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In Vivo Assessment of Neurotransmitters and Modulators with Magnetic Resonance Spectroscopy: Application to Schizophrenia

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Abstract

In vivo measurement of neurotransmitters and modulators is now feasible with advanced proton magnetic resonance spectroscopy (¹H-MRS) techniques. This review provides a basic tutorial of MRS, describes the methods available to measure brain glutamate, glutamine, γ -aminobutyric acid, glutathione, *N*-acetylaspartylglutamate, glycine, and serine at magnetic field strengths of 3Tesla or higher, and summarizes the neurochemical findings in schizophrenia. Overall, ¹H-MRS holds great promise for producing biomarkers that can serve as treatment targets, prediction of disease onset, or illness exacerbation in schizophrenia and other brain diseases.

Keywords

magnetic resonance spectroscopy; glutamate; glutathione; GABA; schizophrenia

1.0 Introduction

Proton magnetic resonance spectroscopy (¹H-MRS) is a noninvasive technique that allows the quantification of certain biochemical concentrations *in vivo*. In the brain, these biochemicals reflect a wide variety of mechanisms that range from cellular function and viability to neurotransmission. The information provided by ¹H-MRS is useful for researchers to understand pathological processes and treatment effects in brain diseases such

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as schizophrenia. The majority of ^1H -MRS studies of schizophrenia measured *N*-acetylaspartate (NAA, a marker of neuronal viability and function), choline + phosphocholine + glycerophosphocholine or choline-containing compounds (Cho, reflective of membrane turnover), and creatine+phosphocreatine (Cr, an index of energy metabolism), simply because these biochemicals yield the most prominent peaks in the ^1H spectrum and therefore are the easiest to quantify. The consistent finding from ^1H -MRS studies in schizophrenia is reduced frontal and temporal lobe NAA (Rowland et al., 2001; Steen et al., 2005; Brugger et al., 2011), plausibly reflecting neuronal dysfunction in these brain regions.

There are several neurochemicals that could prove fruitful in the elucidation of the pathophysiological processes that underlie schizophrenia and have potential to serve as biomarkers for treatment targets, prediction of disease onset, or exacerbation. However, reliable measurement of these biochemicals by ^1H -MRS poses technical challenges because of their low concentrations and low sensitivity due to J-coupling which results in multiplet peaks in the spectrum as well as spectral overlap. Figure 1 provides an illustration of a proton spectrum and corresponding metabolites. Major advances have been made in measuring these biochemicals using ^1H -MRS, and these advances include higher sensitivity and spectral dispersion due to higher magnetic field strengths, greater reliability due to improved MR scanner hardware, new optimized pulse sequences for spectral acquisition, and improved spectral quantification algorithms and commercial software packages. This review will focus on the latest single voxel ^1H -MRS techniques and their applications for measuring such biochemicals as glutamate (Glu), glutamine (Gln), glutathione (GSH), γ -aminobutyric acid (GABA), *N*-acetylaspartylglutamate (NAAG), glycine, and serine in schizophrenia at field strengths of 3T or higher. Although this review focuses on schizophrenia, these ^1H -MRS techniques would be useful in other psychiatric and neurological disorders.

2.0 ^1H -MRS Overview

2.1. ^1H -MRS Signal

^1H -MRS uses the same hardware equipment as standard magnetic resonance imaging (MRI), in which the radiofrequency (RF) coils and receiver channels are all tuned to the proton (^1H) resonance frequency. The external magnetic field generated by the MR scanner is denoted by “ B_0 ”, and the strength typically ranges from 1.5 – 3 Tesla for clinical settings. An explanation of the basic magnetic resonance (MR) principles can be found in (Haacke et al., 1999; Liang et al., 2000; Brown and Semelka, 2010) while a detailed explanation of the principles of ^1H -MRS can be found in (Drost et al., 2002; Zhu and Barker, 2011; De Graaf, 2007). NAA, Cho, and Cr yield the strongest signals in a normal ^1H spectrum of the human brain, and therefore have been most extensively studied using ^1H -MRS. However, with higher magnetic field strengths, glutamate and glutamine that have complex spectral patterns due to coupling between their ^1H nuclei can also be quantified using short echo time (TE) pulse sequences (Gruetter et al., 1998; Petroff et al., 2000; Deelchand et al., 2010; Mекle et al., 2009; Wijtenburg and Knight-Scott, 2011; Mullins et al., 2008) or specifically tailored pulse sequences (Hurd et al., 2004; Schubert et al., 2004; Yang et al., 2008).

2.2 Spectroscopic Localization Techniques

There are two main types of spectroscopic acquisition techniques, single-voxel and spectroscopic imaging (SI) also known as chemical shift imaging (CSI). With single-voxel spectroscopy, the signal is acquired from a 3-dimensional volume called a “voxel” in a region of interest to produce a single spectrum. In contrast, SI is a combination of spectroscopy and imaging, whereby spectral information is acquired in a spatial matrix with corresponding spectra in multiple voxels. There are advantages and disadvantages to both techniques. Single-voxel spectroscopy is ideal for hypothesis-driven questions about specific regions, and to quantify metabolites that have short T_2 relaxation or strongly coupled spin systems such as glutamate, glutamine, and GABA. Commonly used non-editing acquisition sequences include the Stimulated Echo Acquisition Mode (STEAM) and Point Resolved Excitation Spin-echo Sequence (PRESS) sequences (Frahm et al., 1987; Bottomley, 1987). There are two editing sequences commonly used to detect GABA: 2D JPRESS (Ryner et al., 1995) and the more frequently used Mescher-Garwood (MEGA)-PRESS sequence (Mescher et al., 1998). MEGA-PRESS combines the frequency-selective editing technique (MEGA) with a PRESS sequence and is employed for spectral editing of the metabolites with J -coupled spin systems, such as GABA (Terpstra et al., 2002), GSH (Terpstra et al., 2003), and NAAG (Edden et al., 2007). In recent years, improved editing efficiency of the traditional MEGA-PRESS sequence has been reported at higher magnetic fields (Edden and Barker, 2007; Kaiser et al., 2007). Compared to single voxel sequences, the spatial resolution is superior with SI and thus allows the investigator to assess more brain regions and smaller voxels that may allow a distinction between gray and white matter. However, SI is generally limited to the measurement of metabolites with longer T_2 relaxation such as NAA, Cho, and Cr. Metabolites with shorter T_2 s may not be visible using long TE acquisition, which is typical for SI applications. Recent advances in the field do include the applications of short TE SI sequences (Otazo et al., 2007; Gruber et al., 2008), and for more detailed information regarding SI sequences, see (Posse et al., 2013; Zhu and Barker, 2011).

2.3 Spectroscopic Data Considerations

There are several important considerations for the evaluation and interpretation of proton spectroscopic data that include shimming, metabolite quantification, and reliability/reproducibility. An important factor when acquiring spectroscopic data is shimming. Shimming is the act of adjusting the currents of the shim coils in order to make the magnetic field homogeneous in the region of interest (ROI). A smaller line width is always desirable since it translates to better spectral resolution and therefore improved quantification. Often on 3T MR systems, a water peak line width of 12-Hz or less is most desirable, which indicates the acquired spectrum may be of sufficient quality for good quantification. It is common to report the shimming cutoff for inclusion in the data set.

Quantification is typically performed using commercially available software packages such as LCModel or jMRUI (Provencher, 1993; Ratiney et al., 2004) or in-house written programs. A common metric for the goodness of spectral fit is the Cramer Rao Lower Bounds (CRLB). While there is no set standard for CRLB cut-off, CRLBs less than or equal to 20% are generally considered to be of acceptable quality (Cavassila et al., 2001; Provencher, 2014). Generally, metabolites with lower concentrations have higher CRLBs

due to the difficulty in separating the signal from other signals. This difficulty may lead to exclusion of data that may potentially bias the results. In these instances, a CRLBs cut-off greater than 20% such as 25% or 30% may be used as a compromise. Another consideration in quantification is that metabolites must be quantified with respect to a reference. The voxel water and Cr peaks are commonly used as references. The advantage of using Cr as a reference is that it enables comparisons to other studies since utilizing a ratio to Cr removes confounds from the data such as coil loading, scaling factors, and CSF proportion in the voxel. One disadvantage of using Cr as a reference is that Cr may be different across disease states including schizophrenia (Ongur et al., 2009) and changes with age (Danielsen and Ross, 1999). Therefore, alterations in metabolites that are referenced to Cr could actually be due to changes in Cr but not in the metabolite of interest. The use of water as a reference is preferable when metabolites such as NAA and Cr, often used in referencing, vary in different illnesses. There are two main methods for referencing to water that result in relative concentrations in institutional units or absolute concentrations in mmol/kg (Jansen et al., 2006). Relative concentrations are generated when each metabolite is referenced to the entire signal from the water in the voxel whereas absolute concentrations using an internal reference take into account the proportion of the water signal exclusively from the tissue compartment as well as the concentration of water and density of tissue types. There are advantages and disadvantages to water referencing (for review see (Gasparovic et al., 2006; Mullins et al., 2014)). Advantages include the ability to utilize the water reference for eddy-current correction and retention of all metabolite information since one metabolite is used in the ratio denominator resulting in lost information. Disadvantages of utilizing water as a reference include that water is acquired during a separate scan from the metabolites, potentially introducing patient motion into the dataset and increasing scan times, and that the water content can change with different pathologies such as multiple sclerosis (Laule et al., 2004) and stroke (Gideon et al., 1999). Thus, it is highly recommended when using this methodology to perform correction for partial volume to remove effects of CSF from the water signal. Tissue composition correction or relaxation correction are also recommended and are reviewed elsewhere (for details see (Ernst et al., 1993; Gasparovic et al., 2006; Knight-Scott et al., 2003; Jansen et al., 2006)). Another consideration in quantification when using shorter TEs is the macromolecule background signal. The macromolecule background is made up of broad resonances that are underneath the metabolites that visually appear to globally elevate the spectrum (Figure 2). These resonances are short T₂s species and are usually not present at longer TEs since their signals are completely decayed. The specific constituents that contribute to the macromolecule background are unknown, but the characteristic pattern of macromolecule background has been shown in animal and human work (Behar and Ogino, 1993; Behar et al., 1994; Seeger et al., 2003; Gottschalk et al., 2008). There are different methods for accounting for macromolecule background in spectral quantification such as utilizing a program that already incorporates macromolecule handling into fitting such as LCMoDel or jMRUI or to collect a macromolecule spectrum via metabolite nulling for each participant and add it into the specific quantification program.

Assessments of reproducibility for spectroscopic acquisition sequences are also important. Standard sequences that are commercially available such as STEAM and PRESS are used extensively, and reproducibility measures on healthy populations have been well

established. However, reliability assessments may be appropriate for applications to clinical populations in order to show feasibility and reproducibility such as (Mullins et al., 2003). When choosing a newly developed technique, it is important that reproducibility be established. For instance, more recent localization techniques such as MEGA-PRESS (Evans et al., 2010; Bogner et al., 2010; O’Gorman et al., 2011; Geramita et al., 2011), SPECIAL (Near et al., 2013), and PR-STEAM (Wijtenburg et al., 2013) demonstrated good reproducibility for historically difficult to detect metabolites such as GABA and glutamate.

3.0 ¹H-MRS in Schizophrenia

Due to the large number of studies examining glutamate and/or glutamine in SZ, the results will be summarized according to the population under investigation such as high risk (3.1.1), first degree relatives (3.1.2), first episode (3.1.3), chronic (3.1.4), unmedicated state (3.1.5), and anti-psychotic treatment effects (3.1.6). For the GABA, glutathione, NAAG, glycine, and serine sections, the paucity of studies examining these metabolites precludes separation into the different categories similar to the glutamate/glutamine section; thus, results in these sections will include discussions across all illness stages and anti-psychotic medication studies.

