APPROVAL SHEET

Title of Thesis: Neural biomarkers of risk for psychosis

Name of Candidate: Caroline L. Demro
Master of Arts, 2015

Thesis and Abstract Approved:

Jason Schiffman, Ph.D.
Associate Professor
Department of Human Services Psychology

Date Approved: 11-18-2015

ABSTRACT

Title of Document:	NEURAL BIOMARKERS OF RISK FOR PSYCHOSIS.
	Caroline Lisa Demro, M. A., 2015
Directed By:	Professor Jason Schiffman, Department of Psychology

Schizophrenia is a potentially debilitating mental disorder which is usually preceded by one to two years of attenuated psychotic symptoms. The identification of individuals at psychosis-risk has relied on self-report and interview measures, which have limited specificity. Early identification could benefit from the discovery of biomarkers that may add accuracy of identification when used in conjunction with the self-report measures. Proton Magnetic Resonance Spectroscopy is an imaging technique used to quantify brain metabolites. Development of psychosis may be associated with metabolite concentration changes that reflect an alteration in glutamatergic mechanisms. Elevated glutamate levels have been observed in the striatum of individuals at psychosis-risk and individuals in their first episode of schizophrenia as compared to healthy controls. The current study explored glutamatergic metabolite concentrations in the striatal and cingulate gyri as potential biomarkers to aid in the understanding of psychosis-risk symptoms.

NEURAL BIOMARKERS OF RISK FOR PSYCHOSIS.

By

Caroline Demro.

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore County, in partial fulfillment
of the requirements for the degree of
Master of Arts
2015

Acknowledgements

I would like to thank the families who gave their time and energy as study participants to make this work possible.

I am grateful to my mentor, Jason Schiffman, for all his support and his contagious drive to make the world a better place.

I want to thank Laura Rowland for her expertise and guidance.

Thank you to my labmates and my cohort for their encouragement and companionship.

I am grateful for the support of my family and my loving husband. Mom and Dad, thank you for inspiring me to pursue my passion. To Judy and Alan, thank you for motivating me to share what I learn in a meaningful way. Phil, thank you for helping me grow.

Table of Contents

Introduction

Schizophrenia

Schizophrenia is a potentially debilitating mental disorder characterized by positive and negative psychotic symptoms. Positive psychotic symptoms such as hallucinations, delusions, and disorganized speech or behavior reflect an excess or distortion of normal functions as outlined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR). Schizophrenia can also involve negative symptoms, which reflect a loss of normal functions and present in the form of affective flattening (unresponsive facial expression and body language), alogia (poverty of speech), avolition (lack of motivation for goal-directed behavior), or anhedonia (inability to feel pleasure). About 1% of the United States population is diagnosed with schizophrenia (Narrow, Rae, Robins, & Regier, 2002; Tandon, Keshavan, & Nasrallah, 2008). Even though schizophrenia is not one of the more common mental disorders (e.g., depression prevalence of 4.5%-8.3% in the United States; Kessler & Bromet, 2013; Narrow et al., 2002), schizophrenia accounts for a disproportionately large amount of health care costs. An individual with schizophrenia is likely to experience severe distress and require a significant amount of treatment resources. In 2002 schizophrenia accounted for $62.7 billion in health care costs in the United States (Wu et al., 2005). Such health care costs include both direct costs, such as long-term care, medication, and hospital stays, as well as indirect costs, such as unemployment, caregiver burden, and reduced productivity at work (Wu et al., 2005). Additionally, in their cost analysis study, the highest prevalence rate of schizophrenia

was exhibited in the Medicaid population as compared to other health insurance populations (Wu, Shi, Birnbaum, Hudson, & Kessler, 2006), further contributing to societal health care costs. Beyond the financial costs, schizophrenia is associated with significant individual distress and functional impairment. The most pronounced functional decline is observed in the first few years after illness onset (Correll, Hauser, Auther, & Cornblatt, 2010).

The onset of schizophrenia as well as first emerging psychotic symptoms typically occur during late adolescence or early adulthood, and is usually preceded by one to two years of attenuated psychotic symptoms. This period of risk for psychosis is often undetected or misdiagnosed. The onset of psychotic symptoms can be a confusing and frightening experience and adolescents are sometimes unlikely to share their symptoms with anyone and instead are more likely to socially isolate (Møller & Husby, 2000). Adolescence and early adulthood is a critical time for achieving academic milestones and acquiring life skills such as navigating professional and social relationships. Progression of psychotic illness during this time can interfere with these developmental tasks such that young people may be delayed (in some cases, indefinitely) in acquiring important life skills (Röpcke & Eggers, 2005). Further, the impact of psychotic illness on structural and organizational brain maturation that typically occurs during adolescence appears to have important functional consequences for individuals with early-onset disorders (Paus, 2005; Vidal et al., 2006). Identification of psychosis before developmental disruptions have occurred may help youth to avoid developmental delays resulting from interruptions during this critical period.

Early Identification and Intervention

The length of time between onset of psychotic symptoms and the onset of treatment is referred to as the "duration of untreated psychosis" (DUP) and is an indicator of prognosis. Longer DUP is associated with more long-term disability. Individuals with psychosis may not access care for months or even years, as shown by an average DUP of 103 weeks in a review of first episode studies (Marshall et al., 2005). Treatment delay may result from patients hesitating to seek treatment or providers lacking awareness of the symptoms of psychosis or the benefits of early intervention (Norman, Malla, Verdi, Hassall, & Fazekas, 2004). A considerable body of research indicates better prognosis when the length of time between onset of psychotic symptoms and treatment is shorter (Marshall et al., 2005). Evidence suggests that providing treatment interventions to individuals early on, thus reducing the DUP, can result in better outcome (Melle et al., 2004). Furthermore, identifying individuals in the risk stage and providing them with services may lessen the severity of psychosis-risk symptoms and, perhaps in some cases, prevent the onset of psychosis (Wyatt & Henter, 2001). Therefore, early identification of and intervention with individuals at risk for developing psychosis is of paramount interest in the treatment of psychosis and its concomitant concerns.

Challenges of early identification

The phase of risk for psychosis, referred to as psychosis-risk hereafter, is characterized by sub-threshold psychotic symptoms. Such sub-threshold psychotic symptoms do not meet DSM-IV-TR criteria for a psychotic disorder, but yet are associated with psychological distress and need for services. Psychosis-risk

syndromes tend to be identified through semi-structured interviews, with the "gold standard" for use in North America being the Structured Interview for Psychosis-Risk Syndromes (SIPS; Miller et al., 2002).

Heterogeneous symptom presentation as well as high rates of comorbidity with other mental disorders contribute to complications related to diagnosis of psychosis-risk (Wigman et al., 2012). The identification of individuals at psychosis-risk has relied on self-report and structured interview measures. Accuracy of these measures to predict future conversion to psychosis onset ranges from 19 to 36% within two to three years of identifying individuals at psychosis-risk (Cannon, Cadenhead, Cornblatt, Woods, Addington, Walker, et al., 2008; Correll, Hauser, Auther, & Cornblatt, 2010; Fusar-Poli et al., 2013; Simon, Velthorst, Nieman, Linszen, Umbricht, & de Haan, 2011). Thus, interview-based measures identify more individuals at psychosis-risk than the actual number of people that go on to develop psychosis. The apparent low specificity of such tools has raised concerns regarding potential implications of high false positive rates. For example, an individual who is falsely labeled at psychosis-risk based on interview measures may encounter stigma, stress, and misguided treatment as a result.

The accuracy of self-report assessments is limited by methodological issues that influence responding, such as pre-determined response options and social desirability (Schwarz, 1999). Biologically based markers might provide an objective means of overcoming error inherent in self-report assessment methods.

Contributions of biomarkers

Self-report questionnaires and interviews seem to be good first-line identification tools. Early identification, however, could benefit from the discovery of biomarkers that may add accuracy of identification when used in conjunction with the self-report and interview measures. Adding specific biomarkers to the clinical approach could enhance the ability to predict outcomes by reducing bias and honing in on the underlying pathological processes, all of which could provide important steps for early identification and intervention efforts (Keshavan, Berger, Zipursky, Wood, & Pantelis, 2005). One research avenue to isolate biomarkers in an effort to more accurately identify individuals at risk of developing psychosis is technology making use of Magnetic Resonance Imaging (MRI). MRI is used to aid in identifying neurodegenerative disorders such as multiple sclerosis in which gray matter damage can be seen early on in the disease progression (Geurts et al., 2004; Klöppel et al., 2012). The biomarker mechanism discussed in this proposal involves brain chemistry as measured by magnetic resonance.

Proton Magnetic Resonance Spectroscopy

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) is a technique used to measure levels of chemicals in tissues including the brain (Duarte, Lei, Mlynarik, & Gruetter, 2012). ^{1}H-MRS is used to detect metabolite concentrations in specific brain regions, which are identified a priori. ^{1}H-MRS is one of the only in vivo methods to assess the living biochemistry in localized brain regions (Stanley, 2002). Different chemical compounds absorb and emit radio energy at different frequencies. These frequencies are characteristic per chemical and can be used to identify specific

metabolites. ^{1}H-MRS quantifies relative concentrations of: glutamate, the principle excitatory neurotransmitter in the brain; N-acetyl-aspartate (NAA), a marker of neuronal integrity; choline, a measure of cell membrane integrity; and creatine, which supplies energy to cells, among other chemicals. NAA has been implicated in psychopathology research as it generally decreases with any disease that adversely affects neuronal integrity (Moffett, Ross, Arun, Madhavarao, & Namboodiri, 2007).

The role of glutamate

The efficacy of communication across neuronal networks depends critically on the state of the various neurotransmitter systems (Paus, Keshavan, & Giedd, 2008). Neurotransmitters are chemical agents that are created within a presynaptic neuron, released into the synaptic cleft, and bound to receptors on the postsynaptic neuron to facilitate communication by activating that neuron (Lezak, Howieson, & Loring, 2004).The most prominent excitatory neurotransmitter is glutamate, which is an amino acid that can excite nearly all neurons (McDonald & Johnston, 1990). The number of neurons and the number of their connections are influenced by the activity of the glutamate system and its receptors (Konradi & Heckers, 2003). One such receptor is the N-methyl-D-aspartate receptor (NMDAR), which is involved in synaptic plasticity and memory function (Bear, Connors, & Paradiso, 2001). Hypofunction of the NMDAR has been linked to psychosis (Olney & Farber, 1995). NMDAR antagonists such as ketamine have been shown to produce psychotomimetic effects in both healthy individuals and individuals with psychosis (Javitt, 2012; Krystal, D'Souza, Mathalon, Perry, Belger, & Hoffman, 2003; Olney, Newcomer, & Farber, 1999). Novel agents that restore NMDAR function may provide a new avenue

of treatment for psychosis, though efforts thus far have not yet been successful (Tuominen, Tiihonen, & Wahlbeck, 2005).

Glutamate transmission is the primary method of information transfer throughout the brain. Glutamate also contributes to deleterious effects such as excitotoxicity, which is a mechanism that involves the over-activation of NMDARs, permitting calcium ions to enter cells and leading to intracellular cascades that ultimately kill the cells (Konradi & Heckers, 2003). This type of cell death can be linked to neurodegenerative disorders such as multiple sclerosis, Parkinson's disease, or Huntington's disease. Additionally, a hypoactive glutamate system may have a negative impact on neuroplasticity (Konradi & Heckers, 2003). Thus, a dysregulated glutamate system has been linked to a variety of deleterious effects, suggesting that glutamate may affect downstream systems and mechanisms.

Positive symptoms

Theories of the development of psychosis have focused on dopamine involvement in the onset of positive psychotic symptoms. Dopamine (DA) has been linked to the exacerbation of positive symptoms whereby excessive dopamine activity in brain regions with high concentrations of dopamine receptors, such as the striatum, has been found in individuals with schizophrenia (Laruelle & Abi-Dargham, 1999; Laruelle, Abi-Dargham, Gil, Kegeles, & Innis, 1999). Moreover, psychopharmacological research has demonstrated the effectiveness of antipsychotic medication on reducing excessive DA that is evident in schizophrenia (Laruelle, Frankle, Narendran, Kegeles, & Abi-Dargham, 2005). The dopamine hypothesis of schizophrenia indicates that dopamine dysregulation is involved in development of

psychosis (Howes & Kapur, 2009). The dopamine hypothesis has been elaborated upon by the NMDAR hypofunction hypothesis, which posits that upstream neuronal pathway disruption may contribute to the dopaminergic dysregulation and eventual development of psychosis (Schwartz, Sachdeva, & Stahl, 2012). This suggests the involvement of both dopamine and glutamate neurocircuitry in the development of positive symptoms. There is evidence that increased glutamine, the major metabolite of glutamate involved in neurotransmission and a glutamate precursor, occurs early in the course of schizophrenia (Marsman et al., 2011; Theberge et al., 2007). A combination of glutamate and glutamine signals has been shown to be lower in patients with chronic schizophrenia (Rowland et al., 2012).

Negative symptoms

Even though one in three people with schizophrenia experiences significant negative symptoms, which have been linked to poor coping and prognosis, there is a paucity of research on treatment for negative symptoms in schizophrenia spectrum disorders (Makinen, Miettunen, Isohanni, & Koponen, 2008). Research on the development of negative symptoms of schizophrenia is also sparse and somewhat inconclusive. Volume reduction in the anterior putamen of unmedicated individuals with schizophrenia, has been shown to correlate with affective flattening, a core negative symptom of schizophrenia (Ballmaier et al., 2008). The relation between negative symptoms and brain chemistry has not been thoroughly studied. Other research points to glutamate as being involved in the process that "may link excitatory neurotransmission to energy production and usage" (Duarte et al., 2012). Elevated N-acetyl-aspartyl-glutamate (NAAG), which is a precursor of glutamate,

correlated with higher negative symptom severity in patients with schizophrenia who are stabilized on antipsychotic medication (Rowland et al., 2012). NAAG is also considered to be an agonist of certain glutamate receptors (i.e., mGluR) and an antagonist of the NMDA receptor (Tsai et al., 1995). As such, NAAG is thought to modulate the glutamate system.

