duration of untreated illness (probable TR: *M*= 419.52, *SD*= 337.27, non TR: *M*=229.75, *SD*= 215.55), (*t*(41.02)=-2.81, *p*=.008), a lower SAPS score at baseline (probable TR: *M*= 9.2, *SD*=3.45, non TR: *M*= 11.16, *SD*= 3.49; *t*(90)=2.53, *p*=.013) and a lower SANS score at baseline (probable TR: *M*=10.13, *SD*= 4.41, non TR: *M*=13.94, *SD*= 5.09), (*t*(90)= 3.53, *p*= .001).

Table 2-5. Factors Associated with Probable TR (<20% improvement on negative symptoms).

Characteristic	Prob TR	Non TR	*t* or $\chi 2$	df	*p*-value
PAS	0.38/.18	0.28/.14	-2.788	80	0.007*
DUP (Weeks)	89.98/120.79	77.22/117.04	-.485	90	0.629
DUI (Weeks)	419.52/337.27	229.75/215.55	-2.812	41.021	0.008*
Age of Onset	25.11/8.51	23.86/7.91	-.699	90	0.480
SAPS baseline	9.2/3.45	11.16/3.49	2.535	90	0.013*
SANS baseline	10.13/4.41	13.94/5.09	3.53	90	0.001*
Negative ΔSx_{1month} (%)	.12	18.98	1.647	57	0.105
Negative ΔSx_{2month} (%)	14.35	27.47	1.239	60	0.220
Substance abuse/dep. Y/N	7/23	16/46	0.066	1	0.797
Mode of onset I/A	21/7	16/46	0.007	1	0.935
Gender M/F	26/4	47/15	1.455	1	0.228
Family History Y/N	12/14	20/35	0.708	1	0.400
Number of AP switches	0.20/.41	0.32/.47	1.285	65.714	0.203
Cpz eq over 6 months	213.89/170.18	233.77/176.90	0.510	89	0.611

*=p<.005. PAS= premorbid adjustment scale. DUP= duration untreated psychosis. DUI= duration untreated illness. I= insidious. A= acute. Y=yes. N= no. M= male. F= female. Neg symp change ratio is difference between baseline negative symptoms and 1, and 2 month negative symptoms/Baseline negative symptoms. AP= antipsychotic. Cpz eq= chlorpromazine equivalence over 6 months. Note that for all t-tests, Levine's test for homogeneity of variances was conducted. For variables showing significant heterogeneity of variance, the corrected p-value was used.

Finally, a logistic regression analyses was conducted to predict TR negative <20% criteria using all predictors found to be associated with TR in univariate analyses (premorbid adjustment, duration of untreated illness, and SAPS and SANS at baseline). The overall model was significant in its ability to differentially classify probable TR based on negative symptom improvement <20% criterion, from those not meeting this criterion at 6 months ($\chi 2$= 26.80, p<.000, df= 4). The overall prediction success of the model was 79.3% (93.1% for treatment responsiveness and 45.8% for treatment resistance, indicating our model was better at correctly identifying those who were not probable TR). Duration of untreated illness (*OR*= 1.003, 95% CI= 1.001-1.006, p= .008) and SANS score at baseline (OR= .836, CI= .723-.926, p=.015) remained independent predictors of TRS negative symptom <20% criterion at 6 months. We tested for multicolinearity using the variance inflation factor (VIF) among the predictors using a threshold of 2, (with a tolerance of less than 0.9 for all predictors) with no evidence of multicolinearity detected.

Table 2-6. Binary logistic regression analysis of Probable TR negative symptom <20% improvement criteria.

Predictor	Odds Ratio (95% CI)	P-value
Premorbid adjustment	20.38 (.343-1212.99)	.148
Duration of untreated illness	1.003 (1.001-1.006	.008*
SAPS baseline	.896 (.740-1.085)	.261
SANS baseline	.836 (.723-.966)	.015*

*=p<.005. χ^2 = 26.798; P= 0.000; Nagelkerke r^2=0.379; B=-.882; SE= .243; Wald= 13.217; P= 0.000; Exp(B)= .414.

2.3.5 Probable TR and Antipsychotic Trials

Mann-Whitney U tests were used to compare the number of antipsychotic medication trials in individuals with probable and non-TR for each symptom improvement threshold. For probable TR positive, there were no significant differences in number of antipsychotic trials for the 20% improvement threshold (*U*=871.5, *Z*=-.657, p=.511) or the 50% improvement threshold

(*U*= 834.0, *Z*=-.803, *p*=.422). For probable TR negative, there were no differences in the number of antipsychotic medication trials for the 20% improvement threshold (U=744.0, Z=-1.22, p=.223) or the 50% threshold (U=850.0, Z=-.082, p=.935). Similarly, there were no significant differences in the number of antipsychotic medication trials for those with probable and non-TR total symptom improvement for the thresholds of <20% (*U*=853.00, *Z*=-.077, *p*=.939), or <50% improvement (*U*=840.0, *Z*=-.187, *p*=.852).

2.4 Discussion

2.4.1 Summary of Results

This is the first study to our knowledge to investigate the applicability of response thresholds proposed by TRIPP (Howes et al, 2017) in a FES sample. In addition, there have been no other studies looking specifically at the importance of negative symptom persistence in predicting TR in early psychosis.

Our results suggest that at 6 months of treatment, the most ecologically valid definition, based on the expected rate of 30% subjects having a resistant form of schizophrenia, is the failure to improve in total symptoms by 50%, or negative symptoms by 20%. In keeping with our hypothesis, inclusion of negative symptoms in defining TR appears to be crucial to identify the probability of TRS in an acceptable proportion of individuals with FES. Definitions of TR relying solely on positive symptomology were inadequate in identifying cases of probable TR (probable TR rates were 2.2% for the 20% threshold, and 14% for the 50% threshold) likely in keeping with the fact that positive symptom improvement is robust early in the course of schizophrenia (Robinson et al, 1999). The 20% symptom improvement criteria as suggested by

TRIPP (Howes et al, 2017), appears efficacious when considering only negative symptom improvement, but fails to identify the expected proportion of patients when using positive, and total symptoms in a FES sample. Our results provide preliminary evidence that individuals with probable TR can be identified at as early as 6-months following the onset of FES, regardless of the number of antipsychotic trials, and that inclusion of negative symptom improvement is essential in risk-stratification for probable TR in early psychosis.

In retrospective studies on clinical samples, consistent risk factors of TRS have been male sex, earlier age-of onset or longer duration of illness (Lally et al, 2016), poor premorbid functioning, lack of early treatment response, and higher baseline illness severity (indexed by prior hospitalization in some studies) (Schennach et al, 2012). Of these, our 2 prospective categories of probable TR were associated with poor premorbid adjustment, higher severity of symptoms at baseline, longer illness duration as well as lack of early treatment response. The lack of association with male sex is likely to be due to the smaller number of females in the sample; it is also worth noting that a large population-based study failed to note the association of sex and TRS (Wimberley et al, 2017).

2.4.2 Clinical Implications

We have demonstrated the feasibility of prospectively identifying a group of FES subjects that share the risk factors for later TRS, even before 2 antipsychotic failures can be observed. In our sample, lack of early symptom improvement (at months 1, and 2) was a robust predictor of meeting criteria for probable TR at 6 months. Given that TR status at 6 months is associated with poor response at as early as 1 month, clinicians should closely monitor individuals who fail to improve symptomatically over the first couple of months following

antipsychotic initiation. Furthermore, lower premorbid adjustment, and having a longer duration of untreated illness were associated with probable TR at 6 months for both the <50% improvement in total symptoms, and the <20% improvement in negative symptoms criterion, suggesting that individuals with greater impairments at the onset of psychosis are at increased risk for a resistant course. Active monitoring for probable TRS in early intervention programs can aid in treating these individuals at the earliest possible opportunity, and avoid interventional relapse (Emsley et al, 2013) and subsequent resistance (Takeuchi et al, 2018).

Clozapine is the only antipsychotic medication with superior efficacy in TR individuals Siskind et al, 2016) and current guidelines necessitate failure of two antipsychotic agents prior to initiating a trial of clozapine. The requirement of consecutive antipsychotic trials may contribute to delays in clozapine initiation. In addition, a disproportionate focus on positive symptoms in determining clozapine-eligibility, as well as an emphasis on chronicity in defining TR, may also contribute to unnecessary delays in initiating this potentially disease-altering medication. Response rates to clozapine following failed antipsychotic trials have been shown to be more robust in patients with FES (75%) (Agid et al, 2011), relative to chronic samples (40%) (Siskind et al, 2017), and therefore, developing objective clinical indicators of clozapine eligibility earlier-on is of pivotal importance. Our results suggest that TR may be determined by 6 months following FES, and that time-based criteria for TR may be reliable in identifying patients for potential consideration of clozapine. Patients who fail to improve in total symptoms by 50%, or negative symptoms by 20% by 6 months following FES, should be closely assessed for TR. Particular importance should be given to negative symptoms that persist over the first few months following a first episode of psychosis. As positive symptom improvement is significant

at this early stage, focusing exclusively on positive symptom improvement may lead to under-identification of TR individuals.

In a naturalistic practice setting, when considering treatment-compliant subjects, the number of AP trials does not differ between probable TRS and non-TRS. In other words, in the absence of the explicit clinical knowledge of the risk factors, pharmacological practice does not change for patients who are likely to later need clozapine. This is an important aspect to consider in our efforts aimed at mitigating the delay in timely clozapine use. Our results suggest that irrespective of the number of AP trials attempted, if a patient with FES shows less than 50% total symptom burden or 20% total negative symptom improvement by 6 months of early intervention, then fast-tracking to clozapine may be warranted. This highlights the need for measurement-based care in FES settings (Correl et al, 2012).

2.4.3 Strengths and Limitations

The use of standardized improvement thresholds based on severity of initial presentation is a relative strength of our study. Without using objective improvement criteria, individuals presenting with milder symptoms at baseline may be judged as achieving sufficient response, even if their improvement has been sub-par. Similarly, individuals who are severely unwell at baseline, may improve substantially but never meet arbitrary absolute thresholds in terms of symptom severity and be defined as TR despite having improved significantly. In addition, our breakdown of positive and negative symptom domains in defining TR has not previously been employed in a FES sample and contributes important information to the understanding of the early progression of a TR course.

In terms of potential limitations, while formal assessments of medication adherence were completed, we cannot be certain whether or not individuals were compliant as antipsychotic

serum levels were not performed. However, our measure of assessing adherence has been validated, and has been shown to be related to pill counts (Cassidy et al, 2010). In addition, symptom data were available at discrete intervals. However, the relapse status of individuals between assessments was not recorded for the purposes of this analysis. It is possible that individuals who experienced relapse, particularly prior to 6 months when TR status was assessed, may have been mislabelled as having inadequate response simply because their symptoms were higher at the time of assessment secondary to relapse. However, the fact that TR status at 6 months was associated with impaired symptom improvement during the early months, suggests that for the majority, the development of TR was occurring early on, and was not simply related to an acute symptomatic relapse. Finally, because the analysis included only individuals who were rated as being medication adherent at 6 months, our analyses may have missed individuals who were in fact TR if they were not medication-adherent at that particular time point.

2.4.4 Future Directions

Going forward, it would be important to investigate whether individuals identified as being probable TR based on these criteria, also met criteria for more traditional definitions of TR which necessitate two failed antipsychotic trials, and evaluate the ability of these criteria to identify clozapine-eligibility. While informative regarding the clinical characteristics associated with the eventual development of TR in early psychosis, our prediction models demonstrate much better ability to predict response relative to resistance. Future studies are warranted using brain-based measures, in addition to demographic and clinical characteristics, to attempt to improve our ability to prospectively identify TR in FES. Furthermore, prospective studies using

this time-based approach to TR identification are warranted to validate these findings in other samples of patients with FES.

Chapter 3: Neurobiological Markers of Treatment Resistance

Given that TRS remains difficult to define clinically, investigation of underlying neurobiological correlates of a treatment resistant course may improve both understanding of the underlying pathogenesis, and support prospective identification of individuals with TRS. Structural, functional, as well as changes in neural metabolite levels have been demonstrated in TRS individuals relative to general schizophrenia samples. Differential neurobiological patterns in these patients, supports the need for alternative therapeutics targeting individualized brain changes characteristic of a treatment resistant course. In this chapter, we review existing literature on brain abnormalities demonstrated in TRS, with a focus on studies investigating FEP samples.

3.1 Structural Brain Changes in Treatment Resistant Schizophrenia

Structural differences have been demonstrated in the brains of patients with TRS relative to responders since the advent of neuroimaging techniques. However, results have been inconsistent, likely as a result of the use of heterogeneous populations, and variable neuroimaging protocols. While very early Computerized Tomography (CT) studies demonstrated an association between enlarged ventricles and treatment resistance (Weinberger et al, 1979), subsequent studies have not replicated this finding (Friedman et al. 1992; Borgio et al. 2010). Using structural Magnetic Resonance Imaging (MRI), numerous studies have demonstrated reduced frontal grey matter in resistant patients relative to responsive patients (Anderson et al,

2015; Quarentelli et al, 2014; Kubera et al, 2014; Zugman et al, 2013; Mitelman et al, 2005; Molina et al, 2008; Anderson et al, 2015; Quarantelli et al, 2014) and in general, cortical atrophy has been associated with lower rates of response to treatment (Bilder et al, 1994; Stern et al, 1993). Increased white matter volume was also found to differentiate resistant from responsive patients in one study (Molina et al, 2008), although the difference was not significant in another (Anderson et al, 2015). Overall, it appears that in chronic schizophrenia, patients with poor treatment response are more likely to demonstrate volumetric deficits, with these deficits being most specific to frontal areas. However, because this population would have been exposed to numerous neuroleptics, frequently over the course of several years, medication effects on brain volume cannot be ruled out.

Volumetric studies of patients with FEP are important, as treatment exposure is minimal in this group. In an MRI study of patients with FEP, those with increased cortical thickness were more likely to be treatment-responsive, and responded more quickly to antipsychotic medications (Szeszko et al, 2012). Consistent with the concept of impaired neurodevelopment in schizophrenia, Palaniyappan et al (2013) found that non-responders to antipsychotics at 12-weeks displayed hypogyrification in bilateral insular, left frontal, and right temporal regions, relative to responders. Therefore, it appears that cortical thickness, and gyrification patterns may represent biological indicators of poor treatment response in early psychosis. Structural brain changes overall may be longstanding, and support a theory of neurodevelopmental abnormalities in patients that go on to be treatment resistant.

3.2 Functional Brain Changes in Treatment Resistant Schizophrenia

In addition to structural differences between responders and non-responders to

medication, there is evidence for altered neural connectivity in patients with TRS, measured using functional imaging techniques. Reduced white matter integrity in patients with FEP was found to be a predictor of poor treatment response (Reis et al, 2014). In a study of 63 patients with FEP, lower functional anisotropy in the uncinate, cingulum, and corpus callosum at baseline was associated with antipsychotic non-response at 12 weeks, while responders to antipsychotic medication were not significantly different with respect to functional anisotropy relative to healthy controls (Marques et al, 2014). In another study using resting state MRI, greater functional connectivity was demonstrated in treatment resistant patients between the dorsomedial prefrontal cortex and frontotemporal areas, and reduced connectivity was found between the ventromedial prefrontal cortex and the anterior cingulate cortex (Alonso Solis et al, 2015). Deepak et al (2016) investigated striatal functional connectivity nodes and non-response in a sample of FEP patients using resting-state functional MRI and found that greater posterior-striatal connections at baseline were associated with better response, and greater frontal-striatal connections were associated with non-response. Furthermore, antipsychotic response may be associated with progressive changes in functional connectivity in relevant brain areas. In a 12-week prospective study of patients with FEP, a greater reduction in positive symptoms was related to increased functional connectivity between the right dorsal caudate and prefrontal areas including the orbitofrontal cortex, anterior cingulate cortex, and dorsolateral prefrontal cortex (Deepak et al, 2015). Therefore, in addition to structural alterations, treatment resistance appears to be associated with aberrant neuronal connection patterns in both FEP, and chronic schizophrenia samples. While intriguing, findings of global structural and functional alterations in TRS patients do not support the development of alternative treatments. Investigation of the brain chemistry underlying TRS is crucial as determination of metabolite differences may

facilitate targeted treatment development, which is grossly lacking in this population.

3.3 The Dopaminergic Theory of Schizophrenia

For many years, the dopamine hypothesis of schizophrenia represented the field's best understanding of the underlying pathophysiology of schizophrenia. There are several lines of evidence supporting a hyperdopaminergic state as being intrinsically associated with psychosis. First of all, dopamine agonists, such as amphetamines, lead to positive symptoms of psychosis, and exacerbate psychotic symptoms in individuals with established psychotic disorders. In addition, the effectiveness of antipsychotic medications has been directly linked to their capacity to block D2 receptors in the striatum (Seeman et al, 1975; Creese et al, 1976). Individuals with schizophrenia have been found to have increased dopamine synthesis capacity, elevated synaptic dopamine levels, and increased dopamine release (Howes et al, 2012) relative to healthy controls, and elevated presynaptic striatal dopamine has been associated with the severity of positive symptoms (Abi-Dargham & Grace, 2011). Similar findings, albeit to a lesser extent, are seen in individuals at clinical high risk (CHR) for psychosis (Howes et al, 2009), with dopaminergic perturbations being more specific to those eventually going on to develop a psychotic disorder (Howes et al, 2011). Furthermore, in a longitudinal study, dopamine synthesis capacity increased at the onset of acute FEP in patients followed from a clinical high risk state (Howes et al, 2011b). Clearly, dopaminergic abnormalities are implicated in the neurobiological final common pathway in many patients experiencing psychosis. However, more recent evidence suggests that dopaminergic abnormalities are not present in a subset of patients with schizophrenia. In addition, it has become clear that alternative neurotransmitter systems (in addition to dopaminergic abnormalities) are involved in the pathophysiology of schizophrenia and may interact intricately with the dopaminergic system.

As has been described, approximately one third of patients with schizophrenia are categorized as being treatment resistant. Given that all first-line antipsychotic medications act to block dopamine, and that these medications are essentially ineffective for these patients, it has been speculated that poor responders may not have an inherent dopaminergic abnormality (Demjaha et al. 2014). Furthermore, clozapine, the only medication with clinical evidence of effectiveness in TRS patients (Taylor and Duncan-McConnell, 2000; McEvoy et al, 2006), has very little intrinsic dopamine activity. In TRS patients, dopamine-blocking antipsychotic medications may fail as these individuals do not have a hyperdopaminergic state to begin with. Supportive of this, blood homovanillic acid (HVA) levels, the major metabolite of dopamine, are lower in those who do not respond to antipsychotic treatment, and higher in those who demonstrate a good response (Yoshimura et al. 2003; Mazure et al. 1991; Pickar et al. 1984). In addition, increased synaptic dopamine has been associated with better subsequent antipsychotic response (Abi-Dargham et al, 2000). Demjaha et al (2012) found that patients with TRS had decreased striatal dopamine synthesis capacity relative to patients achieving a good response to treatment, and dopamine synthesis capacity did not differ between TRS patients and healthy controls. All in all, the data suggests that while dopamine is inherently involved in the pathophysiology of psychosis for many patients, there appears to be a subset of patients in whose symptomatology cannot be explained exclusively by dopaminergic abnormalities. Furthermore, in those without biological indicators of elevated dopamine activity, response to traditional antipsychotics is not as robust.

3.4 Measuring Brain Metabolites in Schizophrenia

Advances in neuroimaging have granted us the opportunity to assess brain metabolites in vivo. Specifically, ^{1}H-MRS is a non-invasive technique that has contributed significantly to our

understanding of schizophrenia. In this technique, ^{1}H (which has a magnetic dipole moment), can be excited by the use of a magnetic field induced by a radiofrequency head coil (Williamson, 2006). The protons then return to resting state and the time it takes for the protons to return to a low energy state can be measured, and provides information about the environment of the protons in a particular molecule. After being excited by a particular magnetic field, different protons will be affected differently secondarily to varying environments that the proton is in, determining the proton's resonance frequency. A particular molecule will have a specific spectral signature depending on the composition of the hydrogen atoms of that molecule, and can be distinguished this way from other metabolites in a particular voxel, or area of interest, in the brain. Spectral signatures are quantified in parts-per-million (ppm) which provides a relative measurement of the compound of interest. Signals from a generated spectrum are determined based on how far off they are from a chosen reference compound. Two of the molecular compounds that can be measured by this technique, glutamate and glutathione, have been implicated in the pathophysiology of schizophrenia.