3.1 Glutamate and glutamine

Glutamate is the primary excitatory neurotransmitter in the adult brain and released in half of the synapses (Farber et al., 1998). After release into the synapse, glutamate is quickly transported to astrocytes where it is converted to glutamine by glutamine synthetase. Depending on the size of the synapse, number of transporters, and many other factors, removal of glutamate from the synaptic cleft can occur in sub-milliseconds to milliseconds (Danbolt, 2001). This quick transport and conversion helps prevent excitotoxicity. Glutamine is then transported to the neuron and is converted back to glutamate by glutaminase. Glutamate is synthesized from glucose (tricarboxylic cycle) and glutamine. In addition to its role as a neurotransmitter, glutamate also serves as a precursor for the synthesis of GABA and glutathione and as a building block in protein synthesis (Brosnan and Brosnan, 2013; Wu et al., 2004; Duarte and Gruetter, 2013; Mathews and Diamond, 2003). Due to these diverse roles, glutamate is found in multiple cell types, including neurons and glia, and is also found in the extracellular space. ¹H-MRS cannot provide information regarding the amount of glutamate devoted to a specific function or how much is present in a specific cell types. The glutamate signal provided with ¹H-MRS reflects the total amount of glutamate within a specified voxel of interest in the brain. This signal does not simply reflect glutamate neurotransmission, but most likely reflects multiple mechanisms and has been suggested to be an index of cortical excitability (Stagg et al., 2009; Stagg et al., 2011). Although a tight coupling between the neurotransmitter and metabolic glutamate pools has been suggested (Magistretti and Pellerin, 1999; Rothman et al., 1999), the functional relevance of total glutamate assessed through spectroscopy is not clear. It has been suggested that 20–30% of glutamate in tissue is not visible using MRS (Kauppinen et al., 1994; Maddock and Buonocore, 2012), and the MRS invisible glutamate may be part of the neurotransmitter pool (Kauppinen et al., 1994). Approximately 80% of brain glutamine takes part in the glutamatergic neurotransmission cycle (Magistretti and

Pellerin, 1999; Rothman et al., 1999). Hence, glutamine concentration measured with ^1H -MRS may be a good index of the turnover of glutamate involved in neurotransmission. Human brain tissue glutamate levels range from 5–15 mmol per kg wet weight (Danbolt, 2001), and glutamine levels range from 3.0–5.8 mmol per kg wet-weight (Govindaraju et al., 2000). The glutamate signal within the ^1H spectrum occurs at 2.04, 2.11, 2.35, and 3.74 ppm and glutamine occurs at 2.11, 2.13, 2.44, and 3.76 ppm. There are several techniques available to measure glutamate and glutamine (Ramadan et al., 2013). Depending on the TE and the localization technique chosen, glutamate, glutamine, or glutamate+glutamine (Glx) may be reported. In schizophrenia research, glutamate and glutamine are predominantly measured using PRESS or STEAM localization techniques. Short TEs in the range of 20 to 35ms are chosen to minimize the signal losses due to J-coupling and T_2 relaxation. Another TE often chosen is 80 ms since glutamate was shown to be distinguishable from glutamine (Schubert et al., 2004); however, a recent study suggests this TE may be optimal for glutamine detection (Hancu and Port, 2011). MEGA-PRESS and J-editing can also be used to measure Glx; however, the Glx level reported with these techniques consists of only the signal from the multiplets at 3.74 ppm. The advantage of Glx quantification from the edited spectrum is that there is no overlap with other spectral peaks making detection easier; however, a drawback is that the signal-noise ratio is much lower due to the longer TE typically used in these sequences.

There are several lines of evidence that implicate the glutamatergic system in the pathophysiology of schizophrenia. Dissociative anesthetics such as phencyclidine (PCP) and ketamine block the glutamate N-methyl-D-aspartate receptor (NMDAR), and the administration of these drugs produces a state in adults that is phenomenologically similar to schizophrenia (Rowland et al., 2005). Ketamine, when administered to people with stable schizophrenia, produces exacerbations of their unique psychotic content (Lahti et al., 2001) and may increase prefrontal glutamatergic metabolites (Rowland et al., 2005; Stone et al., 2012) similar to preclinical models (Moghaddam et al., 1997). Post-mortem work demonstrates alterations in glutamate receptors (Beneyto et al., 2007; Meador-Woodruff et al., 2003; Zavitsanou et al., 2002), and genetics studies show a link between genes for glutamatergic receptors and risk for schizophrenia (Winchester et al., 2014). These observations led to great interest in the role of glutamate in schizophrenia pathophysiology, as outlined in several previous articles (Poels et al., 2014a; Poels et al., 2014b; Egerton et al., 2012b; Marsman et al., 2013; Stone, 2009). Overall, there is a great emphasis on studying *in vivo* glutamate in those at risk for schizophrenia, first-episode and chronic phases, and the impact of antipsychotic medication treatment.

3.1.1 High Risk—Prior to onset of schizophrenia, there is a prodromal phase or “at-risk mental state” stage where individuals have experienced a decrease in global function, a first-degree relative with a psychotic disorder, and sub-threshold psychotic symptoms (Yung et al., 1996; Yung et al., 2007). These individuals are more likely to convert to a psychotic disorder than the general population (Fusar-Poli et al., 2012). Identifying biomarkers for those at greatest risk for psychosis is imperative for development of treatment interventions to potentially prevent or reduce the incidence of conversion to a psychotic disorder as well as reduce the symptom severity of those that do convert.

The majority of studies that examined frontal regions show no differences between high risk and healthy controls (Fusar-Poli et al., 2011; Valli et al., 2011; Natsubori et al., 2013), but there are two exceptions (Stone et al., 2009; Tibbo et al., 2004). Fusar-Poli et al. (2011) and Valli et al. observed no anterior cingulate glutamate differences between healthy controls and participants at risk for psychosis, who were antipsychotic medication naïve (Fusar-Poli et al., 2011; Valli et al., 2011). In a group of mixed antipsychotic medication use (10 on antipsychotics medications for less than 16 cumulative weeks and 14 were not taking antipsychotic medications), Natsubori et al. reported no differences in medial prefrontal cortex (including the anterior cingulate) Glx levels in ultra-high risk participants compared to controls (Natsubori et al., 2013). Stone et al. (2009) observed no glutamate differences in the anterior cingulate in at-risk participants compared to controls (Stone et al., 2009). In this study, 21 at-risk participants were not currently taking antipsychotic or antidepressant medications, while 6 at-risk participants were taking antipsychotic or antidepressant medications. However, Stone et al. (2009) found elevated glutamine levels in the anterior cingulate in this sample. Similarly, Tibbo et al. found elevated glutamate+glutamine relative to creatine in the right middle frontal region, which encompassed the anterior cingulate cortex (Tibbo et al., 2004).

In temporal regions, the majority of studies of participants at risk for psychosis report no alterations in glutamate or glutamine. Valli et al. observed no glutamate differences between antipsychotic medication naïve, at risk participants and controls in the left hippocampus (Valli et al., 2011). In a mixed antipsychotic medication use sample, Stone et al. (2009) found no glutamate or glutamine differences between groups in the left hippocampus (Stone et al., 2009). In addition, Wood et al. (2010) observed no Glx differences in either left or right medial temporal lobe voxels nor between participants who transitioned to psychosis and those that did not (Wood et al., 2010). Participants in the Wood et al. study did not take antipsychotic medications. However, Fusar-Poli et al. (2011) reported lower glutamate levels in antipsychotic medication naïve at risk participants in the left hippocampus compared to healthy controls at trend level (Fusar-Poli et al., 2011).

In the thalamus, decreased glutamate levels are commonly reported. Antipsychotic medication naïve participants who were at risk for developing a psychotic disorder had significantly lower thalamic glutamate levels compared to controls (Fusar-Poli et al., 2011). Valli et al. reported a trend level decrease in Glu levels in an antipsychotic medication naïve at risk group compared to healthy controls in the left thalamus (Valli et al., 2011). In further support of these findings, Stone et al. (2009) also found decreased glutamate levels in the left thalamus in a mixed antipsychotic medication use, at-risk group compared to controls (Stone et al., 2009). In this same study, no glutamine levels differences were found between groups.

In the dorsal caudate nucleus, de la Fuente-Sandoval et al (2011) showed that 18 antipsychotic medication naïve, ultra high risk participants had elevated glutamate compared to healthy controls (de la Fuente-Sandoval et al., 2011). This same study reported no differences in the right cerebellum between groups. In a follow-up study, de la Fuente-Sandoval et al (2013a) reported that 7 out of 19 of these participants transitioned to a psychotic disorder within two years (de la Fuente-Sandoval et al., 2013a). Those that

transitioned to a psychotic disorder had elevated baseline glutamate levels compared to controls; whereas, there were no glutamate differences between the 12 participants who did not convert to a psychotic disorder and healthy controls.

Thus, decreased thalamic glutamate levels and increased striatal glutamate levels are most often observed in participants at high-risk compared to healthy controls whereas the majority of studies in the frontal and temporal regions report no differences between groups.

3.1.2 First-Degree Relatives—There are two studies examining the neurochemistry of genetic high risk participants with a first-degree family member with schizophrenia. Lutkenhoff et al. found that co-twins (both monozygotic and dizygotic) of participants with schizophrenia had lower glutamate levels than healthy twin pairs across regions, which included mesial prefrontal gray matter, left prefrontal white matter, and left hippocampus (Lutkenhoff et al., 2010). In contrast, Purdon et al. found no Glu/Cr differences in the right and left frontal voxels in adults with a sibling with schizophrenia (Purdon et al., 2008). Thus, there appears to be no differences between first-degree relatives and healthy controls when studying one specific region, while one study suggests overall lower glutamate levels in relatives when combining results from multiple regions.

3.1.3 First Episode Schizophrenia—¹H-MRS studies examining glutamate, separately from glutamine, in antipsychotic medication naïve first episode schizophrenia participants reported no differences between groups in most regions except the striatum. A study by de la Fuente-Sandoval et al. (2011) showed elevated glutamate in the dorsal caudate but not the right cerebellum in antipsychotic medication naïve first episode schizophrenia participants compared to controls (de la Fuente-Sandoval et al., 2011). Several studies with overlapping samples reported no glutamate differences between medication naïve first-episode participants and healthy controls in the left anterior cingulate and left thalamus (Theberge et al., 2002; Theberge et al., 2007; Aoyama et al., 2011). In a cohort of minimally treated first episode schizophrenia participants, Bustillo et al. (2010) observed no differences in glutamate in voxels placed in the bilateral anterior cingulate, left frontal white matter, and left thalamus (Bustillo et al., 2010).

¹H-MRS studies measuring glutamine consistently show elevations in the thalamus and mixed results in the anterior cingulate. In the left anterior cingulate cortex and left dorsomedial thalamus, Theberge et al. (2002) found elevated glutamine concentrations in 21 antipsychotic medication naïve people with first episode schizophrenia compared to 21 controls (Theberge et al., 2002). In overlapping samples with the Theberge et al. (2002) study, Theberge et al. (2007) and Aoyama et al. (2011) both found elevated left thalamic glutamine concentrations in first episode antipsychotic medication naïve schizophrenia participants (Theberge et al., 2007; Aoyama et al., 2011). These same two studies also observed elevated Gln in the left anterior cingulate. Using the sequence implemented by Theberge et al., Bustillo et al. (2010) observed no statistically significant differences between glutamine in the anterior cingulate cortex, which may be due to differences in voxel size (bilateral versus left) and participant age (these participants were older) (Bustillo et al., 2010).