Brain regions of interest

A review of the literature on ^{1}H-MRS focusing on schizophrenia identified twenty-eight studies that examined the medial frontal cortex, hippocampus, and thalamus, and supported a link between glutamate and schizophrenia (Marsman, van den Heuvel, Klomp, Kahn, Luijten, & Hulshoff Pol, 2011). The literature, however, was limited to the above mentioned brain regions, leaving open the possibility for the involvement of other brain regions. The striatum consists of the caudate nucleus and the putamen. The caudate nucleus has been studied in schizophrenia research due to its high concentration of dopamine neurons (Lyon, Abi-Dargham, Moore, Lieberman, Javitch, & Sulzer, 2011). Some studies have argued that striatal dopamine excess in people with schizophrenia is related to glutamatergic mechanisms (Laruelle, Kegeles, & Abi-Dargham, 2003). Significant differences in metabolite concentrations (NAA, phosphocreatine plus creatine, choline-containing compounds, and glutamate plus glutamine) were observed in the caudate of first-degree relatives of people with schizophrenia as compared to healthy comparison participants (Keshavan, Dick, Diwadkar, Montrose, Prasad, & Stanley, 2009). A recent study observed elevated glutamate levels in the striatum of eighteen individuals at psychosis-risk and eighteen individuals in their first episode of schizophrenia as compared to forty age- and sex-

matched healthy controls (de la Fuente-Sandoval et al., 2011). After two years, those initially identified as at psychosis-risk who transitioned to schizophrenia had elevated glutamate levels in the associative striatum in comparison to those who did not transition (de la Fuente-Sandoval et al., 2012). The effects in this study were large, suggesting that striatal glutamate may be a specific biomarker for the development of psychosis, and may also have clinical utility in the prediction of individuals at psychosis-risk.

The striatum and the limbic system communicate with the prefrontal cortex, and both brain regions are implicated in one of several glutamatergic pathways (Schwartz et al., 2012). The limbic system controls internal processes such as emotion, motivation, and memory and consists of the cingulate cortex, amygdala, and hippocampus. The cingulate cortex, specifically the anterior cingulate cortex (ACC) controls behavior by "detecting errors and signaling the occurrence of conflicts during information processing" (Lezak et al., 2004) and is located in the medial frontal region of the brain. The medial frontal area of the brain has high concentrations of dopamine-sensitive neurons and has been implicated in psychosis-risk such that medial frontal glutamate and glutamine were found to be increased in individuals at genetic risk in comparison to healthy controls (Tibbo, Hanstock, Valiakalayil, & Allen, 2004). However, that study did not directly measure, or report, symptoms of psychosis-risk. It is unclear whether the elevated glutamate levels in their genetic risk sample were related to elevated psychosis-risk symptoms. It is possible that elevated glutamate in their sample represents an unrelated pathophysiology. The ACC, which has been implicated in relation to structural

abnormalities in people at psychosis-risk (Jung, Jang, Byun, An, & Kwon, 2010), was a region of interest for the current study in addition to the striatum.

Development

The neurodevelopmental model of schizophrenia indicates biological vulnerability that is present years before psychosis onset (Rapoport, Giedd, & Gogtay, 2012) and has been supported by research on psychosis-risk (Cornblatt, Lencz, Smith, Correll, Auther, & Nakayama, 2003). Some researchers have argued that potential biomarkers need to be "reconceptualized in the context of normal development at the time of illness onset" (Pantelis, Velakoulis, Wood, Yucel, Yung, Phillips, et al., 2007). Complex executive functioning and prefrontal cortex brain structure develop well beyond adolescence (Catts et al., 2013). Glutamate and glutamine may decrease more quickly with age in individuals with schizophrenia as compared to healthy controls (Marsman, van den Heuvel, Klomp, Kahn, Luijten, & Hulshoff Pol, 2011). Glutamate and glutamine concentrations have been shown to vary by age, but this has only been observed in adults (Kaiser, Schuff, Cashdollar, & Weiner, 2005). The relation between age and glutamate is not fully understood in younger populations and those at risk for psychosis.

Present study

As previous research has not reported whether glutamate concentrations in these brain regions correlate with psychotic symptoms in individuals at psychosis-risk, the current study aimed to explore metabolite concentrations in the striatum and cingulate of individuals at psychosis-risk. Specifically, this study explored the

relation between glutamatergic metabolites within the striatal and cingulate gyri and psychosis risk symptoms among adolescents at psychosis-risk.

Specific Aims and Hypotheses

This study aimed to determine whether metabolite concentrations are associated with psychotic-risk symptoms within a group of youth at psychosis-risk with the hypothesis that higher levels of glutamate levels in the striatum and anterior cingulate cortex (ACC) would correlate with higher levels of positive psychotic-risk symptom severity.

Exploratory Aims

Exploratory aims include examining the potential relations between metabolite concentrations and the following: negative symptoms, age, and duration of symptoms. A further exploratory aim was to investigate potential decoupling in the current sample by examining the relation among metabolites within one brain region. Lastly, the relation between psychosis-risk symptoms and metabolites reflecting glial health was also examined.

Inquiry into the relation between negative symptoms and glutamate has no direct evidence base and was thus considered an exploratory aim. A second exploratory aim was based on the adult literature, which suggests that glutamate concentrations differ by age. The current study aimed to determine whether glutamate concentrations systematically differed as a function of age in this adolescent sample. Given the small sample size of the current study, statistical examination of this aim is considered to be exploratory. Several studies have found a relation between duration

of psychosis-risk symptoms and glutamate concentrations. One study found that a compound of both glutamate and glutamine in the medial prefrontal cortex of individuals with first episode psychosis correlated with duration of illness (Natsubori et al., 2014). However, this relation did not remain significant after correction for multiple comparisons. A different study found that glutamate concentrations in the associative striatum correlated significantly with duration of symptoms in a sub-sample of individuals at psychosis risk who later developed psychosis (de la Fuente-Sandoval et al., 2012).

A metabolite that has been hypothesized to function as a "reservoir" for glutamate is NAA (Clark et al., 2006; Moffett et al., 2007). In studies with individuals diagnosed with schizophrenia, NAA and a combination of glutamate and glutamine signals (GLX) have been found to lack a correlation that exists among healthy comparison samples in the dorsolateral prefrontal cortex (dlPFC; Coughlin et al., 2015). This finding may suggest a link between an interrupted glutamate system and oxidative stress that conjointly influences the development of schizophrenia (Coughlin et al., 2015). The same study did not find a correlation between NAA and GLX in the anterior cingulate cortex, suggesting this "decoupling" may be specific to the dlPFC. A separate study found the same pattern in the hippocampus (Kraguljac et al., 2012). The current study aimed to examine the relation between NAA and GLX among individuals who have not yet developed psychosis, but are deemed at risk.

Oxidative stress is often associated with glutathione, an antioxidant, and NAA, an amino acid. In addition to its connection to oxidative stress, NAA has also been conceptualized as a marker of neuronal health and integrity (Moffett et al.,

2007). Inositol and glycine are metabolites that are conceptualized to be markers of glial health (Maddock & Buonocore, 2012). The relation between psychosis risk and metabolites that reflect neuronal as well as glial health, was also examined in the current study.

Methods

Participant Recruitment and Eligibility

The total sample size of the study was 12 participants at psychosis-risk between the ages of 12 and 23. On average, participants were 15.89 years old (SD = 3.10). Six participants had a family history of psychosis within a first degree relative. On average, participants had experienced symptoms of psychosis-risk for a duration of 63.5 weeks (SD = 49.85), with duration of symptoms ranging from seven to 39 months. One participant was prescribed medication at the time of the study. Of the twelve participants, six were male and six were female. All participants identified themselves to be African American with 3 participants identifying as biracial. All but one participant had a family income below $40,000 per year. Participants were recruited from an ongoing research study at the University of Maryland, Baltimore County and the University of Maryland School of Medicine that identifies adolescents who are receiving mental health treatment. To be eligible for the current study, participants met criteria for a SIPS syndrome and had no contra-indications to MRI (see Appendix A). Eligible participants were approached at study visits or, with consent, contacted following study completion to be invited to complete the current study.

Measures

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS)

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) is a non-invasive in vivo technique to measure metabolite concentrations in the brain (Stanley, 2002). ^{1}H-MRS

can be used to detect and quantify brain metabolites (Duarte, Lei, Mlynarik, & Gruetter, 2012) and it has been found to be a stable measure of metabolites (Hoshino et al., 1999). Water constitutes almost 80% of brain tissue, making the scan signal for water much stronger than signals for brain metabolites (Duarte et al., 2012). This requires the suppression of the water signal during data acquisition for in vivo spectra quantification (Duarte et al., 2012).

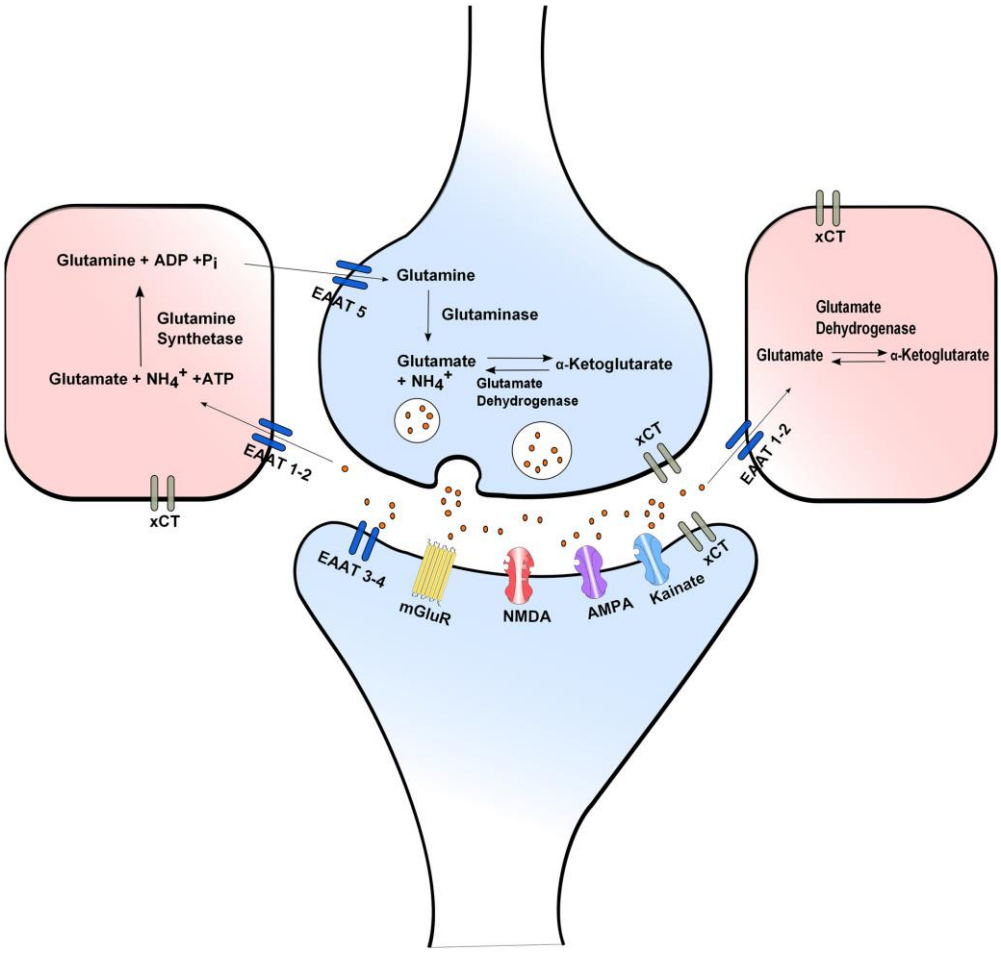
Statistical comparisons of metabolite concentrations between groups of individuals require valid and reliable measurement using ^{1}H-MRS techniques. There is evidence that good precision and reproducibility of ^{1}H-MRS has been found in multiple studies at various magnetic field strengths (Kim, Chang, Na, Song, Kim, Kwon, & Han, 2006) in healthy control samples (Geurts, Barkhof, Castelijns, Uitdehaag, Polman, & Pouwels, 2004) as well as in clinical populations such as samples of people with schizophrenia (Mullins, Rowland, Bustillo, Bedrick, Lauriello, & Brooks, 2003). Reproducibility of MRS measurements is necessary for detecting changes in metabolite concentrations and attributing these concentration differences to disease progression rather than machine error or other methodological limitations such as subject positioning, scanner variability, and physiological variation (Geurts et al., 2004).

Glutamate is challenging to quantify with ^{1}H-MRS due to j-coupling (connectivity of molecules) and overlapping peaks with other metabolites. Glutamate and glutamine are dynamically related (see Figure 1). However, at higher field strengths glutamate separated from glutamine can be reliably quantified. At 4Tesla (4T), the precision in the Anterior Cingulate Cortex was estimated to support a

coefficient of variation of about 9.2%, meaning the standard deviation with respect to the population mean (i.e., the extent of variability in the data), was less than 10% (Venkatraman, Hamer, Perkins, Song, Lieberman, & Steen, 2006). Our pilot data demonstrated low variance (coefficients of variation of 4.1% for glutamate and less than 10% for all assessed metabolites at 3T) that is consistent with the literature.

At 4T, glutamine concentrations as measured by 1H-MRS were found to increase in the anterior cingulate cortex (ACC) of healthy men following administration of ketamine (Rowland, L., Bustillo, J., Mullins, P., Jung, R., Lenroot, R., Landgraf, E., et al., 2005). A 3T study of medial frontal glutamate and glutamine found increased concentrations in individuals at genetic risk in comparison to healthy controls (Tibbo et al., 2004). A more recent study scanned participants at psychosis-risk and healthy controls on a 3T (the strength used in the proposed study) using point-resolved spectroscopy to quantify metabolites and successfully identified elevated glutamate levels in the striatum of individuals at risk for psychosis relative to healthy control glutamate levels (de la Fuente-Sandoval et al., 2012). A 3T 1H-MRS study found a significant negative correlation between severity of negative symptoms and glutathione, an antioxidant involved in modulating NMDA receptors (Matsuzawa et al., 2008).

Figure 1. Glutamate/Glutamine Cycle

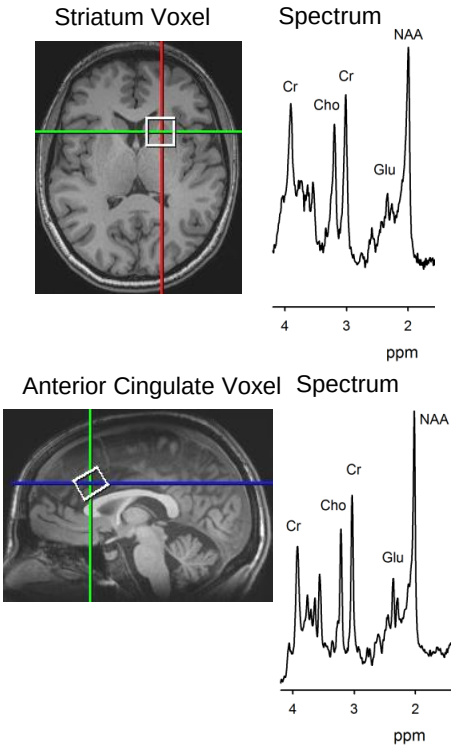


[1]H-MRS data acquisition

All MRI data were acquired on a 3T Siemens Trio scanner at the University of Maryland Center for Brain Imaging Research, located at the Maryland Psychiatric Research Center. A T1-weighted structural image (MP-RAGE: 1 mm isotropic voxels, 256 X 256 mm FOV, TR/TE/TI=1900/3.45/900ms) were acquired for spectroscopic voxel prescription and anatomical reference. Spectra were acquired from voxels in the dorsal striatum (2 x 2 x 2cc) and anterior cingulate cortex (2 x 2 x 1.5cc) regions (see Figure 2) using point-resolved pulse sequence (PRESS;

TR=2000ms, TE=30ms, 2048 points, 2500Hz spectral width, 128 averages). Voxels were positioned to maximize the amount of tissue of interest contained within the voxel. The regions were identified by their shape and position, as compared to a brain atlas. Water suppression were automated using a water suppression enhanced through T1 effects (WET) sequence.