3.5 A Glutamatergic Theory of Schizophrenia

More and more studies have demonstrated that glutamate, the most prominent excitatory neurotransmitter in the brain (Fonnum, 1984), is implicated in the pathophysiology of schizophrenia. It has been hypothesized that NMDAR (N-methyl-D-aspartate receptor; an ionotropic glutamatergic receptor that is ubiquitous throughout the brain) hypofunction on GABAergic interneurons may lead to increased glutamatergic activity (Moghaddam & Krystal, 2012; Lisman et al, 2008; Carlsson et al, 2001). The NMDA glutamate receptor, when antagonized by drugs such as phencyclidine (PCP), leads to the full spectrum of symptoms that are characteristic of schizophrenia, including positive, negative, and cognitive symptoms (Luby

et al, 1962; Javitt and Zukin, 1991), and blockade of the NMDA receptor with ketamine or PCP leads to paradoxical glutamate release (Adams and Moghaddam, 1998; Moghaddam et al, 1997; Kraguljac et al, 2017). Glutamine, the major metabolite of glutamate, is synthesized in astrocytes following the uptake of glutamate from the synaptic cleft (Shen et al, 1999). In the astrocyte, glutamine is subsequently converted back to glutamate via the enzyme glutaminase (Kanamori et al, 2002; Mason et al, 1995; Rothman et al, 2003). It has been shown that approximately 80% of glutamine takes part in the glutamate cycle (Magistretti and Pellerin, 1999; Rothman et al, 1999) and therefore, glutamine levels are often used as a proxy measure of glutamate activity. Glutamate has several important functions including being a precursor for glutathione (GSH), and GABA, and functions as a building block for protein synthesis (Brosnan and Brosnan, 2013; Wu et al, 2004; Duarte and Gruetter, 2013; Matthews and Diamond, 2003). Given the fact that glutamatergic excess leads to symptoms characteristic of schizophrenia, it is reasonable to speculate that perturbations in this system may be implicated in the underlying pathophysiology of psychosis.

While glutamatergic abnormalities theoretically may be involved in the neurobiology of psychosis, findings from different studies have not always been consistent and abnormalities in the glutamatergic system may vary overtime as a function of the stage of illness. In FEP, elevated glutamatergic metabolites have been demonstrated in the prefrontal cortex (Bartha et al, 1997), and ACC (anterior cingulate cortex) (Theberge et al, 2002; Theberge et al, 2007) and have been associated with cognitive impairments (Dempster et al, 2015). However, others have not found significant differences in glutamatergic metabolites between FEP patients who had received minimal treatment and controls (Bustillo et al, 2010). In a comprehensive literature review, it was concluded that in general, glutamatergic metabolites are elevated in FEP (Poels et

al, 2014), and glutamatergic excess has been associated with progressive loss of brain volume (Hulshoff Pol and Kahn, 2008). Some have proposed that this loss of brain volume provides evidence for an excitotoxic effect of glutamatergic excess (Plitman et al, 2016), however, the pathophysiology underlying volumetric loss is difficult to interpret given that the majority of patients included in studies are taking antipsychotic medications, which may contribute to longitudinal grey matter decline. Furthermore, medication status may influence glutamatergic metabolite measurements, even at this early stage. Kegeles et al (2012) found that while unmedicated patients with FEP had higher Glx (a composite measurement of glutamate and glutamine) levels, there was no difference between medicated FEP patients and healthy controls. Glutamatergic abnormalities appear to predate the onset of an acute FEP. Consistent with findings in patients with FEP, glutamate and glutamine may be elevated in those at high risk of developing schizophrenia (de la Fuente-Sandoval et al, 2011; Stone et al, 2009; Tandon et al, 2013; Tibbo et al, 2004), although others have found no difference in Glx levels (Purdan et al, 2008; Keshevan et al, 2009; Yoo et al, 2009), and glutamate levels in the ACC (Fusar Poli et al, 2011; Valli et al, 2011) in patients at clinical high risk for psychosis.

While overall, there appears to be a state of glutamatergic excess in FEP (Poels et al, 2014), findings in chronic schizophrenia have not been as consistent. However, most studies have found glutamatergic metabolites to be unchanged or lower following the FEP (Kraguljac et al, 2012; Lukenhoff et al, 2010; Reid et al, 2010; Rowland et al, 2013; Théberge et al, 2003; Wood et al, 2007; Ohrmann et al, 2000; Tayoshi et al, 2009). Specifically in the ACC, several studies have not found any difference in glutamate between patients with chronic schizophrenia and healthy controls in the dorsal ACC (Wood et al, 2007), bilateral dorsal ACC (Reid et al, 2010); Kraguljac et al, 2012) and bilateral anterior cingulate (Ongur et al, 2010). In one study,

however, glutamine and Gln/Glu ratio were found to be elevated in the dorsal ACC in patients relative to controls, and glutamine levels were correlated with the severity of psychotic symptoms (Bustillo et al, 2014). Overall, it appears that the state of glutamatergic metabolite excess seen in many studies of patients with FEP transitions to a state of lower glutamate levels as patients progress to a more chronic phase of illness. The reason for the differential findings depending on phase of illness are not clear but may be related to medication exposure overtime, or may represent an intrinsic neurobiological progression inherent to the illness itself.

Variable findings from studies analyzing glutamatergic metabolites in schizophrenia may be attributable to several issues including medication usage, age, and the use of different spectroscopic measurement techniques. There is some evidence that medication status may influence glutamate levels, and there are very few studies that have been conducted on completely antipsychotic naïve individuals with schizophrenia. Chronic antipsychotic usage has been shown to decrease glutamate in the frontal cortex (Goto et al, 2012), associative striatum (de la Fuente-Sandoval et al, 2013), and other brain regions (Aoyama et al, 2011; Szulc et al, 2011; de la Fuente-Sandavol et al, 2013) and in the ACC after 6 months (Choe et al, 1996), and 1 month (Egerton et al, 2017) of treatment. However, other studies have found no association between antipsychotic treatment and ACC glutamate levels in patients with FEP after 1 (Szulc et al, 2005), 30 (Theberge et al, 2007) and 80 (Ayoma et al, 2011) months of treatment. For the most part, the effect of specific antipsychotic medications on glutamate status has not been explored.

In addition to medication status, ageing may affect glutamatergic metabolite measurements. Using 3T MRS, Witjenberg et al. (2017) found that ACC glutamate was lower in patients with schizophrenia relative to healthy controls, and that there was a decline with age in

both groups, while the inverse was true of glutamine. Glutamine levels increased in general with age, and were significantly higher in patients with schizophrenia relative to healthy controls. In a meta analysis, glutamate was found to be lower in patients with schizophrenia, while glutamine was elevated in the ACC, although both metabolites declined with age (Marsman et al, 2013). Declining glutamate levels with age in patients with schizophrenia may be related to duration of illness, exposure to medication, or both.

Finally, disparaging findings between MRS studies of glutamate in schizophrenia may not only be related to the effects of antipsychotic usage and ageing, but may be influenced by the technique used to quantify glutamatergic metabolites. The majority of studies to date have used 1.5-4T MRI scanners, and definitively separating glutamate and glutamine at lower field strengths is challenging. More recently, a few studies have taken advantage of the increased precision and ability to definitively separate glutamate and glutamine signals associated with the use of a higher field, 7T MRI (Mekle et al, 2009; Pradhan et al, 2015; Tkac et al, 2009).

To date, only a handful of studies have utilized ultra high-field 7 tesla MRS to investigate patients with schizophrenia, with the majority of these being in patients with chronic schizophrenia. Using 7T MRS, patients with chronic schizophrenia have not been shown to have significantly different glutamate levels relative to healthy controls in the ACC (Brandt et al, 2016) and medial prefrontal cortex (Marsman et al, 2014), although Rowland et al (2016) found higher Gln/Glu ratios in patients relative the healthy controls in the ACC. Findings may vary however, depending on specific characteristics of the sample. Brandt et al (2016) found that ACC glutamate decreased with age in patients with chronic schizophrenia, while levels remained more constant in healthy controls. Kumar et al (2018) found decreased glutamate levels in patients with stable schizophrenia relative to healthy controls, however, much of this effect was

related to a portion of the sample with residual schizophrenia. Takkhar et al (2017) found decreased occipital cortex glutamine in patients with chronic schizophrenia relative to their unaffected siblings, with both groups having decreased levels relative to controls. No differences in glutamatergic metabolites were observed in the basal ganglia. In a 7T MRS study of patients within the first two years following an initial episode of psychosis, patients had significantly lower levels of glutamate relative to controls (Reid et al, 2018). Although these patients were considered "first episode", the majority of patients had already been receiving antipsychotic medications for close to one year at the time of scanning. To date, no studies have been published examining glutamatergic metabolites in first-episode, antipsychotic-naïve patients using a 7T MRI.

Another potential explanation for the apparent inconsistencies in studies assessing glutamatergic metabolites in schizophrenia, may be related to the fact that various subgroups of patients with schizophrenia may have distinct glutamatergic abnormalities, while others may not. Therefore, depending on the characteristics of the overall sample, group differences from healthy controls may or may not be evident. Specifically, TRS may be associated with glutamatergic abnormalities that are distinct from the overall population with schizophrenia. Several studies have found higher levels of glutamate in the ACC to be associated with lack of treatment response, in both samples of chronically unwell, and in FEP, patients. In patients with chronic schizophrenia, non-responders to antipsychotic medications have been shown to have significantly higher glutamate to creatine levels (Mouchlianitis et al, 2016), and Glx/Cr levels (Szulc et al., 2013) relative to responders in the ACC. Demjaha et al (2014) found that patients with resistant schizophrenia had higher ACC glutamate than healthy controls, and that glutamate levels in healthy controls were not significantly different from patients with good treatment

response. Interestingly, in the same group of patients, they were also able to demonstrate that those with TRS had lower striatal dopamine synthesis. This represents the only study examining both dopaminergic and glutamatergic neurotransmission in the same cohort of patients, and suggests that in TRS, glutamatergic abnormalities may be prominent, while dopaminergic excess may not be. Only a couple of studies have investigated glutamate and response to treatment in FEP samples. In patients with FEP who were treated with antipsychotic medications for at least 6 months, higher levels of glutamate in the ACC were found in non-remitters, relative to those achieving remission (Egerton et al, 2012). However, even at this early stage of illness, medication effects cannot be ruled out. Only one study has examined the relationship between ACC glutamate prior to antipsychotic usage and treatment response prospectively. Using a 3T MRI, Egerton et al (2018) found that higher levels of Glu/Cr in the ACC were associated with non-remission at one month. However, no studies have investigated this association using a higher resolution 7T MRI. Furthermore, this study did not describe any measures for assessing medication compliance which may have contributed to lack of remission in some patients. Further studies are warranted to evaluate the association between ACC glutamate and treatment response prospectively, ideally with efforts to control for medication adherence which may greatly impact response and remission rates.

3.6 Glutathione Abnormalities in Schizophrenia

Along with abnormalities in the glutamatergic system, oxidative stress (Wood et al, 2009) and exaggerated neural free radical production (Mahadik and Mukherjee, 1996; Reddy and Yao, 1996) have been hypothesized to be intricately involved in the pathophysiology of psychotic disorders. Specifically, glutathione (GSH), the brain's primary intracellular antioxidant, has been speculated to play a role in maintaining neural health by protecting against damage from reactive

oxygen species (Cohen, 1983; Meister and Anderson, 1983), and is thought to be implicated in neuropsychiatric disorders, including schizophrenia (Mahadik and Mukherjee, 1996). There is some evidence that schizophrenia may be associated with an impaired ability to synthesize GSH as a neuroprotective defense against oxidative stress (Gysin et al, 2007). Animal studies have demonstrated that under normal conditions, GSH acts as an agonist at NMDA receptors (Kohr et al, 1994; Leslie et al, 1992). Under conditions of oxidative stress, GSH levels may become depleted which may lead to NMDA hypofunction (Steullet et al, 2007, Kantrowitz and Javitt, 2010; Stone et al, 2009), and reduced GSH synthesis may in turn lead to increased glutamate concentrations (Koga et al, 2011). Glutamatergic excess has been shown to activate free radical pathways (Bains and Shaw, 1997), potentially leading to neural damage due to oxidative stress (Volterra et al, 1994). Without sufficient GSH activity to combat oxidative stress and free radical formation, this may result in a cycle of ongoing toxicity. While the specific role of GSH in the pathophysiology of schizophrenia has not been fully delineated, there is sufficient evidence to suggest that GSH is important for neutralizing oxidative stress that may be perpetuated by glutamatergic dysfunction.

There have been a few studies measuring GSH levels in patients with schizophrenia, although most have been completed in samples of chronically unwell patients. In patients with schizophrenia who were currently antipsychotic-free (the sample consisted of patients in varying phases of illness), GSH was found to be reduced in the cerebrospinal fluid (CSF) and medial prefrontal cortex relative to healthy controls (Do et al, 2000), and post-mortem in the prefrontal cortex (Gawryluk et al, 2011). However, other studies have found no significant differences in GSH levels in patients relative to controls in the posterior medial frontal cortex (Monin et al, 2015), the mPFC (Matsuzawa et al, 2008; Xin et al, 2016), and the ACC (Terpstra et al, 2005;

Brandt et al. 2016), although low GSH levels have been associated with the severity of negative symptoms (Matsuzawa et al, 2008).

In FEP, elevated GSH has been demonstrated in the medial temporal lobe (Wood et al, 2009), and decreased GSH levels have been associated with cognitive impairments (Martinez-Cengotitabengoa et al, 2012) and grey matter volumetric deficits longitudinally (Fraguas et al, 2012), and at initial presentation (Langbein et al, 2017). However, other studies have found decreased GSH in the brain (Mico et al, 2011) and in erythrocytes and plasma in FEP patients (Pavlovic et al, 2002; Altunas et al, 2000; Raffa et al, 2011). In one study comparing drug-naïve patients with FEP to healthy controls, plasma GSH reductase activity, an enzyme essential for catalyzing the reaction converting oxidized GSH to reduced GSH, was lower in patients, and inversely correlated with negative symptoms (Langbein et al, 2017). Overall, the findings are mixed regarding GSH perturbations in schizophrenia and may depend on factors specific to the population being studied such as the severity of illness, the duration of untreated illness, and the overall burden of negative symptoms. Further studies are warranted to elucidate the role of GSH in schizophrenia, and determine whether specific subgroups may be characterized by abnormalities in GSH.

As described above, glutamate and GSH interact reciprocally with eachother, although, only a few studies have investigated glutamate and GSH in the same group of patients. In one study using 7T MRS, glutamate and GSH in the ACC were significantly positively correlated in a sample of patients with schizophrenia, and both were reduced relative to healthy controls (Kumar et al, 2018). The known close association of glutamate and GSH levels in vivo supports the notion that these two systems interact interchangeably. As may be the case with glutamate, differential GSH findings in schizophrenia may relate to neurobiological variations inherent in

specific subgroups of patients; certain individuals may be adept at mounting a GSH response in the face of early oxidative stress, whereas others may not. In addition, the ability to synthesize GSH overtime may diminish under conditions of chronic, longitudinal oxidative stress. Given the evidence that glutamatergic dysfunction may be implicated in treatment resistance, abnormalities in mounting a GSH response may be expected in patients with TRS. However, to date, no studies have specifically examined the role of GSH in TRS.

An improved understanding of the neurobiological correlates of TRS is grossly needed in order to enhance our ability to accurately characterize TRS at earlier stages, and to target new treatments for this subgroup of patients. The identification of early biomarkers suggestive of treatment resistance may support selective treatments for individuals at risk for a resistant course. For example, preliminary results have suggested that N-acetyl cysteine (NAC) treatment, an antioxidant that is thought to be neuroprotective (Dean et al, 2011), may increase GSH in the brain (Dringen and Hirrlingen, 2003), and is thought to play a role in glutamatergic function (Baker et al, 2002). Unfortunately, GSH itself is not bioavailable, and therefore, cannot be administered directly. There is some evidence that NAC may be therapeutic in patients with schizophrenia and in early psychosis patients, it has been shown to increase GSH brain levels (Conus et al, 2018). Furthermore, NAC has been shown to be efficacious in treating total (Zeng et al, 2018), negative (Berk et al, 2008; Farokhnia et al, 2014; Breier et al, 2018) as well as cognitive symptoms (Conus et al, 2018; Rapado-Castro et al, 2017), and has even demonstrated some benefit in TRS (Bulut et al, 2009). While promising, it remains unclear which patients may benefit from NAC treatment. One randomized-placebo controlled study found that those with a longer duration of untreated psychosis benefited most from NAC adjuvant therapy (Rapado-Castro et al, 2015). It is possible that early in the course of illness, most individuals are able to

mount a response to cellular antioxidant stress, by increasing GSH in some capacity, but that overtime, their capacity to defend against reactive oxygen species decreases. In addition, there may be a subgroup of patients with FEP whom for various reasons, are not able to mount a neuroprotective response by increasing GSH. The establishment of an association between GSH and treatment response may support the use of NAC treatment (and potentially other anti-oxidant therapies), particularly in patients demonstrating abnormalities in GSH at their first episode of psychosis.

Chapter 4: Anterior Cingulate Cortex Glutamate and Glutathione at First-Episode Psychosis and Subsequent Antipsychotic Treatment Response

4.1 Introduction

Early treatment response has been identified as one of the most robust predictors of longer-term clinical outcomes in schizophrenia (Agid et al, 2003; Emsley et al, 2007; Schennach-Wolff et al, 2010). In particular, the lack of an early response to antipsychotic treatment appears to be strongly indicative of subsequent non-response (Stauffer et al, 2011), failure to achieve full remission (Emsley et al, 2006), as well as higher rates of treatment discontinuation (Kinon et al, 2008). Approximately one third of patients with schizophrenia are considered to be treatment resistant (Meltzer, 1997; Lindenmayer, 2000), with the majority of these failing to respond appreciably to dopamine-blocking antipsychotic medications from their first episode of psychosis (Agid et al, 2011; Lally et al, 2016; Demjaha et al, 2017). The majority (84%) of patients who eventually develop treatment-resistant schizophrenia are poor responders to first-line antipsychotics even during the first episode (Demjaha et al, 2017). Nevertheless, the

neurochemical mechanism of early response is poorly understood, precluding efforts to prevent or reduce the rates of treatment failure and persistent disability.

Individuals who respond poorly to dopamine blocking medications may be characterized by alternative neurochemical profiles relative to patients who demonstrate an adequate response. The first episode of non-affective psychosis (FEP) is characterized by a relative state of glutamatergic excess (Kegeles et al, 2012; Poels et al, 2014; Merritt et al 2016; Marsman et al, 2013). Elevated anterior cingulate cortex (ACC) glutamate has been associated with lack of remission in certain samples of chronic (Mouchlianitis et al, 2016; Szulc et al., 2013; Demjaha et al, 2014) or first-episode schizophrenia (Egerton et al, 2012; 2018 [UK sample]), but this has not been a consistent observation. For example, in a sample of patients with established schizophrenia Iwata et al (2018) reported no difference in dorsal ACC glutamate levels between treatment-responsive and resistant groups. Similarly, the samples in 2 out of 3 sites in Egerton et al (2018) study showed no glutamate excess in patients with FEP who do not achieve remission by 1 month. Nevertheless, the relative glutamatergic excess appears to be specific to early stages of illness (Marsman et al, 2013), and relates to more severe symptoms at presentation (Egerton 2018), as well as subsequent longitudinal grey matter decline (Aoyoma et al, 2011), cognitive (Dempster et al, 2015) and functional (Egerton et al, 2012; 2018) impairments in schizophrenia.

Glutathione (GSH), the brain's most prominent intracellular antioxidant has been suspected to play a key protective role in free-radical-mediated damage to neurons (Mahadik and Mukherjee, 1996), giving rise to the redox dysregulation hypothesis of schizophrenia (Do et al, 2009). MRS studies have found a small but significant GSH deficit in the ACC in patients with

schizophrenia (Gawryluk et al, 2011; Wang et al., 2019), indicating the presence of subgroups of patients with different redox profile (Das et al, 2018). A recent observation indicates that the most prominent reduction in GSH seems to occur particularly in patients with persistent residual symptoms, indicating that low levels of GSH may be associated with poor response to antipsychotics (Kumar et al 2018). Furthermore, N-acetyl-cysteine (NAC), precursor of GSH, appears to increase the rate of symptomatic response when used as an adjunct to antipsychotics in early stages of psychosis (Klauser et al, 2018).

Glutamate is a precursor of GSH (Persson et al, 2006) while GSH acts as a neuronal reservoir for glutamate synthesis (Sedlak et al, 2019). As a result, when neuro-glial metabolic integrity is intact, glutamate and glutathione levels remain tightly linked in the brain. Glutamatergic excess can result in neurotoxic oxidative stress (Volterra et al, 1994), while a concomitant elevation of GSH may offer a neuroprotective 'gate-keeping' effect (Frade et al, 2008), thus a strong covariance between the two may be a marker of a healthy state. Nevertheless, repeated or prolonged exposure to excess glutamate can deplete GSH levels (Shih et al, 2006), resulting in a state of reduced GSH-glutamate covariance. Furthermore, the GSH-glutamate homeostasis can also be disrupted in patients with schizophrenia due to deficiencies in GSH synthesis (Fournier et al, 2017), thus leading to a reduced GSH-glutamate covariance in patients with FEP compared to healthy controls.

4.2 Purpose of Study

In this study, we use ultra-high field 7T MRS for the first time to test the relative contribution of anterior cingulate GSH deficiency and glutamatergic excess in predicting the early treatment response in FEP. Given the gatekeeper role of GSH in tackling oxidative stress that results from various converging processes (Steullet et al, 2016), we expected GSH to be a

more critical determinant of early treatment response in FEP. We hypothesized that patients with FEP with higher GSH levels will be able to mount a swift response to increased oxidative stress, and thus show a rapid symptom reduction upon starting antipsychotic treatment (hypothesis 1). As not all patients with FEP will be able to increase GSH in accordance with glutamate levels, we expected a reduction in the strength of correlation between the GSH and glutamate levels in patients compared to healthy controls (hypothesis 2). Furthermore, in light of the excitotoxic theory of acute schizophrenia (Plitman et al, 2016), we expected both reduced GSH and increased glutamate levels to predict impaired Social and Occupational Functioning at the onset of illness (hypothesis 3).