However, compared to healthy controls, Bustillo et al. (2010) found elevations of Gln/Glu ratio in bilateral anterior cingulate cortex in a minimally treated schizophrenia group with a lifetime exposure to antipsychotic medications of less than three weeks (Bustillo et al., 2010). This group reported no glutamate or glutamine differences in thalamic or frontal white matter voxels between patients and controls. Since glutamine is a precursor of glutamate, Gln/Glu ratio may be a good measure of glutamatergic neurotransmission turnover (Bustillo et al., 2010; Ongur et al., 2008; Shirayama et al., 2010). Another study reported elevated Glx/tCr in the left basal ganglia of 16 first episode schizophrenia participants, and no differences in the frontal lobe or parieto-occipital lobe compared to controls (Goto et al., 2012). This study did not provide detailed information regarding the medication status of participants with these results or size and placement of the frontal lobe voxel so it is unclear if the frontal lobe voxel incorporated the anterior cingulate cortex.

In contrast, a study comparing controls with first-episode schizophrenia participants where 18 out of 19 were on antipsychotic medications found no differences in Glx levels in the medial prefrontal cortex, which included the anterior cingulate (Natsubori et al., 2013). In a study comparing medicated, treated with at least one antipsychotic medication, remitted and non-remitted first episode schizophrenia participants, Egerton et al. (2012) found anterior cingulate cortex Glu/Cr elevations in a group of first episode schizophrenia participants still experiencing symptoms despite receiving at least one antipsychotic medication compared to a group of first episode schizophrenia participants who were not experiencing symptoms and on at least one antipsychotic medication (Egerton et al., 2012a). This study found no Glx/Cr differences between groups in this region as well as no Glu/Cr or Glx/Cr differences in the left thalamus. The authors also found that higher Glu/Cr in the anterior cingulate cortex was associated with greater negative symptom severity and lower global functioning. Since a healthy control group was not included in the Egerton et al. study, it is difficult to gauge whether the Glu/Cr levels in the medicated, symptomatic group were higher than normal controls or whether Glu/Cr levels were reduced in medicated, non-symptomatic group.

Overall, in antipsychotic naïve participants experiencing their first episode, glutamate is elevated only in the striatum, and glutamine is elevated in the left thalamus with mixed results in the anterior cingulate. Longitudinal studies at first episode with administration of antipsychotic medications suggest a reduction in glutamate and glutamine levels over years.

3.1.4 Chronic Schizophrenia—Differences between chronic participants with schizophrenia (an illness duration of one year or longer) and healthy controls vary depending upon the region under investigation. In the anterior cingulate, two studies observed lower glutamate levels in chronic, medicated schizophrenia participants compared to controls (Tayoshi et al., 2009; Theberge et al., 2003). In addition, Theberge et al. (2003) also showed decreased glutamine in this population as well. In the medial prefrontal cortex (which contained the anterior cingulate), Natsubori et al. showed that chronic, medicated participants with schizophrenia had lower Glx than healthy controls, ultra-high risk participants, and first-episode schizophrenia participants (Natsubori et al., 2013). In this study, half of participants in the ultra-high risk group and the majority of participants in the first episode schizophrenia group were on antipsychotic medications. Rowland et al. (2013) reported a main effect of diagnostic group, which showed chronic, medicated schizophrenia

participants had lower Glx levels compared to healthy controls across regions, which were the anterior cingulate cortex and centrum semiovale (Rowland et al., 2013). Lutkenhoff et al. showed that glutamate was significantly lower in medicated schizophrenia participants compared to healthy controls overall, but not in specific regions (Lutkenhoff et al., 2010). In an exploratory analysis, this study reported significantly reduced glutamate in adults with schizophrenia and their co-twin compared to healthy controls in the mesial prefrontal cortex. In another cohort, Chang et al. (2007) utilized an older sample of chronic, medicated schizophrenia participants with a mean age of 66.3 ± 7.2 years and observed elevated Glx in right prefrontal white matter (Chang et al., 2007). In contrast, several studies reported no differences in Glx, Glx/Cr, or Glu/Cr ratios between chronic, medicated schizophrenia participants and healthy controls in several regions, specifically the left and right rostral and dorsal anterior cingulate (Wood et al., 2007), bilateral dorsal anterior cingulate (Reid et al., 2010; Kraguljac et al., 2012), left middle prefrontal region (Rowland et al., 2009), left anterior cingulate or left frontal lobe (Jessen et al., 2013), and bilateral anterior cingulate (Ongur et al., 2010). Another study by Terpstra et al. (2005) reported no differences in glutamate or glutamine from the anterior cingulate between medicated adults with schizophrenia and controls; however, this study did not provide information regarding illness stage, illness duration, or type of medications currently being taken (Terpstra et al., 2005). In a mixed illness stage sample with the majority taking antipsychotic medications, Bustillo et al. (2014) showed that glutamine and Gln/Glu ratio are elevated in the dorsal anterior cingulate in adults with schizophrenia compared to controls, and in particular, that glutamine levels relates to severity of psychotic symptoms (Bustillo et al., 2014). Contrary to a meta-analysis (Marsman et al., 2013), Bustillo et al. (2014) observed increased glutamine with aging in both groups; thus, more comprehensive aging studies are needed to further investigate how these metabolites change with age. Interestingly, the studies that observed no Glx differences had younger cohorts on average with shorter illness durations than the Chang et al. (2007) or Rowland et al. (2013) studies, suggesting that there may be glutamatergic alterations later in the illness.

In temporal lobe regions, $^1\text{H-MRS}$ results may depend on medication status and cohort age. Two studies examining the left hippocampus reported no Glx/Cr differences between healthy controls and chronic, medicated schizophrenia participants (Hutcheson et al., 2012; Kraguljac et al., 2012). In another medicated sample, Lutkenhoff et al. found no significant glutamate differences between groups in the hippocampal region (Lutkenhoff et al., 2010). In left medial temporal lobe white matter, another study with an older medicated cohort (mean age 66.3 ± 7.2 years old) showed elevated Glx in the chronic schizophrenia participants compared to healthy controls (Chang et al., 2007).

Four studies utilized $^1\text{H-MRS}$ to examine neurochemistry of the parietal and occipital lobes. Rowland et al. (2009) showed no Glx differences between medicated schizophrenia participants and healthy controls in the left inferior parietal region while Ongur et al. (2010) observed no Glu/Cr differences between chronic, medicated participants and controls in the parietal-occipital cortex (Rowland et al., 2009; Ongur et al., 2010). Chang et al. (2007) showed elevated Glx in occipital white matter of chronic, older medicated schizophrenia participants (Chang et al., 2007). A third study showed no Glx/Cr differences between schizophrenia participants and controls in the calcarine sulci (Yoon et al., 2010). The cohort

in this study comprised stable chronic and recent onset schizophrenia participants who had differing medication statuses, from unmedicated for at least one month to minimal antipsychotic medication exposure to medicated using antipsychotics.

Thus far, few studies reported results from subcortical regions. In the substantia nigra and the basal ganglia, two studies of chronic, medicated schizophrenia participants and healthy controls reported no differences glutamate or Glx/Cr (Tayoshi et al., 2009; Reid et al., 2013). In a study of the left dorsomedial thalamus, Theberge et al. (2003) observed elevated glutamine in chronic, medicated participants and healthy controls, but no Glu differences (Theberge et al., 2003).

Overall, in gray matter or mixed frontal, temporal, parietal, and occipital regions, results are mixed showing either no differences or lower glutamatergic metabolites in chronic SZ compared to healthy controls. In exclusively white matter regions and in the left thalamus, glutamatergic metabolites are elevated in chronic SZ compared to healthy controls.

3.1.5 Unmedicated State—To date, there are eight studies examining glutamate, glutamine, or Glx in unmedicated participants with schizophrenia. Six studies with exclusively first-episode participants with schizophrenia showed mixed regional results. In overlapping samples, Theberge et al. (2002), Theberge et al. (2007), and Aoyama et al. (2011) showed that antipsychotic medication naïve first-episode participants had elevated glutamine in the left anterior cingulate and left thalamus and showed no differences in glutamate in either region (Theberge et al., 2002; Theberge et al., 2007; Aoyama et al., 2011). Two studies reported elevated glutamate in the right dorsal caudate nucleus in antipsychotic medication naïve participants (de la Fuente-Sandoval et al., 2011; de la Fuente-Sandoval et al., 2013b) and either no differences in Glx levels (de la Fuente-Sandoval et al., 2011) or elevated Glx levels (de la Fuente-Sandoval et al., 2013b) compared to controls. In the right cerebellum, these studies reported either elevated glutamate and Glx levels (de la Fuente-Sandoval et al., 2013b) or no differences (de la Fuente-Sandoval et al., 2011) between first episode participants and controls. Goto et al. (2012) found no differences in Glx/Cr in the frontal or parieto-occipital lobe and elevated Glx/Cr in the basal ganglia (Goto et al., 2012). Overall, these data suggest higher glutamatergic metabolites in unmedicated, first-episode schizophrenia but caution is warranted since there is discrepancy among the specific glutamatergic metabolites quantified (glutamate, glutamine, or Glx) and brain regions.

Two other unmedicated studies included participants with mixed previous exposure to antipsychotic medications. Each of these studies required participants to be unmedicated for a minimum of 14 days prior to the study. Kegeles et al. reported significantly elevated medial prefrontal cortex Glx in unmedicated participants with schizophrenia compared to healthy controls, and trend level elevations compared to medicated participants (Kegeles et al., 2012). In addition, this same study observed no Glx alterations between groups in the left dorsolateral prefrontal cortex in this same study. The unmedicated group was antipsychotic medication free for a mean of 21 ± 17 months, ranging from 4 days to 4 years. The schizophrenia group included both first-episode and chronic illness stage, with a mean duration of illness of 7 ± 7 years. Kraguljac et al. (2013) found elevated Glx/Cr in the left

hippocampus of unmedicated schizophrenia participants compared to controls (Kraguljac et al., 2013). In this study, the schizophrenia cohort included eleven antipsychotic medication naïve participants and sixteen other participants who were medication free for a mean of 24.7 ± 45 months. In addition, this study included participants at varying illness stages, including first episode and chronic participants with a mean illness duration of 10.5 ± 8.7 years. Overall, statistical analyses in the Kraguljac et al. (2013) study showed that Glx/Cr from first episode, antipsychotic medication naïve, and chronic participants were not significantly different.

The majority of unmedicated studies reported increased glutamatergic metabolite levels. However, when considering the exceptions that found no differences in glutamatergic levels (Goto et al., 2012; de la Fuente-Sandoval et al., 2011) and the studies of antipsychotic medicated patients that reported higher glutamatergic levels (Chang et al., 2007; Aoyama et al., 2011; Egerton et al., 2012a), it is premature to conclude that glutamatergic levels are only higher in the unmedicated state. Inclusion of mixed illness stage studies appears to complicate interpretation since there is heterogeneity early in the illness, there are no studies of exclusively unmedicated, chronic participants, and the effect of illness duration and age on glutamate and glutamine levels later in the illness is unknown. More studies are needed in both unmedicated first-episode and chronic participants with lengthy illness durations, especially since recent data suggest that glutamatergic levels differ later in the illness. In particular, a meta-analysis suggested that medial frontal glutamate decreases across the illness (Marsman et al., 2013), and other data showed that glutamate levels decrease with age in healthy controls (Kaiser et al., 2005; Chang et al., 2009). In contrast, Chang et al. (2007) reported elevated Glx in white matter regions of medicated participants with mean illness durations of 43.1 ± 5.4 years (Chang et al., 2007), suggesting that there may be altered glutamatergic patterns in white versus gray matter in older adults with schizophrenia. Providing further support to an altered glutamatergic state later in the illness is that less severe psychopathology is observed and lower dosages of medication are required in older adults with schizophrenia (Jeste et al., 2003; Jeste et al., 2011). Thus, more off-medication research is needed to further our understanding glutamatergic levels throughout the illness.