Figure 2: Voxel Placement

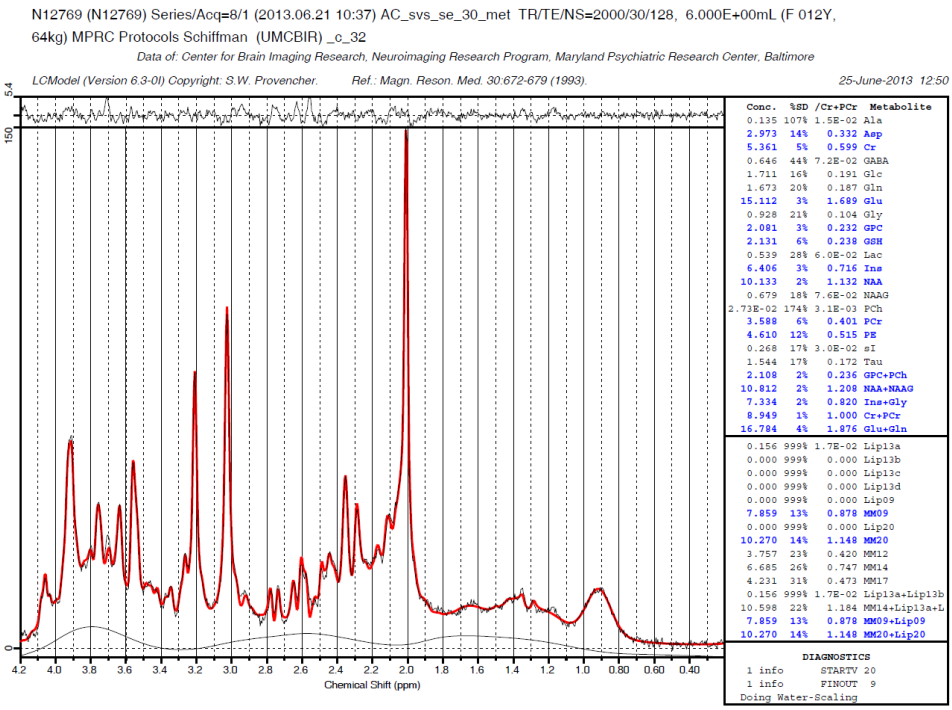


^{1}H-MRS data analysis

Spectra were analyzed using the fully automated, standard curve-fitting software, LCModel (Provencher, 2001), that fits in-vivo spectra as a linear combination of the spectra of pure compounds (the "basis set"). The simulated basis set will include peaks for gamma-Aminobutyric acid (GABA), glutamate, glutamine, alanine, aspartate, choline, creatine, glutathione, lactate, myo-inositol, n-acetylaspartylglutamate, phenylalanine, and taurine. The program also provides

automated phase-correction and baseline correction, and estimates various macromolecule resonances. Only spectra of good quality as determined by LCModel metabolite fits of Cramer-Rao Lower Bound (CRLB) of less than 20% were included (see Figure 3). Spectroscopic voxels were segmented into cerebrospinal fluid (CSF), gray, and white matter tissue using a Statistical Parametric Mapping program (SPM8) and in-house Matlab code. Spectra were CSF-corrected. Previous research has noted brain structure morphometry in the striatum and ACC, and as is standard with ^{1}H-MRS, any volumetric reductions in the specified brain regions were accounted for in quantification of metabolites (Marsman et al., 2011).

Figure 3: Spectra



Structured Interview for Psychosis-Risk Symptoms (SIPS)

The Structured Interview for Psychosis-risk Symptoms (SIPS; Miller et al., 2002) is considered the "gold standard" technique for assessing sub-threshold psychotic symptoms in individuals at risk for developing psychosis. The clinician-administered SIPS was found to have rater agreement of 93% (kappa = 0.81, 95% CI = 0.55-0.93) and to predict development of psychosis at 6 months (46%) and at 12 months (54%) in an initial validity study (Miller et al., 2002). The reported percentages were based on longitudinal data indicating that 6 of the 13 individuals initially rated as at psychosis-risk developed schizophrenia within six months and 7 of the 13 individuals developed the illness within a year (Miller et al., 2002). The results of this initial validity study reflect the high false positive rate in the field of psychosis-risk identification. More recently, one of the largest psychosis-risk studies found that 35% of individuals identified as at psychosis-risk using the SIPS developed schizophrenia at 2.5 year follow-up (Cannon et al., 2008).

The SIPS assesses the following symptom groupings: positive (hallucinations, delusional ideas, etc.), negative (anhedonia, avolition, etc.), disorganized (odd behavior, bizarre thinking, etc.), and general (sleep disturbance, motor disturbances, etc.) symptoms. SIPS ratings are based on a 7-point Likert-type symptom scale ranging from "absent" to "severe and psychotic". Psychosis-risk status is assessed based on symptom severity, intensity, and duration.

The SIPS categorizes endorsed symptoms into: psychotic syndrome (i.e. schizophrenia), three psychosis-risk syndromes, schizotypal personality disorder, or no psychotic syndrome. The three SIPS psychosis-risk syndromes include: Brief

Intermittent Psychotic Symptom syndrome (BIPS), Attenuated Positive Symptom syndrome (APS), and the Genetic Risk and Deterioration syndrome (GRD). The BIPS syndrome is characterized by recent onset of frank psychotic symptoms that are present infrequently and for short periods of time, APS is characterized by the onset or worsening of attenuated symptoms that are present at least once a week in the past month, and GRD is characterized by having a first-degree relative with a psychotic disorder or meeting criteria for schizotypal personality disorder and a significant (>30%) drop in functioning scores (Miller et al., 2003).

The date of onset of each psychosis-risk symptom was recorded within the SIPS for each participant. The duration of symptoms was calculated by subtracting this date of onset from the date that the participant completed MRI scanning.

Staff training for the SIPS administration involved attending a two day training with the SIPS authors at which staff were 'certified' to use the instrument by achieving 90% agreement with gold-standard scores on practice cases. Staff members unable to attend the training completed a process in which they read vignettes provided by the SIPS authors (McGlashan, Walsh, & Woods, 2010), practiced rating taped interviews, observed two or more interviews, and led at least two interviews while being observed by an experienced interviewer. New interviewers were considered reliable once their ratings and diagnoses matched those of the observing interviewer over at least two cases or until an Intra Class Correlation of 80% was reached. A reliability check of 10 randomly selected audio recordings revealed intra-class coefficients of $\alpha = .83$ for symptoms and $\kappa = 1$ for SIPS diagnosis across all

interviewers. Weekly case reviews in team meetings also contributed to agreement on ratings and diagnoses.

Within the current study, the positive symptoms subscale of the SIPS was used as a continuous measure of psychotic symptom severity. Diagnostic groups were dichotomized based on SIPS diagnoses, with any psychosis-risk diagnosis representing a positive case.

Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS)

The Kiddie-Schedule for Affective Disorders and Schizophrenia, present and lifetime version (K-SADS-PL) is a semi-structured interview to assess current and lifetime episodes of psychopathology based on Diagnostic Statistical Manual (DSM-IV) criteria (Kaufman & Schweder, 2004). A substantial evidence base for inter-rater reliability ($k > .95$ for bipolar disorder) and concurrent validity (internal consistency coefficient = .82 - .90 with a range of depression questionnaires) have established the K-SADS as the "gold standard" technique for assessing a wide array of child psychiatric diagnoses (Frazier et al., 2007; Ambrosini, 2000).

Procedures

Participants and, if under 18 years old their legal guardian(s), provided consent to complete the study (minors provided assent before participating). Following the informed consent procedure, all participants completed a brief safety screen that assesses for any metal objects in or on the body that would prevent them from safely completing the MRI procedure (see Appendix A). Participants were asked to respond to the SIPS structured interview as well as a battery of self-report

measures. Participants, and a legal guardian for participants under the age of 18, also completed a K-SADS-PL interview to identify any current mental illness diagnoses. Legal guardian interviews were conducted separately from the participant interviews.

Prior to the MRI scan, participants completed a mock scan with a fake MRI machine that allows participants the opportunity to habituate to the scanner environment. Female participants were asked to provide a urine sample for a pregnancy test. MRI was not to be completed with any pregnant participants. No female participants were pregnant at the time of MRI. Participants were asked to lie still in the MRI for 30 minutes to allow for data acquisition of the T1-weighted structural image, the spectra for the striatum, and the spectra for the anterior cingulate cortex. Following the MRI, participants completed neurocognitive tasks such as mazes, drawing, card games, and matching games as part of the larger study though beyond the scope of this proposal. Participants received compensation for their time and effort.

Data Analysis

Primary Study Aim

This study aimed to determine whether attenuated psychotic symptoms are associated with brain metabolite concentrations. Specifically, glutamate concentrations in the striatum and anterior cingulate cortex (ACC) were hypothesized to positively correlate with severity of positive symptoms within our sample of youth at psychosis-risk.

Analyses for Study Aim

Pearson correlations between attenuated positive symptoms and glutamate concentrations in the striatum and anterior cingulate cortex were conducted. These analyses attempted to assess a potential relation between these two continuous variables in each brain region.

Assumptions of Pearson correlation analysis

1) Glutamate and attenuated positive symptoms are measured with minimal error.
2) The true relation between glutamate and attenuated positive symptoms is linear.
3) Observations are independent.
4) At each value of glutamate, symptoms are normally distributed and at each value of symptoms, glutamate is normally distributed.

Glutamate is expected to be measured with limited error, but measurement error may still emerge. ^{1}H-MRS is a technique that is optimized to limit error to acceptable levels. Should anomalously large measurement error occur, as detected by visual inspection of the spectra, affected data points were to be removed from analyses. No data points were removed in the current sample because all spectra were of good quality. Correlation analysis is robust to violations of the third and fourth assumptions. Regression diagnostics were conducted to identify outlying data points and to visually inspect the nature of the relation between symptoms and glutamate. In the case of non-normality, data transformations were to be performed and non-parametric alternatives were to be explored. This was not necessary in the current sample.

Exploratory Analyses

Exploratory analyses examined whether glutamate concentrations in the striatum or ACC share a relation with negative symptoms, age, and duration of symptoms. Additionally, the relation between certain metabolites as well as the relation between symptoms and metabolites reflecting glial health was also examined. All exploratory aims were analyzed using Pearson correlations.

Results

Spectrum quality

The quality of the MRS data in this study was excellent. There are two measures commonly used to determine the quality of spectroscopy data: Signal-to-Noise Ratio (SNR) and the signal's Full Width at Half Maximum (FWHM). Signal-to-Noise Ratio is a value indicating the ratio of metabolite signal (peak height) to random noise (standard deviation of noise that results from randomly fluctuating signals related to either the brain tissue or scanner performance; Alger, 2010). The average signal-to-noise ratio was 46.08 (SD 9.29) in the ACC and 27.25 (SD 7.51) in the caudate. Signal-to-noise ratios above 10 are generally considered good quality in the literature (Fusar-Poli et al., 2011; Lutkenhoff et al., 2010; Natsubori et al., 2014; O'Neill et al., 2004). The second indicator of spectroscopy measurement precision is the width of a spectrum curve, known as the Full Width at Half Maximum (FWHM) or simply, line width (Alger, 2010). FWHM and SNR are connected such that wider metabolite peaks are associated with smaller signal-to-noise ratios (Alger, 2010). FWHM values above 0.10 ppm are generally considered poor quality (Bustillo et al., 2010; de la Fuente-Sandoval et al., 2011; Fusar-Poli et al., 2011; Lutkenhoff et al., 2010; Natsubori et al., 2014; O'Neill et al., 2004). In the current study, the average FWHM line width was 0.03 (SD 0.01) Hz in the ACC and 0.04 (SD 0.01) Hz in the caudate. The tissue underlying each voxel placement was segmented into % gray matter, % white matter, and % cerebrospinal fluid (CSF). CSF levels in the current study never exceeded 0.20% in the ACC and 0.11% in the caudate.