4.3 Methods

4.3.1 Participants

The sample consisted of 37 consecutive new referrals to the PEPP (Prevention and Early Intervention for Psychosis Program) at London Health Sciences Centre between April, 2017 and January, 2018. The PEPP program provides assessment and treatment to individuals 16-39 experiencing FEP using an assertive case-management model. All potential participants provided written, informed consent prior to participation as per the approval provided by the Western University Health Sciences Research Ethics Board, London, Ontario. Inclusion criteria for study participation were as follows: individuals experiencing a first episode of psychosis, and having received antipsychotic treatment for less than 14 days in their lifetime. Both inpatients and outpatients were eligible to participate, so long as they could provide informed consent, and could safely participate in the MRI protocol. A consensus diagnosis was established using the best estimate procedure (as described in Leckman et al, 1982) for all participants after approximately 6 months by 3 psychiatrists (KD/LP and the primary treatment provider) based on

the Structured Clinical Interview for DSM-5 (APA, 2013; First et al, 2016). Based on the 6-month consensus diagnosis, participants meeting criteria for bipolar disorder with psychotic features, major depressive disorder with psychotic features, or suspected drug-induced psychoses were excluded from further analyses. Study participants received care-as-usual through their psychiatrist and other allied health members within the PEPP program. Antipsychotic medications were chosen by the treating psychiatrist and the patient and/or their substitute decision maker in a collaborative manner. In accordance with current national guidelines for the treatment of FEP, individuals were offered the option of treatment with a long acting injectable at the earliest opportunity (Remington et al, 2017).

Healthy control subjects were recruited through the use of posters advertising the opportunity to participate in a neuroimaging study involving tracking outcomes following FEP. Healthy control subjects had no personal history of mental illness, and no family history of psychotic disorders. Group matching with the FEP cohort for age, sex, and parental education was maintained. Exclusion criteria for both the FEP and healthy control groups involved meeting criteria for a substance use disorder in the past year according to DSM-5 criteria (DSM-5; APA, 2013), having a history of a major head injury (leading to a significant period of unconsciousness or seizures), having a significant, uncontrolled medical illness, or having any contraindications to undergoing MRI.

4.3.2 Medication Adherence

Individuals were treated with long-acting injectable (LAI) medications whenever clinically appropriate. Patients taking LAI's received their injection from a nurse at the PEPP clinic and therefore, it was known if an individual had missed, or was late for their scheduled

dose. Assessments of medication adherence were also recorded at each clinical encounter, taking into account information provided by the patient, their family, and/or case manager using a 5-point rating scale (ranging from 0 for individuals not taking medication to 4 for those being adherent 75–100% of the time). This measure has been found to correlate with pill counts (Cassidy et al, 2010). We only included subjects who had >75% recorded adherence, and thus patients who dropped out due to medication intolerance were not included in this study.

4.3.3 Measures of Treatment Response

The proportion of patients with FEP in remission at any given time appears to be relatively consistent longitudinally, but it is often not the same individuals who remain in remission at each time point (Norman et al, 2018). Additionally, while remission reflects a specific clinical status, it is not necessarily informative of direct treatment effects. The use of absolute criteria in defining remission is highly dependent on initial illness severity, with individuals with a higher initial symptom burden being much less likely to achieve remission (Harvey and Bellack, 2009). As a result, we studied the continuous measure of time to response as the primary clinical outcome of interest, and used the cross-sectional remission criterion (Andreasen et al, 2005) as a secondary measure of interest, as well as the percent symptom reduction at 1 month.

The 8 items of the Positive and Negative Syndrome Scale capturing the core symptoms critical in defining remission (PANSS-8; Andreasen et al, 2005) was administered at the baseline, 2 weeks, 4 weeks, and at every clinical encounter thereafter on a 2-4 weekly basis. The PANSS-8 has acceptable internal consistency and comparable sensitivity to early improvement in psychotic symptoms (Lin et al, 2018) relative to the PANSS-30 (Kay et al, 1987). The time to

achieve a 50% PANSS-8 improvement from baseline (as per Leucht et al, 2007), sustained for at least 2 consecutive visits 2 weeks apart, was used as a continuous measure of treatment response. A 50% symptom improvement from baseline roughly equates to a Clinical Global Impression-Schizophrenia (CGI-S; Haro et al, 2003) scale score of "much improved" thus, is clinically meaningful (Correll et al, 2011). Relative PANSS8 improvement was calculated as $(PANSS8_{baseline} - PANSS8_{endpoint})/(PANSS8_{baseline} - 8)$ in order to adjust for the minimal possible PANSS8 score is 8 (as per Obermeier et al, 2010). All patients were observed clinically for a period of at least 6 months, and no patients failed to reach this milestone within this time frame.

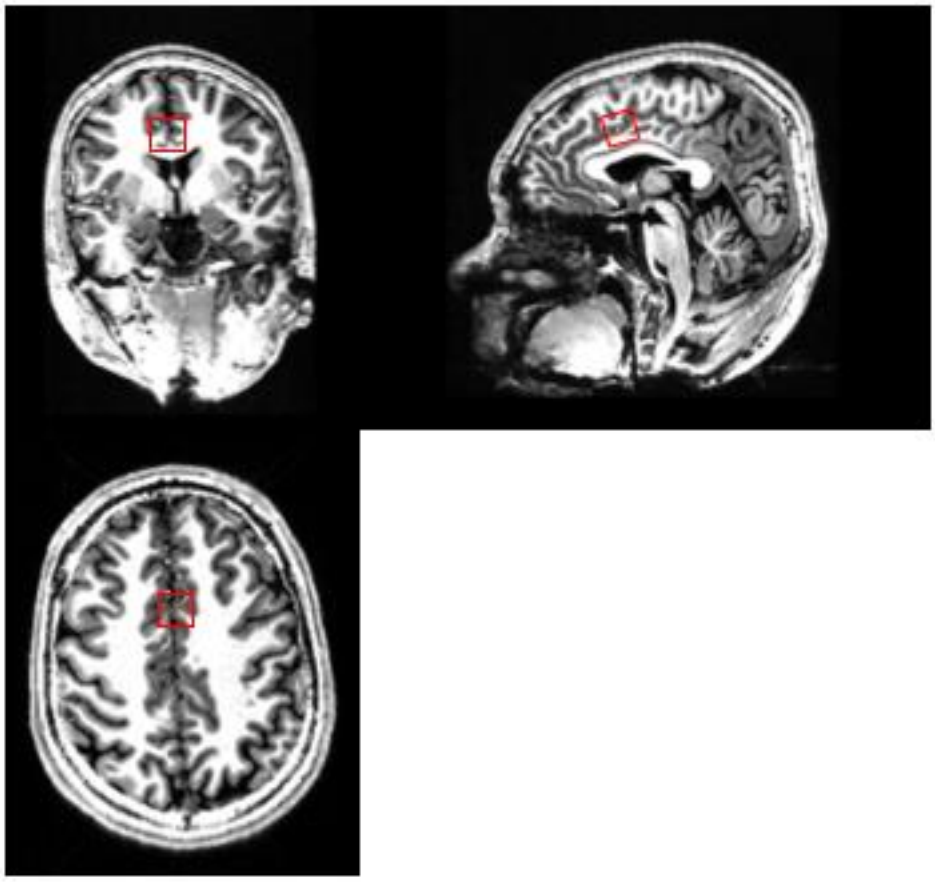
In addition to continuous treatment response measurements, we also assessed binary remission status after the first month of treatment (remission or not in remission). Symptomatic remission was allocated based on remission criteria proposed by Andreasen et al (2005) which categorize remission as achieving scores of mild (3) or less on all PANSS8 items, without any stipulation of a duration criteria, in line with Egerton et al (2012, 2018). Finally, social functioning was assessed at baseline using the Social and Occupational Functioning Assessment Scale (SOFAS, Goldman et al, 1992).

4.3.4 ^{1}H-MRS

Metabolite concentrations (glutamate and GSH) were estimated using single-voxel 1H-MRS data acquired with a Siemens/Agilent MAGNETOM 7.0T head-only MRI (Siemens, Erlangen, Germany; Agilent, Walnut Creek, California, USA) using an 8-channel transmit/32-channel receive head coil at the Centre for Functional and Metabolic Mapping of Western University in London, Ontario. A 2.0 x 2.0 x 2.0 cm ($8cm^3$) ^{1}H-MRS voxel was placed in the bilateral dorsal ACC (see figure 1) using a two-dimensional anatomical imaging sequence in the sagittal direction (37 slices, TR=8000ms, TE=70ms, flip-angle (α)=120°, thickness = 3.5mm,

field of view = 240×191mm). The posterior end of the voxel was set to coincide with the precentral gyrus and the caudal face of the voxel coincided with the most caudal location not part of the corpus callosum. The angulation of the voxel was determined to be tangential to the corpus callosum. A total of 32 channel-combined, water-suppressed spectra were acquired using a semi-LASER ^{1}H-MRS pulse sequence (TR=7500ms, TE=100ms) during each scan session, while participants were at rest and asked to stare at a white cross on a black screen for 4 minutes. Water suppression was achieved using the VAPOR preparation sequence (Tkáć et al, 2005), and water-unsuppressed spectra were acquired for spectral quantification and line shape deconvolution reference. The 32 spectra were corrected for frequency and phase drifts as described in Near et al, (2015) prior to averaging and lineshape deconvolution using QUECC (Bartha et al, 2000). Residual water peaks were removed from the averaged spectrum using HSVD[57] (Bartha et al, 1999). Metabolite quantification was acquired using Barstool (Wong, Schranz and Bartha, 2018). Water-subtracted spectra were modelled using the fitMAN (Bartha et al, 1999), a-prior-knowledge based minimization algorithm, and a quantification template including 17 metabolite spectral signatures derived from simulation (Wong, Schranz and Bartha, 2018). Our fitting template included 17 metabolites (alanine, aspartate, choline, creatine, GABA, glucose, glutamate, glutamine, glutathione, glycine, lactate, myo-inositol, N-acetyl aspartate, N-acetyl aspartyl glutamate, phosphorylethanolamine, scyllo-inositol, and taurine). Importantly, at this long echo time, no macromolecules were included in the spectra as their signal had decayed below noise level. Metabolite concentrations were corrected for gray and white matter volumes using the anatomical MRI images and previously described methods (Stanley et al, 1995). All spectra and spectral fit were inspected visually for quality and Cramer-Rao lower bounds (CRLB) were assessed for each metabolite.

Figure 4-1. Voxel in Dorsal ACC.



Glutamate, glutamine, and GSH were measured in the ACC due to considerable evidence implicating this brain area in the pathophysiology of schizophrenia (Roberts et al, 2015; Fornito et al, 2009; Lahti et al, 2006; Wood et al, 2007). Specifically, the ACC plays a critical role in attention, emotion, and cognition (Benes, 2009; Reid et al, 2010), is thought to be important for mediating executive functioning (Barch et al, 2001; Braver et al, 2001) and has been shown to be impaired during cognitive processing tasks in schizophrenia (Quintana et al, 2004; Dehaene et al, 2003; Carter et al, 2001; Laurens et al, 2003; Heckers et al, 2004; Kerns et al, 2005; Stern et al, 2009; Minzberg et al, 2009; Salgada-Pineda et al, 2004; Snitz et al, 2005; Laurens et al, 2005). Furthermore, previous studies have demonstrated elevated glutamatergic metabolites in the ACC

in patients with FEP (Theberge et al, 2002, 2007), and elevated ACC glutamate has previously been associated with TRS (Mouchlianitis et al, 2016; Szulc et al., 2013; Demjaha et al, 2014) and with poor response in FEP (Egerton et al, 2012; Egerton et al, 2018).

4.3.5 Statistical Analyses

All statistical tests were performed using IBM SPSS Statistics version 24. Differences in demographic and baseline factors between patients and controls were calculated using t-tests for continuous variables, and chi-square analyses for dichotomous variables. A linear regression analysis was used to assess the association between metabolites (glutamate and GSH), and both time to response, and social functioning (Hypotheses 1 and 3). Using ANOVA, we then compared glutamate and GSH measures among patients achieving remission at one month, no remission at one month, and healthy controls. Finally, Pearson correlation coefficients were used to assess the association between glutamate and GSH in patients and healthy controls. Differences in the magnitude of these correlations were then evaluated using Fisher's r-to-Z transformation (Hypothesis 2).

4.4 Results

4.4.1 Patient Characteristics

37 patients completed baseline scanning. Of these, 27 met criteria for a schizophrenia spectrum disorders (SSD: schizophrenia, schizoaffective disorder, or schizophreniform disorder). Of the patients not included in the analysis, 3 met criteria for major depressive disorder with psychotic features, 4 for bipolar disorder with psychotic features, and 3 for unspecified psychotic disorders. Follow-up outcome data were not available for one female patient who was transferred

to a different hospital shortly after scanning. In one male patient, time to response was not available due to irregular follow-up however, remission status at one month was obtained. Therefore, the final sample consisted of 26 patients with SSD, with time to response measures available for 25 patients (see Table 4-1).

9 patients (34.6%) were antipsychotic naïve at the time of scanning. Of those who had already started antipsychotic treatment, (17; 65.4%), the median days of treatment was 6 (range of 3-12 days). The mean total defined daily dose-days (DDD X days on medication) for antipsychotic use was 2.27 days. At one month, 11 (42.31%) patients met remission criteria, while 15 (57.69%) did not, and 12 (46.15%) were taking a long acting injectable medication.

Table 4-1. Sample Demographic and Clinical Characteristics.

Characteristic	**Patient Group (N=26)**	**Healthy Controls (N=27)**	***t* orχ2**	***p*-value**
Gender (Male/Female)	21/5	17/10	2.07	0.15
Diagnosis (S/SA/SF)	21/2/3			
Marital Status (M/S)	3/23	1/26	1.17	0.28
Inpatient (Y/N)	13/13			
Family Hx (Y/N/DK)	10/12/4			
AP Dur (M/SD; days)	6.94/3.3			
Total DDD-days at scan (M/SD)	2.27/2.7			
DUP (weeks) (M/SD)	28.34/65.03			
Drug use past year (Y/N)	18/8	8/16	6.44	0.01*
Age (M/SD)	24.04/5.4	21.48/3.57	-2.05	0.05*
SOFAS (M/SD)	38.12/10.29	80.56/4.41	19.07	0.00*
PANSS-8 Total (M/SD)	25.23/5.08			
Time to res (M/SD; weeks)	6.6/5.4			
On LAI 1 month (Y/N)	12/14			
AP 1 month (O/A/P/B/M/S/C/NM)	7/2/3/1/4/7/1/1			
Glutamate (M/SD)	8.51/2.05	8.35/2.30	-2.66	0.79
Glutamine (M/SD)	1.25/0.56	1.27/0.50	0.14	0.89
Glutathione (M/SD)	1.74/0.39	1.68/0.52	-0.41	0.68

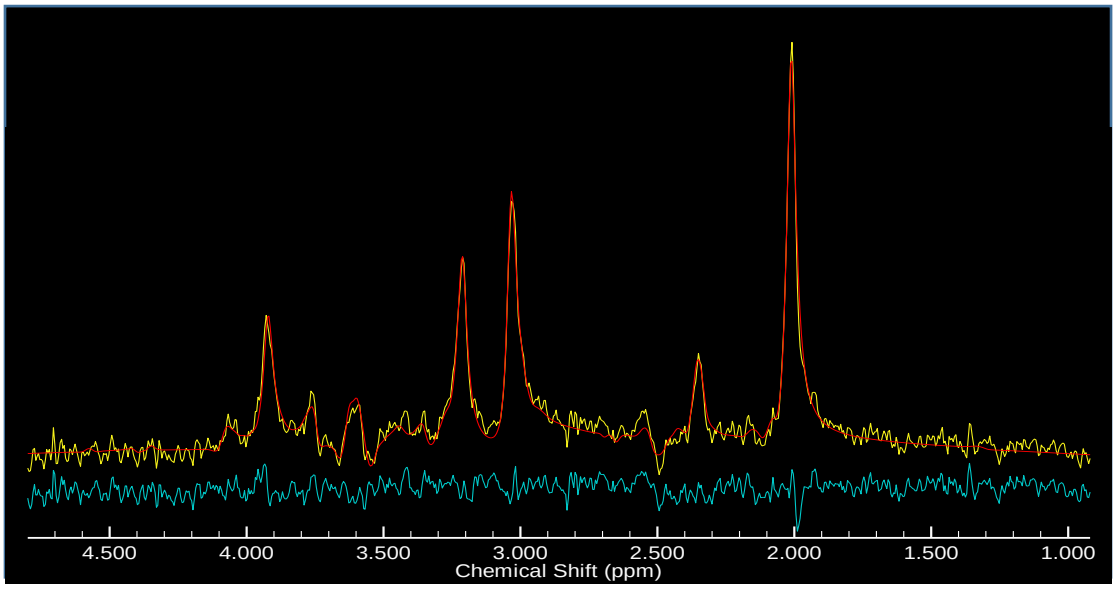
P-values for differences between groups were calculated using chi-square analyses for categorical variables, and independent t-tests for continuous variables. S= schizophrenia; SA= schizoaffective disorder; SF= schizophreniform disorder; Mar= married; S= single; Hx= history; Y= yes; N= no; DK= don't know; AP= antipsychotic; Dur=duration;

DDD= defined daily dose; M= mean; SD= standard deviation; DUP= duration untreated psychosis; res= response. O= olanzapine; A= Aripiprazole; P= paliperidone; B= brexiprazole; M= Aripiprazole LAI; S= paliperidone LAI; C= risperidone LAI; NM= not taking meds.

4.4.2 ^{1}H-MRS Data Quality

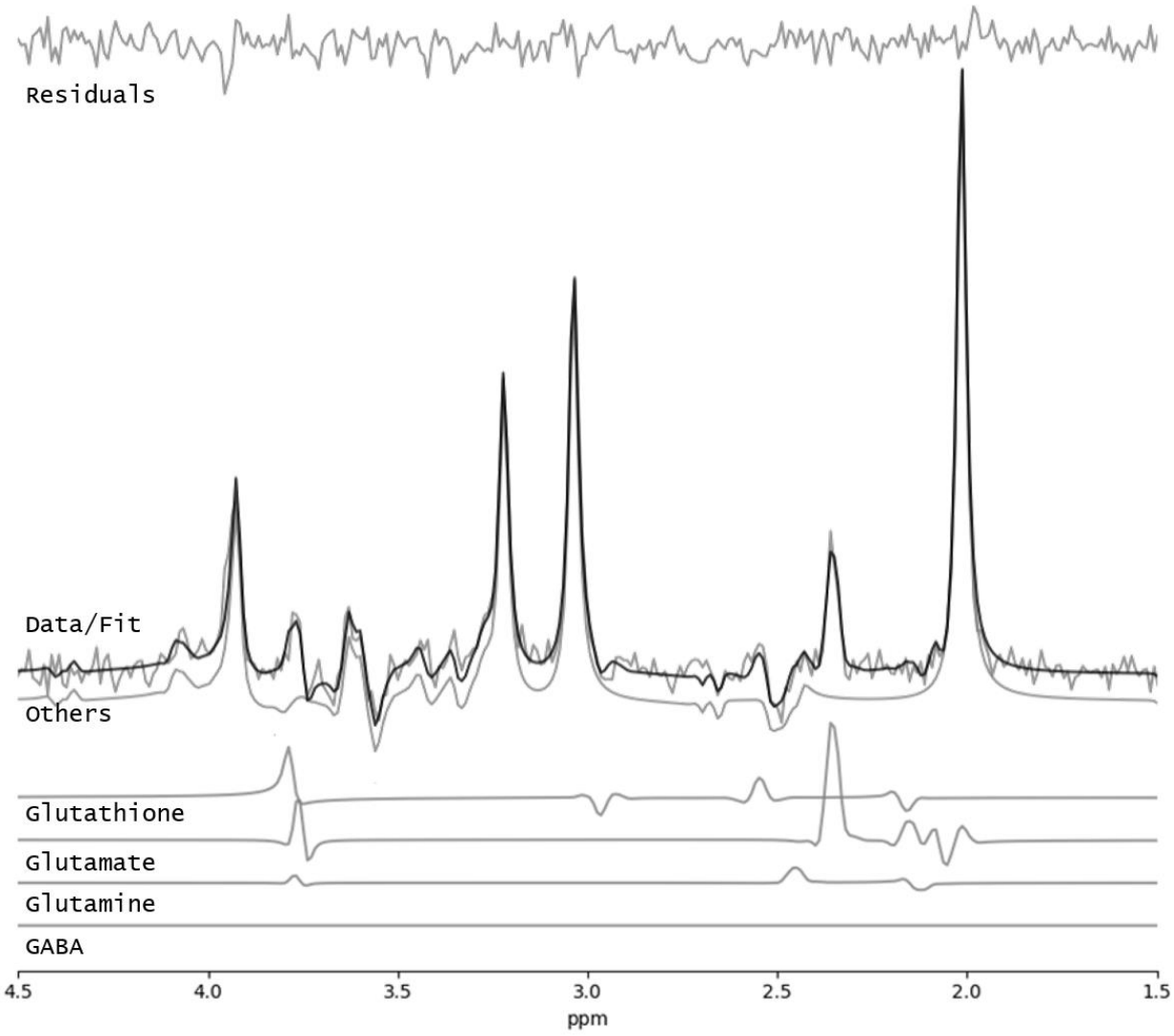
The mean glutamate CRLB percentages did not differ between healthy controls and patients (mean(SD) in % = 3.4 (1.0) in controls; 3.7(1.2) in FEP; t=1.16, p=0.25). Mean GSH CRLBs were (mean(SD) in % = 10.5(3.9) in controls; 11.5(4.9) in FEP; t=0.81, p=0.42). The percent coefficient of variation (%CV), calculated as the standard deviation divided by the mean of a sample, was 20.4% and 24.1% for healthy control and FEP glutamate measurements, respectively and 24.8% and 22.6% for healthy control and FEP GSH measurements, respectively (control vs FEP - p>0.6 for both metabolites). The average line width of the water-unsuppressed spectra did not differ between the 2 groups (mean(SD) = 7.6(1.2) in controls; 7.5(1.4) in FEP; t=0.4,p=0.7). The NAA peak-area signal-to-noise ratio was also not different (mean(SD) = 109.9 (18.4) in controls; 102.2 (24.5) in FEP; t=1.29,p=0.20).