3.1.6 Antipsychotic Treatment Effects—Antipsychotic medications are a first-line treatment for adults with schizophrenia, and several studies examined the effects of medication treatment both in the short term (4 weeks) and long term (80 months) on glutamate levels in several regions. After 6 months of atypical antipsychotic treatment in first episode schizophrenia participants, Goto et al. (2012) observed a decrease in frontal lobe Glx/Cr from baseline to the study end point (Goto et al., 2012). However, this study did not provide details regarding medication status of participants at baseline or voxel placement within the frontal lobe. Similarly, de la Fuente-Sandoval et al. (2013b) reported a decrease in glutamate levels in the right dorsal caudate nucleus after 4 weeks of treatment with risperidone in antipsychotic medication naïve first episode psychosis participants (de la Fuente-Sandoval et al., 2013b). At baseline, first-episode participants showed elevated glutamate levels compared to controls, and after treatment, this study found no differences in glutamate levels between groups. In the right cerebellar cortex, de la Fuente-Sandoval et al. (2013b) observed elevated glutamate levels in first episode psychosis participants compared

to controls, and this effect remained after treatment with risperidone unlike the associative striatum (de la Fuente-Sandoval et al., 2013b). In another study that performed assessments at baseline on minimally treated first episode schizophrenia participants and at follow-up (mean of 7.3 months later) with participants on antipsychotic therapy, Bustillo et al. (2010) reported no differences in glutamate, glutamine, or Gln/Glu ratio between groups in bilateral anterior cingulate cortex, left frontal white matter, or left thalamus (Bustillo et al., 2010). Compared to a treatment as usual in an ultra-high risk group, Glx levels in participants receiving low dose lithium remained the same in the left or right medial temporal lobe (predominantly containing the hippocampus) over a three month interval (Berger et al., 2012).

In two follow-up studies (Theberge et al., 2007; Aoyama et al., 2011), antipsychotic medication naïve participants with first episode schizophrenia were treated and scanned at 10, 30, and 80 months after initial visit. At each time interval, the majority of schizophrenia participants were taking antipsychotic medications. In participants with schizophrenia, glutamine levels remained the same after 10 months of treatment in the left thalamus; however, glutamine levels at 30 months decreased significantly compared to glutamine levels at baseline and 10 months (Theberge et al., 2007). This study observed no differences in glutamine levels in the left anterior cingulate over these time intervals or for glutamate levels over time in either the left anterior cingulate or the left thalamus. After 80 months of treatment, Aoyama et al. (2011) reported that thalamic glutamine levels decreased compared to baseline at trend level, and that thalamic glutamate+glutamine levels decreased significantly compared to baseline (Aoyama et al., 2011). Thalamic glutamate+glutamine correlated negatively with social functioning despite treatment with antipsychotic medications. Comparisons at 80 months between groups showed no differences in glutamate or glutamine in either region.

Overall, these studies suggest that glutamate and glutamine levels decrease or remain stable with treatment depending upon the region examined and the variety of medications utilized, and that over a longer period of time, glutamate and glutamine levels decrease compared to baseline. The study by de la Fuente-Sandoval et al. (2013b) was the only study to examine the treatment effect of one antipsychotic medication whereas the other treatment studies utilized several different medications within their cohort. The de la Fuente-Sandoval et al. (2013b) study also examined spectroscopic differences following treatment after a short period of time compared to all other studies that ranged from 6 to 80 months. Longitudinal studies that more densely sample the time course of glutamatergic levels as a function of treatment would be informative.

3.1.7 Summary of Glutamatergic Alterations in Schizophrenia—Glutamate and glutamine levels depend upon illness stage, brain region, and medication status, and results of the studies previously discussed are summarized in Table 1. In the prodromal stage, thalamic glutamate levels are lower in at risk participants while in the striatum, glutamate is elevated in this same population. Frontal and temporal regions do not appear greatly affected in the prodromal stage. In antipsychotic naïve participants experiencing their first episode, glutamate is elevated in the striatum while increased glutamine and no glutamate differences are observed in the anterior cingulate and thalamus. At first episode, longitudinal studies

with administration of antipsychotic medications suggest a reduction in glutamate and glutamine levels over years. Several studies with chronic participants with a longer duration of illness suggest that Glx is decreased in either gray matter or mixed regions. To date, there is one study that examined an older cohort of medicated adults with schizophrenia, and in this study, elevated Glx was observed in three white matter regions (Chang et al., 2007). More studies focused on old adults with schizophrenia who have longer durations of illness are needed to understand whether the glutamatergic system is altered late in life. Since studies in older adults with schizophrenia report less general psychopathology and the need for lower dosages of medication (Jeste et al., 2003), new information regarding the glutamatergic system would be useful and may directly inform treatment interventions.

3.2 Gamma-aminobutyric acid (GABA)

GABA is the primary inhibitory neurotransmitter in the brain and occurs at approximately 1 mmol/kg concentration. Altered GABAergic function is implicated in the pathophysiology of schizophrenia as evident through post-mortem studies (Hashimoto et al., 2008; Deng and Huang, 2006; Beneyto and Lewis, 2011) and challenge studies (Ahn et al., 2011; Taylor et al., 2013). Within the ^1H spectrum, GABA peaks arise at 1.89, 2.28, and 3.01 ppm, and GABA concentrations typically range from 1.3–1.9 mM (Govindaraju et al., 2000). GABA levels are often reported relative to either water (GABA/W) or creatine (GABA/Cr). In addition, GABA is commonly measured with specifically tailored editing sequences such as MEGA-PRESS, which has been previously discussed. However, recent studies utilizing non-editing sequences with very short TEs demonstrate the ability to detect GABA as well (Near et al., 2013; Wijtenburg et al., 2013).

To date, seven studies utilized spectral editing to study GABA concentrations in schizophrenia (Table 2). In frontal regions, GABA levels differ based on illness stage and medication status. Goto et al. (2010) studied first-episode participants prior to and after treatment with antipsychotic medications and found no differences in GABA/Cr between controls and participants with SZ at baseline and after 6 months of treatment (Goto et al., 2010). In chronic, medicated participants with schizophrenia, Tayoshi et al. (2010) reported no differences in GABA levels between groups in the anterior cingulate, but this same study found a significant negative correlation between GABA levels and antipsychotic medication dose (Tayoshi et al., 2010). In a mixed illness stage sample, Kegeles et al. reported elevated GABA/W in unmedicated adults with schizophrenia compared to healthy controls in the medial prefrontal cortex, and no differences between medicated adults with schizophrenia and healthy controls in this same region (Kegeles et al., 2012). In addition, Kegeles et al. showed no differences in GABA/W levels between any of the three groups in the left dorsolateral prefrontal cortex. Rowland et al. (2013) reported no GABA/W group differences in the anterior cingulate or centrum semiovale, with no participants taking benzodiazepines or mood stabilizers. When broken down into age and diagnostic groups, this study reported lower GABA/W levels in older adults with schizophrenia (mean age of 51.1 ± 4.0 years old) compared to older healthy controls (Rowland et al., 2013). When comparing age groups by combining diagnoses, Rowland et al. (2013) observed higher GABA/W levels in younger participants compared to older participants across regions. In conjunction with a study by Gao et al. (2013), these data suggest that GABA levels may

decrease due to age and illness (Rowland et al., 2013; Gao et al., 2013). In contrast, Ongur et al. (2010) showed elevated GABA/Cr ratios in the anterior cingulate cortex in chronic, medicated schizophrenia participants compared to healthy controls (Ongur et al., 2010). Ten adults with schizophrenia took anticonvulsants. When this sample was removed from analyses, Ongur et al. (2010) reported no GABA/Cr differences between groups. Thus, concomitant usage of anticonvulsants and liberal criteria for GABA quantification (CRLB cutoff of 50%) may have influenced the results of this study.

Two studies examined GABA levels in the left basal ganglia of schizophrenia participants and controls. Goto et al. (2010) reported reduced GABA/Cr concentrations in the left basal ganglia in medicated subjects with early-stage schizophrenia compared to healthy control subjects at baseline and following six-months of antipsychotic treatment (Goto et al., 2010). Tayoshi et al. (2010) found no differences in GABA levels in the left basal ganglia between chronic, medicated participants and healthy controls (Tayoshi et al., 2010). When broken down according to typical versus atypical antipsychotic medication being taken, increased GABA was observed in participants taking typical antipsychotic medications versus atypical antipsychotic medications, and both groups included equal numbers of participants taking benzodiazepines.

In more posterior regions of the brain, results for GABA levels are mixed, most likely due to concomitant medication and mixture of illness stage. In early stage schizophrenia, Goto et al. (2010) reported no differences in GABA/Cr between medicated adults with schizophrenia and healthy controls in the parieto-occipital lobe (Goto et al., 2012). Keleman et al. (2013) found decreased GABA/Cr levels in the bilateral calcarine sulci of antipsychotic medication naïve, first-episode participants compared to controls, and this effect remained after 8 weeks of treatment with antipsychotic medications (Keleman et al., 2013). Ongur et al. (2010) reported elevated GABA/Cr in the parieto-occipital cortex in chronic, medicated schizophrenia participants and healthy controls (Ongur et al., 2010). However, when participants who were taking concomitant anticonvulsants were removed from analyses, the effect was no longer significant. In another study by Yoon et al., GABA/Cr levels were decreased in the bilateral calcarine sulci (Yoon et al., 2010). The schizophrenia group included recent onset and chronic individuals, and the medication status of this group ranged from not taking antipsychotic medications for at least one month to medicated.

With advanced MR techniques, it is possible to measure both GABA and glutamate or glutamine within a study session and ratios of these may reflect excitatory and inhibitory neurotransmission balance. This may be useful since it is believed that an imbalance in excitatory and inhibitory neurotransmission occurs in schizophrenia (Lewis et al., 2012). To date, there have been no studies examining the ratios of GABA to glutamate or glutamine. Another approach to provide insight into excitatory-inhibitory balance is to investigate the relationship between GABA and glutamate or glutamine (i.e correlation analysis). However, caution is warranted if the metabolites are acquired with the same sequence and hence the same reference (water or Cr) is utilized which inflates r values and must be controlled (Maddock, 2014)

There have been four few studies that measured both GABA and glutamate metabolites within the same session. Ongur et al (2010), Yoon et al (2010), and Kegeles et al (2012) used spectral editing to quantify GABA and Glx relative to creatine or water whereas Rowland et al (2013) used spectral editing for GABA and single voxel spectroscopy for Glx, both referenced to water (Ongur et al., 2010; Yoon et al., 2010; Kegeles et al., 2012; Rowland et al., 2013). Based on approximate mean GABA/Glx or GABA/Glu ratios calculated from these studies, there appears to be an imbalance in the ratio of GABA to Glx or Glu in SZ participants versus healthy controls. In three of these studies, ratios of GABA/Glx or GABA/Glu are elevated in frontal and posterior brain regions in both medicated and unmedicated SZ subjects, consisting of either all chronic or mixed illness duration cohorts (Ongur et al., 2010; Kegeles et al., 2012; Rowland et al., 2013) whereas in one study, GABA/Glx is reduced in a mixed illness duration, medicated SZ cohort in the bilateral calcarine sulci (Yoon et al., 2010). It is important to note that it is not known whether these differences are significant between groups. In addition to ratios of GABA to Glx, Ongur et al (2010) and Kegeles et al (2012) studies performed correlation analyses between GABA and Glx or Glu measures and did not observe differences between healthy controls and participants with SZ. Thus, in terms of ratios of GABA to Glu or Glx, the few studies conducted thus far extend the potential for an inhibitory/exhibitory imbalance in SZ; however, more extensive research is needed to evaluate regional, illness stage, and medication differences.

In summary, the majority of studies focusing on GABA observed lower GABA levels than healthy controls regardless of region and illness stage. Interestingly, antipsychotic treatment had no effect on GABA levels in two studies of first-episode schizophrenia (Goto et al., 2010; Kelemen et al., 2013). Two other studies observed elevated GABA levels in frontal and posterior regions (Ongur et al., 2010; Kegeles et al., 2012), but when the effects of concomitant anticonvulsants were considered in the Ongur et al. (2010) study, the increased GABA/Cr levels were no longer significant. Since benzodiazepines and anticonvulsants are known to affect GABA(A) receptors and increase the observable concentration levels of GABA in the brain (Goddard et al., 2004), it is important to take the benzodiazepine and anticonvulsant medication usage into consideration when evaluating results for GABA. Thus, more studies with full concomitant medication disclosure are needed to fully determine GABA alterations throughout the illness.