Table 1. Correlation matrix for the anterior cingulate cortex

Symptoms:	**GLU**	**GSH** n=11	**GLN** n=5	**GLX**	**NAA+NAAG**	**INS+GLY**
Positive						
P1	.077	**-.718***	**.818+**	-.061	.001	-.083
P2	.451	-.124	-.060	.220	.387	.285
P3	**.518+**	-.470	-.273	**.504+**	**.609***	.081
P4	.211	-.204	-.108	-.090	.020	.249
P5	-.021	**.606***	-.565	-.163	.002	.032
Psum	.394	-.229	-.198	.099	.297	.223
Negative						
N1	-.147	.508	.377	-.156	-.215	.210
N2	.216	**.667***	-.108	-.026	.164	**.512+**
N3	-.483	**.720***	.143	-.436	-.371	-.155
N4	.093	**.636***	.323	.002	.132	.330
N5	-.010	-.099	-.714	-.422	-.122	-.016
N6	.002	.145	.665	.113	.323	-.235
Nsum	-.096	**.675***	.279	-.220	-.018	.149
Disorganized						
D1	-.335	.322	-.144	-.071	-.079	-.433
D2	.237	**.634***	-.526	.037	.297	.223
D3	-.070	.448	-.508	-.366	-.202	.223
D4	.386	-.076	.459	.349	.167	**.603***
Dsum	.030	**.606***	-.395	-.094	.039	.198

+ $p < .10$; * $p < .05$; ** $p < .01$

Note: P1=Unusual thought content/Delusional ideas; P2=Suspiciousness/Persecutory ideas; P3=Grandiosity; P4=Perceptual abnormalities/Hallucinations; P5=Disorganized communication; Psum=Sum of all positive symptoms; N1=Social anhedonia; N2=Avolition; N3=Expression of emotion; N4=Experience of emotion and self; N5=Ideational richness; N6=Occupational functioning; Nsum=Sum of all negative symptoms; D1=Odd behavior/appearance; D2=Bizarre thinking; D3=Focus/attention; D4=Hygiene; Dsum=Sum of all disorganized symptoms

Table 2. Correlation matrix for the caudate

	GLU	**GSH** n=10	**GLN** n=5	**GLX**	**NAA+NAAG**	**INS+GLY**
P1	.175	-.497	.702	-.214	-.155	-.227
P2	.079	-.278	-.034	-.445	-.195	-.233
P3	.096	**-.816****	.619	-.123	.379	**-.601***
P4	-.009	-.204	-.232	-.376	-.448	-.070
P5	-.115	.313	-.371	-.264	.018	.137
Psum	.047	-.422	.015	-.481	-.199	-.277
N1	-.063	**.573+**	-.326	.030	-.255	.489
N2	-.060	.533	**-.838+**	-.384	-.200	.433
N3	-.369	**.852****	-.735	.062	.016	.283
N4	-.208	**.584+**	-.411	-.271	-.140	.258
N5	.223	-.010	-.678	-.193	.066	-.084
N6	-.349	.002	.495	-.233	.430	-.464
Nsum	-.238	**.647***	-.684	-.242	-.011	.225
D1	-.205	.279	-.205	.327	**.528+**	-.198
D2	.000	.384	-.380	-.393	.085	.105
D3	-.356	.162	-.551	**-.515+**	-.448	.330
D4	.108	-.260	.087	-.016	-.412	.326
Dsum	-.246	.266	-.444	-.283	-.076	.225

+ $p < .10$; * $p < .05$; ** $p < .01$

Note: P1=Unusual thought content/Delusional ideas; P2=Suspiciousness/Persecutory ideas; P3=Grandiosity; P4=Perceptual abnormalities/Hallucinations; P5=Disorganized communication; Psum=Sum of all positive symptoms; N1=Social anhedonia; N2=Avolition; N3=Expression of emotion; N4=Experience of emotion and self; N5=Ideational richness; N6=Occupational functioning; Nsum=Sum of all negative symptoms; D1=Odd behavior/appearance; D2=Bizarre thinking; D3=Focus/attention; D4=Hygiene; Dsum=Sum of all disorganized symptoms

Glutamate

Glutamate (Glu) is an amino acid and the most prominent excitatory neurotransmitter (McDonald & Johnston, 1990). Analyses for Aim 1 did not reveal statistically significant correlations between psychosis-risk symptoms and glutamate concentrations in either brain region (significance ranging from $p = .141$ to $p = .978$). However, one correlation in the predicted direction did reach a trend level towards significance: glutamate concentrations in the anterior cingulate cortex shared a positive correlation with grandiosity ($r = .518$, $p = .084$). Glutamate is only one of

several chemicals within the glutamatergic system. We were able to obtain data from three such glutamatergic neurotransmitters: glutathione, glutamine, and N-acetyl-aspartyl-glutamate (NAAG).

Glutathione

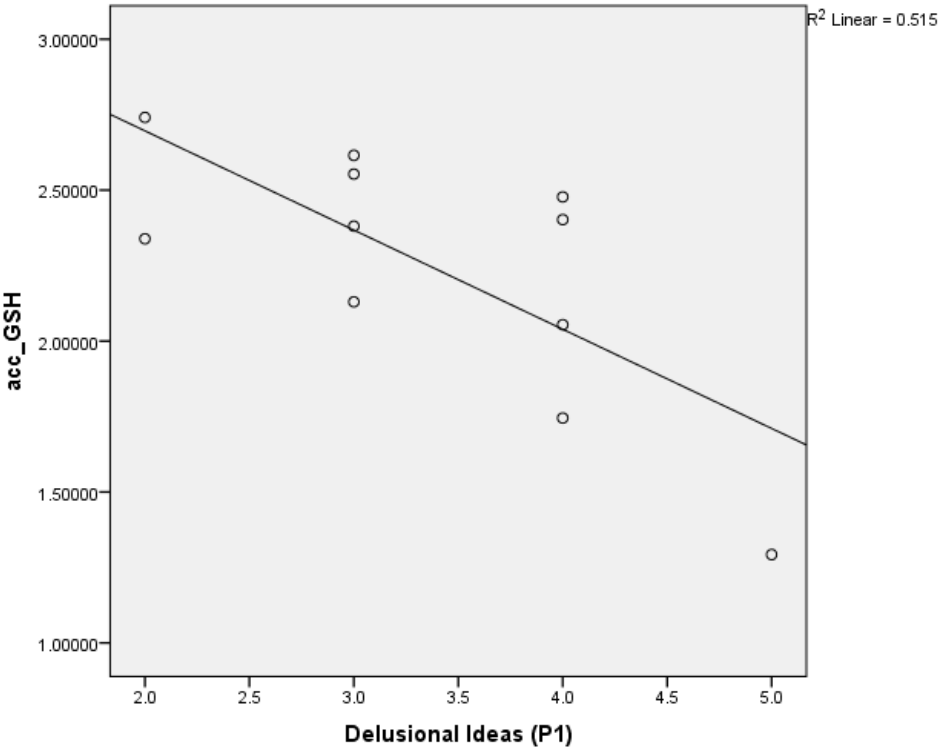
Glutathione (GSH) is an antioxidant involved in NMDAr neurotransmission. When GSH becomes depleted, it results in the cell's lowered ability to eliminate free radicals and makes cells vulnerable to cell death (Kritis, Stamoula, Paniskaki, & Vavilis, 2015). Glutathione deficit in schizophrenia has been linked to neurodegenerative processes in dopaminergic terminals and impairment NMDA receptor response to glutamate (Do et al., 2000).

Glutathione concentrations in the anterior cingulate cortex significantly correlated with Delusional Ideas (r = -.718, p = .013) and Disorganized Communication (r = .606, p = .048) of the SIPS. Additionally in this brain region, glutathione shared a statistically significant correlation with several negative symptoms including Avolition (r = .667, p = .025), Expression of Emotion (r = .720, p = .012), Experience of Emotion and Self (r = .636, p = .035) and a summary score of all negative symptoms combined (r = .675, p = .023; see Table 1). Glutathione in the ACC also correlated with a summary score of all disorganized symptoms (r = .606, p = .048) and Bizarre Thinking (r = .634, p = .036) specifically.

In the caudate, glutathione concentrations correlated significantly with Grandiosity (r = -.816, p = .004), Expression of Emotion (r = .852, p = .002), and a summary measure of negative symptoms (r = .647, p = .043). At a trend level,

glutathione in the caudate correlated with Social Anhedonia (r = .573, p = .084) and Experience of Emotion and Self (r = .584, p = .076).

Figure 4. Scatterplot of Delusional Ideas and Glutathione in the ACC



Glutamine

Glutamine (Gln) is the major metabolite of glutamate and is considered to be a glutamate precursor. Glutamine signals are generally small and were difficult to capture reliably in the current study using a 3T magnet. After excluding participants whose data did not meet the reliability threshold (Cramer-Rao Lower Bound < 20%), only five data points remained. Among these five individuals, glutamine concentrations in the anterior cingulate cortex correlated with Delusional Ideas at a trend level (r = .818, p = .090). Also at a trend level, glutamine concentrations in the

caudate correlated with Avolition, a negative symptom measured by the SIPS (r = -.838, p = .076).

When examining a combination of glutamine and glutamate signals, we found a positive correlation between this complex in the ACC and Grandiosity symptoms (r = .504, p = .095) and in the caudate we found a negative correlation with a disorganized symptom (Trouble with Focus and Attention; r = -.515, p = .087) that each reached a trend level towards significance.

Total NAA+NAAG

N-acetyl-aspartyl-glutamate (NAAG) is also a precursor to glutamate. NAAG is considered to be an agonist of certain glutamate receptors (i.e., mGluR) and an antagonist of the NMDA receptor (Tuominen, Tiihonen, & Wahlbeck, 2005). As such, NAAG is thought to modulate the glutamate system. In the current study, a measure of NAAG combined with NAA significantly correlated with Grandiosity (r = .609, p = .036) when looking at the ACC. The same measure in the caudate correlated with a disorganized symptom (Odd Behavior or Appearance) at a trend level (r = .528, p = .077).

Exploratory Analyses

Exploratory analyses examined relations between metabolite concentrations and the following: negative symptoms, age, and duration of symptoms. Additional exploratory analyses examined decoupling among metabolites as well as relations between symptoms and glial health (see Tables 1 and 2).

Negative symptoms

Negative symptoms significantly correlated with several metabolites. Avolition, Expression of Emotion, Experience of Emotion and Self, and a summary measure of negative symptoms significantly correlated with glutathione concentrations in the ACC (see Table 1). Expression of Emotion and the summary measure also correlated with glutathione concentrations in the caudate at a significant level. At a trend level, Social Anhedonia and Experience of Emotion and Self correlated with glutathione. Whereas correlations between negative symptoms and glutathione were in the positive direction, Avolition was inversely correlated with glutamine in the caudate.

Age

Age did not significantly correlate with glutamate or any other metabolite in either brain region. Of note, the majority of participants (83.3%) were between 12 and 15 years old, and only two participants were older (20 and 23 years old). Thus, with such a limited range of ages we were unable to detect an effect of age on metabolite concentrations in this study. However, one study examining glutamatergic metabolites over the course of 30 months did not find change in concentrations over time in either the first episode psychosis or healthy control groups (Theberge et al., 2007), suggesting that glutamatergic metabolites may not fluctuate as a factor of age but rather illness progression or medication effects.

Duration of Symptoms

Duration of psychosis-risk symptoms correlated with glutamate concentrations in the caudate at a trend level (n = 10, r = .589, p = .073). Duration of symptoms did not correlate significantly with any other metabolite concentrations in either brain region.

Decoupling

We examined the relations between metabolites, specifically the correlation between NAA and GLX in the anterior cingulate cortex, to probe for decoupling. In our sample, NAA+NAAG and GLX shared a statistically significant positive correlation (r = .746, p = .005) in the anterior cingulate cortex. These two metabolites were not correlated significantly across brain regions.

Glial Health

In addition to the glutamatergic system, overall glial health was of interest in this study. A marker of glial health, the combined concentrations of inositol and glycine, correlated significantly with Grandiosity (r = -.601, p = .039) when measured in the caudate. This finding suggests that lower glial health is associated with more grandiose ideas and beliefs. When measured in the ACC, this marker correlated significantly with Personal Hygiene (a symptom on the disorganization scale; r = .603, p = .038) and at a trend level with Avolition (r = .512, p = .089) such that higher concentrations of this glial health marker were related to more problems with hygiene and more difficulties with motivation.

Discussion

Findings support the connection between aspects of the glutamatergic system and psychosis-risk symptoms. As guided by prior research, the hypothesis that glutamate in both the ACC and caudate would share a positive correlation with symptoms was partially supported as glutamate concentrations in the ACC shared a trend-level correlation in the predicted direction with one positive symptom (Grandiosity). Thus, among our sample of individuals at psychosis-risk, those with higher glutamate concentrations in the ACC also tended to report more severe grandiosity symptoms. Expanding beyond glutamate to include related metabolites revealed several notable relations between psychosis-risk symptom severity and the glutamatergic system. The most robust associations were between positive and negative symptoms and glutathione and glutamine.

Schizophrenia has been associated with reduced levels of glutathione (Kantrowitz & Javitt, 2010). A similar pattern emerged in the current study such that more severe psychosis-risk symptoms generally correlated with lower levels of glutathione. This was observed in both brain regions that were measured, the anterior cingulate cortex and the caudate, providing some evidence for consistency across brain regions. Glutathione, an antioxidant, has been linked to oxidative stress and the pathogenesis of schizophrenia (Matsuzawa et al., 2008). More specifically, the oxidized form of glutathione modulates NMDA receptors (Kantrowitz & Javitt, 2010). Thus, glutathione deficit has been interpreted as one potential causal factor for the hypofunction of NMDA receptors in schizophrenia (Steullet et al., 2006). The

connection between glutathione concentration and psychosis-risk symptoms observed in this study therefore may reflect an early dysregulation of the glutamatergic system.

Trends towards significance emerged between both glutamine concentrations and GLX concentrations in the ACC, and core positive symptoms of psychosis (Delusional Ideas and Grandiosity, respectively). These trends emerged despite significant reduction of sample size due to reliability thresholds. However, findings were limited to one of the two brain regions studied, as correlations in the caudate did not reflect a clear pattern with positive symptoms. Our analyses yielding positive correlations between glutamine in the ACC and positive symptoms are consistent with prior research indicating elevated glutamine in the ACC of psychosis-risk individuals (Stone et al., 2009) and un-medicated individuals experiencing their first episode of psychosis (Theberge et al., 2002; Theberge at al., 2007). Thus, in our sample of individuals at psychosis-risk, those with more severe psychosis-risk symptoms also tended to have higher glutamine levels in the ACC. Further, findings from the current study regarding GLX are also consistent with previous studies, including studies demonstrating that GLX in the ACC was significantly elevated in individuals at genetic risk for schizophrenia (Tibbo et al., 2004), in individuals with schizophrenia who had received no treatment (Bartha et al., 1997) or minimum treatment (Bustillo et al., 2010) as well as more chronic samples (Bustillo et al., 2014; Chang et al., 2007; Choe et al., 1994). Further, two studies demonstrated a similar pattern but at a trend levels towards significance (Öngür et al., 2008; Shirayama et al., 2010). A recent meta-analysis summarizes findings relevant to glutamine and GLX in the ACC among people with psychosis, indicating that results are mixed across

studies (Marsman et al., 2011). Although evidence from several studies suggests a link between elevated glutamine and psychosis, such a pattern has not consistently been observed (see Bartha et al., 1999; Galinska et al., 2009; Keshavan et al., 2009; Reid et al., 2010; Rowland et al., 2008; Tayoshi et al., 2009; Theberge et al., 2003; Wood et al., 2007; Yoo et al., 2009). The variability in findings may stem from differences in measurement accuracy, such as field strength, or other imaging technique differences. Alternatively, inconsistent findings may reflect the effects of other mechanisms at play. Our robust findings within a small sample add to this growing literature suggesting that the relation between glutamine as well as GLX in the ACC and psychosis risk symptoms exists among individuals at clinical high risk.

The direction of correlations between glutathione in the ACC and Delusional Ideas versus Disorganized Communication differed such that the correlation was positive for Delusions and negative for Disorganized Communication. Even though both the Delusional Ideas scale and the Disorganized Communication scale are captured within the same "positive symptoms" category within the SIPS, they did not significantly correlate to one another in our study (r = -.204, p = .524), suggesting that they may represent disparate constructs that could uniquely influence risk for psychosis. Similarly, in the larger study of adolescents at risk for psychosis (n=57) from which the current sample was accrued, Disorganized Communication was the only positive symptom that did not correlate with other positive symptoms (see Appendix D). Further, in a sample of 94 individuals at risk for psychosis all the positive symptom scales loaded onto a single factor, except Disorganized Communication which loaded onto a factor shared with several disorganized and

negative symptoms (McGlashan, Walsh, & Woods, 2010). In the current sample Disorganized Communication did not significantly correlate with any other positive symptom. It was also the only positive symptom that shared a correlation with glutathione in the ACC in the opposite direction of the remaining positive symptoms. Disorganized Communication (P5) did, however, significantly correlate with a symptom named Bizarre Thinking (D2), which is classified under the Disorganized symptom scale on the SIPS (see Appendix E; $r = .862$, $p = .001$). Thus, while it is possible that the disparate directions of the correlations between positive symptoms and glutathione may suggest a spurious finding, it could also reflect the possibility that each positive symptom represents a unique aspect of the overall category and that the glutamatergic system is related to both. Disorganized communication is one of the key predictors of conversion to psychosis (Cornblatt et al., 2015). Regardless of whether Disorganized Communication is conceptualized as a positive or disorganized symptom, its relation to glutathione suggests that early psychosis-risk symptoms co-occur with changes in the glutamatergic system.