Figure 4-2. Example of Spectral Fit.



The figure above is an example of a spectral fit using the fitMAN software. The yellow line represents the raw data, the red line represents the fitted data, and the teal line represents the residual.

Figure 4-3. Metabolite Breakdown from Spectral Fit.



The figure above depicts metabolite components of interest used in the Barstool software. The first line represents the residual between the raw and the fitted data. The lines marked as Data/Fit represent the raw (grey line) and the fitted data (superimposed black line). The grey line marked 'Others' represents the sum of 14 metabolites used in the fitting model; the next four grey lines represent the separated glutathione, glutamate, glutamine and GABA contributions toward the total fit data.

4.4.3 Glutamate and Treatment Outcomes

We first examined the association between glutamate and measures of treatment response (time to improve from baseline by 50% on the PANSS-8, and percent symptom reduction at one month) using correlational analyses. Scatterplots of the data were obtained and as there was evidence of non-normality of distribution, Spearman rank order correlations were used. While ACC glutamate was not significantly associated with time (in weeks) to improve by 50%, rho= -.366, *p*= .072 (see figure 4-4), higher glutamate was significantly associated with a greater reduction in psychotic symptoms at one month relative to baseline; rho = .470, *p*= .027 (see figure 4-5).

Figure 4-4. Baseline ACC Glutamate and Time to Response.

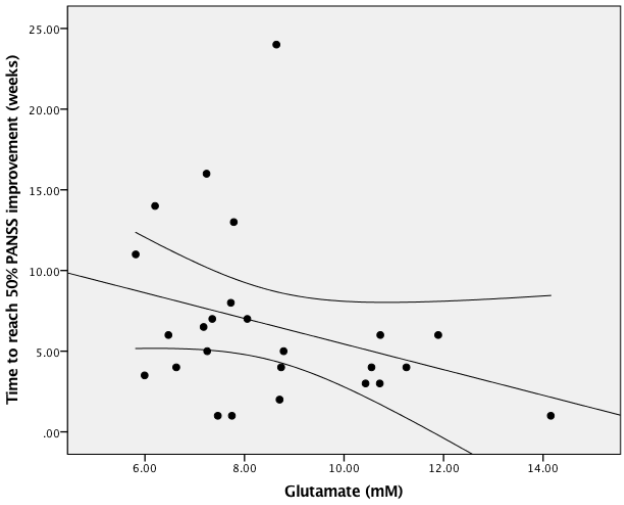
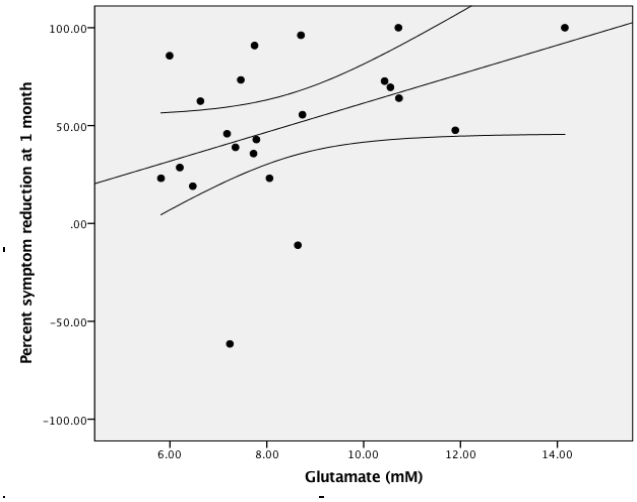
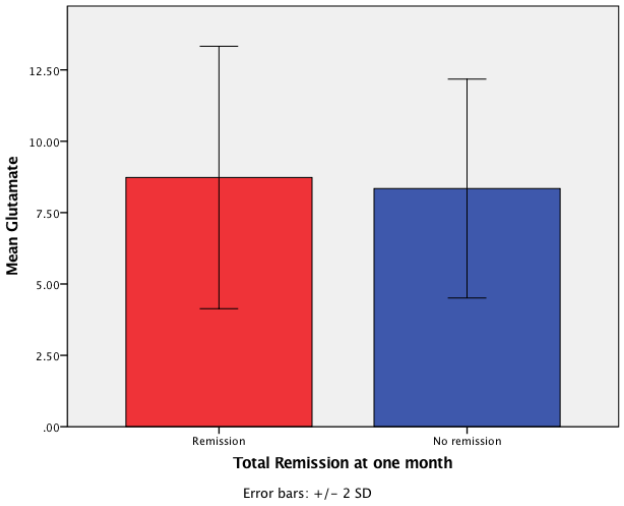


Figure 4-5. Baseline ACC Glutamate and Percent Symptom Reduction 1 Month.



The association between ACC glutamate and remission status at one month was explored using binary logistic regression analyses and was not found to be significant (χ^2= .236; p= .627; Nagelkerke R^2= .012; B=-.096; SE= .198; Wald= .234; *p*= .628; Exp(B)= .909) (see figure 4-6).

Figure 4-6. Baseline ACC Glutamate Levels in Remission Versus Not at 1 Month.



4.4.4 Glutamine and Treatment Outcomes

While glutamine was not found to be significantly associated with time to improve by 50% (rho = -.356, *p*= .081) (see figure 4-7), higher glutamine at baseline was significantly associated with a greater percent symptom reduction at one month (rho= .553, *p*=.008) (see figure 4-8).

Figure 4-7. Baseline ACC Glutamine and Time to Response.

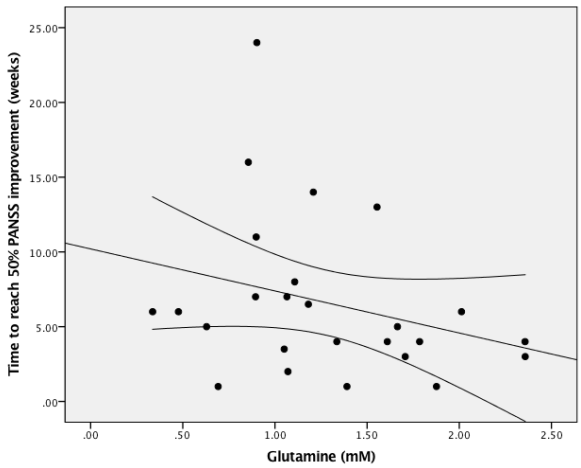
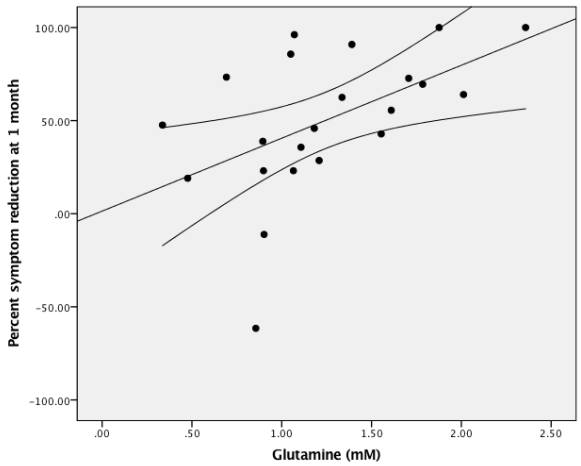
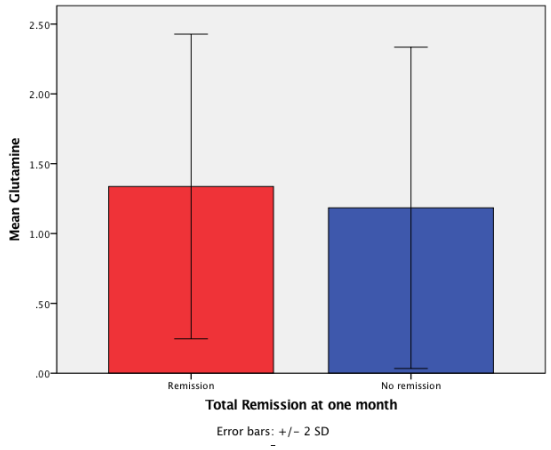


Figure 4-8. Baseline ACC Glutamine and Percent Symptom Reduction 1 Month.



Based on binary logistic regression analyses, glutamine was not significantly associated with remission status at one month (χ^2= .499; p= .480; Nagelkerke R^2= .026; B= -.517; SE= .738; Wald= .490; *p*=.484; Exp(B)= .587) (see figure 4-9).

Figure 4-9. Baseline ACC Glutamine Levels in Remission Versus Not at 1 Month.



4.4.5 Glutathione and Treatment Outcomes

Higher GSH was associated with both time to improve symptomatically by 50% (rho= -.535, *p*= .006) (see figure 4-10) and percent symptom reduction at one month (rho= .594; *p*= .004) (see figure 4-11).

Figure 4-10. Baseline ACC GSH and Time to Response.

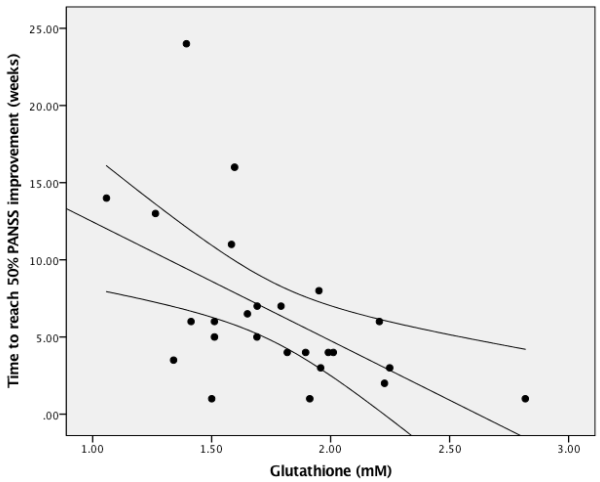
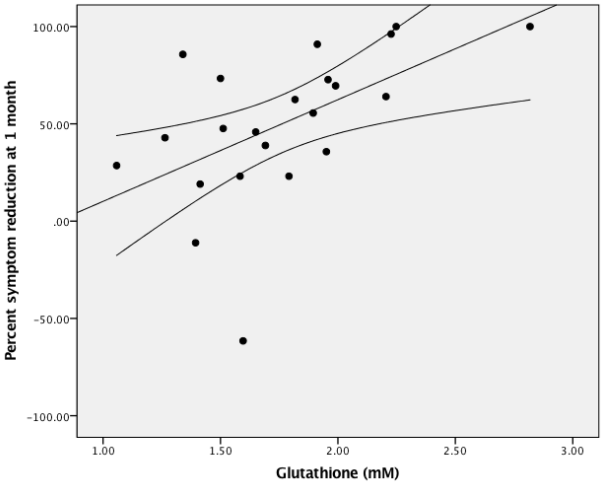
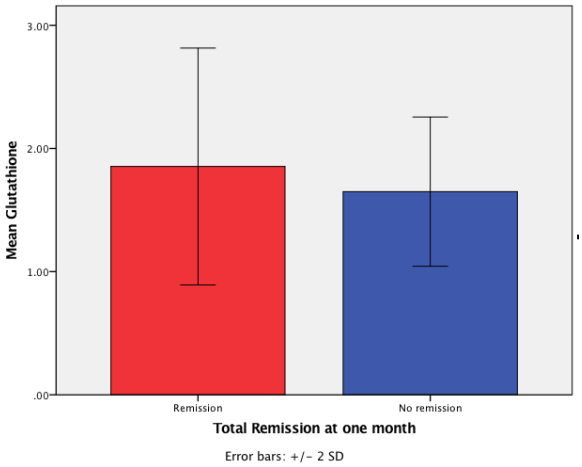


Figure 4-11. Baseline ACC GSH and Percent Symptom Reduction 1 Month.



However, GSH was not significantly associated with remission status at one month using binary logistic regression analyses (χ^2= 1.829; p= .176; Nagelkerke R^2= .091; B= -1.463; SE= 1.143; Wald= 1.638; p=.201; Exp(B)= .231) (see figure 4-12).

Figure 4-12. Baseline ACC GSH Levels in Remission Versus Not at 1 Month.



4.4.6 Model Using Baseline Metabolite Levels to Predict Time to Response

Multiple regression analysis was used to test if GSH and glutamate significantly predicted the time taken by patients with FEP to respond to antipsychotic treatment. The results of the regression indicated the two predictors explained 31% of the variance (R2 =.0.31, F(2,24)=4.86, p=0.018). Higher levels of GSH predicted a shorter time to response (β = -0.65, p=0.017) while glutamate was not a significant predictor (β = 0.15, p=0.563). A very low level of multicollinearity was present (VIF = 1.98 for both GSH and glutamate). Results remained unchanged after controlling for age, sex, and daily dose of antipsychotics.

4.4.7 Metabolites and Social Functioning

Multiple regression analysis was used to test if GSH and glutamate significantly predicted the SOFAS scores in patients with FEP. The results of the regression indicated the two predictors explained 33% of the variance (R^2 =.0.33, F(2,24)=5.33, p=0.013). Higher levels of glutamate predicted lower SOFAS scores (β = -0.70, p=0.008) (see figure 4-13) while GSH was

not a significant predictor (β = 0.22, p=0.376). A very low level of multicollinearity was present (VIF = 1.89 for both GSH and glutamate). Results remained unchanged after controlling for age, sex, and daily dose of antipsychotics.

Figure 4-13. Baseline ACC Glutamate and Social Functioning.

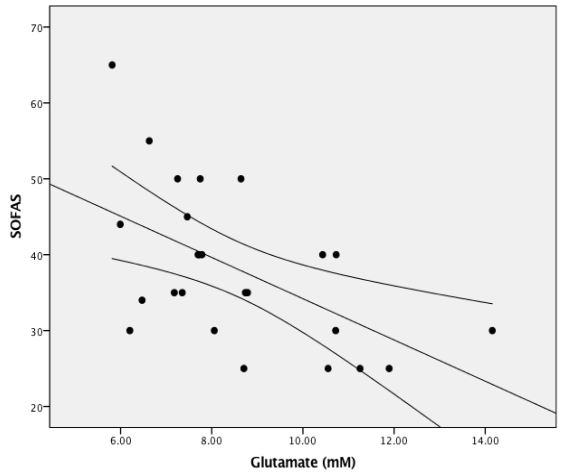


Table 4-2. Summary of Correlational Findings.

Metabolite Significant (Yes or No)	Time to Response	Percent Symptom Improvement 1 Month	Remission at 1 month
Glutamate	No	Yes	No
Glutamine	No	Yes	No
Glutathione	Yes	Yes	No

4.4.8 Metabolites in Healthy Controls versus Patients in Remission and Not

One-way ANOVAs were conducted to evaluate the differences in metabolite levels among patients in remission or non-remission at one month and healthy control subjects. There were no significant difference between groups for glutamate ($F(2,50)=.134$, $p=.875$) or GSH ($F(2,50)=.712$, $p=.496$) (see Table 4-2). There were no significant differences between patients

(as a single group) and controls on measures of glutamate (t(51)= -.266, *p*= .791) or GSH (t(51)= -.412, *p*=.682.

Table 4-3. Baseline Metabolite Levels in Patients in Remission, Not in Remission, and Healthy Controls.

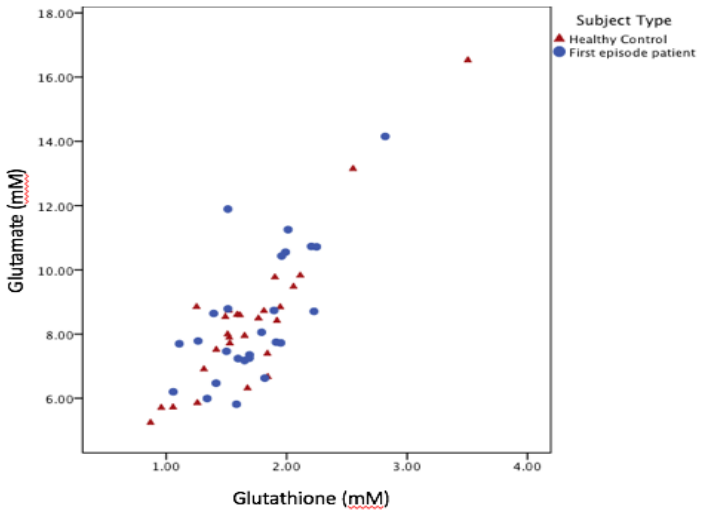
Metabolite (M/SD)	Total Group (N=53)	Remission (N=11)	No Remission (N=15)	HC (N=27)
Glutamate	8.43/2.16	8.73/2.30	8.34/1.91	8.35/2.30
Glutamine	1.26/0.50	1.34/0.55	1.18/0.57	1.27/0.50
Glutathione	1.71/0.46	1.85/0.48	1.65/0.30	1.68/0.52

Remission status was calculated at one month. HC= healthy controls.

4.4.9 Correlations Between Metabolite Levels

The association between glutamate and GSH was tested using Pearson correlation coefficients. There was a positive association between levels of ACC glutamate and GSH in both healthy control subjects (*r*= .91, *p*< .001), and in patients with FEP (r= .69, *p*< .001). We then used Fisher's r-to-z transformation to test the significance of difference between the correlations, and found that the correlation between glutamate and GSH was significantly weaker in patients compared to the healthy control subjects (Z= 2.26, *p*= .023) (see figure 4-14).

Figure 4-14. Correlation between Glutamate and Glutathione in Patients and Healthy Controls.



4.5 Discussion

4.5.1 Summary of Results

Contrary to our original hypothesis, we did not find an association between elevated glutamate (or glutamine) at baseline, and poorer subsequent treatment response. In fact, higher glutamate and glutamine were both associated with greater relative percent symptom reduction at one month. When included in a linear regression model with GSH however, neither glutamate or glutamine were independently associated with time to response in weeks. In keeping with our original hypothesis, higher baseline ACC glutamate was associated with worse social functioning at baseline, while glutamine and GSH were not independent predictors in the overall model. GSH was found to be significantly associated with both time to improve by 50% and relative psychotic symptom reduction at 1 month. GSH remained a significant predictor of time to response when included in a model with glutamate and glutamine. None of the metabolites were associated with binary remission status at one month and there were no significant differences in

glutamate, glutamine, or GSH between healthy controls, patients in remission at one month, and patients not in remission. Finally, glutamate, glutamine, and GSH were all significantly positively correlated in the overall sample, including healthy controls, however, when compared to healthy controls, GSH levels in patients are dissociated from glutamate levels.

This is the first study to use ultra high-field 7T MRS to investigate the role of glutamate and GSH in early response to antipsychotics. This is also the first 7T MRS study on minimally medicated FEP subjects. A previous 7T MRS study included FEP subjects with an average of 55 weeks of antipsychotic exposure (Reid et al, 2018; Overbeek et al, 2018), compared to 6 days of median exposure in our sample. A more recent study (Wang et al, 2019) included FEP subjects with up to 2 years of illness duration, while we recruited all subjects during the acute first episode (mean SOFAS score of 38.1). We report 3 major findings (1) Patients with FEP with higher GSH levels in ACC show a rapid symptom reduction upon starting antipsychotic treatment (2) When compared to healthy controls, GSH levels in patients are dissociated from glutamate levels (3) Glutamate excess predicts the degree of Social and Occupational dysfunction seen at the time of presentation with FEP. Taken together, these results indicate that markers of cortical redox integrity influence the putative glutamatergic toxicity and early treatment response in psychosis.