3.3 Glutathione (GSH)

Glutathione (GSH) is a major intracellular antioxidant that protects the brain against oxidative stress and may play a role in the pathophysiology of schizophrenia (O'Donnell, 2011; Wu et al., 2013). Flatow et al. conducted a meta-analysis of serum and plasma oxidative stress markers measured in participants with schizophrenia and concluded that oxidative stress may be a potential biomarker of schizophrenia (Flatow et al., 2013). Further evidence stems from studies in adults with schizophrenia showing reduced GSH concentrations in CSF (Do et al., 2000), reduced GSH blood levels (Raffa et al., 2009), and reduced GSH concentrations in the post-mortem caudate nucleus (Yao et al., 2006) and pre-frontal cortex (Gawryluk et al., 2011). Flatow et al. also suggested oxidative stress abnormalities may be independent of antipsychotic medication status supported by the

abnormalities observed in first episode participants (Flatow et al., 2013). Ballesteros et al. ruled out exogenous factors such as diet, smoking, and medication status from causing the lower GSH concentrations observed in adults with schizophrenia (Ballesteros et al., 2013).

GSH levels measured by ¹H-MRS originate from resonances at 2.15, 2.55, 3.77 (glutamate-moiety), 2.93, 2.98, 4.56 (cysteine-moiety), and 3.77 (glycine-moiety) ppm. The concentration of GSH in the brain is approximately 2.0mM. To date, in vivo measurements of GSH concentrations are conducted either utilizing a specifically tailored pulse sequence or relying on improved MRI hardware by using standard localization sequences such as STEAM or PRESS. Using MEGA-PRESS, only the 2.95 ppm multiplet of GSH is observable and quantifiable whereas with PRESS or STEAM localization, the entire GSH spectrum is quantified. One previous study utilized a PRESS localization sequence to detect GSH in adults with schizophrenia (Wood et al., 2009), and a recent study by Wijtenburg et al. (2013) shows that GSH can be detected reproducibly in healthy controls without the use of spectral editing technique such as MEGA-PRESS (Wijtenburg et al., 2013).

Three ¹H-MRS studies measured GSH in adults with schizophrenia (Table 3). In the frontal lobe, two studies measured GSH levels using MEGA-PRESS from medicated participants with schizophrenia and controls. Matsuzawa et al. observed no GSH differences between chronic participants with schizophrenia and controls in the posterior medial frontal cortex (Matsuzawa et al., 2008). Matsuzawa et al. also reported a negative correlation between medial prefrontal GSH concentrations and severity of negative symptoms, such that lower levels of GSH were associated with greater negative symptoms. Terpstra et al. (2005) reported no GSH differences in the anterior cingulate; however, this study noted that participants were medicated but did not provide information regarding illness stage or type of medication being currently taken (Terpstra et al., 2005). Interestingly, this study compared GSH quantification from a very short TE STEAM sequence and a GSH MEGA-PRESS sequence, and concluded that the techniques were comparable. Lastly, Woods et al. (2009) observed an increase in medial temporal lobe GSH in first-episode schizophrenia compared to control subjects (Wood et al., 2009). The medication status of the first-episode participants was mixed in that 13 participants were antipsychotic medication naïve while the remaining participants received at least one dose of atypical antipsychotic medication for a mean treatment duration of six days. Further investigation indicated that GSH increased only in those subjects with schizophrenia (approximately 1/2 of the sample) that were not responsive to niacin, and a non-responsive niacin skin test is related to increased oxidative stress. Despite the liberal criteria for inclusion of GSH metabolite fits (i.e. CRLB threshold of 50%), these exciting results provide important incentive for further investigation.

In the two studies at 3 T that provide participant illness status, it appears as though GSH is elevated earlier in the illness (with minimal medication exposure) in the temporal lobe, and this may relate to increased oxidative stress. Meanwhile, there are no observable differences in the posterior medial prefrontal cortex in chronic, medicated participants with schizophrenia. This suggests that earlier in the illness, GSH is elevated while compensatory mechanisms are still in place prior to chronic oxidative stress in the latter stage of the illness. This ¹H-MRS data seems to further support prior findings in blood chemistry which show that GSH decreases from first episode to chronic (Ruiz-Litago et al., 2012). Overall,

additional studies assessing GSH are needed to better characterize how GSH changes throughout the brain in different stages of the illness and whether these alterations are associated with any symptoms or outcomes.

3.4 N-acetylaspartylglutamate (NAAG)

NAAG is an abundant neuropeptide (0.5–3 mM) in the human brain, and is a highly selective agonist at the group II metabotropic glutamate subtype 3 receptor (mGluR3) that presynaptically inhibits glutamate release (Shave et al., 2001; Tsai, 2005; Wroblewska et al., 1997; Wroblewska et al., 2006; Neale et al., 2011; Bergeron and Coyle, 2012) and a NMDAR antagonist (Bergeron and Coyle, 2012). Post-mortem studies in schizophrenia report alterations in NAAG (Reynolds and Reynolds, 2011; Ghose et al., 2009), indicating that NAAG may play a pivotal role in the pathophysiology of schizophrenia. Additional evidence stems from studies utilizing the PCP drug model of schizophrenia revealing that NAAG peptidase inhibitors prevent the behavioral and physiological effects of NMDA receptor antagonism in rodents (Olszewski et al., 2008; Olszewski et al., 2012; Zuo et al., 2012).

Despite occurring at concentrations of 1.0–3.0 mM (Dringen, 2000; Govindaraju et al., 2000), NAAG has been exceedingly difficult to quantify with ¹H-MRS since it has resonances at 2.04, 2.6, and 4.6 ppm which overlap with many other metabolites. NAAG's concentration is higher in white matter versus gray matter (Pouwels and Frahm, 1997). Due to the fact that NAAG resonances overlap with many other metabolites, several different techniques are utilized to identify NAAG from NAA, glutamate, and other metabolites. Jessen et al. utilized a long TE PRESS sequence since simulations showed that contributions from overlapping resonances of glutamate and glutamine were minimized due to J-coupling and T₂ relaxation (Jessen et al., 2013). Edden et al. (2007) optimized a MEGA-PRESS sequence specifically tailored to separate NAA from NAAG by employing a long TE of 140 ms and applying specific editing pulses that detect the 2.5 and 2.6 ppm resonances of NAA and NAAG, respectively (Edden et al., 2007). This method ensures separation of NAA and NAAG through editing, but the results suffer from a low signal-to-noise ratio. In addition, Zhang et al. utilized TE-Averaged PRESS and line shape deconvolution (Zhang et al., 2011). TE-Averaging acquires spectroscopic data at varying TEs and averages the data together. The resultant concentrations are typically lower than standard PRESS sequences due to T₂ weighting and signal cancellation as a result of averaging across multiple TEs. The technique has been shown to enable detection of glutamate previously (Hurd et al., 2004). The purpose of line shape deconvolution is to improve the spectral resolution without sacrificing signal-to-noise ratio since standard techniques using Gaussian or exponential filters negatively impact spectral line widths. As a result, this technique will need additional steps in a spectroscopy processing pipeline. Thus, there are several techniques currently available to measure NAAG.

Recently, two studies quantified NAAG in vivo in schizophrenia (Table 4). Using a PRESS sequence at a TE of 140ms, Jessen et al. showed that NAAG was decreased at trend level in the left anterior cingulate of chronic, medicated patients with schizophrenia compared to healthy controls, but not in the left frontal lobe (Jessen et al., 2013). A negative correlation

between NAAG levels and negative symptoms and total symptom score from the PANSS (Kay et al., 1987) was observed. Rowland et al. (2013) utilized the MEGA-PRESS editing technique and showed that NAAG decreased across age groups in the centrum semiovale in schizophrenia participants, but NAAG increased with age in healthy controls (Rowland et al., 2013). In younger schizophrenia participants, this study reported higher NAAG levels in the centrum semiovale compared to controls. In contrast, this same study found the opposite effect in the older cohort, suggesting that NAAG is higher earlier in the illness and declines over time. Rowland et al. (2013) did not observe a decrease in NAAG levels in the anterior cingulate that Jessen et al. did, and they reported a different directional relationship between NAAG and negative symptom severity. Direct comparisons between the studies are difficult due to different localization methodologies, quantification software packages, and brain regions assessed. Overall, these results suggest that NAAG levels change over the course of the illness, and further investigation is needed to determine whether NAAG levels are altered in first episode and whether NAAG peptidase inhibitors are beneficial for schizophrenia participants.

3.5 Glycine

Glycine is an amino acid neurotransmitter that is an allosteric modulator of the glutamate NMDAR. Glycine acts on the glycine/D-serine site located on the NR1 subunit of the NMDAR. If NMDAR hypofunction is involved in the pathophysiology of schizophrenia, then a possible treatment target is the glycine system (Coyle and Tsai, 2004). Oral glycine administration improves negative symptoms in schizophrenia (Javitt et al., 1994; Heresco-Levy et al., 1996; Heresco-Levy et al., 1999; Heresco-Levy and Javitt, 2004) and has been explored as a possible drug target (Hashimoto, 2011; Cioffi, 2013). While a recent meta-analysis found no observable differences in serum glycine levels between healthy controls and adults with schizophrenia (Brouwer et al., 2013), a recent ¹H-MRS study, in which healthy adult males were given two weeks of high dose oral glycine, showed elevated levels of Gly/Cr in 10 out of 11 participants in the occipital cortex (Kaufman et al., 2009). Even though it crosses the blood brain barrier poorly (Hashimoto et al., 2013), the ability to quantify cerebral glycine levels after treatment is exciting and is applicable to the study of schizophrenia.

Although two uncoupled methylene protons of glycine generate a simple singlet resonance at 3.55 ppm, the reliable detection of glycine *in vivo* with ¹H-MRS is difficult. This is primarily due to its low concentration (0.4–1.0 mM) and the spectral overlap with myo-Inositol (mI), which usually yields much higher spectral resonances at 3.55 ppm. In order to reliably measure glycine with ¹H-MRS, it is necessary to suppress the resonances of mI around 3.55 ppm. This can be achieved by exploiting the differences in spin coupling between the metabolites. The two glycine proton spins are uncoupled while the proton spins of mI are scalar coupled (Govindaraju et al., 2000). Metabolites with coupled spins undergo signal loss due to J-coupling and T₂ relaxation, compared to metabolites with uncoupled spins that undergo signal loss due to T₂ relaxation. Thus, at longer TEs, it is more feasible to separate glycine and mI than at short TEs (Govindaraju et al., 2000). TE-Averaging PRESS is a method to detect glycine (Kaufman et al., 2009; Prescott et al., 2006) where data are acquired over a range of TEs and then averaged together. While the signal from glycine and

mI is expected to decrease due to T_2 relaxation observed in the multiple TEs, the signal from mI is expected to be greatly reduced due to J-coupling, allowing detection of glycine. Another study by Choi et al. (2008) utilized multiple refocusing pulses at a long TE to suppress the mI signal (Choi et al., 2008). By using a long TE, both of these sequences remove the signal contributions from macromolecules from the spectral data. These techniques are employed at 3T and 4T, but at 7T, where the frequency separation between metabolites is improved, two other sequences are used to measure glycine in vivo: a long TE PRESS sequence (Choi et al., 2009b) and a short TE spin echo, full intensity acquired localized (SPECIAL) sequence (Gambarota et al., 2009). To date, there are no published reports of ^1H -MRS glycine measurements in schizophrenia. Applying these techniques to study participants with schizophrenia would provide new information regarding cerebral glycine levels, insight into NMDAR function, an additional mechanism for monitoring treatment response, and overall, improve our understanding of the pathophysiology of schizophrenia.