Whereas previous research with patients with schizophrenia found a negative correlation between posterior medial frontal glutathione and negative symptoms (Matsuzawa et al., 2008), results of the current study indicate statistically significant positive correlations between glutathione and several negative symptoms in both brain regions surveyed. In Matsuzawa and colleagues' (2008) study glutathione did not correlate with positive symptoms. It is possible that the metabolic profile of individuals at psychosis-risk differs from that of individuals with full psychosis, as in schizophrenia. Even though that study controlled for antipsychotic medication

exposure, their sample was chronically ill with full psychosis for seven years on average. Thus, it is possible that medication effects altered the metabolic profile of their participants such that positive symptoms were dampened through dopaminergic processes that in turn affected the functioning of the glutamatergic system. If that was the case, there would be insufficient variability in positive symptoms to detect a relation with glutathione. Further, negative symptoms would be unchanged and likely quite elevated, which would allow negative symptoms to correlate with glutathione. Studies with antipsychotic-naïve individuals with schizophrenia are necessary to understand the connection between glutathione and negative symptoms in relation to positive symptoms. It is also possible that rates of depression were higher in their adult sample, which may have systematically inflated their negative symptom ratings. Finally, it is possible that in our younger, at-risk sample the negative symptoms have not yet expressed themselves fully or systematically. For example, in our sample the sum of all positive symptoms did not significantly correlate with the sum of all negative symptoms ($r = 0.154$, $p = .632$). Likewise, disrupted glutathione concentration may be the result of ongoing illness progression rather than a risk factor for psychosis. For example, it is possible that our finding of higher glutathione concentrations correlating with more severe negative symptoms may reflect an over-activation of antioxidant effects such that glutathione, an antioxidant, is elevated as an attempt to repair tissue damage related to pathophysiological processes of the illness (e.g., excitotoxicity). It is unclear whether our findings are spurious or a true reflection of a relation between negative symptoms and glutathione.

We found a correlation between two metabolites within the ACC that have been found to show a disruption in their relation to one another, known as decoupling, in studies with patients with chronic schizophrenia (Coughlin et al., 2015). While it is possible that this finding reflects intact coupling or relation between these metabolites early in the phase of illness, there are several methodological factors that should be considered to appropriately interpret these results with caution. For example, differences in sample demographics across groups, differences in voxel size, and differences in how metabolites are referenced (e.g., relative concentrations to water vs. creatine) could all impact differences in metabolite relations with one another across studies.

When measured in the caudate, a marker of glial health correlated significantly with Grandiosity. This finding suggests that lower glial health is associated with more grandiose ideas and beliefs in an area of the brain that is responsible for learning, memory, and social behavior (among other functions). Conversely, higher concentration of the same marker in the ACC was related to more severe problems with hygiene and more difficulties with motivation. It is possible that a higher concentration of the marker reflects an over-activation of the glial support cells in response to cell stress. This finding could reflect brain changes underlying early cognitive and social difficulties that are evident in individuals at psychosis-risk.

Limitations

The main limitation of the current study was the small sample size. However, that evidence of relations of any kind were detected suggests that the connection between the glutamatergic system and psychosis-risk symptoms is strong. Another

aspect of the study that limited the range of questions that could be addressed was the fact that it lacked a control group. In the future, we plan to collect data from a typically developing comparison sample in order to examine whether differences in glutamatergic system exist between the two groups. Such a design will allow for the assessment of whether glutamatergic metabolite concentrations can predict group membership such that imaging data can help identify those at risk for developing psychosis.

Future Directions

Findings from the current study partially support study hypotheses, independently replicating findings from recent research (by the de la Fuente-Sandoval group) to some degree. This replication supports evidence that aberrant glutamatergic processes may be involved in the pathophysiology of the early stages of psychosis, providing further information on the etiology of psychosis. However, whether disrupted glutamatergic processes are truly specific to psychosis development in at-risk samples remains to be determined. Future studies could examine metabolic parameters of individuals at risk for psychosis compared to those of other help-seeking individuals rather than healthy controls to help elucidate this.

A potential implication of this research involves the power to accurately identify individuals who will go on to develop schizophrenia. Because glutamatergic neurotransmitters are related to psychotic symptoms and can contribute to predicting psychosis-risk status, measurement of concentrations of these neurotransmitters may add incremental value to the interview-based psychosis-risk assessments by reducing the high false-positive rate. The discovery of biomarkers of risk for psychosis

presents the possibility of the field having powerful tools to more accurately identify individuals at risk of developing schizophrenia.

Results may inform future treatment development, particularly pharmacological treatments targeting the glutamate pathway. Such treatment may be able to alleviate symptoms for individuals for whom other antipsychotic medication, which traditionally targets the dopamine system, has not been effective. A link between glutathione and negative symptoms was supported in this study. Future treatments influencing the glutamatergic pathway thus may have implications for negative symptoms, addressing an important functional impairment associated with psychosis that is not currently addressed by dopaminergic antipsychotic medications. However, whether medications should aim to increase or decrease concentrations of glutathione is not yet clearly understood, as different patterns have emerged across samples and brain regions. Studies aiming to increase the concentration of glutathione in the brain are ongoing with animal models (Matsuzawa et al., 2008). Further, it is as yet unknown whether targeting the glutamatergic system will result in clinical benefit, as for instance, it is possible that glutamatergic dysfunction is a downstream mechanism of another system that could be more proximally targeted by medications.

One important goal of this line of research is to use imaging techniques in conjunction with interview tools to more accurately identify individuals at risk of developing psychosis. Future clinical directions may involve creating specialized psychosis-risk clinics that combine all available tools (self-report screeners, structured interview methods, neural biomarkers) to ultimately help prevent psychosis as well as reduce associated health care costs. Biomarkers may contribute to the

development of a psychosis-risk detection algorithm that could incorporate clinical assessment and measurement of brain mechanisms to improve early identification of psychosis and thus decrease duration of untreated psychosis by connecting those at highest risk with services.

Appendices

Appendix A. MRI Safety Screen

UNIVERSITY of MARYLAND SCHOOL OF MEDICINE

MRI Screening Form

UM Center for Brain Imaging Research
Maryland Psychiatric Research Center
University of Maryland
55 wade Ave, Catonsville MD 21228

Date _____/_____/_____ Patient Number _____

Name _____ DOB_____ Male____Female_____
Last name First name Middle Initial

Height_____ Weight_____

---

1. Have you had prior surgery or an operation (e.g., arthroscopy, endoscopy, etc.) of any kind? _ No _ Yes
   If yes, please indicate the date and type of surgery:

   Date____/____/_____ Type of surgery: _____
   Date____/____/_____ Type of surgery: _____

2. Have you experienced any problem related to a previous MRI examination or MR procedure? _No _Yes
   If yes, please describe: _____

3. Have you had an injury to the eye involving a metallic object or fragment (e.g., metallic slivers, shavings, foreign body, etc.)? _ No _ Yes
   If yes, please describe: _____

4. Have you ever been injured by a metallic object or foreign body (e.g., BB, bullet, shrapnel, etc.)? _ No _ Yes
   If yes, please describe: _____

5. Are you allergic to any medication? _ No _ Yes
   If yes, please list: _____

6. Do you have a history of asthma, allergic reaction, respiratory disease, or reaction to a contrast medium or dye used for an MRI, CT, or X-ray examination? _ No _ Yes

7. Do you have tattoos, permanent make-up done in the last 2 months? _No _Yes
   If yes, location?_____

8. Do you have hair extensions? _No _Yes

9. Do you have any non-removable piercings? _No _Yes
   If yes, location? _____

10. Do you have metal in your body (**pacemaker**, plates, aneurysm clips/coils, deep brain stimulator, pins, rods, joints, pellets, cochlear implants, etc.)? _No _Yes
    If yes, please describe: _____

11. Have you ever been employed as a farm worker, metal grinder, or welder? _No _Yes

12. Do you wear dentures, partials, braces, or a non-removable orthodontic retainer? _No _Yes

13. Have you ever considered yourself to be claustrophobic? _No _Yes

For female patients:

14. Are you pregnant or could you possibly be pregnant? _ No _ Yes

UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

MRI Screening Form

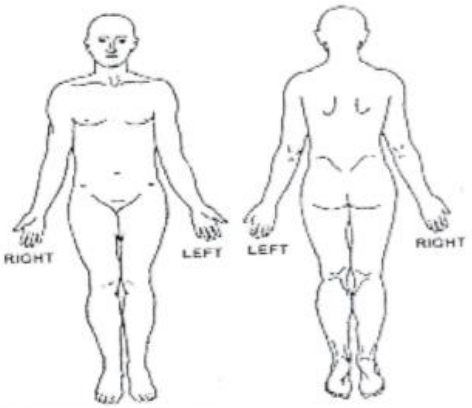
UM Center for Brain Imaging Research
Maryland Psychiatric Research Center
University of Maryland
55 wade Ave, Catonsville MD 21228

WARNING: Certain implants, devices, or objects may be hazardous to you and/or may interfere with the MR procedure (i.e., MRI, MR angiography, functional MRI, MR spectroscopy). **Do not enter** the MR system room or MR environment if you have any question or concern regarding an implant, device, or object. Consult the MRI Technologist or Radiologist BEFORE entering the MR system room. The MR system magnet is ALWAYS on.

Please indicate if you have any of the following:

_Yes _No Aneurysm clip(s) or coils
_Yes _No Cardiac pacemaker
_Yes _No Implanted cardioverter defibrillator (ICD)
_Yes _No Electronic implant or device
_Yes _No Magnetically-activated implant or device
_Yes _No Neurostimulation system (Deep Brain Stimulator)
_Yes _No Spinal cord stimulator
_Yes _No Internal electrodes or wires
_Yes _No Bone growth/bone fusion stimulator
_Yes _No Cochlear, otologic, or other ear implant
_Yes _No Insulin or other infusion pump
_Yes _No Implanted drug infusion device
_Yes _No Any type of prosthesis (eye, penile, etc.)
_Yes _No Heart valve prosthesis
_Yes _No Eyelid spring or wire
_Yes _No Artificial or prosthetic limb
_Yes _No Metallic stent, filter, or coil
_Yes _No Shunt (spinal or intraventricular)
_Yes _No Vascular access port and/or catheter
_Yes _No Radiation seeds or implants
_Yes _No Swan-Ganz or thermodilution catheter
_Yes _No Medication patch (Nicotine, Nitroglycerine)
_Yes _No Any metallic fragment or foreign body
_Yes _No Wire mesh implant
_Yes _No Tissue expander (e.g., breast)
_Yes _No Surgical staples, clips, or metallic sutures
_Yes _No Joint replacement (hip, knee, etc.)
_Yes _No Bone/joint pin, screw, nail, wire, plate, etc.
_Yes _No IUD, diaphragm, or pessary
_Yes _No Dentures or partial plates
_Yes _No Tattoo or permanent makeup
_Yes _No Body piercing jewelry
_Yes _No Hearing aid
(Remove before entering MR system room)
_Yes _No Other implant _____
_Yes _No Breathing problem or motion disorder
_Yes _No Claustrophobia

Please mark on the figure(s) below the location of any implant or metal inside of or on your body.



IMPORTANT INSTRUCTIONS

Before entering the MR environment or MR system room, you must remove all metallic objects including hearing aids, dentures, partial plates, keys, beeper, cell phone, eyeglasses, hair pins, barrettes, jewelry, body piercing jewelry, watch, safety pins, paperclips, money clip, credit cards, bank cards, magnetic strip cards, coins, pens, pocket knife, nail clipper, tools, clothing with metal fasteners, & clothing with metallic threads.

Please consult the MRI Technologist or Radiologist if you have any question or concern BEFORE you enter the MR system room.

NOTE: You are required to wear earplugs or other hearing protection during the MRI procedure

I attest that the above information is correct to the best of my knowledge. I read and understand the contents of this form and had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

Signature of Person Completing Form: _____ Date _____/_____/_____
Signature Initials

Screened By: _____ Date_____/____/_____
Signature Initials

Appendix B. SIPS Symptom Scales

SUMMARY OF SIPS DATA

Positive Symptom Scale

0 Absent	1 Questionably Present	2 Mild	3 Moderate	4 Moderately Severe	5 Severe but Not Psychotic	6 Severe and Psychotic

Positive Symptoms

P1. Unusual Thought Content/Delusional Ideas (p. 11)	0	1	2	3	4	5	6
P2. Suspiciousness/Persecutory Ideas (p. 13)	0	1	2	3	4	5	6
P3. Grandiosity (p. 15)	0	1	2	3	4	5	6
P4. Perceptual Abnormalities/Hallucinations (p. 18)	0	1	2	3	4	5	6
P5. Disorganized Communication (p. 20)	0	1	2	3	4	5	6

Negative, Disorganized, General Symptom Scale

0 Absent	1 Questionably Present	2 Mild	3 Moderate	4 Moderately Severe	5 Severe	6 Extreme

Negative Symptoms

N1. Social Anhedonia (p. 21)	0	1	2	3	4	5	6
N2. Avolition (p. 22)	0	1	2	3	4	5	6
N3. Expression of Emotion (p. 23)	0	1	2	3	4	5	6
N4. Experience of Emotions and Self (p. 24)	0	1	2	3	4	5	6
N5. Ideational Richness (p. 25)	0	1	2	3	4	5	6
N6. Occupational Functioning (p. 26)	0	1	2	3	4	5	6

Disorganization Symptoms

D1. Odd Behavior or Appearance (p. 27)	0	1	2	3	4	5	6
D2. Bizarre Thinking (p. 28)	0	1	2	3	4	5	6
D3. Trouble with Focus and Attention (p. 29)	0	1	2	3	4	5	6
D4. Personal Hygiene (p. 30)	0	1	2	3	4	5	6

General Symptoms

G1. Sleep Disturbance (p. 31)	0	1	2	3	4	5	6
G2. Dysphoric Mood (p. 32)	0	1	2	3	4	5	6
G3. Motor Disturbances (p. 33)	0	1	2	3	4	5	6
G4. Impaired Tolerance to Normal Stress (p. 34)	0	1	2	3	4	5	6

Appendix C. SIPS prompts

P. 1. UNUSUAL THOUGHTS/DELUSIONAL IDEAS

1.1. Have you had the feeling that something odd is going on or that something is wrong that you can't explain?
1.2. Have you ever been confused at times whether something you have experienced is real or imaginary?
1.3. Do familiar people or surroundings ever seem strange? Confusing? Unreal? Not a part of the living world? Alien? Inhuman? Evil?
1.4. Does your experience of time seem to have changed? Unnaturally faster or slower?
1.5. Do you ever seem to live through events exactly as you have experienced them before?
1.6. Have you felt that you are not in control of your own ideas or thoughts?
1.7. Do you ever feel as if somehow thoughts are put into your head or taken away from you? Do you ever feel that some person or force may be controlling or interfering with your thinking?
1.8. Do you ever feel as if your thoughts are being said out loud so that other people can hear them?
1.9. Do you ever think that people might be able to read your mind?
1.10. Do you ever think that you can read other people's minds?
1.11. Do you ever feel the radio or TV is communicating directly to you?
1.12. Do you have strong feelings or beliefs that are very important to you, about such things as religion, philosophy, or politics?
1.13. Do you daydream a lot or find yourself preoccupied with stories, fantasies, or ideas? Do you ever feel confused about whether something is your imagination or real?
1.14. Do you know what it means to be superstitious? Are you superstitious? Does it affect your behavior?
1.15. Do other people tell you that your ideas or beliefs are unusual or bizarre? If so, what are these ideas or beliefs?
1.16. Do you ever feel you can predict the future?
1.17. Do you ever worry that something might be wrong with your body or your health?
1.18. Have you ever felt that you might not actually exist? Do you ever think that the world might not exist?
1.19. Do you ever find yourself thinking a lot about how to be good or begin to believe that you deserve to be punished in some way?
1.20. Have you felt that things happening around you have a special meaning for just you?
1.21. Have you had the sense that you are often the center of people's attention? Do you feel they have hostile or negative intentions?