4.5.2 Glutamatergic Metabolites and Treatment Response

Given the mounting lack of evidence for dopaminergic abnormalities in individuals with TRS (Yoshimura et al, 2003; Mazure et al, 1991; Pickar et al, 1984; Abi-Dargham et al, 2000; Demjaha, 2012), it has been speculated that alternative neurobiological mechanisms may be implicated in the approximately one third of individuals who do not respond to dopamine-

blocking antipsychotic medications. To date, the most compelling support exists for a glutamatergic theory of treatment resistance. Several studies have found treatment resistant individuals to have higher ACC glutamate in both chronic schizophrenia (Mouchlianitis et al, 2016; Szulc et al, 2013) and in FEP (Egerton et al, 2012; Egerton et al, 2018). Tying the dopaminergic and glutamatergic findings together, Demjaha et al (2014) investigated both striatal dopamine synthesis, and ACC glutamate, in the same population of individuals with schizophrenia and found those with TRS to be characterized by lower striatal dopamine synthesis, and higher ACC glutamate. Only a few studies have examined the association between ACC glutamate and treatment response in FEP samples. Egerton et al (2012) found that individuals with FEP who had not achieved remission after 6 months demonstrated higher levels of ACC glutamate than those achieving remission. While interesting, these results may have been influenced by medication effects as all individuals were assumed to have been taking prescribed antipsychotic medications. In the first study to examine the association of ACC glutamate and subsequent treatment response prospectively, Egerton et al (2018) found that baseline ACC glutamate was associated with lack of remission at one month. While providing valuable information regarding the relationship between treatment response and ACC glutamate, the exclusive use of binary measures of response may be problematic as response to treatment is more likely represented on a spectrum, with some individuals being very responsive to treatment, and others being very resistant, with the majority falling somewhere in between. Furthermore, the decision to assess remission status at one month is relatively arbitrary; an individual not in remission at one month may achieve remission at 6 weeks, for example. Finally, the use of criteria applying absolute thresholds in defining remission without regard for baseline severity presents the risk of biasing individuals with milder illness presentations as being more likely to

achieve remission, despite potentially improving very little from baseline. Ours was the first study to apply both continuous and binary measures in evaluating the association between ACC glutamate and treatment response.

Given we did not find an association between high ACC glutamate and early treatment response (or lack thereof), our results differ from all studies published to date. Furthermore, we found that higher ACC glutamate and glutamine were both associated with greater symptom reduction at one month from baseline, although neither was significantly associated with time to improve by 50%, or with binary remission status at one month. Only one study has similarly investigated the association of ACC glutamate and subsequent treatment response prospectively (Egerton et al, 2018), and there are several differences between this study and ours that are worth noting, and may underlie differences reported. Our study was the first to use a 7T MRI to investigate the association of ACC glutamate and treatment response. The use of a scanner of higher field strength may have contributed to increased precision in separating glutamate from glutamine (Mekle et al, 2009; Pradhan et al, 2015; Tkáč et al, 2009). Second, while we used water as a reference for measuring metabolites, the Egerton et al (2018) study calculated a ratio of glutamate to creatine. Though frequently employed in spectroscopy research, the use of creatinine as a reference may be problematic if creatine values vary systematically in individuals with schizophrenia, or in individuals with treatment resistant disease. While some studies have found significant differences in absolute creatine levels in individuals with schizophrenia relative to healthy controls in the ACC (Ongur et al, 2009), others have not (Deiken et al, 1997; Prenkumar at al, 2010). No studies have investigated whether there are any differences in creatine between patients with good and poor antipsychotic response. The population of FEP patients in our studies may have also varied significantly. With a higher proportion of

outpatients, and no patients receiving compulsory treatment, it is reasonable to speculate that the Egerton et al (2018) study may have been analyzing a less severely unwell population of individuals with FEP.

Elevated ACC glutamate levels may be characteristic of a more severe presentation of psychotic illness, and general decreased tendency to comply with treatment. All patients in the Egerton et al (2018) study were administered oral antipsychotic medications, and no methods of assessing treatment adherence are described. Given the generally high rates of medication non-adherence in schizophrenia (one study of patients with FEP found that only 40% of individuals persisted with recommended treatment for 30 days or more (Tiihonen et al, 2011)), it is reasonable to speculate that many individuals in their sample may have not been compliant with oral medication as prescribed. Lack of treatment adherence may have varied systematically between remission subgroups, presenting a major confounding variable in interpreting results. In fact, failure to comply with medication treatment may underlie metabolite differences reported, wherein the non-remitted group may have higher rates of non-adherence. Indeed, individuals who do not achieve remission of positive and negative symptoms have been shown to be more likely to discontinue medication (Mustafa et al, 2017). Egerton et al (2018) found that glutamate levels in the ACC decreased after one month relative to baseline, suggesting that antipsychotic administration may lead to a dampening of glutamate levels, even over the short-term. Individuals who are not adherent with treatment, would not be expected to demonstrate this lowering of glutamate from baseline, and would also be more likely to continue to experience psychotic symptoms. Therefore, the apparent association between elevated glutamate and lack of remission reported in previous studies may be reflective of early medication adherence patterns (or lack thereof) and not indicative of a general association between high glutamate and poor

response. Due to our increased use of injectable antipsychotic medications, as well as frequent clinical assessments of treatment adherence, we can be more confident in ruling out the confounding effect of non-adherence.

While we did not find evidence to support an association between elevated ACC glutamate and treatment response in FEP, our results do support a hyperglutamatergic state as being associated with a generally worse prognosis, particularly with regards to social functioning. In our study, higher ACC glutamate and glutamine were both associated with greater symptom reduction at one month. Individuals who are more unwell initially, would be expected to demonstrate greater improvements in absolute symptoms from baseline. Comparatively fewer studies have investigated associations of glutamatergic metabolites and social and/or functional outcomes. Egerton et al (2012) similarly found that in a sample of patients with FEP, higher levels of ACC glutamate were associated with worse social functioning. However, in another longitudinal study, while decline in glutamate and glutamine in the thalamus was associated with impaired function, no such relationship was found for the ACC (Aoyama et al, 2011). The association of elevated glutamate and poorer social functioning may be in keeping with a hyperglutamatergic state as being a biomarker of a more severe presentation of illness, with poor functioning being just one manifestation. As has been discussed, individuals who are more severely unwell, may additionally struggle with reliable medication adherence, leading to poorer outcomes, and lack of remission.

4.5.3 Glutathione and Treatment Response

Given that oxidative stress (Wood et al, 2009), and excess free radical production (Mahadik and Mkherjee, 1996; Reddy and Yao, 1996), have been hypothesized as being

implicated in the pathophysiology of schizophrenia, GSH, an intracellular antioxidant, may play a role in early treatment response. GSH appears to be an important regulator of glutamatergic activity. Specifically, it has been hypothesized that a state of low GSH can contribute to glutamatergic excess (Koga et al, 2011), and that under oxidative stress, GSH levels may become deplete and lead to NMDA receptor dysfunction (Steullet et al, 2007). Glutamatergic excess in turn, has been shown to activate free radical pathways (Bains and Shaw, 1997), leading to further oxidative stress (Volterra et al, 1994). Therefore, any association of glutamate and eventual treatment response, may also depend on the activity of GSH, and specifically, on the balance and interaction between glutamate and GSH. No studies to date have examined the association of GSH, in addition to glutamate, with early treatment response.

For the first time, we report a positive association between GSH in the ACC at initial presentation of FEP, and treatment response. The overall pathophysiology of GSH in schizophrenia remains unclear as previous studies have produced inconsistent results regarding alterations in this key neural regulator of the glutamatergic system. In chronic schizophrenia, while some studies have found deficits in GSH relative to controls (Do et al, 2000; Gawryluk at al, 2011, Das et al, 2018), others have not (Monin et al, 2015; Matsuzawa et al, 2008; Xin et al, 2016; Terpstra et al, 2005; Brandt et al, 2016). One study of patients with FEP found GSH to be elevated (Wood et al, 2009), with lower GSH levels being associated with cognitive impairment (Martinez-Cengotitabengoa et al, 2012) and grey matter volume deficits (Fraguas et al, 2012; Langbein et al, 2017). However, others have found decreased GSH in FEP patients relative to controls (Mico et al, 2011). It may be that GSH abnormalities are uniquely present in sub-populations of patients with schizophrenia, as opposed to characterizing a unifying neurobiological signature of all patients with this heterogeneous illness. In addition, GSH levels

may vary depending on the stage of illness. Our results suggest that some individuals with FEP appear able to mount a compensatory response to psychosis by increasing GSH in some capacity. It is unclear whether GSH levels are higher in these individuals even before the onset of psychosis, or if levels increase secondarily to the development of a psychotic episode and associated neurobiological perturbations. Regardless, in individuals with higher GSH levels, the response to antipsychotic medications appears to be more robust, and there are several potentially promising clinical implications of this finding.

Specifically, our finding of increased GSH being associated with better early treatment response may have direct implications for the development of innovative treatments for psychosis. Given that GSH is a major intracellular antioxidant and is thought to be neuroprotective (Cohen, 1983; Meister and Anderson, 1983), our results suggest that antioxidant activity may be important for response/remission in early FEP. In contrast to agents targeting glutamate excess which have generally not proven successful to date, targeting GSH as a treatment mechanism may be more feasible in terms of both efficacy and safety. In particular, N-acetyl cysteine (NAC) treatment has demonstrated benefit for schizophrenia in some trials (Berk et al, 2008, Farokhnia et al, 2014; Brier et al, 2018; Conus et al, 2018; Zeng et al, 2018), and has been shown to increase GSH in the brain (Dringen and Hirrlingen, 2003). It remains unclear which patients specifically may benefit from treatments like NAC. Perhaps those patients who are unable to mount a compensatory elevation in GSH would benefit from additional therapeutics, such as NAC, while others may not. GSH may be particularly important at earlier illness stages when glutamatergic excess is present (Poels et al, 2014), as there is some evidence that ongoing glutamatergic activity may be inherently neurotoxic (Plitman et al, 2014) and the FEP may represent a particularly vulnerable period in terms of potential neurodegenerative

effects. To date, no studies have investigated whether agents increasing GSH may protect against neurodegeneration and longitudinal grey matter decline. As there are currently no available neuroprotective agents for the treatment of early schizophrenia, further studies investigating the potential of NAC and other agents targeting GSH may lead to revolutionary advancements in our understanding and treatment of FEP.

We found evidence that despite their significant within-group correlation, when compared to healthy controls, glutamate and GSH levels were less tightly correlated among patients with FEP. A similar dissociation was also reported by Xin et al, (2016) in a first episode sample, especially among patients with a GCLC-risk genotype affecting GSH synthesis. These results indicate that in a subset of patients with FEP, concomitant GSH response fails to occur when demands arise due to glutamatergic excess. Such patients are likely to be vulnerable to neurotoxic damage (Hulshoff Pol and Kahn, 2008), poor treatment response, and greater functional decline as a result of unchecked neuronal/glial damage (Steullet et al, 2006; Kantrowitz and Javitt, 2010; Stone et al, 2009). Interestingly in healthy controls, when glutamatergic synapses are active due a task demand, GSH levels appear to increase concomitantly with glutamate (Lin et al, 2012). It remains to be seen if the resting-state dissociation between GSH and glutamate levels in FEP persists even when task demands arise. Such a persistent dissociation, if observed in FEP, may allow us to infer disruptions in the metabolic pathways linking synaptic glutamate dynamics to GSH synthesis.

4.5.4 Strengths, Limitations, and Future Directions

One of the biggest strengths of our study was our use of a 7T MRI scanner. There are only a limited number of studies that have examined glutamatergic perturbations in FEP using a

scanner of this field strength, and no studies have specifically explored treatment response and resistance in early psychosis. There is evidence that the use of higher field strengths allows for increased precision in measuring glutamate, and distinguishing it from glutamine (Mekle et al, 2009; Pradhan et al, 2015; Tkáč et al, 2009). Second, our sample size was reasonably large for a neuroimaging study, and participants received frequent clinical monitoring to allow for detection of early symptom improvement at discrete intervals. This close clinical monitoring allowed us to determine treatment response measures in a continuous manner. To date, all other studies of treatment response and resistance in FEP have applied dichotomous outcomes in measuring treatment response. In reality, response to treatment is more likely represented on a spectrum, with individuals varying between both extremes (no response, and complete response). Furthermore, the chosen timing of assessing dichotomous responsiveness is arbitrary; an individual who is not in remission at 4 weeks, may be in remission at 6 weeks, for example. Finally, the use of binary criteria with an absolute threshold for defining remission runs the risk of over-categorizing those with milder presentations as being in remission. Individuals with higher symptom scores at baseline will be much less likely to meet remission criteria, even if they display significant symptomatic improvement from baseline, particularly if a short follow-up interval is employed. An additional strength of our study was the fact that our sample consisted of patients with a wide spectrum of illness severities. Many other neuroimaging studies of TRS have consisted primarily of outpatients. The use of a sample that is less “severely unwell” may skew results, and would be less likely to include patients with severe treatment refractoriness. Because we incorporated a relatively balanced mix of inpatients and outpatients, including those requiring compulsory treatment, our sample may have been more closely representative of the general population of patients with FEP treated in clinical settings. Finally,

our use of long acting injectable antipsychotic medications represents an additional strength of our study as these patients could be assumed to be adherent to their medication. Many other studies of TRS have not employed reliable means of assessing medication adherence, and in these studies, "pseudo-resistance" cannot be ruled out.

While our results contribute considerably to the understanding of the early neurobiology of treatment resistant schizophrenia, there are some limitations that should be noted. First, although our population consisted of minimally-treated patients with FEP, it is possible that antipsychotic usage, although limited, may have influenced metabolite levels. The research is conflicting regarding the effect of antipsychotic administration and changes in glutamate levels longitudinally. Chronic antipsychotic usage has been associated with decreased glutamate in the ACC after six months (Choe et al, 1996), and one month (Egerton et al, 2017) of treatment. However, other studies have found no association between antipsychotic treatment and ACC glutamate levels in patients with FEP after one (Szulc et al, 2005), thirty (Theberge et al, 2007) and eighty (Ayoma et al, 2011) months of treatment. Importantly, changes in glutamate levels overtime cannot be assumed to be solely related to antipsychotic usage and may be a result of physiological changes related to the underlying illness itself. Furthermore, acute glutamatergic effects of short-term antipsychotic usage (as employed in our study) are unclear as no studies have assessed the association between same-day antipsychotic administration and immediate glutamatergic change. In addition, no studies have examined the effect of antipsychotic treatment on GSH levels in the ACC. Overall, while it is unlikely that the use of low dose antipsychotic medications for less than fourteen days would have an acute effect on brain glutamate, glutamine, and GSH levels, this possibility cannot be ruled out definitively. Under ideal conditions, all patients would have been scanned in a completely drug-naïve state, however, for

ethical and practical reasons, this was not possible for all cases.

Similarly, the fact that patients in our sample were treated with several different antipsychotic medications, may have influenced the results of our study, although no studies have examined brain glutamatergic changes in relation to specific antipsychotic agents. While controlling for this potential confounding variable by treating all patients with the same medication according to a standardized algorithm would have been ideal, this would have decreased our ability to recruit patients into the study. In addition, in real-world clinical practice, patients are treated with a variety of antipsychotic medications and therefore, our results may have been more generalizable. That said, because clinicians could choose to increase medication doses at their discretion (no algorithm was used), patients treated more aggressively (ie, having their medication increased at a faster rate), may have improved more quickly.

Although we employed methods to attempt to ensure medication compliance, non-adherence with antipsychotic medication may have influenced results. Rates of antipsychotic non-adherence are high in schizophrenia; 74% of patients were non-adherent to medications within eighteen months in the CATIE study (Lieberman et al, 2005) and similarly high rates have been reported in FEP (Hill et al, 2010; Levy et al, 2012; Miller at al, 2011). Non-adherence may lead to falsely identifying a particular person as being resistant to treatment (Correll et al, 2011). Many studies to date that have examined neurobiological correlates of treatment resistance have employed limited, or have used no, assessments of adherence. Without protocols for monitoring adherence, lack of improvement may be simply due to failure to take medication. Furthermore, recent evidence has demonstrated that patients who obtain remission of positive and negative symptoms are most likely to remain adherent to treatment in FEP (Mustafa et al, 2018), suggesting that TRS patients are the most likely to demonstrate poor compliance patterns with

medication recommendations. We attempted to improve adherence in our sample by offering all patients the option to be treated with long acting injectable antipsychotic medications. Because our clinic protocol is to provide injections on-site, we are immediately aware if someone has missed, or is late for a dose of their injection. However, many patients elected to remain on oral antipsychotic medications. In these patients, adherence status at each clinical visit was assessed, incorporating feedback from the patient, their family (if involved in their care), and other allied mental health professionals. Although all efforts were employed to ensure compliance, we cannot be definitively certain that patients on oral medications were adhering to treatment as prescribed, and cannot rule out non-adherence as a potential confounder of our results.

In addition to non-adherence with medication, substance use may influence the early clinical trajectory of FEP. Rates of substance use in patients with schizophrenia are high ranging between 40% (Ziedonis and Fisher, 1994), and 70% (Strakowski et al, 1993). Although our sample did not include patients meeting criteria for DSM-5 (APA, 2013) substance use disorders, patients may have been using drugs recreationally during the study period. We did inquire about, and record use of, substances at each clinical visit however, urine drug screens were not performed as part of the study protocol. It is possible that ongoing drug use may have both influenced metabolite levels, and contributed to poorer treatment response. Particularly, as is seen in the general population of patients with FEP (estimates range between 8% (Strakowski et al, 1994) and 71% (Lambert et al, 2005)), many patients in our study used marijuana recreationally. While an entire sample of non-marijuana users would not be representative of today's general FEP patient, we cannot rule out the effect of marijuana specifically on our results. There has been one study demonstrating that chronic cannabis users (without other mental health diagnoses) had decreased glutamate in the ACC relative to those who had never

used cannabis (Prescott et al 2011; 2013), although these results were not replicated in another study (Sung et al, 2013). That said, no studies have specifically examined the effects of acute, same-day marijuana usage on glutamate or GSH levels, and no studies have examined this association in a schizophrenia or FEP sample.

In the future, there will be several opportunities to expand upon our current findings. All study participants continue to be monitored clinically, with symptom scales being completed at each clinical encounter. Going forward, we plan to continue to analyze outcomes in these patients to assess the association of baseline glutamate and GSH with longer term treatment response and remission. Specifically, it would be interesting to assess whether baseline metabolite levels have any association with eventual clozapine-eligibility, or clozapine usage. For individuals with TRS, clozapine has been shown to be more effective than other first-line antipsychotic medications (Chakos et al, 2011; Lewis et al, 2006; McEvoy et al, 2006). Currently in Canada, there remain significant delays in initiating clozapine therapy in suitable patients, and overall, this medication is thought to be grossly under-utilized (Bogers et al, 2016). While the reasons for this are multifactorial, some of the delay may be associated with the difficulty in determining treatment resistance early-on, and the lack of objective biomarkers suggestive of clozapine-eligibility. The ability to demonstrate an association between neurobiological alterations at baseline and eventual clozapine use would contribute considerably to the understanding of treatment resistance, and may promote reformatting of current clozapine guidelines. While current guidelines mandate a trial of two antipsychotic medications prior to clozapine being initiated (NICE, 2014), the necessity of two medication trials has been questioned (Remington et al, 2013; Kahn et al, 2018), particularly as recent evidence has indicated that a treatment resistant course can be reliably predicted within weeks following a

FEP (Agid et al, 2003; Suzuki et al, 2011; Samra et al, 2015; Derks et al, 2010; Ucock et al, 2011). Recently, a large, multi-center, three-phase switching study found that there was no added benefit to a second antipsychotic trial in patients with FEP who did not achieve remission after one month (Kahn et al, 2018). The authors go onto suggest that these results support reformulation of guidelines such that only one failed antipsychotic trial should be required prior to determining clozapine-eligibility. Neurobiological parameters indicative of a higher risk of resistance may further support triaging of certain patients towards clozapine at an earlier stage.

In keeping with phase-specific neurobiological alterations in early psychosis, future studies investigating the association of glutamate and GSH in those at clinical high risk (CHR) for psychosis are warranted. It would be beneficial to evaluate whether abnormalities in these metabolites prior to the development of acute psychosis have any association with eventual treatment response once FEP occurs (in those patients who go onto develop psychosis). To date, no studies have examined GSH in a CHR sample, and its association with developing psychosis. A demonstrated association between GSH levels in the at-risk state, and development of psychosis, would support the potential for use of antioxidant treatments, and other therapeutics that may increase GSH, at this vulnerable stage. Disease-modifying treatments for individuals at risk for schizophrenia are currently lacking, and are grossly needed.

4.5.5 Conclusion

While we did not replicate previous findings that have demonstrated an association between elevated ACC glutamate and lack of treatment response in FEP, we are the first to report an association between levels of GSH and early treatment response. We found that higher ACC glutamate was associated with worse baseline social functioning, supporting the

proposition that elevated glutamate may be neurotoxic, and associated with a more severe presentation of illness. Our results suggest that neuroinflammation may be implicated in early lack of treatment response. The finding of an association between GSH and response to treatment is clinically useful as this may promote the development of novel treatment mechanisms. Specifically, agents with a targeted mechanism to increase GSH may be beneficial as adjuvants to antipsychotic medications, particularly in the one third of patients who fail to respond well to these agents alone.

References

Abi-Dargham A, Grace, A. Dopamine and schizophrenia. In: Weinberger DR, Harrison P, ed. *Schizophrenia*. 3rd ed. Oxford, Wiley-Blackwell, 2011, pp 413- 432.

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles L, et al. (2000). Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proceedings of the National Academy of Sciences, USA,* 97, 8104–8109.

Adams B, Moghaddam B. (1998). Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *Journal of Neuroscience*, 18, 5545–5554.