3.6 Serine

Serine (Ser) is an amino acid in the brain acting as a co-agonist of glutamate transmission through the interactions with the glycine site of the NMDA receptor (Van Horn et al., 2013). Serine exists in two forms: L-serine and D-serine, and each isomer has similar chemical and physical properties (Bardaweel et al., 2014). While both forms exist in the brain, D-serine distribution is parallel to the distribution of NMDA glutamate receptors (Bardaweel et al., 2014), and D-serine binds three hundred times more strongly than L-serine at the NR1 subunit of NMDAR (Furukawa and Gouaux, 2003). Serum D-serine is typically reduced in adults with schizophrenia (Hashimoto et al., 2003; Calcia et al., 2012), and a recent meta-analysis found that serum levels of total serine are elevated in adults with schizophrenia (Brouwer et al., 2013). In addition, D-serine has been investigated as a monotherapy (Ermilov et al., 2013) and as adjunctive therapy (Heresco-Levy et al., 2005) in treatment-refractory adults with schizophrenia and found to be helpful in relieving negative symptoms.

The coupled proton spins of the CH_2 and CH group of serine yield resonances at 3.98, 3.94, and 3.83 ppm (Govindaraju et al., 2000). Measurement of serine with ^1H -MRS is significantly impaired by the spectral overlap with the prominent resonance of creatine (Cr) at 3.91 ppm as well as the low in vivo concentration of serine (around 0.4 mM), making quantification very difficult. To date, there are no ^1H -MRS studies reporting serine at 3T or 4T; however, there is one study at 7T that was able to measure serine in healthy controls. Choi et al (2009a) utilized a constant-TE triple-refocusing difference editing method for detecting serine separate from Cr (Choi et al., 2009a). In this sequence, an additional 180° RF pulse was added to a PRESS sequence, and this RF pulse was spectrally-selective, in that it is tuned to 3.0 ppm and only excites resonances from 1.8 ppm to 4.2 ppm. This technique also utilized a subtraction method that maintains a fixed TE and varied sub-TEs in order to cancel out the overlapping creatine signal and provide the maximum signal difference for serine. Using a long TE attenuates the macromolecule signal, which is beneficial, but it also leads to significant signal loss due to T_2 relaxation. This technique is not suitable for shorter TEs where the editing efficiency is poor and requires excellent water suppression due to serine's proximity to the water peak within the spectrum. Since TE simulations for the

maximal serine signal were performed at 7T, another simulation for detection of serine using the triple-refocusing technique would need to be conducted to determine the optimal TE and sub-TEs for 3T. To date, there have been no ^1H -MRS schizophrenia studies that reported serine, but this technique by Choi et al. holds promise for acquisition at high fields, and would provide new and valuable information regarding NMDAR function in schizophrenia.

4.0 Discussion

In conclusion, ^1H -MRS has provided invaluable information regarding the pathophysiology of schizophrenia. It has enhanced our understanding of metabolite differences between brain regions, stages of the illness, and pharmacological treatments, especially regarding glutamate, glutamine, and glutamate+glutamine. There is still much knowledge to be gained regarding historically difficult to detect metabolites, and currently, there are methods available to expand our understanding of the illness in terms of GABA, GSH, NAAG, glycine, and serine. Application of ^1H -MRS to the study of schizophrenia has also elicited new questions regarding off-medication metabolite differences in participants with longer illness durations, effects of short and long term antipsychotic medication treatment on different brain regions, whether GABA and GSH levels change in different stages of the illness, and whether metabolite levels change later in life. Overall, ^1H -MRS is a powerful technique that holds great promise for producing biomarkers that can serve as intervention targets and predict disease onset or illness exacerbation for schizophrenia and other brain diseases.

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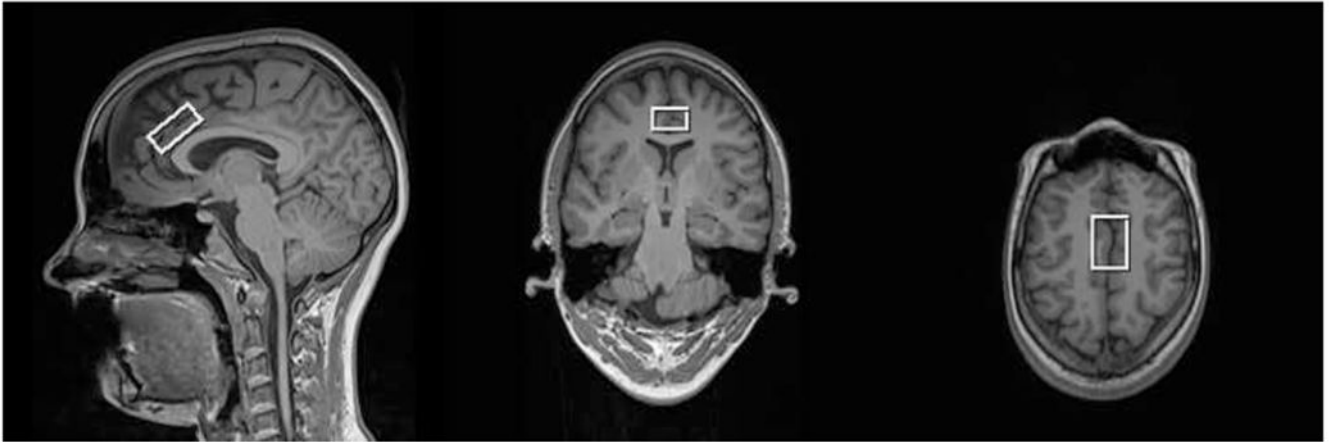
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Highlights

- MRS is a powerful technique used to assess neurotransmitters and modulators in vivo
- MRS techniques enable detection of Glu, GABA, GSH, NAAG, glycine, and serine
- MRS studies of schizophrenia show alterations in Glu, GABA, GSH and NAAG
- Metabolite differences depend on brain region, illness stage and medication status
- MRS shows great promise for understanding glutamatergic systems in schizophrenia

Figure1A



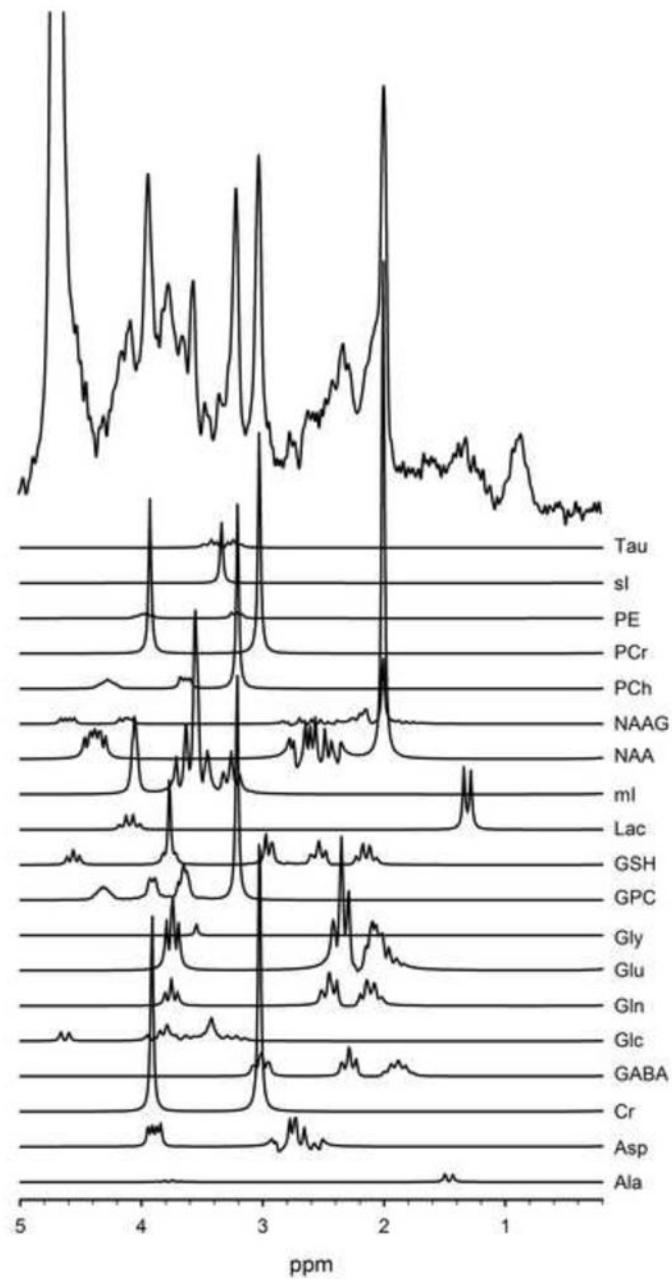
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Figure1B

**Figure 1.**

T_1 -weighted voxel images acquired at 3T with a MP-RAGE imaging sequence (TR/TE/TI=1900/3.45/900-ms, FOV 256×256 mm, 1-mm isotropic voxels, flip angle = 9°). These images show that the voxel was placed in the anterior cingulate (a). A very short echo time (TE) spectrum with corresponding plots of each metabolite in the spectrum shown below (b). These metabolites are included in the basis set used for quantification. The metabolites were simulated at a frequency of 123.56-MHz using a STEAM sequence (TR/TM/TE=2000/10/6.5ms).

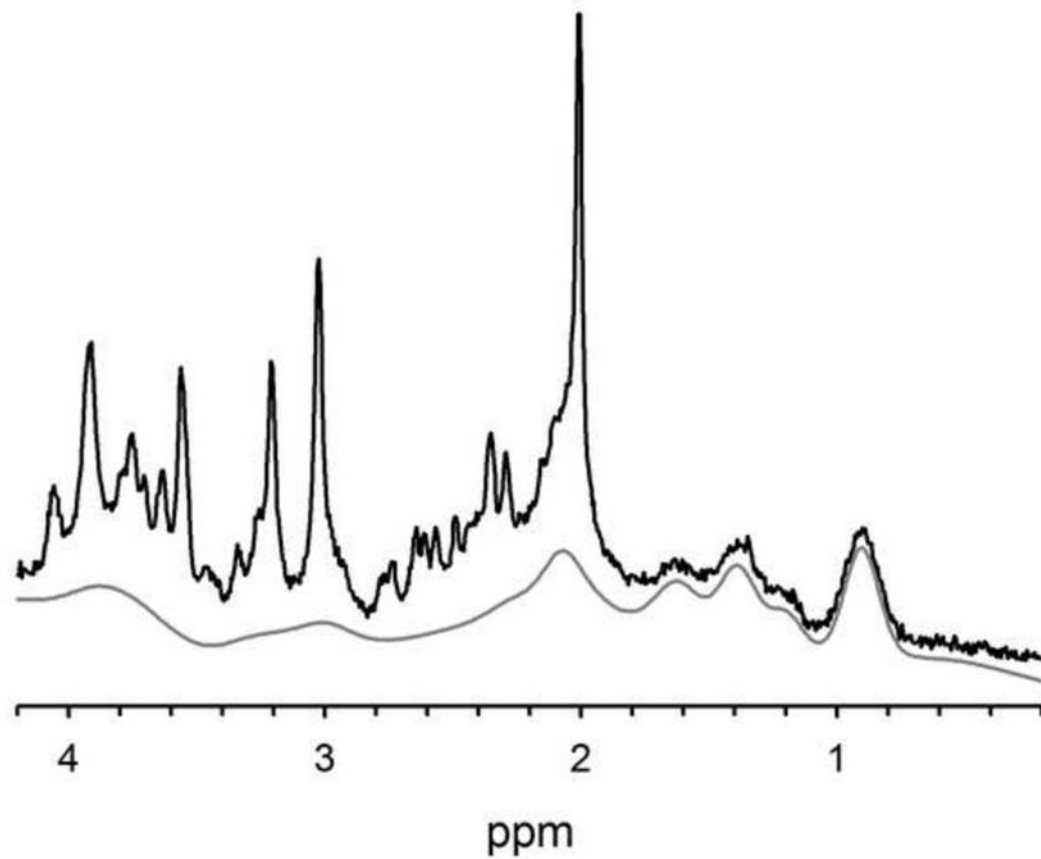


Figure 2.