0 Absent	1 Questionably Present	2 Mild	3 Moderate	4 Moderately Severe	5 Severe but Not Psychotic	6 Severe and Psychotic
	"Mind tricks" that are puzzling. Sense that something is different.	Overly interested in fantasy life. Unusually valued ideas/ beliefs. Some superstitions beyond what might be expected by the average person but within cultural norms.	Unanticipated mental events that are puzzling, unwilled, but not easily ignored. Experiences seem meaningful because they will recur and will not go away. Functions mostly as usual.	Sense that ideas/ experience/ beliefs may be coming from outside oneself or that they may be real, but doubt remains intact. Distracting, bothersome. May affect functioning	Experiences familiar, anticipated. Doubt can be induced by contrary evidence and others' opinions. Distressingly real. Affects daily functioning	Delusional conviction (with no doubt) at least intermittently. Interferes persistently with thinking, feeling, social relations, and/or behavior.

P. 2. SUSPICIOUSNESS/PERSECUTORY IDEAS

2.1. Do you ever feel that people around you are thinking about you in a negative way? Have you ever found out later that this was not true or that your suspicions were unfounded?
2.2. Have you ever found yourself feeling mistrustful or suspicious of other people?
2.3. Do you ever feel that you have to pay close attention to what's going on around you in order to feel safe?
2.4. Do you ever feel like you are being singled out or watched?
2.5. Do you ever feel people might be intending to harm you?
Do you have a sense of who that might be?

0 Absent	1 Questionably Present	2 Mild	3 Moderate	4 Moderately Severe	5 Severe but Not Psychotic	6 Severe and Psychotic
	Wariness.	Concerns about safety. Hypervigilance without clear source of danger.	Concerns that people are untrustworthy and/or may harbor ill will. Sense of unease and need for vigilance (often unfocused). Mistrustful. Recurrent (yet unfounded) sense that people might be thinking or saying negative things about person.	Thoughts of being the object of negative attention. Sense that people may wish harm. Self-generated skepticism present. Pre-occupying, distressing. May affect daily functioning. May appear defensive in response to questioning.	Beliefs about danger from hostile intentions of others. Skepticism and perspective can prevail with non-confirming evidence or other's opinion. Anxious, unsettled. Daily functioning affected. Guarded presentation may diminish information gathered in the interview.	Delusional paranoid conviction (no doubt) at least intermittently. Frightened, avoidant, watchful. Interferes persistently with thinking, feeling, social relations, and/or behavior.

P. 3 GRANDIOSE IDEAS

3.1. Do you feel you have special gifts or talents? Do you feel as if you are unusually gifted in any particular area? Do you talk about your gifts with other people?
3.2. Have you ever behaved without regard to painful consequences? For example, do you ever go on excessive spending sprees that you can't afford?
3.3. Do people ever tell you that your plans or goals are unrealistic? What are these plans? How do you imagine accomplishing them?
3.4. Do you ever think of yourself as a famous or particularly important person?
3.5. Do you ever feel that you have been chosen by God for a special role? Do you ever feel as if you can save others?

0 Absent	1 Questionably Present	2 Mild	3 Moderate	4 Moderately Severe	5 Severe but Not Psychotic	6 Severe and Psychotic
	Private thoughts of being better than others.	Mostly private thoughts of being talented, understanding or gifted.	Notions of being unusually gifted, powerful, or special and have exaggerated expectations. May be expansive but can redirect to the everyday on own.	Beliefs of talent, influence, and abilities. Unrealistic goals that may affect plans and functioning, but responsive to other's concerns and limits.	Compelling beliefs of superior intellect, attractiveness, power, or fame. Skepticism and modesty can only be elicited by the efforts of others. Affects functioning.	Delusions of grandiosity with conviction (no doubt) at least intermittently. Interferes persistently with thinking, feeling, social relations, or behavior.

P. 4. PERCEPTUAL ABNORMALITIES/HALLUCINATIONS

4.1. Do you ever feel that your mind is playing tricks on you?
4.2. Do you ever feel that your ears are playing tricks on you?
4.3. Have you been feeling more sensitive to sounds? Have sounds seemed different? Louder or softer?
4.4. Do you ever hear unusual sounds like banging, clicking, hissing, clapping, ringing in your ears?
4.5. Do you ever think you hear sounds and then realize that there is probably nothing there?
4.6. Do you ever hear your own thoughts as if they are being spoken outside your head?
4.7. Do you ever hear a voice that others don't seem to or can't hear? Does it sound clearly like a voice speaking to you as I am now? Could it be your own thoughts or is it clearly a voice speaking out loud?
4.8. Do you ever feel your eyes are playing tricks on you?
4.9. Do you seem to feel more sensitive to light or do things that you see ever appear different in color, brightness or dullness; or have they changed in some other way?
4.10. Have you ever seen unusual things like flashes, flames, vague figures or shadows out of the corner of your eye?
4.11. Do you ever think you see people, animals, or things, but then realize they may not really be there?
4.12. Do you ever see things that others can't or don't seem to see?
4.13. Have you noticed any unusual bodily sensations such as tingling, pulling, pressure, aches, burning, cold, numbness, vibrations, electricity, or pain?
4.14. Do you ever smell or taste things that other people don't notice?

0 Absent	1 Questionably Present	2 Mild	3 Moderate	4 Moderately Severe	5 Severe but Not Psychotic	6 Severe and Psychotic
	Minor, but noticeable perceptual sensitivity (e.g. heightened, dulled, distorted, etc.).	Unformed perceptual experiences/ changes that are noticed but are not considered to be significant.	Recurrent, unformed, images (e.g., shadows, trails, sounds, etc.), illusions, or persistent perceptual distortions that are puzzling and experienced as usual.	Illusions or momentary formed hallucinations that are ultimately recognized as unreal yet can be distracting, curious, unsettling. May affect functioning.	Hallucinations experienced as external to self though skepticism can be induced by others. Mesmerizing, distressing. Affects daily functioning.	Hallucinations perceived as real and distinct from the person's thoughts. Skepticism cannot be induced. Captures attention, frightening. Interferes persistently with thinking, feeling, social relations, and/or behavior.

P. 5. DISORGANIZED COMMUNICATION

5.1. Do people ever tell you that they can't understand you? Do people ever seem to have difficulty understanding you?
5.2. Are you aware of any ongoing difficulties getting your point across, such as finding yourself rambling or going off track when you talk?
5.3. Do you ever completely lose your train of thought or speech, like suddenly blanking out?

0 Absent	1 Questionably Present	2 Mild	3 Moderate	4 Moderately Severe	5 Severe but Not Psychotic	6 Severe and Psychotic
	Occasional word or phrases doesn't make sense.	Speech is slightly vague, muddled, overelaborate or stereotyped.	Incorrect words, irrelevant topics. Goes off track, but redirects on own.	Speech is clearly circumstantial (i.e. eventually getting to the point). Difficulty in directing sentences toward a goal. Sudden pauses. Can be redirected with occasional questions and structuring.	Speech tangential (i.e. never getting to the point). Some loosening of associations or blocking. Can reorient briefly with frequent prompts or questions.	Communication persistently loose, irrelevant or blocked and unintelligible when under minimal pressure or when the content of the communication is complex. Not responsive to structuring of the interview.

Appendix D. Correlation Matrix of Positive Symptoms

Current Sample (*n*=12)

	P1	P2	P3	P4	P5
P1	-				
P2	.512+	-			
P3	.143	.020	-		
P4	.440	.839**	-.200	-	
P5	-.204	.411	-.323	.276	-

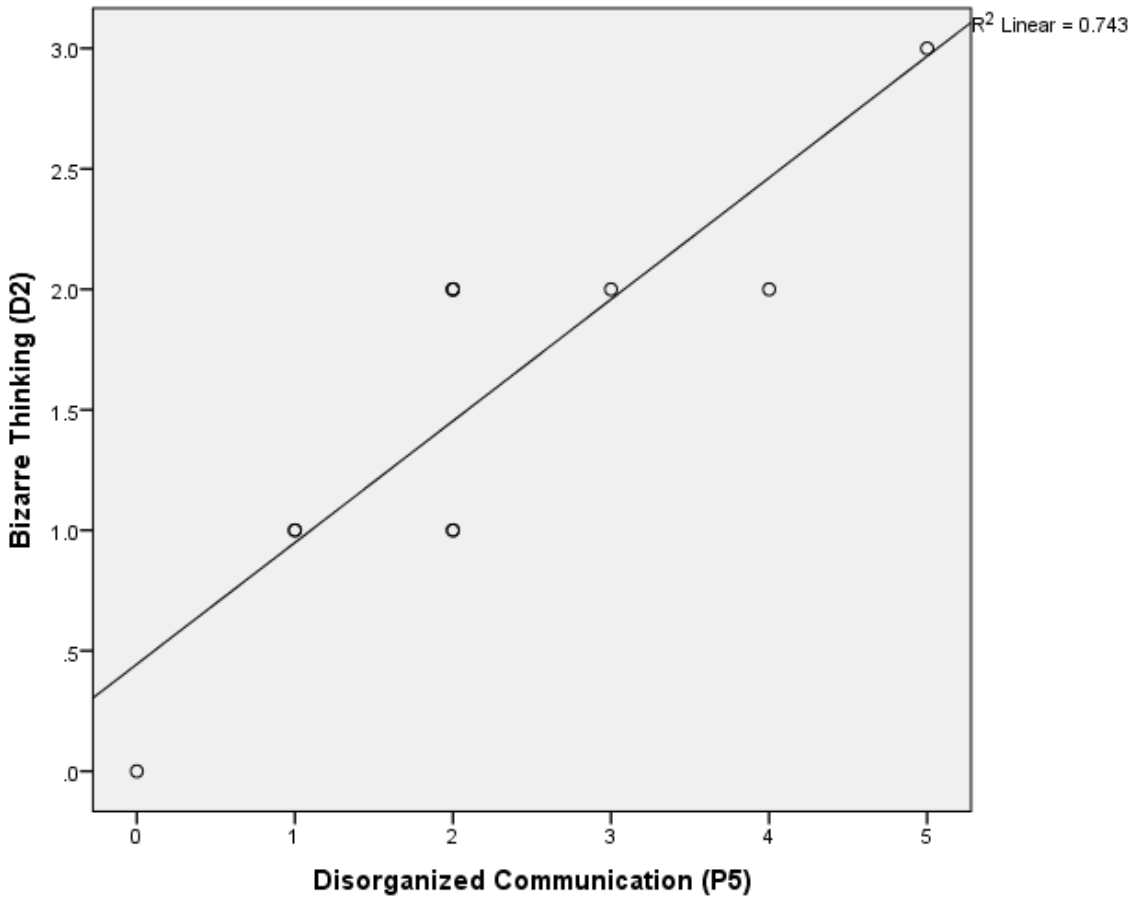
Full Sample

	P1	P2	P3	P4	P5
P1	-				
P2	.575***	-			
P3	.287*	.161	-		
P4	.542***	.455***	.184	-	
P5	.139	.194	.019	.119	-

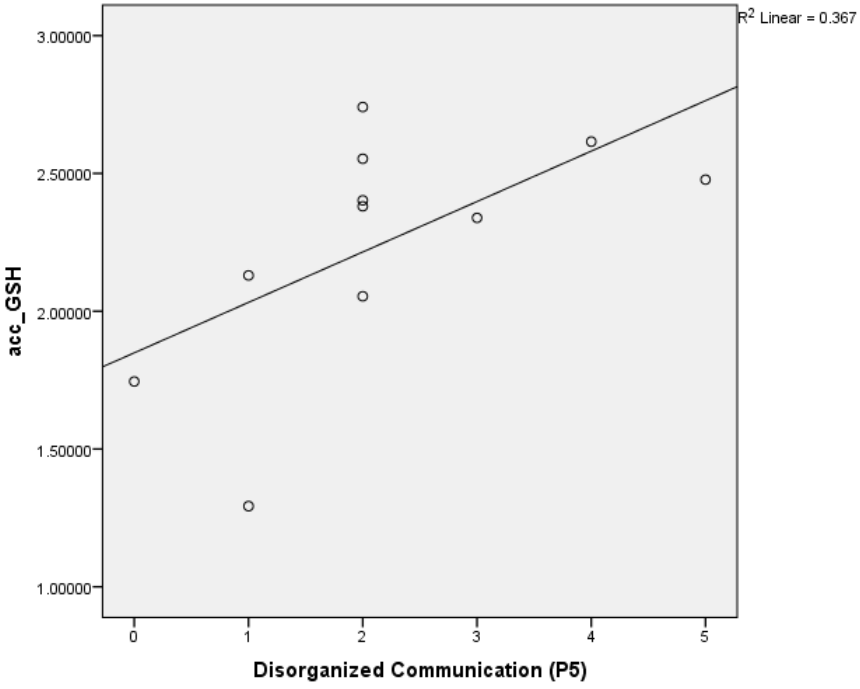
+ $p < .10$; * $p < .05$; ** $p < .01$; *** $p < .001$