Agid O, Kapur S, Arenovich T, Zipursky RB. (2003). Delayed-onset hypothesis of antipsychotic action: a hypothesis tested and rejected. *Archives of General Psychiatry*, 60, 1228–1235

Agid O, Arenovich T, Sanjeev G, Zipursky RB, Kapur S, Foussias G, et al. (2011). An algorithm based approach to first-episode schizophrenia: response rates over 3 prospective antipsychotic trials with a retrospective data analysis. *Clinical Journal of Psychiatry*, 72(11), 1439-1444.

Alathiaki AS, Czernikiewicz A. (2005). The effect of risperidone on metabolite measures in the frontal lobe, temporal lobe, and thalamus in schizophrenic patients. A proton magnetic resonance spectroscopy (1H MRS). *Pharmacopsychiatry*, 38(5), 214–219.

Alonso-Solís A, Vives-Gilabert Y, Grasa E, Poretella MJ, Rabella M, Sauras RB, et al. (2015). Resting-state functional connectivity alterations in the default network of schizophrenia patients with persistent auditory verbal hallucinations. *Schizophrenia Research*, 161, 261–268.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Arlington, VA: American Psychiatric Publishing.

Anderson VM, Goldstein ME, Kydd RR, Russell BR. (2015). Extensive gray matter volume reduction in treatment-resistant schizophrenia. *International Journal of Neuropsychopharmacology*, 18(7), 1-10.

Andreasen NC, Carpenter Jr WT, Kane JM, Lasser RA, Marder SR, Weinberger DR (2005). Remission in schizophrenia: proposed criteria and rationale for consensus. *American Journal of Psychiatry*, 162, 441–449.

Andreasen NC, Liu D, Ziebell S, Vora A, Ho BC. (2013). Relapse duration, treatment intensity, and brain tissue loss in schizophrenia: A prospective longitudinal MRI study. *American Journal of Psychiatry*, 170(6), 689.

Andreasen NC, Carpenter WT Jr, Kane JM, Lasser RA, Marder SR, Weinberger DR. (2005). Remission in schizophrenia: proposed criteria and rationale for consensus. *American Journal of Psychiatry*, 162(3), 441–449.

Aoyama N, Theberge J, Drost DJ, Manchanda R, Northcott S, Neufeld RWJ, et al. (2011). Grey matter and social functioning correlates of glutamatergic metabolite loss in schizophrenia. *British Journal of Psychiatry*, 198(6), 448–5610.

Altuntas I, Aksoy H, Coskun I, Caykoylu A, Akcay F. (2000). Erythrocyte superoxide dismutase and glutathione peroxidase activities, and malondialdehyde and reduced glutathione levels in schizophrenic patients. *Clinical Chemical Laboratory Medicine*, 38, 1277–1281.

Bains JS, Shaw CA. (1997). Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. *Brain Research Review*, 25, 335–58.

Baker DA, Shen H, Kalivas PW. (2002). Cystine/glutamate exchange serves as the source for extracellular glutamate: modifications by repeated cocaine administration. *Amino Acids*, 23, 161–162.

Barch DM, Braver TS, Akbudak E, Conturo T, Ollinger J, Snyder A. (2001). Anterior cingulate cortex and response conflict: Effects of response modality and processing domain. *Cerebral Cortex*, 11, 837– 848.

Bartha R, Drost DJ, Menon RS, Williamson PC. (2000). Spectroscopic lineshape correction by QUECC: combined QUALITY deconvolution and eddy current correction. *Magnetic Resonance Medicine*, 44, 641–645.

Bartha R, Drost DJ, Williamson PC. (1999). Factors affecting the quantification of short echo in vivo 1 H MR spectra: prior knowledge, peak elimination, and filtering. *NMR Biomedicine*, 12, 205–216.

Benes, F.M. (2009). Amygdalocortical circuitry in schizophrenia: from circuits to molecules. *Neuropsychopharmacology*, 35(1), 239–257.

Berk M, Copolov D, Dean O, Lu K, Jeavons S, Schapkaitz I, Anderson-Hunt M, Judd F, Katz F, Katz P, Ording-Jespersen S, Little J, Conus P, Cuenod M, Do KQ, Bush AI. (2008). N-acetyl cysteine as a glutathione precursor for schizophrenia—a double- blind, randomized, placebo-controlled trial. *Biological Psychiatry*, 64, 361–368.

Birchwood M, Todd P, Jackson C. (1998). Early intervention in psychosis: the critical period hypothesis. *British Journal of Psychiatry Supplement*, 172(33), 53–59

Bertolote J, McGorry P. (2005). Early intervention and recovery for young people with early psychosis: consensus statement. *British Journal of Psychiatry Supplement*, 187(suppl 48), s116–s119.

Black K, Peters L, Rui Q, Milliken H, Whitehorn D, Kopala LC. (2001). Duration of untreated psychosis predicts treatment outcome in an early psychosis program. *Schizophrenia Research,* ***47,*** 215–22.

BleulerM. (1972). The Schizophrenic Disorders. Yale University Press, NewHaven.

Bogers JP, Schilte PF, Van Diijk D, Bakker B, Cohen D. (2016). Clozapine underutilization in the treatment of schizophrenia: how can clozapine prescription rates be improved? *Journal of Clinical Psychopharmacology*, 36(2), 109-111.

Borgio J, Rocha D, Elkis H, Bressan R. (2010) Neuroimagning in treatment-resistant schizophrenia. In: Elkis H, Meltzer H (eds) Therapy-resistant schizophrenia. Karger, Basel, pp 63–73.

Brandt AS, Unschuld PG, Pradhan S, Lim IA, Churchill G, Harris AD et al. (2016). Age- related changes in anterior cingulate cortex glutamate in schizophrenia: A ^{1}H MRS Study at 7 Tesla. *Schizophrenia Research*, 172(1), 101-5.

Braver TS, Barch DM, Gray JR, Molfese DL, Snyder A. (2001). Anterior cingulate cortex and response conflict: Effects of frequency, inhibition and errors. *Cerebral Cortex,* 11,825– 836.

Breier A, Leffick E, Hummer TA, Vohs JL, Yang Z, Mehdiyoun NF, Visco AC, Metzler E, Zhang Y, Francis MM. (2018). Effects of 12-month double-blind N-acetyl cysteine on symptoms, cognition and brain morphology in early phase schizophrenia-spectrum disorders. *Schizophrenia Research*, 199, 395-402.

Brosnan JT, Brosnan ME. (2013). Glutamate: a truly functional amino acid. *Amino Acids*, 45, 413–418.

Brown MA. (2004). Time-domain combination of MR spectroscopy data acquired using phased-array coils. *Magnetic Resonance Medicine*, 52, 1207–1213.

Bulut M, Savas HA, Altindag A, Virit O, Dalkilic A. (2009). Beneficial effects of N-acetylcysteine in treatment resistant schizophrenia. *World Journal of Biological Psychiatry*, 10, 626–628.

Bustillo JR, Rowland LM, Mullins P, Jung R, Chen H, Qualls C, Hammond R, Brooks WM, Lauriello J. (2010). 1H-MRS at 4 tesla in minimally treated early schizophrenia. *Molecular Psychiatry*, 15(6), 629–636.

Bustillo JR, Chen H, Jones T, Lemke N, Abbott C, Qualls C, Canive J, Gasparovic C. (2014). Increased glutamine in patients undergoing long-term treatment for schizophrenia: a proton magnetic resonance spectroscopy study at 3 T. *JAMA Psychiatry*, 71, 265–272.

Bustillo JR, Chen H, Gasparovic C, Mullins P, Caprihan A, Qualls C, Apfeldorf W, Lauriello J, Posse S. (2011). Glutamate as a marker of cognitive function in schizophrenia: a proton spectroscopic imaging study at 4 Tesla. *Biological Psychiatry*, 69(1), 19–27.

Carter CS, MacDonald AW, 3rd, Ross LL, Stenger VA. (2001). Anterior cingulate cortex activity and impaired self-monitoring of performance in patients with schizophrenia: An event-related fMRI study. *American Journal of Psychiatry*, 158, 1423–1428.

Caseiro O, Perez-Iglesias R, Mata I, Martinez-Garcia O, Pelayo-Teran JM, Tabares-Seisdedos R,

Crespo-Facorro B. (2012). Predicting relapse after a first episode of non-affective psychosis: a three-year follow-up study. *Journal of Psychiatric Research*, 46(8), 1099–1105.

Cervellione KL, Burdick KE, Cottone JG, Rhinewine JP, Kumra S. (2007). Neurocognitive deficits in adolescents with schizophrenia: longitudinal stability and predictive utility for short-term functional outcome. *Journal of the American Academy of Child and Adolescent Psychiatry*, 46(7), 867–878.

Chakos M, Lieberman J, Hoffman E, Bradford D, Sheitman B. (2001). Effectiveness of second-generation antipsychotics in patients with treatment-resistant schizophrenia: a review and meta-analysis of randomized trials. *American Journal of Psychiatry*, 158(4), 518-26.

Chen H. (2017). Altered glutamate and regional cerebral blood flow levels in schizophrenia: A 1H-MRS and pCASL study. *Neuropsychopharmacology,* 42, 562-517.

Choe BY, Suh TS, Shinn KS, Lee CW, Lee C, Paik IH. (1996). Observation of metabolic changes in chronic schizophrenia after neuroleptic treatment by in vivo hydrogen magnetic resonance spectroscopy. *Investigational Radiology*, 31(6), 345–352.

Cohen G. (1983) Catalase, glutathione peroxidase, superoxide dismutase and cytochrome P-450. In Lajtha, A. (ed.), Handbook of Neurochemistry. Plenum Press, New York, pp. 315-328.

Conus P, Seidman LJ, Fournier M, Xin L, Cleusix M, Baumann PS, Ferrari C, Cousins A, Alameda L, Gholam-Rezaee M, Golay P, Jenni R, Woo TW, Keshavan MS, Eap CB, Wojcik J, Cuenod M, Buclin T, Gruetter R, Do KQ. (2018). N- acetylcysteine in a double-blind randomized placebo-controlled trial: toward biomarker-guided treatment in early psychosis. *Schizophrenia Bulletin*, 44, 317–327.

Correll C, Kishimoto T, Nielsen J, Kane J. (2012). Quantifying clinical relevance in the treatment of schizophrenia. *Clinical Therapeutics*, 33(12), B16–B39

Coyle JT. (2012). NMDA receptor and schizophrenia: A brief history. *Schizophrenia Bulletin*, 38, 920–926.

Creese I, Burt D, Snyder SH. (1976). Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science*, 192, 481–483.

Curtis AT, Gilbert KM, Klassen LM, Gati JS, Menon RS. (2012). Slice-by-slice B1+ shimming at 7T. *Magnetic Resonance Medicine*, 68, 1109–1116.

Das TK, Javadzadeh A, Dey A, Sebasan P, Theberge J, Radua J, Palaniyappan P. (2018). Antioxidant defense in schizophrenia and bipolar disorder: A meta-analysis of MRS studies of anterior cingulate glutathione. *Prog Neuropsychopharmacol Biol Psychiatry* (in press).

Dean O, Giorlando F, Berk M. (2011). N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. *Journal of Psychiatry and Neuroscience*, 36, 78–86.

De Beer R, Van Ormondt D. (1992). Analysis of NMR data using time domain fitting procedures. *NMR Basic Principles and Progress*, 26, 201–248.

Dehaene S, Artiges E, Naccache L, Martelli C, Viard A, Schurhoff F, et al. (2003). Conscious and subliminal conflicts in normal subjects and pa- tients with schizophrenia: The role of the anterior cingulate. *Proceedings of the National Academy of Sciences USA,* 100, 13722–13727.

Deicken RF, Zhou L, Schuff N, Weiner MW. (1997). Proton magnetic resonance spectroscopy of the anterior cingulate region in schizophrenia. *Schizophrenia Research,* 27, 65–71.

de la Fuente-Sandoval C, Leon-Ortiz P, Favila R, Mamo D, Ramirez-Bermudez J, Graff-Guerrero. (2011). Higher levels of glutamate in the associative-striatum of subjects with prodromal symptoms of schizophrenia and patients with first-episode psychosis. *Neuropsychopharmacology*, 36, 1781–1791.

Demjaha A, Murray RM, McGuire PK, Kapur S, Howes OD. (2012). Dopamine synthesis capacity in patients with treatment-resistant schizophrenia. *American Journal of Psychiatry,* 169, 1203–10.

Demjaha A, Egerton A, Murray RM, Kapur S, Howes OD, Stone JM, McGuire PK. (2014). Antipsychotic treatment resistance in schizophrenia associated with elevated glutamate levels but normal dopamine function. *Biological Psychiatry* 75, e11–13.

Demjaha A, Lappin JM, Stahl D, Patel MX, MacCabe JH, Howes OD, et al. (2017). Antipsychotic treatment resistence in first-episode psychosis: prevalence, subtypes and predictors. *Psychological Medicine*, 47, 1981-1989.

Derks EM, Fleischhacker WW, Boter H, Peuskens J, Kahn RS. (2010). Antipsychotic drug treatment in first-episode psychosis: should patients be switched to a different antipsychotic drug after 2, 4, or 6 weeks of nonresponse? *Journal of Clinical Psychopharmacology*, 30(2), 176-180.

Do KQ, Trabesinger AH, Kirsten-Krüger M, Lauer CJ, Dydak U, Hell D et al. (2000). Schizophrenia: glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. *European Journal of Neuroscience,* 12(10), 3721–8.

Dringen R, Hirrlinger J. (2003). Glutathione pathways in the brain. *The Journal of Biological Chemistry*, 384, 505–516.

Duarte JM, Gruetter R. (2013). Glutamatergic and GABAergic energy metabolism measured in the rat brain by (13) C NMR spectroscopy at 14.1 T. J. *Neurochemistry*, 126, 579–590.

Egerton A, Brugger S, Raffin M, Barker GJ, Lythgoe GJ, McGuire PK, Stone JM. (2012). Anterior cingulate glutamate levels related to clinical status following treatment in first-episode schizophrenia. *Neuropsychopharmacology*, 37, 2515–2521.

Egerton A, Broberg BV, Van Haren N, Merrit K, Barker GJ, Lythcoe DJ, Perez-Iglesias R, Baandrup L, During SW, Sendt KV, Stone JM, Rostrup E, Sommer IE, Glenthoj B, Kahn RS, Dazzan P, McGuire P. (2018). Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: A multicenter 1H-MRS study (OPTiMiSE). *Molecular Psychiatry* (in press).

Egerton A, Bhachu A, Merritt K, McQueen G, Szulc A, McGuire P. (2017). Effects of antipsychotic administration on brain glutamate in schizophrenia: A systematic review of longitudinal ₁H-MRS studies. *Frontiers in Psychiatry*, 8, 7.

Emsley R, Chiliza B, Asmal L, Harvey BH. (2013). The nature of relapse in schizophrenia. *BMC Psychiatry* 13 (50).

Farokhnia M, Sabzabadi M, Pourmahmoud H, Khodaie-Ardakani MR, Hosseini SM, Yekehtaz H, Tabrizi M, Rezaei F, Salehi B, Akhondzadeh S. (2014). A double- blind, placebo controlled, randomized trial of Riluzole as an adjunct to risperidone for treatment of negative symptoms in patients with chronic schizophrenia. *Psycho-pharmacology*, 231, 533–542.

Farooq S, Agid A, Foussias G, Remington G. (2013). Using treatment response to subtype schizophrenia: Proposal for a new paradigm in classification. *Schizophrenia Bulletin*, 39(6), 1169-1172.

First MB, Williams JBW, Karg RS, Spitzer RL. (2016). Structured Clinical Interview for DSM-5 Disorders, Clinician Version (SCID-5-CV). Arlington, VA, American Psychiatric Association.

Fonnum F. (1984). Glutamate: a neurotransmitter in mammalian brain. Journal of *Neurochemistry*, 42 (1), 1–11.

Fornito A, Yucel M, Dean B, Wood SJ, Pantelis C. (2009). Anatomical abnormalities of the anterior cingulate cortex in schizophrenia: bridging the gap between neuroimaging and neuropathology. *Schizophrenia Bulletin*, 35(5), 973–993.

Fraguas D, Gonzalez-Pinto A, Mico JA, Reig S, Parellada M, Martinez-Cengotitabengoa M, et al. (2012). Decreased glutathione levels predict loss of brain volume in children and adolescents with first-episode psychosis in a two-year longitudinal study. *Schizophrenia Research*, 137(1–3), 58–65.

Friedman L, Lys C, Schulz SC. (1992). The relationship of structural brain imaging parameters to antipsychotic treatment response: a review. *Journal of Psychiatry and Neuroscience*, 17(2), 42–54

Fujii DE, Wylie AM. (2003). Neurocognition and community outcome in schizophrenia: long-term predictive validity. *Schizophrenia Research*, 59(2–3), 219–223.

Fusar-Poli P, Stone JM, Broome MR, Valli I, Mechelli A, McLean MA, Lythgoe DJ, O'Gorman RL, Barker GJ, McGuire PK. (2011). Thalamic glutamate levels as a predictor of cortical response during executive functioning in subjects at high risk for psychosis. *Archives of General*

Psychiatry, 68, 881–890.

Gallego JA, Robinson DG, Sevy SM, Napolitano B, McCormack J, Lesser ML, Kane JM. (2011). Time to treatment response in first-episode schizophrenia: should acute treatment trials last several months? *Journal of Clinical Psychiatry*, 72(12), 1691-1696.

Gawryluk JW, Wang JF, Andreazza AC, Shao L, Young LT. (2011). Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. *International Journal of Neuropsychopharmacol*ogy, 14, 123–130.

Gilbert KM, Curtis AT, Gati JS, Klassen LM, Menon RS. (2011). A radiofrequency coil to facilitate B 1+shimming and parallel imaging acceleration in three dimensions at 7T. *NMR Biomedicine*, 24, 815–823.

Goldman HH, Skodol AE, Lave TR. (1992). Revising axis V for DSM-IV: a review of measures of social functioning. *American Journal of Psychiatry*, 149(9), 1148–1156.

Goto N, Yoshimura R, Kakeda S, Nishimura J, Moriya J, Hayashi K, et al. (2012). Six-month treatment with atypical antipsychotic drugs decreased frontal-lobe levels of glutamate plus glutamine in early-stage first-episode schizophrenia. *Neuropsychiatric Disease Treatment*, 8:119–122.

Guy W. ECDEU. Assessment manual for psychopharmacology. Washington, DC. DHEW Publication 76338; 1976.

Gysin R, Kraftsik R, Sandell J, Bovet P, Chappuis C, Connus P, Deppen P, Preisig M, Ruiz V, Steullet P, Tosic M, Werge T, Cuenod M, Do K. (2007). Impaired glutathione synthesis in schizophrenia: Convergent genetic and functional evidence. *Proceedings of the National Academy of Sciences USA*, 104(42), 16621-16626.

Hafner H, van der Heiden W, Behrens S, Gattaz WF, Hambrecht M, Loffler W, et al. (1998). Causes and consequences of the gender differences in age of onset of schizophrenia. *Schizophrenia Bulletin*, 24, 99–113.

Harding CM, Brooks GW, Ashikaga T, Strauss JS, Breier A. (1987). The Vermont longitudinal study of persons with severe mental illness, II: Long-term outcome of subjects who retrospectively met DSM-III criteria for schizophrenia. *American Journal of Psychiatry*, 144, 727–735.

Harris AD, Saleh MG, Edden RA. (2017). Edited 1 H magnetic resonance spectroscopy in vivo: methods and metabolites. *Magnetic Resonance Medicine*, 77:1377–1389.

Heckers S, Weiss AP, Deckersbach T, Goff DC, Morecraft RJ, Bush G (2004). Anterior cingulate cortex activation during cognitive interfer- ence in schizophrenia. *American Journal of Psychiatry,* 161, 707–715.

Hill M, Crumlish N, Whitty P, Clarke M, Browne S, Kamali M et al. (2010). Nonadherence to medication four years after a first episode of psychosis and associated risk factors. *Psychiatric Services, 61,* 189-192.

Horsdal HT, Wimberley T, Kohler-Forsberg O, Baandrup L, Gasse C. Association between global functioning at first schizophrenia diagnosis and treatment resistance. *Early Intervention in Psychiatry 2018*; **12**:1198-1202.

Howes OD, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A et al. (2012) The nature of dopamine dysfunction in schizophrenia and what this means for treatment: meta-analysis of imaging studies. *Archives of General Psychiatry*, 69(8), 776–786

Howes OD, Montgomery AJ, Asselin MC, Murray RM, Valli I, Tabraham P et al. (2009b). Elevated striatal dopamine function linked to prodromal signs of schizophrenia. *Archives of General Psychiatry*, 66 (1), 13–20

Howes OD, Bose S, Valli I, Turkheimer F, Egerton A, Valmaggia L et al. (2011a). Dopamine synthesis capacity before onset of psychosis: a prospective [18]-DOPA PET imaging study. *American Journal of Psychiatry*, 168(12), 1311–1317

Howes O, Bose S, Turkheimer F, Valli I, Egerton A, Stahl D et al (2011b) Progressive increase in striatal dopamine synthesis capacity as patients develop psychosis: a PET study. Mol Psychiatry 16(9):885–886

Howes OD, McCutcheon R, Agid O, deBartolomeis A, van Beveren NJM, Birnbaum ML et al. (2017). Treatment-Resistant Schizophrenia: Treatment Response and Resistance in Psychosis (TRIPP) Working Group Consensus Guidelines on Diagnosis and Terminology. *American Journal of Psychiatry*, 174(3), 216-227.