The macromolecule background is only present at shorter TEs due to its short T₂ constituents, and it can be a complicating factor in quantification. This is an example short TE spectrum (black) with its macromolecule background fit spectrum shown underneath (gray) as fit by LCMoel. These data were acquired at 3T using a very short TE phase rotation sequence (TR/TM/TE=2000/10/6.5-ms, 256 excitations, 2.5-kHz spectral width, 2048 complex points, and phases: $\phi_1=135^\circ$, $\phi_2=22.5^\circ$, $\phi_{13}=112.5^\circ$, $\phi_{ADC}=0^\circ$).

Table 1

Summary of Glutamatergic Findings in Ultra High Risk (UHR) or At Risk Mental State (ARMS), First Episode (FEP), and Chronic Participants with Schizophrenia at 3 T or Higher

Stage of Illness	Region	Authors	Scanner & Method	Participants (SZ/HC)	Antipsychotic Medication Use	SZ Mean Age (yrs±std or yrs (range))	Results (vs. HC)
UHR	right medial frontal region	Tibbo et al. (2004)	3T STEAM (TE=20ms)	20/22	None	16.4±1.99	↑ Glx/Cr in high risk adolescents
UHR	medial prefrontal cortex	Natsubori et al. (2013)	3T STEAM (TE=15ms)	24/26	Yes	21.7 (16–29)	no Glx diff.
ARMS	anterior cingulate	Stone et al. (2009)	3T PRESS (TE=30ms)	27/27	Yes, ^g	25±5	no Glu diff. and ↑ Glx in ARMS
ARMS	anterior cingulate	Fusar-Poli et al. (2011)	3T PRESS (TE=30ms)	24/17	None (naïve)	26.6±5	no Glu diff.
ARMS	anterior cingulate	Valli et al. (2011)	3T PRESS (TE=30ms)	22/16	None (naïve)	25.72±4.97	no Glu diff.
UHR	left and right medial temporal lobe (mostly anterior hippocampus)	Wood et al. (2010)	3T PRESS (TE=30ms)	66/29	None	19.18±3.17	no Glx diff.
ARMS	left hippocampus	Stone et al. (2009)	3T PRESS (TE=30ms)	27/27	Yes, ^g	25±5	no Glu or Glx diff.
ARMS	left hippocampus	Fusar-Poli et al. (2011)	3T PRESS (TE=30ms)	24/17	None (naïve)	26.6±5	↓ Glu in ARMS (trend level)
ARMS	left hippocampus	Valli et al. (2011)	3T PRESS (TE=30ms)	22/16	None (naïve)	25.72±4.97	no Glu diff.
UHR	left and right temporal lobe (largely anterior hippocampus)	Berger et al. (2012)	3T PRESS (TE=30ms)	11/10	Baseline: None (naïve); Treatment: Low-Dose Lithium for 3 months	20.74±3.32	no Glx diff. between UHR on lithium and UHR on treatment as usual only
UHR	right dorsal caudate nucleus	de la Fuente-Sandoval et al. (2011)	3T PRESS (TE=35ms)	18/40, ^a	None (naïve)	19.56±3.46	↑ Glu and no Glx diff. in UHR; no diff. between UHR and FEP
ARMS	left thalamus	Stone et al. (2009)	3T PRESS (TE=30ms)	27/27	Yes, ^g	25±5	↓ Glu and no Glx diff in ARMS
ARMS	left thalamus	Fusar-Poli et al. (2011)	3T PRESS (TE=30ms)	24/17	None (naïve)	26.6±5	↓ Glu in ARMS (trend level)
ARMS	left thalamus	Valli et al. (2011)	3T PRESS (TE=30ms)	22/16	None (naïve)	25.72±4.97	↓ Glu in ARMS (trend level)

Stage of Illness	Region	Authors	Scanner & Method	Participants (SZ/HC)	Antipsychotic Medication Use	SZ Mean Age (yrs±std or yrs (range))	Results (vs. HC)
UHR	right cerebellum	de la Fuente-Sandoval et al. (2011)	3T PRESS (TE=35ms)	18/40, ^a	None (naïve)	19.56±3.46	no Glu and Glx diff.; no diff. between UHR and FEP
FEP	left anterior cingulate	Theberge et al. (2002)	4T STEAM (TE=20ms)	21/21	None (naïve)	26±7	no Glu diff and ↑ Glx in FEP
FEP	left anterior cingulate	Theberge et al. (2007)	4T STEAM (TE=20ms)	16/16	Baseline: none (naïve); 10 months: Yes; 30 months: Yes	baseline: 25±8, 10m: 25±8, 30m: 27±8	no Glu diff at baseline, 10m, and 30m; ↑ Glx at baseline
FEP	left anterior cingulate	Aoyama et al. (2011)	4T STEAM TE=20ms	17/17	Baseline: none (naïve); 10 months: Yes; 80 months: Yes	32±9	↑ Glx at baseline and 80m in SZ; no Glu diff.
FEP	frontal lobe	Goto et al. (2012)	3T MEGA-PRESS (TE=68ms)	16/18	Baseline: No Information; Treatment: AP for 6 months	30±11	Baseline: no Glx/Cr diff.; 6m: ↓ Glx/Cr compared to baseline (trend level)
FEP	medial prefrontal cortex	Natsubori et al. (2013)	3T STEAM (TE=15ms)	19/19	Yes	25.4 (17–37)	no Glx diff.
FEP	bilateral anterior cingulate and left frontal white matter	Bustillo et al. (2010)	4T STEAM (TE=20ms)	14/10	Baseline: minimal exposure (< 3 weeks); Treatment: AP and 7/u at 1, 6, and 12 months	27.2±8.9	no Glu and Glx diff. in both regions at baseline and following treatment; baseline: ↑Glu/Glx ratio in anterior cingulate of FEP
FEP	anterior cingulate cortex	Egerton et al. (2012)	3T PRESS (TE=30ms)	32/0, ^b	Yes	remission: 30±6; non-remission: 25±7	↑Glu/Cr in non-remission (symptomatic) versus remission; no Glx/Cr diff.; higher Glu/Cr was associated with greater negative symptom severity and lower global functioning
FEP	left thalamus	Egerton et al. (2012)	3T PRESS (TE=30ms)	32/0, ^b	Yes	remission: 30±6; non-remission: 25±7	no Glu/Cr and Glx/Cr diff.
FEP	left medial thalamus	Theberge et al. (2002)	4T STEAM (TE=20ms)	21/21	None (naïve)	26±7	no Glu diff and ↑ Glx in FEP

Stage of Illness	Region	Authors	Scanner & Method	Participants (SZ/HC)	Antipsychotic Medication Use	SZ Mean Age (yrs=std or yrs (range))	Results (vs. HC)
FEP	left thalamus	Theberge et al. (2007)	4T STEAM (TE=20ms)	16/16	Baseline: none (naïve); 10 months: Yes; 30 months: Yes	baseline: 25±8, 10m: 25±8, 30m: 27±8	No Glu diff. at baseline, 10m, and 30m; ↑ Gln at baseline; In SZ, Gln at 30m was reduced compared to baseline and 10m
FEP	left thalamus	Aoyama et al. (2011)	4T STEAM TE=20ms	17/17	Baseline: none (naïve); 10 months: Yes; 80 months: Yes	32±9	↑ Gln at baseline in SZ; no Glu diff.; ↓ Glx over 80m
FEP	left thalamus	Bustillo et al. (2010)	4T STEAM (te=20ms)	14/10	Baseline: minimal exposure (< 3 weeks); Treatment: AP and f/u at 1, 6, and 12 months	27.2±8.9	no Glu and Gln diff. at baseline or following treatment
FEP	basal ganglia	Goto et al. (2012)	3T MEGA-PRESS (TE=68ms)	16/18	Baseline: No Information; Treatment: AP for 6 months	30±11	Baseline: ↑ Glx/Cr in SZ; 6m: no change Glx/Cr
FEP	right dorsal caudate nucleus	de la Fuente-Sandoval et al. (2011)	3T PRESS (TE=35ms)	18/40, ^a	None (naïve)	23.44±4.93	↑ Glu and no Glx diff. in FEP; no diff. between UHR and FEP
FEP	right dorsal caudate nucleus	de la Fuente-Sandoval et al (2013a)	3T PRESS (TE=35ms)	19/26	None (naïve)	not available	↑ Glu in UHR who transitioned to psychotic disorder compared to UHR who did not transition and controls
FEP	right dorsal caudate nucleus	de la Fuente-Sandoval et al (2013b)	3T PRESS (TE=35ms)	28/18	Baseline: none (naïve); Treatment: risperidone for 4 weeks	26.58±8.49	Baseline: ↑ Glu and ↑ Glx in FEP; Post-treatment (4-weeks); no Glu and Glx diff.; ↓ in Glu after 4-weeks was significant
FEP	parieto-occipital lobe	Goto et al. (2012)	3T MEGA-PRESS (TE=68ms)	16/18	Baseline: No Information; Treatment: AP for 6 months	30±11	Baseline: no Glx/Cr diff.; 6m: no change in Glx/Cr
FEP	right cerebellum	de la Fuente-Sandoval et al. (2011)	3T PRESS (TE=35ms)	18/40, ^a	None (naïve)	23.44±4.93	no Glu and Glx diff.; no diff. between UHR and FEP
FEP	right cerebellum	de la Fuente-	3T PRESS (TE=35ms)	28/18	Baseline: none (naïve); Treatment: risperidone for 4 weeks	26.58±8.49	Baseline: ↑ Glu and ↑ Glx in FEP; Post-treatment (4-weeks)

Stage of Illness	Region	Authors	Scanner & Method	Participants (SZ/HC)	Antipsychotic Medication Use	SZ Mean Age (yrs±std or yrs (range))	Results (vs. HC)
		Sandoval et al (2013b) Sandoval et al (2013b)					weeks): no Glu diff. and ↑ Glx remained
Chronic	left and right rostral and dorsal anterior cingulate	Wood et al. (2007)	3T PRESS (TE=30ms)	15/14	Yes	31.5±7.5	no Glx diff. in either of the 4 voxels
Chronic	anterior cingulate	Tayoshi et al (2009)	3T STEAM (TE=18ms)	30/25	Yes	33.8±9.5	↓ Glu and no Glx diff in SZ, driven by diff. in males
Chronic	left anterior cingulate	Theberge et al. (2003)	4T STEAM (TE=20ms)	21/21	Yes	37±11	↓ Glu and ↓ Glx in SZ
Chronic	anterior cingulate cortex	Kraguljac et al. (2012)	3T PRESS (TE=80ms)	48/46	Yes	38.02±12.4	no Glx/Cr diff.
Chronic	bilateral dorsal anterior cingulate cortex	Reid et al. (2010)	3T PRESS (TE=80ms)	28/25	Yes	40.4±13.1	no Glx/Cr diff.; negative correlation between Glx/Cr and negative symptoms from BPRS
Chronic	left middle frontal	Rowland et al. (2009)	3T PRESS (TE=35ms)	20/10, ^c	Yes	Deficit: 43±6, Non-deficit: 40±6	No Glx diff.
Chronic	mesial prefrontal gray matter and left prefrontal white matter	Lutkenhoff et al (2010)	3T PRESS TE=30ms	14/26, ^d	Yes	48.8±11.5	across frontal and temporal regions, ↓ Glu in SZ; exploratory analysis; ↓ Glu in mesial prefrontal cortex
Chronic	left and right prefrontal white matter	Chang et al. (2007)	4T PRESS (TE=30ms)	23/22	Yes	66.3±7.2	↑ Glx in right prefrontal white matter in SZ; overall, ↑ Glx across frontal, temporal, and occipital
Chronic	anterior cingulate and centrum semiovale	Rowland et al. (2013)	3T PRESS (TE=35ms)	21/20, ^e	Yes	YSZ: 30.2±6.6; OSZ: 51.1±4.0	no Glx diff. in either region; overall effect of ↓ Glx in SZ
Chronic	anterior cingulate	Ongur et al. (2010)	3T MEGA-PRESS (TE=68ms)	21/19	Yes	39.0±10.8	no Glu/Cr diff.
Chronic	left anterior cingulate cortex and left frontal lobe	Jessen et al. (2013)	3T PRESS (TE=30ms)	20/20	Yes	34.5±10.2	no Glu/Cr and Glx/Cr diff. in both regions