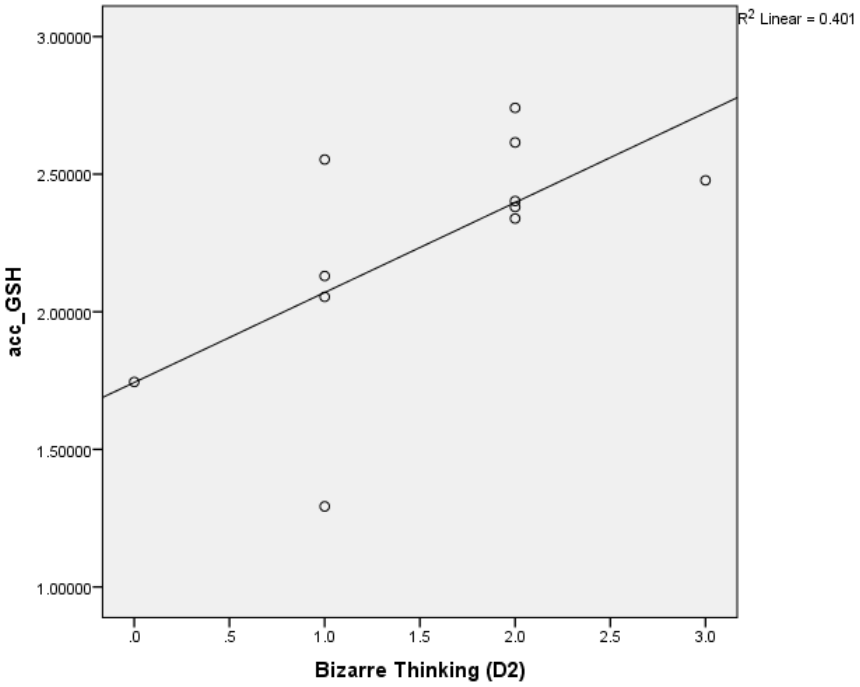
Note: n=57 individuals who met criteria for psychosis (12), a psychosis risk syndrome (42), or schizotypal personality disorder (3) on the SIPS

Appendix E: Scatterplot of the relation between Disorganized Communication and Bizarre Thinking



Appendix F: Scatterplot of Disorganized Symptoms and Glutathione in the ACC





References

Alger, J.R. (2010). Quantitative Proton Magnetic Resonance Spectroscopy and Spectroscopic Imaging of the Brain: A Didactic Review. *Topics in Magnetic Resonance Imaging, 21 (2), 115-128.* doi:10.1097/RMR.0b013e31821e568f

Ambrosini, P.J. (2000). Historical Development and Present Status of the Schedule for Affective Disorders and Schizophrenia for School-Age Children (K-SADS). *Journal of the American Academy of Child and Adolescent Psychiatry, 39,* 49-58.

Ballmaier, M., Schlagenhauf, F., Toga, A., Gallinat, J., Koslowski, M., Zoli, M. … Heinz, A. (2008). Regional patterns and clinical correlates of basal ganglia morphology in non-medicated schizophrenia. *Schizophrenia Research, 106,* 140-147. doi:10.1016/j.schres.2008.08.025.

Bartha, R., Williamson, P. C., Drost, D. J., Malla, A., Carr, T. J., Cortese, L., … Neufeld, R. W. J. (1997). Measurement of glutamate and glutamine in the medial prefrontal cortex of never-treated schizophrenic patients and healthy controls by proton magnetic resonance spectroscopy. *Archives of General Psychiatry, 54,* 959-965.

Bartha, R., Al-Semaan, Y. M., Williamson, P. C., Drost, D. J., Malla, A. K., Carr, T. J., … Neufeld, R. W. J. (1999). A short echo proton magnetic resonance spectroscopy study of the left mesial-temporal lobe in first-onset schizophrenic patients. *Biological Psychiatry, 45,* 1403-1411.

Bear, M., Connors, B., & Paradiso, M. (2001). *Neurotransmitter systems.* In Neuroscience. Exploring the Brain (2nd ed), (pp 131-162), Baltimore, MD:

Lippincott, Williams, & Wilkens.

Brugger, S., Davis, J. M., Leucht, S., & Stone, J. M. (2011). Proton magnetic resonance spectroscopy and illness stage in schizophrenia--A systematic review and meta-analysis. *Biological Psychiatry, 69,* 495-503. doi: 10.1016/j.biopsych.2010.10.004.

Bustillo, J. R., Rowland, L. M., Mullins, P., Jung, R., Chen, H., Qualls, C., … Lauriello, J. (2010). 1 H-MRS at 4 Tesla in minimally treated early schizophrenia. *Molecular Psychiatry, 15(6),* 629-636. doi:10.1038/mp.2009.121.

Bustillo, J. R., Chen, H., Jones, T., Lemke, N., Abbott, C., Qualls, C. … Gasparovic, C. (2014). Increased glutamine in patients undergoing long-term treatment for schizophrenia: A proton magnetic resonance spectroscopy study at 3T. *JAMA Psychiatry, 71,* 265-72. doi: 10.1001/jamapsychiatry.2013.3939.

Cannon, T. D., Cadenhead, K., Cornblatt, B., Woods, S. W., Addington, J., Walker, E., et al. (2008). Prediction of psychosis in youth at high clinical risk: A multisite longitudinal study in North America. *Archives of General Psychiatry, 65,* 28-37. doi: 10.1001/archgenpsychiatry.2007.3.

Catts, V., Fung, S., Long, L., Joshi, D., Vercammen, A., Allen, K., … Weickert, C. (2013). Rethinking schizophrenia in the context of normal neurodevelopment. *Frontiers in Cellular Neuroscience, 7,* 1-27. doi: 10.3389/fncel.2013.00060.

Chang, L., Friedman, J., Ernst, T., Zhong, K., Tsopelas, N. D., & Davis, K. (2007). Brain metabolite abnormalities in the white matter of elderly schizophrenic subjects: Implication for glial dysfunction. *Biological Psychiatry, 62,* 1396-

1404.

Choe, B. Y., Kim, K. T., Suh, T. S., Lee, C., Paik, I. H., Bahk, Y. W., … Lenkinski, R. E. (1994). 1H magnetic resonance spectroscopy characterization of neuronal dysfunction in drug-naive, chronic schizophrenia. *Academic Radiology, 1(3),* 211-216.

Clark, J. F., Doepke, A., Filosa, J. A., Wardle, R. L., Lu, A., Meeker, T. J., & Pyne-Geithman, G. J. (2006). N-acetylaspartate as a reservoir for glutamate. *Medical Hypotheses, 67(3*): 506-12.

Cohen, J. (1983). The cost of dichotomization. *Applied Psychological Measurement, 7,* 249-253. doi:10.1177/014662168300700301.

Cornblatt, B., Lencz, T., Smith, C., Correll, C., Auther, A., & Nakayama, E. (2003). The schizophrenia prodrome revisited: A neurodevelopmental perspective. *Schizophrenia Bulletin, 29,* 633-651.

Cornblatt, B., Carrion, R. E., Auther, A., McLaughlin, D., Olsen, R. H., John, M. & Correll, C. U. (2015). Psychosis prevention: A modified clinical high risk perspective from the Recognition and Prevention (RAP) Program. *American Journal of Psychiatry*. doi: 10.1176/appi.ajp.2015.13121686.

Correll, C. U., Hauser, M., Auther, A. M., & Cornblatt, B. A. (2010). Research in people with psychosis risk syndrome: A review of the current evidence and future directions. *Journal of Child Psychology and Psychiatry, 51,* 390-431. doi: 10.1111/j.1469-7610.2010.02235.

Coughlin, J.M., Tanaka, T., Marsman, A., Wang, H., Bonekamp, S., Kim, P.K. … Sawa, A. (2015). Decoupling of N-Acetyl-Aspartate and glutamate within the

dorsolateral prefrontal cortex in schizophrenia. *Current Molecular Medicine, 15,* 176-183.

de la Fuente-Sandoval, C., León-Ortiz, P., Favila, R., Stephano, S., Mamo, D., Ramírez-Bermúdez, J., & Graff-Guerrero, A. (2011). Higher levels of glutamate in the associative-striatum of subjects with prodromal symptoms of schizophrenia and patients with first-episode psychosis. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 36,* 1781-1791. doi:10.1038/npp.2011.65.

de la Fuente-Sandoval, C., Leon-Ortiz, P., Azcarraga, M., Favila, R., Stephano, S., & Graff-Guerrero, A. (2012). Striatal glutamate and the conversion to psychosis: A prospective ^{1}H-MRS imaging study. *International Journal of Neuropsychopharmacology*, 1-5. doi:10.1017/S1461145712000314.

De Silva, P. N. (2011). Does the association with diabetes say more about schizophrenia and its treatment?--The GLUT hypothesis. *Medical Hypotheses, 77,* 529-531. doi: 10.1016/j.mehy.2011.06.022.

Do, K. Q., Trabesinger, A. H., Kirsten-Krüger, M., Lauer, C. J., Dydak, U., Hell, D., … Cuénod, M. (2000). Schizophrenia: Glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. *European Journal of Neuroscience, 12,* 3721-3728.

Duarte, J. M., Lei, H., Mlynarik, V., & Gruetter, R. (2012). The neurochemical profile quantified by in vivo ^{1}H NMR spectroscopy. *NeuroImage, 61,* 342-362. doi:10.1016/j.neuroimage.2011.12.038.

Faul, F., Erdfelder, E., Buchner, A., & Lang, A.-G. (2009). Statistical power analyses

using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods, 41,* 1149-1160.

Frazier, T., Demeter, C., Youngstrom, E., Calabrese, J., Stansbrey, R., McNamara, N., & Findling, R. (2007). Evaluation and comparison of psychometric instruments for pediatric bipolar spectrum disorders in four age groups. *Journal of Child And Adolescent Psychopharmacology, 17,* 853-866. doi:10.1089/cap.2007.0057.

Fusar-Poli, P., Stone, J. M., Broome, M. R., Valli, I., Mechelli, A., McLean, M. A., … McGuire, P. K. (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(9),* 881-890.

Fusar-Poli, P., Bonoldi, I., Yung, A. R., Borgwardt, S., Kempton, M. J., Valmaggia, L. … McGuire, P. (2013). Predicting psychosis: Meta-analysis of transition outcomes in individuals at high clinical risk. *Archives of General Psychiatry, 69,* 220-229.

Galinska, B., Szulc, A., Tarasow, E., Kubas, B., Dzienis, W., Czernikiewicz, A., & Walecki, J. (2009). Duration of untreated psychosis and proton magnetic resonance spectroscopy (1 H-MRS) findings in first-episode schizophrenia. *Medical Science Monitor, 15(2),* CR82-88.

Geurts, J.J., Barkhof, F., Castelijns, J.A., Uitdehaag, B.M., Polman, C.H., & Pouwels, P.J. (2004). Quantitative 1H-MRS of healthy human cortex, hippocampus, and thalamus: metabolite concentrations, quantification precision, and reproducibility. *Journal of Magnetic Resonance Imaging, 20,* 366-71.

Howes O., & Kapur S. (2009). The dopamine hypothesis of schizophrenia: Version III–the final common pathway. *Schizophrenia Bulletin, 35,* 549–562.

Javitt D.C. (2012). Twenty-five years of glutamate in schizophrenia: Are we there yet? *Schizophrenia Bulletin, 38,* 911-913.

Jung, W. H., Jang, J. H., Byun, M. S., An, S. K., & Kwon, J. S. (2010). Structural brain alterations in individuals at ultra-high risk for psychosis: A review of magnetic resonance imaging studies and future directions. *Journal of Korean Medical Science, 25*, 1700-1709. doi: 10.3346/jkms.2010.25.12.1700.

Kaiser, L. G., Schuff, N., Cashdollar, N., & Weiner, M. W. (2005). Age-related glutamate and glutamine concentration changes in normal human brain: 1H MR spectroscopy study at 4 T. *Neurobiology of Aging, 26,* 665-672.

Kantrowitz, J. T., & Javitt, D. C. (2010). Thinking glutamatergically: Changing concepts of schizophrenia based upon changing neurochemical models. *Clinical Schizophrenia & Related Psychoses, 4(3),* 189-200. doi: 10.3371/CSRP.4.3.6.

Kaufman, J., & Schweder, A. E. (2004). The Schedule for Affective Disorders and Schizophrenia for School-Age Children: Present and Lifetime version (K-SADS-PL). In M. J. Hilsenroth, D. L. Segal (Eds.), Comprehensive handbook of psychological assessment, Vol. 2: Personality assessment (pp. 247-255). Hoboken, NJ US: John Wiley & Sons Inc.

Keshavan, M. S., Berger, G., Zipursky, R. B., Wood, S. J., & Pantelis, C. (2005). Neurobiology of early psychosis. *The British Journal of Psychiatry, 187,* 8-18. doi:10.1192/bjp.187.48.s8.

Keshavan, M. S., Dick, R. M., Diwadkar, V. A., Montrose, D. M., Prasad, K. M., & Stanley, J. A. (2009). Striatal metabolic alterations in non-psychotic adolescent offspring at risk for schizophrenia: A (1)H spectroscopy study. *Schizophrenia Research, 115*, 88-93. doi: 10.1016/j.schres.2009.08.012.

Kessler, R., & Bromet, E. (2013). The epidemiology of depression across cultures. *Annual Review of Public Health*, 34119-138. doi:10.1146/annurev-publhealth-031912-114409

Kim, J.H., Chang, K.H., Na, D.G., Song, I.C., Kim, S.J., Kwon, B.J., & Han, M.H. (2006). Comparison of 1.5T and 3T 1H MR spectroscopy for human brain tumors. *Korean Journal of Radiology, 7,* 156-61.

Klöppel, S., Abdulkadir, A., Jack, C., Koutsouleris, N., Mourao-Miranda, J., & Vemuri, P. (2012). Diagnostic neuroimaging across diseases. *Neuroimage, 61,* 457-463.

Konradi, C., & Heckers, S. (2003). Molecular aspects of glutamate dysregulation: Implications for schizophrenia and its treatment. *Pharmacology & Therapeutics, 97,* 153-179.

Kraguljac, N.V., Reid, M.A., White, D.M., den Hollander, J., & Lahti, A.C. (2012). Regional decoupling of N-acetyl-aspartate and glutamate in schizophrenia. *Neuropsychopharmacology, 37,* 2635-2642.

Kritis, A. A., Stamoula, E. G., Paniskaki, K. A., & Vavilis, T. D. (2015). Researching glutamate – induced cytotoxicity in different cell lines: A comparative/collective analysis/study. *Frontiers in Cellular Neuroscience, 9,* 1-18. doi: 10.3389/fncel.2015.00091.