Hulshoff Pol HE, Kahn RS. (2008). What happens after the first episode? A review of progressive brain changes in chronically ill patients with schizophrenia. *Schizophrenia Bulletin*, 34, 354–366.

Hukkanen J, Jacob P, Peng M, Dempsey D, Benowitz NL. (2011). Effect of nicotine on cytochrome P450 1A2 activity. *British Journal of Clinical Pharmacology*, 72(5):836-8.

Itil TM, Keskiner A, Fink M. (1966). Therapeutic studies in “therapy resistant” schizophrenic patients. *Comprehensive Psychiatry*, 7(6), 488–493

Javitt DC (2004). Glutamate as a therapeutic target in psychiatric disorders. *Molecular Psychiatry*, 9, 984–997.

Kahn RS, van Rossum W, Leucht S, McGuire P, Lewis SW, Leboyer M, Arango C, et al. (2018). Amisulpride and olanzapine followed by open-label treatment with clozapine in first-episode schizophrenia and schizophreniform disorder (OPTIMISE): A three-phase switching study. *The Lancet Psychiatry* (Aug 13), https://www-lib-uwo-ca.proxy1.lib.uwo.ca/cgi-bin/ezpauthn.cgi?url=http://search.proquest.com.proxy1.lib.uwo.ca/docview/2089165460?accountid=15115 (accessed August 17, 2018)

Kanamori K, Ross BD, Kondrat RW. (2002). Glial uptake of neurotransmitter glutamate from the extracellular fluid studied in vivo by microdialysis and (13)C NMR. *Journal of Neurochemistry,* 83, 682–695.

Kane J, Honigfeld G, Singer J, Meltzer H. (1988) Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. *Archives of General Psychiatry*, 45(9), 789–796

Kantrowitz JT, Javitt DC. (2010). N-methyl-D-aspartate (NMDA) receptor dysfunction or dysregulation: The final common pathway on the road to schizophrenia? *Brain Research Bulletin*, 83, 108–121.

Kay SR, Fiszbein A, Opler LA. (1987). The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. *Schizophrenia Bulletin,* 13, 261–276.

Kegeles LS, Mao X, Stanford AD, Girgis R, Ojeil N, Xu X, Gil R, Slifstein M, Abi-Dargham, A, Lisanby SH, Shungu DC. (2012). Elevated prefrontal cortex gamma-aminobutyric acid and glutamate-glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy. *Archives of General Psychiatry*, 69(5), 449–459.

Kennedy JL, Altar CA, Taylor DL, Degtiar I, Hornberger JC. (2014). The social and economic burden of treatment-resistant schizophrenia: A systematic literature review. *International Clinical Psychopharmacology*, 29(2), 63-76

Kerns JG, Cohen JD, MacDonald AW, 3rd, Johnson MK, Stenger VA, Aizenstein H, Carter CS (2005). Decreased conflict- and error-related activity in the anterior cingulate cortex in subjects with schizophrenia. *American Journal of Psychiatry,* 162, 1833–1839.

Keshavan MS, Dick RM, Diwadkar VA, Montrose DM, Prasad KM, Stanley JA. (2009). Striatal metabolic alterations in non-psychotic adolescent offspring at risk for schizophrenia: a (1)H spectroscopy study. *Schizophrenia Research*, 115(1), 88–93.

Kinon BJ, Kane JM, Johns C, Perovich R, Ismi M, Koreen A, Weiden P. (1993). Treatment of neuroleptic-resistant schizophrenic relapse. *Psychopharmacology Bull*etin, 29(2), 309-314.

Klassen LM, Menon RS. (2004). Robust Automated Shimming Technique Using Arbitrary Mapping Acquisition Parameters (RASTAMAP). *Magnetic Resonance Medicine*, 51, 881–887.

Koga M, Serritella AV, Messmer MM, Hayashi-Takagi A, Hester LD, Snyder SH, Sawa A,

Sedlak TW. (2011). Glutathione is a Physiologic Reservoir of Neuronal Glutamate. *Biochemical and Biophysical Research Communication*, 409, 596–602.

Kohr G, Eckardt S, Luddens H, Monyer H, Seeburg PH. (1994). NMDA receptor channels: subunit-specific potentiation by reducing agents. *Neuron,* 12, 1031–1040.

Kraguljac NV, Frölich MA, Tran S, White DM, Nichols N, Barton-McArdle A, et al. (2017). Ketamine modulates hippocampal neurochemistry and functional connectivity: a combined magnetic resonance spectroscopy and restingstate fMRI study in healthy volunteers. *Molecular Psychiatry,* 22, 562–569.

Kraguljac NV, Reid MA, White DM, den Hollander J, Lahti AC. (2012). Regional decoupling of N-acetyl-aspartate and glutamate in schizophrenia. *Neuropsychopharmacology*, 37, 2635–2642.

Kubera KM, Sambataro F, Vasic N, Wolf ND, Frasch K, Hirjak D, et al. (2014). Source-based morphometry of gray matter volume in patients with schizophrenia who have persistent auditory verbal hallucinations. *Progress in Neuropsychopharmacology and Biological Psychiatry,* 50, 102–09.

Kumar J, Liddle EB, Fernandes CC, Palaniyappan L, Hall EL, Robson SE, Simmonite M, Jan F, Katshu MZ, Qureshi A, Skelton M, Christodoulou NG, Brookes MJ, Morris PG, Liddle PF. (2018). Glutathione and Glutamate in schizophrenia: A 7T MRS Study. *Molecular Psychiatry*, (in press).

Lahti AC, Weiler MA, Holcomb HH, Tamminga CA, Carpenter WT, McMahon R. (2006). Correlations between rCBF and symptoms in two independent cohorts of drug-free patients with schizophrenia. *Neuropsychopharmacology*, 31, 221–230.

Lally J, Ajnakina O, Di Forti M, Trotta A, Demjaha A, Kolliakou A, Mondelli V, Reis Marques T, Pariante C, Dazzan P, Shergil SS, Howes OD, David AS, MacCabe JH, Gaughran F, Murray RM. (2016). Two distinct patterns of treatment resistence in first-episode schizophrenia-spectrum psychoses. *Psychological Medicine,* 46, 3231-3240.

Lambert M, Conus P, Lubman DI, Wade D, Yuen H, Moritz S, et al. (2005). The impact of substance use disorders on clinical outcome in 643 patients with first-episode psychosis. *Acta Psychiatrica Scandinavica,* 112, 141–8.

Langbein K, Hesse J,Gussew E, Milleit B, Lavoie S, Amminger GP, Gaser C, Wagner G, Reichenbach JR, Hipler UC, Winter D, Smensy S. (2017). Disturbed glutathione antioxidant defense is associated with structural brain changes in neuroleptic-naïve first episode psychosis patients. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, (in press).

Laurens KR, Ngan ET, Bates AT, Kiehl KA, Liddle PF. (2003). Rostral anterior cingulate cortex dysfunction during error processing in schizophrenia. *Brain,* 126, 610–622.

Laurens KR, Kiehl KA, Ngan ET, Liddle PF. (2005). Attention orienting dysfunction during

salient novel stimulus processing in schizophrenia. *Schizophrenia Research,* 75, 159-171.

Leucht S, Wahlbeck K, Hamann J, et al. (2003). New generation antipsychotics versus low-potency conventional antipsychotics: a systematic review and meta-analysis. *Lancet* 361, 1581–1589.

Leucht S, Beitinger R, Kissling W. (2007). On the concept of remission in schizophrenia. *Psychopharmacology* 194, (4) (11), 453-461

Leslie SW, Brown LM, Trent RD, Lee YH, Morris JL, Jones TW, et al. (1992). Stimulation of N-methyl-D-aspartate receptor-mediated calcium entry into dissociated neurons by reduced and oxidized glutathione. *Molecular Pharmacology,* 41, 308–314.

Levine SZ, Rabinowitz J, Engel R, Etschel E, Leucht S. (2008). Extrapolation between measures of symptom severity and change: an examination of the PANSS and CGI. *Schizophrenia Research,* 98, 318–322.

Levy E, Pawliuk N, Joober R, Abadi S, Malla A. (2012). Medication-adherent first-episode psychosis patients also relapse: why? *Canadian Journal of Psychiatry*, 57, 78-84.

Lewis SW, Barnes TR, Davies L, Murray RM, Dunn G, Hayhurst KP, et al. (2006). Randomized controlled trial of effect of prescription of clozapine versus other second-generation antipsychotic drugs in resistant schizophrenia. *Schizophrenia Bulletin*, 32(4), 715-23.

Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO. (2005). Clinical Antipsychotic Trials of Intervention Effectiveness, Investigators (2005) Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *New England Journal of Medicine,* 353(12), 1209–1223.

Lindenmayer JP. (2000). Treatment refractory schizophrenia. *Psychiatry Quarterly*, 71, 373–384.

Lin CH, Lin HS, Kuo CC, Wang FC, Huang YH. (2018). Early Improvement in PANSS-30, PANSS-8 and PANSS-6 scores predicts ultimate response and remission during acute treatment of schizophrenia. *Acta Psychiatrica Scandinavica,* 137, 98-108.

Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S, Grace AA. (2008). Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends in Neurosciences,* 31, 234–242

Lutkenhoff ES, van Erp TG, Thomas MA, Therman S, Manninen M, Huttunen MO, et al. (2010). Proton MRS in twin pairs discordant for schizophrenia. *Molecular Psychiatry*, 15, 308–318.

Magistretti PJ, Pellerin L. (1999). Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. *Philosophical Transactions of the Royal Society London*

B: Biological Sciences, 354, 1155–1163.

Mahadik, S.P. & Mukherjee, S. (1996). Free radical pathology and antioxidant defense in schizophrenia ± a review. *Schizophrenia Research*, IW, 1-17.

Malla AK, Norman RM, Manchanda R, Ahmed MR, Scholten D, Harricharan R, et al. (2002). One year outcome in first episode psychosis: influence of DUP and other predictors. Schizophrenia Research, 54(3), 231–242.

Malla A, Norman R, Bechard-Evans L, Schmitz N, Manchanda R, Cassidy C. (2008). Factors influencing relapse during a two-year followup of first-episode psychosis in a specialized early intervention service. *Psychological Medicine*, 38, 1585-1593.

Marques TR, Arenovich T, Agid O, Sajeev G, Muthe B, Chen L, Kinon BJ, Kapur S. (2011). The different trajectories of antipsychotic response: Antipsychotics versus placebo. *Psychological Medicine,* 41, 1481-1488.

Marsman A, Mandl RCW, Klomp DWJ, Bohlken MM, Boer VO, Andreychenko A, Cahn W, Kahn RS, Luijten PR, Hulshoff Pol HE. (2014). GABA and glutamate in schizophrenia: A 7T 1H-MRS Study. *Neuroimage: Clinical,* 6, 398-407.

Marsman A, van den Heuvel MP, Klomp DW, Kahn RS, Luijten PR, Hulshoff Pol HE. (2013). Glutamate in schizophrenia: a focused review and meta-analysis of ^{1}H- MRS studies. *Schizophr Bulletin,* 39(1), 120–9.

Martinez-Cengotitabengoa M, Mico JA, Arango C, Castro-Fornieles J, Graell M, Paya B, Leza JC, Zorrilla I, Parellada M, Lopez MP, Baeza I, Moreno C, Rapado-Castro M, Gonzalez-Pinto A (2014). Basal low antioxidant capacity correlates with cognitive deficits in early onset psychosis. A 2-year follow-up study. *Schizophrenia Research*, 156 ,23–29.

Marwaha S, Johnson S. (2004). Schizophrenia and employment - a review. *Society of Psychiatry and Psychiatric Epidemiology,* 39(5), 337–349.

Mason GF, Gruetter R, Rothman DL, Behar KL, Shulman RG, Novotny NG. (1995) Simultaneous determination of the rates of the TCA cycle, glucose utilization, alpha- ketoglutarate/glutamate exchange, and glutamine synthesis in human brain by NMR. *Journal of Cerebral Blood Flow and Metabolism,* 15, 12–25.

Matsuzawa D, Obata T, Shirayama Y, Nonaka H, Kanazawa Y, Yoshitome E et al. (2008). Negative correlation between brain glutathione level and negative symptoms in schizophrenia: a 3T 1H-MRS study. *PLoS One,* 3(4), e1944.

Mathews GC, Diamond JS. (2003). Neuronal glutamate uptake contributes to GABA synthesis and inhibitory synaptic strength. *Journal of Neuroscience*, 23, 2040–2048.

Mazure CM, Nelson JC, Jatlow PI, Bowers MB. (1991), Plasma free homovanillic acid (HVA) as a predictor of clinical response in acute psychosis. *Biological Psychiatry,* 30(5), 475–482

McEvoy JP, Lieberman JA, Stroup TS, Davis SM, Meltzer HY, Rosenheck RA, Swartz MS, et al. (2006). Effectiveness of clozapine versus olanzapine, quetiapine, and risperidone in patients with chronic schizophrenia who did not respond to prior atypical antipsychotic treatment. *The American Journal of Psychiatry*, 163, (4) (04), 600-610.

McGlashan TH. (2006). Schizophrenia in translation: Is active psychosis neurotoxic? *Schizophrenia Bulletin*, 32(4), 609-613.

McGrath J, Saha S, Chant D, Welham J. (2008). Schizophrenia: a concise overview of incidence, prevalence, and mortality. *Epidemiology Review*, 30, 67–76.

Meister, A. & Anderson, M.E. (1983). Glutathione. *Annual Review of Biochemistry*, SP, 711-760.

Meltzer HY. (1997). Treatment resistant schizophrenia- the role of clozapine. *Current Medical Research and Opinion*, 14(1),1-20.

Mekle R, Mlynárik V, Gambarota G, Hergt M, Krueger G, Gruetter R. (2009). MR spectroscopy of the human brain with enhanced signal intensity at ultrashort echo times on a clinical platform at 3 T and 7 T. *Magnetic Resonance Medicine*, 61(6), 1279–1285.

Mesholam-Gately RI, Giuliano AJ, Goff KP, Farone SV, Seidman LJ. (2009). Neurocognition in first episode schizophrenia: a meta-analytic review. *Neuropsychology*, 23(3), 315–336.

Mico JA, Rojas-Corrales MO, Gibert-Rahola J, Parellada M, Moreno D, Fraguas D, et al. (2011). Reduced antioxidant defense in early onset first-episode psychosis: a case–control study. *BMC Psychiatry*, 11, 26.

Miller BJ, Bodenheimer CB, Crittenden K. (2011). Second-generation antipsychotic discontinuation in first episode psychosis: an updated review. *Clinical Psychopharmacology and Neurosci*ence, 9, 45-53.

Minzenberg MJ, Laird AR, Thelen S, Carter CS, Glahn DC. (2009). Meta-analysis of 41 functional neuroimaging studies of executive function in schizophrenia. *Archives of General Psychiatry*, 66, 811– 822.

Mitelman SA, Shihabuddin L, Brickman AM, Hazlett EA, Buchsbaum MS. (2005). Volume of the cingulate and outcome in schizophrenia. *Schizophrenia Research*, 72, 91–108.

Modestin J, Huber A, Satirli E, Malti D, Hell D. (2003). Long-term course of schizophrenia reconsidered: Bleuler's study reconsidered. *American Journal of Psychiatry*, 160, 2202–2208.

Moghaddam B, Adams B, Verma A, Daly D. (1997). Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to

dopaminergic and cognitive disruptions associated with the prefrontal cortex. *Journal of Neuroscience*, 17, 2921–2927.

Moghaddam B, Krystal JH. (2012). Capturing the angel in "angel dust": twenty years of translational neuroscience studies of NMDA receptor antagonists in animals and humans. *Schizophrenia Bulletin*, 38(5), 942-9.

Mouchlianitis E, Bloomfield MAP, Law V, Beck K, Selvaraj S, Rasquinha N, Waldman A, et al. (2016). Treatment-resistant schizophrenia patients show elevated anterior cingulate cortex glutamate compared to treatment-responsive. *Schizophrenia Bulletin*, 42, (3)(05), 744-752

Molina V, Reig S, Sanz J, Paloma T, Benito C, Sarramea, et al. (2008). Differential clinical, structural and P300 parameters in schizophrenia patients resistant to conventional neuroleptics. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 32, 257–66.

Monin A, Baumann PS, Griffa A, Xin L, Mekle R, Fournier M et al. (2015). Glutathione deficit impairs myelin maturation: relevance for white matter integrity in schizophrenia patients. *Molecular Psychiatry*, 20(7), 827–38.

Murray CJ, Vos T, Lozano R, Naghavi N, Flaxman AD, Michaud C, et al. (2012). Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*, 380, 2197–2223.

Mustafa S, Joober R, Lepage M, Iyer S, Shah J, Malla A. (2018). Predictors of 'all cause discontinuation' of initial oral medication in first episode psychosis. *Schizophrenia Research*. (In press).

Near J, Edden R, Evans CJ, Paquin R, Harris A, Jezzard P. (2015). Frequency and phase drift correction of magnetic resonance spectroscopy data by spectral registration in the time domain. *Magnetic Resonance in Medicine*, 73(1), 44-50.

NICE guidelines [CG178]. (2014), Psychosis and schizophrenia in adults: prevention and management. London: National Institute For Clinical Excellence. http://www.nice.org.uk/guidance/cg178 (accessed Jan 7, 2016).

Ohrmann P. Siegmund A, Suslow T, Spitzberg K, Kersting A, Arolt V, Heindel W, Pfleiderer B. (2005). Evidence for glutamatergic neuronal dysfunction in the prefrontal cortex in chronic but not in first-episode patients with schizophrenia: a proton magnetic resonance spectroscopy study. *Schizophrenia Research*, 73(2–3), 153–157.

Ongur D, Prescot AP, Jensen JE, Cohen BM, Renshaw PF. (2009). Creatine abnormalities in schizophrenia and bipolar disorder. *Psychiatry Research Neuroimaging*, 172, 44–48.

Ongur D, Prescot AP, McCarthy J, Cohen BM, Renshaw PF. (2010). Elevated gamma-aminobutyric acid levels in chronic schizophrenia. *Biological Psychiatry*, 68, 667–670.

Palaniyappan L, Liddle PF. (2012). Does the salience network play a cardinal role in psychosis? An emerging hypothesis of insular dysfunction. *Journal of Psychiatry and Neuroscience,* 37(1), 17–27.

Pavlovic D, Tamburic V, Stojanovic I. (2002). Oxidative stress as markers of positive symptoms in schizophrenia. *Physics in Medicine and Biology*, 9, 157–161.

Pickar D, Labarca R, Linnoila M, Roy A, Hommer D, Everett D et al. (1984). Neuroleptic-induced decrease in plasma homovanillic acid and antipsychotic activity in schizophrenic patients. *Science*, 225(4665), 954–957

Plitman E, Patel R, Chung JK, Pipitone J, Chavez S, Reyes-Madrigal F, Gomez-Cruz G, Leon-Ortiz P, Chakravarty MM, de la Fuente-Sandoval, Graff-Guerro A. (2016). Glutamatergic Metabolites, Volume, and Cortical Thickness in Antipsychotic-Naïve Patients with First Episode Psychosis: Implications for Excitotoxicity. *Neuropsychopharmacology*, 41(10), 2606-2613.

Plitman E, Nakajima S, la Fuente de PG, Chakravarty MM, Kobylianskii J, Chung JK, et al. (2014). Glutamate-mediated excitotoxicity in schizophrenia: A review. *European Neuropsychopharmacology,* 24, (10)(10), 1591-1605

Poels EMP, Kegeles LS, Kantrowitz JT, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis, RR. (2014). Glutamatergic abnormalities in schizophrenia: A review of proton MRS findings. *Schizophrenia Research,* 152, (2-3)(02), 325-332

Pradhan S, Bonekamp S, Gillen JS, Rowland LM, Wijtenburg SA, Edden RAE, Barker PB, (2015). Comparison of single voxel brain MRS AT 3 T and 7 T using 32- channel head coils. *Magnetic Resonance Imaging,* 33(8), 1013–1018.

Premkumar P, Parbhakar VA, Fannon D, Lythgoe D, Williams SC, Kuipers E et al. (2010). N-acetyl aspartate concentration in the anterior cingulate cortex in patients with schizophrenia: a study of clinical and neuropsychological correlates and preliminary exploration of cognitive behaviour therapy effects. *Psychiatry Research: Neuroimaging*, 182, 251–260.

Prescot AP, Locatelli AE, Renshaw PF, Yurgelun-Todd DA. (2011.) Neurochemical alterations in adolescent chronic marijuana smokers: a proton MRS study. *Neuroimage*, 57, 69–75.

Prescot AP, Renshaw PF, Yurgelun-Todd DA. (2013). Amino butyric acid and glutamate abnormalities in adolescent chronic marijuana smokers. *Drug and Alcohol Dependence*, 129, 232–239.

Purdon SE, Valiakalayil A, Hanstock CC, Seres P, Tibbo P. (2008). Elevated 3 T proton MRS glutamate levels associated with poor Continuous Performance Test (CPT-0X) scores and genetic risk for schizophrenia. *Schizophrenia Research*, 99 (1–3), 218–224.

Quarantelli M, Palladino O, Prinster A, Schiavone V, Carotenuto B, Brunetti A, et al. (2014). Patients with poor response to antipsychotics have a more severe pattern of frontal atrophy: a

voxel-based morphometry study of treatment resistance in schizophrenia. *Biomed Research International,* 325052.

Quintana J, Wong T, Ortiz-Portillo E, Marder SR, Mazziotta JC. (2004). Anterior cingulate dysfunction during choice anticipation in schizophrenia. *Psychiatry Research,* 132, 117–130.

Raffa M, Atig F, Mhalla A, Kerkeni A, Mechri A. (2011). Decreased glutathione levels and impaired antioxidant enzyme activities in drug-naive first-episode schizophrenic patients. *BMC Psychiatry,* 11, 124.

Rapado-Castro M, Berk M, Venugopal K, Bush AI, Dodd S, Dean OM. (2015). Towards stage specific treatments: Effects of duration of illness on therapeutic response to adjuvant treatment with N-acetyl cysteine in schizophrenia. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 57, 69-75.

Rapado-Castro M, Dod S, Bush AI, Skvarc DR, On ZX, Berk M, Dean OM. (2017). Cognitive effects of adjunctive N-acetyl cysteine in psychosis. *Psychological Medicine*, 47(5), 866-876.

Reddy RD, Yao JK. (1996). Free radical pathology in schizophrenia: a review. *Prostaglandins Leukotrienes & Essential Fatty Acids*, SS, 33-43.

Reid MA, Stoeckel LE, White DM, Avsar KB, Bolding MS, Akella NS, et al. (2010). Assessments of function and biochemistry of the anterior cingulate cortex in schizophrenia. *Biological Psychiatry,* 68, 625–633.

Reid MA, Salibi N, White DM, Gawne TJ, Denney TS, Lahti AC. (2018). 7T Proton Magnetic Spectroscopy of the Anterior Cingulate Cortex in First Episode Schizophrenia. *Schizophrenia Bulletin*, (in press).

Remington G, Addington D, Honer W, Ismail Z, Raedler T, Teehan M. (2017). Guidelines for the Pharmacotherapy of Schizophrenia in Adults. *Canadian Journal of Psychiatry*, 62(9), 604-616.

Remington G, Agid O, Foussias G, Hahn M, Rao N, Sinyor M. (2013). Clozapine's role in the treatment of first episode schizophrenia. *The American Journal of Psychiatry*, 170, 146-151.

Roberts RC, Barksdale KA, Roche JK, Lahti AC. (2015). Decreased synaptic and mitochondrial density in the postmortem anterior cingulate cortex in schizophrenia. *Schizophrenia Research,* 168(1–2), 543–553.

Rossler AR, Rossler W. (1998). The course of schizophrenic psychoses: what do we really know? A selective review from the epidemiological perspective. *European Archives of Psychiatry and Clinical Neuroscience,* 248, 189–202.

Rothman DL, Behar KL, Hyder F, Shulman RG. (2003). In vivo NMR studies of the glutamate neurotransmitter flux and neuroenergetics: Implications for brain function. *Annual Review of Physiology,* 65, 401–427.

Rowland LM, Kontson K, West J, Edden RA, Zhu H, Wijtenburg SA, et al. (2013). In vivo measurements of glutamate, GABA, and NAAG in schizophrenia. *Schizophrenia Bulletin,* 39, 1096–1104.

Salgado-Pineda P, Junque C, Vendrell P, Baeza I, Bargallo N, Falcon C, Bernardo M. (2004). Decreased cerebral activation during CPT performance: Structural and functional deficits in schizophrenic patients. *Neuroimage,* 21, 840 – 847.

Samra MT, Leucht C, Leeflang MM, Anghelescu IG, Chung YC, Crespo-Faccoro et al. (2015). Early improvement as a predictor of later response to antipsychotics in schizophrenia: A diagnostic test review. *American Journal of Psychiatry*, 172, 617-629.

Schennach R, Riedel M, Musil R, Moller HG**.** (2012). Treatment response in first-episode schizophrenia. *Clinical Psychopharmacology and Neuroscience* 10, 78-87.

Schennach-Wolff R, Seemuller FH, Mayr A, Maier W, et al. (2010). An early improvement threshold to predict response and remission in first-episode schizophrenia. *The Bristish Journal of Psychiatry*, 196(6), 460-466.

Schultze-Lutter F. (2009). Subjective symptoms of schizophrenia in research and the clinic: the basic symptom concept. *Schizophrenia Bulletin*, 35, 5–8.

Sedlak TW, Paul BD, Parker GM, Hester LD, Snowman AM, Taniguchi Y, *et al.* The glutathione cycle shapes synaptic glutamate activity. *PNAS* 2019; (in press).

Seeman P, Chau-Wong M, Tedesco J, Wong K. (1975). Brain receptors for antipsychotic drugs and dopamine: direct binding assays. *Proceedings of the National Academy of Sciences of the USA*, 72: 4376–4380.

Siskind D, Siskind V, Kisely S. (2017). Clozapine response among people with treatment-resistant schizophrenia: data from a systematic review and meta-analysis. *Canadian Journal of Psychiatry,* 62(11), 772-777.

Siskind D, McCartney L, Goldschlager R, Kisely S. (2016). Clozapine v. first-and second-generation antipsychotics in treatment-refractory schizophrenia: systematic review and meta-analysis. *British Journal of Psychiatry*, 209(5), 385-392.

Smith TE, Hull JW, Huppert JD, Silverstein SM. (2002). Recovery from psychosis in schizophrenia and schizoaffective disorder: symptoms and neurocognitive rate-limiters for the development of social behavior skills. *Schizophrenia Research*, 55(3), 229–237.

Snitz BE, MacDonald A, 3rd, Cohen JD, Cho RY, Becker T, Carter CS. (2005). Lateral and medial hypofrontality in first-episode schizophrenia: Functional activity in a medication-naive state and effects of short- term atypical antipsychotic treatment. *American Journal of Psychiatry* 162, 2322–2329.

Stanley JA, Drost DJ, Williamson PC, Thompson RT. (1995). The use of a priori knowledge to quantify short echo in vivo 1H MR spectra. *Magnetic Resonance Medicine*, 34: 17–24.

Stentebjerg-Olesen M, Jeppesen P, Pagsberg AK, Fink-Jensen A, Kapoor S, Chekuri R, et al. (2013). Early non response determined by the clinical global impressions scale predicts poorer outcomes in youth with schizophrenia spectrum disorders naturalistically treated with second-generation antipsychotics. *Journal of Child and Adolescent Psychopharmacology*, 23, 665-675.

Stern ER, Welsh RC, Fitzgerald KD, Taylor SF. (2009). Topographicanal-ysis of individual activation patterns in medial frontal cortex in schizophrenia. *Human Brain Mapping,* 30, 2146 – 2156.

Steullet P, Neijt HC, Cuénod M, Do KQ. (2006). Synaptic plasticity impairment and hypofunction of NMDA receptors induced by glutathione deficit: relevance to schizophrenia. *Neuroscience,* 137(3), 807-19.

Stone JM, Raffin M, Morrison P, McGuire PK. (2010). Review: the biological basis of antipsychotic response in schizophrenia. *Journal of Psychopharmacology*, 24, 953–964.

Stone JM, Day F, Tsagaraki H, Valli I, McLean MA, Lythgoe DJ, et al. (2009). Glutamate dysfunction in people with prodromal symptoms of psychosis: relationship to gray matter volume. *Biological Psychiatry*, 66, 533–539.

Stone JM, Bramon E, Pauls A, Sumich A, McGuire PK. (2010). Thalamic neurochemical abnormalities in individuals with prodromal symptoms of schizophrenia–relationship to auditory event-related potentials. *Psychiatry Research*, 183, 174–176.

Strakowski SM, Tohen M, Stoll AL, Faedda GL, Mayer PV, Kolbrener ML, Goodwin DC. (1993). Comorbidity in psychosis at first hospitalization. *American Journal of Psychiatry*, 150, 752–757.

Strakowski SM, Tohen M, Flaum M, Amador X. (1994). Substance abuse in psychotic disorders: associations with affective syndromes. DSM-IV Field Trial Work Group. *Schizophrenia Research*, 14, 73–81.

Sukuki T, Remington G, Mulsant BH, Rajji TK, Uchida H, Graff-Gruererro A, Mamo DC. (2011). Treatment resistant schizophrenia and response to antipsychotics: A review. *Schizophrenia Research*, 133, 54-62.

Sung YH, Carey PD, Stein DJ, Ferrett HL, Spottiswoode BS, Renshaw PF, Yurgelun-Todd DA. (2013). Decreased frontal N-acetylaspartate levels in adolescents concurrently using both methamphetamine and marijuana. *Behavior and Brain Research*, 246, 154–161.

Szulc A, Konarzewska B, Galinska-Skok B, et al. (2013). Proton magnetic resonance spectroscopy measures related to short-term symptomatic outcome in chronic schizophrenia.

Neuroscience Letter, 547, 37–41.

Szulc A, Galinska B, Tarasow E, Lazarczyk J, Waszkiewicz N, Tarasow E, et al. (2011). Proton magnetic resonance spectroscopy study of brain metabolite changes after antipsychotic treatment. *Pharmacopsychiatry*, 44, 148–157.

Szulc A, Galinska B, Tarasow E, Dzienis W, Kubas B, Konarzewska B, Walecki J, Yoo SY, Yeon S, Choi CH, Kang DH, Lee JM, Shin NY, Jung WH, Choi JS, Jang DP, Kwon JS. (2009). Proton magnetic resonance spectroscopy in subjects with high genetic risk of schizophrenia: investigation of anterior cingulate, dorsolateral prefrontal cortex and thalamus. *Schizophrenia Research*, 111(1–3), 86–93.

Tandon N, Bolo NR, Sanghavi K, Mathew IT, Francis AN, Stanley JA, et al. (2013). Brain metabolite alterations in young adults at familial high risk for schizophrenia using proton magnetic resonance spectroscopy. *Schizophrenia Research*, 148, 59–66.

Tandon R, Nasrallah HA, Keshavan MS. (2009). Schizophrenia, "just the facts" 4. Clinical features and conceptualization. Schizophrenia Research, 110(1–3), 1–23.

Taylor R, Neufeld R, Schaefer B, Densmore M, Rajakumar N, Osuch EA, Williamson PC, Theberge J. (2015). Functional magnetic resonance spectroscopy of glutmate in schizophrenia and major depressive disorder: anterior cingulate activity during a color-word stroop task. *NPJ Schizophrenia*, 15028.

Tayoshi S, Sumitani S, Taniguchi K, Shibuya-Tayoshi S, Numata S, Iga J, Nakataki M, Ueno S, Harada M, Ohmori T. (2009). Metabolite changes and gender differences in schizophrenia using 3-Tesla proton magnetic resonance spectroscopy (^{1}H-MRS). *Schizophrenis Research*, 108, 69–77.

Takeuchi H, Siu C, Remington G, Fervaha G, Zipursky RB, Foussias G, Agid A**.** (2018). Does relapse contribute to treatment resistance? Antipsychotic response in first- vs. second-episode schizophrenia. Neuropsychopharmacology doi: 10.1038/s41386-018-0278-3

Terpstra M, Vaughan TJ, Ugurbil K, Lim KO, Schulz SC, Gruetter R. (2005). Validation of glutathione quantitation from STEAM spectra against edited 1H NMR spectroscopy at 4T: application to schizophrenia. *MAGMA*, 18(5), 276– 82.

Thakkar KN, Rösler L, Wijnen JP, Boer VO, Klomp DWJ, Cahn W, Kahn RS, and Neggers SFW. (2017). 7T Proton Magnetic Resonance Spectroscopy of Gamma-Aminobutyric Acid, Glutamate, and Glutamine Reveals Altered Concentrations in Patients With Schizophrenia and Healthy Siblings. *Biological Psychiatry*, 81, 525-535.

Thara R. (2004). Twenty-year course of schophrenia: the Madras Longitudinal Study. *Canadian Journal of Psychiatry*, 49, 564–569.

Théberge J, Al-Semaan Y, Williamson PC, Menon RS, Neufeld RW, Rajakumar N, et al. (2003). Glutamate and glutamine in the anterior cingulate and thalamus of medicated patients with

chronic schizophrenia and healthy comparison subjects measured with 4.0-T proton MRS. *American Journal of Psychiatry*, 160, 2231–2233.

Theberge J, Bartha R, Drost DJ, Menon RS, Malla A, Takhar J, Neufeld RW, Rogers J, Pavloski W, Schaefer B, Densmore M, Al-Serman Y, Williamson P. (2002). Glutamate and glutamine measured with 4.0T proton MRS in never-treated patients with schizophrenia and healthy volunteers. *American Journal of Psychiatry*, 159, 144-146.

Théberge J, Williamson KE, Aoyama N, et al. (2007). Longitudinal grey- matter and glutamatergic losses in first-episode schizophrenia. *British Journal of Psychiatry,* 191, 325–334.

Tibbo P, Hanstock C, Valiakalayil A, Allen P. (2004). 3-T proton MRS investigation of glutamate and glutamine in adolescents at high genetic risk for schizophrenia. *American Journal of Psychiatry*, 161, 1116–1118.

Tiihonen J, Haukka J, Taylor M, Haddad PM, Patel MX, Korhonen P. (2011). A nationwide cohort study of oral and depot antipsychotics after first hospitalization for schizophrenia. *American Journal of Psychiatry*, 168(6), 603-9.

Tkáč I, Öz G, Adriany G, Uğurbil K, Gruetter R. (2009). In vivo 1H NMR spectroscopy of the human brain at high magnetic fields: metabolite quantification at 4 T vs. 7 T. *Magnetic Resonance Medicine*, 62(4), 868–879.

Tkáć I, Gruetter R. (2005). Methodology of 1 H NMR spectroscopy of the human brain at very high magnetic fields. *Applied Magnetic Resonance*, 29, 139–157.

Tkáč I, Öz G, Adriany G, Uğurbil K, Gruetter R. (2009). In vivo 1H NMR spectroscopy of the human brain at high magnetic fields: metabolite quantification at 4 T vs. 7 T. *Magnetic Resonance Medicine*, 62(4), 868–879.

Valli I, Stone J, Mechelli A, Bhattacharyya S, Raffin M, Allen P, Fusar-Poli P, Lythgoe D, O'Gorman R, Seal M, McGuire P. (2011). Altered medial temporal activation related to local glutamate levels in subjects with prodromal signs of psychosis. *Biological Psychiatry*, 69, 97–99.

Van den Boogaart A, Ala-Korpela M, Jokisaari J, Griffiths JR. (1994). Time and frequency domain analysis of NMR data compared: an application to 1D 1H spectra of lipoproteins. *Magnetic Resonance Medicine*, 31, 347–358.

Volterra A, Trotti D, Tromba C, Floridi S, Racagni G. (1994). Glutamate uptake inhibition by oxygen free radicals in rat cortical astrocytes. *Journal of Neuroscience,* 14, 2924–32.

Wang AM, Pradhan S, Coughlin JM, Trivedi A, DubBois SL, Crawford JL, et al. Assessing brain metabolism with 7-T proton magnetic resonance spectroscopy in patients with first-episode psychosis. JAMA Psychiatry 2019; (in press).

Weinberger DR, Torrey EF, Neophytides AN, Wyatt RJ. (1979). Lateral cerebral ventricular enlargement in chronic schizophrenia. *Archives of General Psychiatry*, 36(7), 735–739

Wijtenburg SA, Wright SN, Korenic SA, Gaston FE, Ndubuizu N, Chiappelli J, McMahon RP,

Wood SJ, Yücel M, Pantelis C, Berk M. (2009). Neurobiology of schizophrenia spectrum disorders: the role of oxidative stress. *Annual Academy of Medicine Singapore,* 38(5), 396–6.

Wimberley T, Gasse C, Meier SM, Agerbo E, MacCabe JH, Horsdal HT. (2017). Polygenic risk scores for schizophrenia and treatment-resistant schizophrenia. *Schizophrenia Bulletin* **43**(5), 1064-1069.

Wood SJ, Yücel M, Wellard RM, Harrison BJ, Clarke K, Fornito A, et al. (2007). Evidence for neuronal dysfunction in the anterior cingulate of patients with schizophrenia: a proton magnetic resonance spectroscopy study at 3 T. *Schizophrenia Research*, 94, 328–331.

Wijtenburg SA, Yang S, Fischer BA, Rowland LM. (2015). In vivo assessment of neurotransmitters and modulators with magnetic resonance spectroscopy: Application to schizophrenia. *Neuroscience and Biobehavioral Reviews,* 51(04), 276-295

Wood SJ, Berger GE, Wellard RM, Proffitt TM, McConchie M, Berk M et al. (2009). Medial temporal lobe glutathione concentration in first episode psychosis: a 1H- MRS investigation. *Neurobiological Disorders,* 33(3), 354–7.

Wood SJ, Yucel M, Wellard RM, Harrison BJ, Clarke K, Fornito A et al. (2007). Evidence for neuronal dysfunction in the anterior cingulate of patients with schizophrenia: a proton magnetic resonance spectroscopy study at 3T. *Schizophrenia Research*, 94, 328–331.

Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. (2004). Glutathione metabolism and its implications for health. *Journal of Nutrition*, 134, 489–492.

Wyatt RJ. (1991). Neuroleptics and the natural course of schizophrenia. *Schizophrenia Bulletin*, 17(2), 325.

Wyatt RJ. (1995). Early intervention for schizophrenia: can the course of the illness be altered? *Biological Psychiatry,* 38(1), 1–3

Wyatt RJ. (1991). Early intervention with neuroleptics may decrease the longterm morbidity of schizophrenia. *Schizophrenia Research*, 5(3), 201-202.

Xin L, Mekle R, Fournier M, Baumann PS, Ferrari C, Alameda L, Jenni R, et al. (2016). Genetic polymorphism associated prefrontal glutathione and its coupling with brain glutamate and peripheral redox status in early psychosis. *Schizophrenia Bulletin,* 42(5)(09), 1185-1196

Yoshimura R, Ueda N, Shinkai K, Nakamura J. (2003). Plasma levels of homovanillic acid and the response to risperidone in first episode untreated acute schizophrenia. *International Clinical Psychopharmacology*, 18(2), 107–111

Zeidler J, Slawik L, Fleischmann J, Greiner W. (2012). The costs of schizophrenia and predictors of hospitalisation from the statutory health insurance perspective. *Health Economics Review,* 2(1), 9.

Zheng W, Zhang QE, Cai DB, Yang XH, Qiu Y, Ungvari GS, Ng CH, Berk M, Ning YP, Xiang YT. (2018). N-acetylcysteine for major mental disorders: a systematic review and meta-analysis of randomized controlled trials. *Acta Psychiatrica Scandinavica*, 137, 391–400.

Ziedonis DM, Fisher W. (1994). Assessment and treatment of comorbid substance abuse in individuals with schizophrenia. *Psychiatric Annals*, 24, 477–483.

Zipursky RB, Reilly TJ, Murray RM. (2012). The myth of schizophrenia as a progressive brain disease. Schizophrenia Bulletin, 39(6), 1363-1372.

Zugman A, Gadelha A, Assunção I, Sato J, Oto VK, Rocha DL, et al. (2013). Reduced dorso-lateral prefrontal cortex in treatment resistant schizophrenia. *Schizophrenia Research,* 148, 81–86.

Curriculum Vitae

Name: Kara Dempster

Post-secondary Education and Degrees:

Western University
London, Ontario, Canada
2017-2019 MSc, Neurosciences, Clinical Investigator Program

Western University
London, Ontario, Canada
2013-2017, Psychiatry Residency, FRCPC certified

Western University
London, Ontario, Canada
2009-2013, Doctor of Medicine

Acadia University
Nova Scotia, Canada
2004-2008 BSc (honours) in Psychology

Honours and Awards:

CIHR Canada Graduate Student Scholarship (CGSM)
2018-2019

Western University Department of Psychiatry Seed Funding
2017-2018

American Psychiatric Association Junior Research Colloquium
May, 2017

Paul Patterson Resident Award, Western University
May, 2016

Junior Resident of the Year Award, Western University
May, 2016

Canadian Psychiatric Association Resident Research Colloquium
September, 2014

PSI Foundation Resident Research Award
2014

Ontario Medical Student Bursary
2011-2012

SROP Grant Recipient
2011-2012

Meds Class of 1958 Award
2010-2011

Ellen Tiedge Memorial Award
2009-2010

Publications:

1. Dempster K, Norman R, Theberge J, Densmore M, Schaefer B & Williamson P. 2015. Glutamatergic Metabolite Correlations with Neuropsychological Tests in First-Episode Schizophrenia. Journal of Psychiatry Research: Neuroimaging 233(2), 180-185.
2. Dempster K, Norman R, Theberge J, Densmore M, Schaefer B & Williamson P. 2017. Cognitive Performance is Associated with Gray Matter Decline in First-Episode Psychosis. Journal of Psychiatry Research: Neuroimaging 264, 46-51.
3. Palaniyappan P, Dempster K, Luo Q. 2017. A Brain Network-Based Grading of Psychosis: Could Resting Functional Magnetic Resonance Imaging Become a Clinical Tool? JAMA Psychiatry 74(6), 613-614.
4. Palaniyappan L, Das T, Dempster K. 2017. The neurobiology of transition to psychosis: Are we there yet? Journal of Psychiatry and Neuroscience 42(5), 294-299.