Krystal, J.H., D'Souza, D.C., Mathalon, D., Perry, E., Belger, A., & Hoffman, R. (2003). NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: Toward a paradigm shift in medication development. *Psychopharmacology, 169*, 215-233.

Laruelle, M., & Abi-Dargham, A. (1999). Dopamine as the wind of the psychotic fire: New evidence from brain imaging studies. *Journal of Psychopharmacology, 13*, 358-371.

Laruelle, M., Abi-Dargham, A., Gil, R., Kegeles, L., & Innis, R. (1999). Increased dopamine transmission in schizophrenia: Relationship to illness phases. *Biological Psychiatry, 46*, 56-72.

Laruelle, M., Kegeles, L. S., & Abi-Dargham, A. (2003). Glutamate, dopamine, and schizophrenia: From pathophysiology to treatment. Annals of the New York Academy of Sciences, 1003, 138-158.

Laruelle, M., Frankle, W. G., Narendran, R., Kegeles, L. S., & Abi-Dargham, A. (2005). Mechanism of action of antipsychotic drugs: From dopamine D(2) receptor antagonism to glutamate NMDA facilitation. *Clinical Therapeutics, 27*, 16-24.

Lezak, M., Howieson, D. & Loring, D. (2004). *The behavioral geography of the brain.* In Neuropsychological Assessment (4th ed), (pp. 39 – 85) New York, NY: Oxford University Press.

Lutkenhoff, E. S., van Erp, T. G., Thomas, M. A., Therman, S., Manninen, M., Huttunen, M. O., … Cannon, T. D. (2010). Proton MRS in twin pairs discordant for schizophrenia. *Molecular Psychiatry, 15*, 308-318.

Lyon, G.J., Abi-Dargham, A., Moore, H., Lieberman, J.A., Javitch, J.A., & Sulzer, D. (2011). Presynaptic regulation of dopamine transmission in schizophrenia. *Schizophrenia Bulletin, 37,* 108-117. doi: 10.1093/schbul/sbp010.

Maddock, R. J., & Buonocore, M. H. (2012). MR spectroscopic studies of the brain in psychiatric disorders. *Current Topics in Behavioral Neurosciences, 11,* 199-251. doi: 10.1007/7854_2011_197.

Makinen, J., Miettunen, J., Isohanni, M., & Koponen, H. (2008). Negative symptoms in schizophrenia: A review. *Nordic Journal of Psychiatry, 62,* 334-341. doi: 10.1080/08039480801959307.

Marshall, M., Lewis, S., Lockwood, A., Drake, R., Jones, P., & Croudace, T. (2005). Association Between Duration of Untreated Psychosis and Outcome in Cohorts of First-Episode Patients. *Archives of General Psychiatry, 62,* 975-983. doi:10.1001/archpsyc.62.9.975.

Marsman, A., van den Heuvel, M. P., Klomp, D. W., Kahn, R. S., Luijten, P. R., & Hulshoff Pol, H. E. (2011). Glutamate in schizophrenia: a focused review and meta-analysis of (1)H-MRS studies. *Schizophrenia Bulletin, 39,* 120-129. doi: 10.1093/schbul/sbr069.

Matsuzawa, D., Obata, T., Shirayama, Y., Nonaka, H., Kanazawa, Y., Yoshitome, E., … Hashimoto, K. (2008). Negative correlation between brain glutathione level and negative symptoms in schizophrenia: A 3T 1H-MRS study. *PloS One, 3(4),* 1-6. doi: 10.1371/journal.pone.0001944.

McDonald, J., & Johnston, M. (1990). Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Research Reviews, 15,* 41-70.

McGlashan, T., Walsh, B., & Woods, S. (2010). *The psychosis-risk syndrome.* New York, NY: Oxford University Press.

Melle, I., Larsen, T.K., Haahr, U., Friis, S., Johannessen, J.O., Opjordsmoen, S., … McGlashan, T. (2004). Reducing the duration of untreated first-episode psychosis. *Archives of General Psychiatry, 61,* 143-50.

Miller, T. J., McGlashan, T. H., Rosen, J. L., Somjee, L., Markovich, P. J., Stein, K., & Woods, S.W. (2002). Prospective diagnosis of the initial prodrome for schizophrenia based on the Structured Interview for Prodromal Syndromes: Preliminary evidence of interrater reliability and predictive validity. *American Journal of Psychiatry, 159*, 863-865.

Miller, T. J., McGlashan, T. H., Rosen, J. L., Cadenhead, K., Cannon, T., Ventura, J., ... Woods, S. W. (2003). Prodromal assessment with the structured interview for prodromal syndromes and the scale of prodromal symptoms: predictive validity, interrater reliability, and training to reliability. *Schizophrenia Bulletin, 29,* 703-715.

Moffett J., Ross B., Arun P., Madhavarao, C., & Namboodiri, M. (2007). N-Acetylaspartate in the CNS: From neurodiagnostics to neurobiology. *Progress in Neurobiology, 81,* 89–131.

Møller, P., & Husby, R. (2000). The initial prodrome in schizophrenia: Searching for naturalistic core dimensions of experience and behavior. *Schizophrenia Bulletin, 26,* 217-232.

Mullins, P.G., Rowland, L., Bustillo, J., Bedrick, E.J., Lauriello, J., & Brooks, W.M. (2003). Reproducibility of 1H-MRS measurements in schizophrenic patients. *Magnetic Resonance in Medicine, 50,* 704-7.

Narrow, W.E., Rae, D. S., Robins, L. N., Regier, D. A. (2002). Revised prevalence estimates of mental disorders in the United States: Using a clinical significance criterion to reconcile 2 surveys estimates. *Archives of General Psychiatry, 59,* 115-123.

Norman, R.M.G., Malla, A.K., Verdi, M.B., Hassall, L.D., & Fazekas, C. (2004). Understanding delay in treatment for first-episode psychosis. *Psychological Medicine, 34,* 255-266.

Olney, J.W., & Farber, N.B. (1995). Glutamate receptor dysfunction and schizophrenia. *Archives of General Psychiatry, 52,* 998-1007.

Olney, J.W., Newcomer, J.W., & Farber, N.B. (1999). NMDA receptor hypofunction model of schizophrenia. *Journal of Psychiatric Research, 33,* 523-533.

O'Neill, J. O., Levitt, J., Caplan, R., Asarnow, R., McCracken, J. T., Toga, A. W., & Alger, J. R. (2004). 1H MRSI evidence of metabolic abnormalities in childhood-onset schizophrenia. *NeuroImage, 21,* 1781-1789.

Öngür, D., Jensen, J. E., Prescot, A. P., Stork, C., Lundy, M., Cohen, B. M., & Renshaw, P. F. (2008). Abnormal glutamatergic neurotransmission and neuronalglial interactions in acute mania. *Biological Psychiatry, 64(8),* 718-

726. doi:10.1016/j.biopsych.2008.05.014.

Pantelis, C., Velakoulis, D., Wood, S. J., Yucel, M., Yung, A. R., Phillips, L. J., et al. (2007). Neuroimaging and emerging psychotic disorders: The Melbourne ultra-high risk studies. *International Review of Psychiatry, 19*, 371-381.

Paus, T., Keshavan, M., & Giedd, J.N. (2008). Why do many psychiatric disorders emerge during adolescence? *Nature Reviews, Neuroscience, 9,* 947-57. doi: 10.1038/nrn2513

Provencher SW. (2001) Automatic quantitation of localized in vivo 1H spectra with LCModel. NMR in Biomedicine,14, 260-264.

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

Rapoport, J., Giedd, J., & Gogtay, N. (2012). Neurodevelopmental model of schizophrenia: Update 2012. *Molecular Psychiatry, 17,* 1228-38. doi: 10.1038/mp.2012.23.

Reid, M. A., Stoeckel, L. E., White, D. M., Avsar, K. B., Bolding, M. S., Akella, N. S., … Lahti, A. C. (2010). Assessments of function and biochemistry of the anterior cingulate cortex in schizophrenia. *Biological Psychiatry, 68(7),* 625-633. doi:10.1016/j.biopsych.2010.04.013.

Röpcke, B. & Eggers, C. (2005). Early-onset schizophrenia: a 15-year follow-up. *European Child & Adolescent Psychiatry, 14,* 341-50.

Rowland, L., Bustillo, J., Mullins, P., Jung, R., Lenroot, R., Landgraf, E., et al., (2005). Effects of Ketamine on Anterior Cingulate Glutamate Metabolism in Healthy Humans: A 4-T Proton MRS Study. *American Journal of Psychiatry, 162,* 394-396.

Rowland, L. M., Spieker, E. A., Francis, A., Barker, P. B., Carpenter, W. T., & Buchanan, R. W. (2009). White matter alterations in deficit schizophrenia. *Neuropsychopharmacology, 34(6),* 1514-1522. doi:10.1038/npp.2008.207.

Rowland, L., Kontson, K., West, J., Edden, R., Zhu, H., Wijtenburg, A., Holcomb, H., & Barker, P. (2012). In vivo measurements of glutamate, GABA, and NAAG in schizophrenia. *Schizophrenia Bulletin,* epub ahead of print. doi: 10.1093/schbul/sbs092.

Shirayama, Y., Obata, T., Matsuzawa, D., Nonaka, H., Kanazawa, Y., Yoshitome, E., … Iyo, M. (2010). Specific metabolites in the medial prefrontal cortex are associated with the neurocognitive deficits in schizophrenia: A preliminary study. *NeuroImage, 49,* 2783-2790.

Schwarz, N. (1999). Self-reports. *American Psychologist, 54,* 93-105.

Schwartz, T. L., Sachdeva, S., & Stahl, S. M. (2012). Glutamate neurocircuitry: Theoretical underpinnings in schizophrenia. *Frontiers in Pharmacology, 3,* 195. doi: 10.3389/fphar.2012.00195.

Simon, A., Velthorst, E. Nieman, D., Linszen, D., Umbricht, D. & de Haan, L. (2011). Ultra high-risk state for psychosis and non-transition: A systematic review. *Schizophrenia Research, 132,* 8-17. doi:10.1016/j.schres.2011.07.002.

Stanley, J. A. (2002). In vivo magnetic resonance spectroscopy and its application to

neuropsychiatric disorders. *Canadian Journal of Psychiatry, 47,* 315-326.

Steullet, P., Neijt, H. C., Cuenod, M., & Do, K. Q. (2006). Synaptic plasticity impairment and hypofunction of NMDA receptors induced by glutathione deficit: Relevance to schizophrenia. *Neuroscience, 137,* 807-819. doi:10.1016/j.neuroscience.2005.10.014

Stone, J.M., Day, F., Tsagaraki, H., Valli, I., McLean, M.A., Lythgoe, D.J. … McGuire, P.K. (2009). Glutamate dysfunction in people with prodromal symptoms of psychosis: Relationship to gray matter volume. *Biological Psychiatry, 66,* 533–539.

Stone, J. M., Howes, O. D., Egerton, A., Kambeitz, J., Allen, P., Lythgoe, D. J., et al. (2010). Altered relationship between hippocampal glutamate levels and striatal dopamine function in subjects at ultra high risk of psychosis. *Biological Psychiatry, 68,* 599-602. doi: 10.1016/j.biopsych.2010.05.034.

Tandon, R., Keshavan, M., & Nasrallah, H. (2008). Schizophrenia, "just the facts" what we know in 2008. 2. Epidemiology and etiology. *Schizophrenia Research, 102,* 1-18.

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

Theberge, J., Bartha, R., Drost, D. J., Menon, R. S., Malla, A., Takhar, J., … Williamson, P. C. (2002). Glutamate and glutamine measured with 4.0 T proton MRS in never-treated patients with schizophrenia and healthy

volunteers. *American Journal of Psychiatry, 159,* 1944-1946.

Theberge, J., Al-Semaan, Y., Williamson, P. C., Menon, R. S., Neufeld, R. W. J., Rajakumar, N., … Drost, D. J. (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., Williamson, K., Aoyama, N., Drost, D., Manchanda, R., Malla, A. … Williamson, P. (2007). Longitudinal grey-matter and glutamatergic losses in first-episode schizophrenia. *British Journal of Psychiatry, 191,* 325-334. doi: 10.1192/bjp.bp.106.033670.

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.

Tsai G., Passani L. A., Slusher, B. S., Carter, R., Baer, L., Kleinman, J. E., & Coyle, J. T. (1995). Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. *Archives of General Psychiatry, 52,* 829–836.

Tuominen, H. J., Tiihonen, J., & Wahlbeck, K. (2005). Glutamatergic drugs for schizophrenia: A systematic review and meta-analysis. *Schizophrenia Research, 72,* 225-34.

Venkatraman, T. N., Hamer, R. M., Perkins, D. O., Song, A. W., Lieberman, J. A., & Steen, R. G. (2006). Single-voxel 1H PRESS at 4.0 T: Precision and variability of measurements in anterior cingulate and hippocampus. *NMR in Biomedicine, 19,* 484-491.

Vidal, C. N., Rapoport, J. L., Hayashi, K. M., Geaga, J. A., Sui, Y., McLemore, L. E. … Thompson, P. M. (2006). Dynamically spreading frontal and cingulate deficits mapped in adolescents with schizophrenia. *Archives of General Psychiatry, 63,* 25-34.

Wigman, J. W., van Nierop, M., Vollebergh, W. M., Lieb, R., Beesdo-Baum, K., Wittchen, H., & van Os, J. (2012). Evidence that psychotic symptoms are prevalent in disorders of anxiety and depression, impacting on illness onset, risk, and severity-Implications for diagnosis and ultra–high risk research. *Schizophrenia Bulletin, 38,* 247-257. doi:10.1093/schbul/sbr196.

Wood, S. J., Yuecel, M., Wellard, R. M., Harrison, B. J., Clarke, K., Fornito, A., … Pantelis, C. (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.

Wu, E. Q., Birnbaum, H. G., Shi, L., Ball, D. E., Kessler, R. C., Moulis, M., et al. (2005). The economic burden of schizophrenia in the United States in 2002. *Journal of Clinical Psychiatry, 66,* 1122-1129.

Wu, E. Q., Shi, L., Birnbaum, H., Hudson, T., & Kessler, R. (2006). Annual prevalence of diagnosed schizophrenia in the USA: A claims data analysis approach. *Psychological Medicine, 36,* 1535-1540. doi:10.1017/S0033291706008191.

Wyatt, R.J., & Henter, I. (2001). Rationale for the study of early intervention. *Schizophrenia Research, 51,* 69-76.

Yoo, S.Y., Yeon, S., Choi, C., Kang, D., Lee, J., Shin, N.Y., … Kwon, J.S. (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,* 86-93. doi:10.1016/j.schres.2009.03.036.