Stage of Illness	Region	Authors	Scanner & Method	Participants (SZ/HC)	Antipsychotic Medication Use	SZ Mean Age (yrs±std or yrs (range))	Results (vs. HC)
Chronic	medial prefrontal cortex	Natsubori et al. (2013)	3T STEAM (TE=15ms)	25/28	Yes	32.7 (17–50)	↓ Glx in SZ; ↓ Glx in chronic compared to UHR and FEP groups ↑ Glx in unmedicated SZ compared to HC in medial prefrontal cortex; ↑ Glx in unmedicated versus medicated participants (trend level) in medial prefrontal cortex; no Glx diff between medicated SZ and HC in medial prefrontal cortex; no Glx diff in dorsolateral prefrontal cortex between groups
Mixed	medial prefrontal cortex and left dorsolateral prefrontal cortex	Kegeles et al. (2012)	3T J-edited (TE=68ms)	32/22 ^f	Yes & No	unmedicated: 32±11, medicated: 32±10	↑ Glx in SZ and ↓ Glx in HC in temporal white matter in SZ; overall, ↑ Glx across frontal, temporal, and occipital
Mixed	dorsal anterior cingulate	Bustillo et al. (2014)	3T PRESS (TE=40ms)	84/81	Yes	36.7±13.9 (16–64)	↑ Glx in SZ and with aging (both groups); no Glu diff; ↑ Glx/Glu no Glx/Cr diff.
Chronic	left hippocampus	Hutcherson et al. 2012	3T PRESS (TE=80ms)	39/41	Yes	36.7±12.2	no Glx/Cr diff.
Chronic	left hippocampus	Kragljac et al. (2012)	3T PRESS (TE=80ms)	48/46	Yes	38.02±12.4	no Glx/Cr diff.
Chronic	left hippocampus	Lutkenhoff et al. (2010)	3T PRESS TE=30ms	14/26, ^d	Yes	48.8±11.5	No diff. in Glu; across frontal and temporal regions, ↓ Glu in SZ
Chronic	left and right medial temporal lobe white matter	Chang et al. (2007)	4T PRESS (TE=30ms)	23/22	Yes	66.3±7.2	↑ Glx in left medial temporal white matter in SZ; overall, ↑ Glx across frontal, temporal, and occipital
Mixed	left hippocampus	Kraguljac et al. (2013)	3T PRESS (TE=80ms)	27/27	None (unmedicated at least 2 weeks)	32.63±9.28	↑ Glx/Cr in SZ
Chronic	left thalamus	Theberge et al. (2003)	4T STEAM (TE=20ms)	21/21	Yes	37±11	no Glu and ↑ Glx in SZ

Stage of Illness	Region	Authors	Scanner & Method	Participants (SZ/HC)	Antipsychotic Medication Use	SZ Mean Age (yrs±std or yrs (range))	Results (vs. HC)
Chronic	left basal ganglia	Tayoshi et al (2009)	3T STEAM (TE=18ms)	30/25	Yes	33.8±9.5	no Glu diff.; no Gln diff. (including both genders); Gln was lower in SZ females versus SZ males
Chronic	substantia nigra	Reid et al. (2013)	3T PRESS (TE=80ms)	35/22	Yes	37.9±12	no Glx/Cr diff.
Chronic	left inferior parietal	Rowland et al. (2009)	3T PRESS (TE=35ms)	20/10, ^c	Yes	Deficit: 43±6, Non-deficit: 40±6	no Glx diff.
Chronic	left and right occipital white matter	Chang et al. (2007)	4T PRESS (TE=30ms)	23/22	Yes	66.3±7.2	↑ Glx in SZ; overall, ↑ Glx across frontal, temporal, and occipital
Mixed	bilateral calcarine sulci	Yoon et al (2010)	3T MEGA-PRESS (TE=68ms)	13/13	Yes	27.5±8.8	no Glx/Cr diff.
Chronic	parietal-occipital cortex	Ongur et al. (2010)	3T MEGA-PRESS (TE=68ms)	21/19	Yes	39.0±10.8	no Glu/Cr diff.
Unknown	anterior cingulate	Terpstra et al. (2005)	4T STEAM (TE=5ms)	13/9	Yes	26±5	no Glu and Gln diff.
Relatives							
Sibling	left and right frontal	Purdon et al. (2008)	3T STEAM (TE=20ms)	19/16	None	46.32±6.09	no Glu/Cr and Glx/Cr diff.
Co-twin	mesial prefrontal cortex, left prefrontal white matter, left hippocampus	Lutkenhoff et al (2010)	3T PRESS (TE=30ms)	15/26, ^d	None	49.5±10.0	↓ Glu overall (mesial prefrontal cortex, left prefrontal white matter, and left hippocampus)
Child of Parent with SZ	right medial frontal region	Tibbo et al. (2004)	3T STEAM (TE=20ms)	20/22	None	16.4±1.99	↑ Glx/Cr in high risk adolescents

↑ - increased, ↓ - decreased, Cr - creatine, diff - difference, Glu - glutamate, Gln - glutamine, Glx - glutamate+glutamine, HC - healthy controls, m - months, ms - millisecond

OHC - older healthy controls, OSZ, - older schizophrenia, SZ - schizophrenia, TE - echo time, YHC - younger healthy controls, YSZ - younger schizophrenia

^a one control group for comparison to UHR and FEP

^b 16 patients in remission, 16 patients in non-remission

^c SZ group of 20: 10 deficit and 10 non-deficit

^d 14 SZ, 15 co-twins, and same 26 HC (13 twin pairs)

g on current AP or Antidepressant Medication (do not separate the two) types of meds)

f 16 unmedicated and 16 medicated SZ

e YSZ: 11; YHC: 10; OSZ: 10; OHC: 10

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Table 2
 Summary of GABA Findings in First Episode (FEP) and Chronic Participants with Schizophrenia at 3T or higher

Stage of Illness	Region	Authors	Scanner & Method	Participants (SZ/HC)	Antipsychotic Medication Use	SZ Mean Age (yrs±std or yrs (range))	Results (vs. HC)
FEP	frontal lobe	Goto et al. (2010)	3T MEGA-PRESS (TE=68ms)	16/18	Baseline: No Information; Treatment: AP for 6 months	30±10.0	Baseline: No GABA/Cr diff.; 6 months: No GABA/Cr diff. & no change
FEP	left basal ganglia	Goto et al. (2010)	3T MEGA-PRESS (TE=68ms)	16/18	Baseline: No Information; Treatment: AP for 6 months	30±10.0	Baseline: ↓ GABA/Cr in SZ; 6 months: ↓ GABA/Cr in SZ remained & no diff. between baseline and 6 months
FEP	parieto-occipital lobe	Goto et al. (2010)	3T MEGA-PRESS (TE=68ms)	16/18	Baseline: No Information; Treatment: AP for 6 months	30±10.0	Baseline: No GABA/Cr diff.; 6 months: No GABA/Cr diff. & no change
FEP	bilateral calcarine sulci	Keleman et al. (2013)	3T MEGA-PRESS (TE=68ms)	28/20	Baseline: None (naïve); 8 weeks: Yes	24.9±8.3	Baseline: ↓GABA/Cr in SZ; 8 weeks: ↓ GABA/Cr in SZ
Chronic	anterior cingulate	Tayoshi et al. (2010)	3T MEGA-PRESS (TE=68ms)	38/29	Yes	34.0±10.0	No GABA diff; negative correlation between GABA level and antipsychotic dose
Chronic	anterior cingulate	Ongur et al. (2010)	4T MEGA-PRESS (TE=68ms)	21/19	Yes	39.0±10.08	↑ GABA/Cr in SZ
Mixed	medial prefrontal cortex and left dorsolateral prefrontal cortex	Kegeles et al. (2012)	3T MEGA-PRESS (TE=68ms)	32/22, ^a	Yes & No	unmedicated: 32±11, medicated: 32±10	↑ GABA/W in unmedicated versus HC in medial prefrontal cortex; no GABA/W diff in medicated versus HC in medial prefrontal cortex; no GABA/W diff in left dorsolateral prefrontal cortex
Chronic	anterior cingulate and centrum semiovale	Rowland et al. (2013)	3T MEGA-PRESS (TE=68ms)	21/20, ^b	Yes	YSZ: 30.2±6.6; OSZ: 51.1±4.0	↓ GABA/W in OSZ compared to OHC in anterior cingulate; No GABA/W diff in centrum semiovale; Younger group (SZ+HC) had higher GABA than older group (SZ+HC) across region

Stage of Illness	Region	Authors	Scanner & Method	Participants (SZ/HC)	Antipsychotic Medication Use	SZ Mean Age (Yrs±std or yrs (range))	Results (vs. HC)
Chronic	left basal ganglia	Tayoshi et al. (2010)	3T MEGA-PRESS (TE=68ms)	38/29	Yes	34.0±10.0	No GABA diff; ↑ GABA in SZ taking typical APs compared to SZ taking atypical APs; positive correlation between GABA level and anticholinergic dose
Chronic	parieto-occipital cortex	Ongur et al. (2010)	4T MEGA-PRESS (TE=68ms)	21/19	Yes	39.0±10.08	↑ GABA/Cr in SZ
Mixed	bilateral calcarine sulci	Yoon et al. (2010)	3T MEGA-PRESS (TE=68ms)	13/13	Yes	27.5±8.8	↓ GABA/Cr in SZ

↑ - increased, ↓ - decreased, AP - antipsychotic medications, Cr - creatine, diff - differences, GABA - γ -aminobutyric acid, HC - healthy controls, ms - millisecond

OHC - older healthy controls, OSZ - older schizophrenia, SZ - schizophrenia, TE - echo time, W - water, YHC - younger healthy controls, YSZ - younger schizophrenia

^a 16 unmedicated SZ & 16 medicated SZ

^b YSZ: 11; YHC: 10; OSZ: 10; OHC: 10

Table 3

Summary of GSH Findings in First Episode (FEP) and Chronic Participants with Schizophrenia at 3T or higher

<u>Stage of Illness</u>	<u>Region</u>	<u>Authors</u>	<u>Scanner & Method</u>	<u>Participants (SZ/HC)</u>	<u>Antipsychotic Medication Use</u>	<u>SZ Mean Age (yrs ± std)</u>	<u>Results (vs. HC)</u>
FEP	medial temporal lobe	Wood et al. (2009)	3T PRESS (TE=30ms)	30/18	Yes	19.4±3.4	↑ GSH in SZ
Chronic	posterior medial prefrontal cortex	Matsuzawa et al. (2008)	3T MEGA-PRESS (TE=94ms)	20/16	Yes	30.7±5.8	No GSH diff.; negative correlation between GSH level and negative symptoms
Unknown	anterior cingulate	Terpsira et al. (2005)	4T STEAM (TE=5ms) & MEGA-PRESS (TE=68ms)	13/9	Yes	26±5	No GSH diff.

↑ - increased, diff. - difference, GSH - glutathione, HC - healthy controls, ms - millisecond, std - standard deviation, SZ - schizophrenia, TE - echo time, yrs - years

Table 4
 Summary of NAAAG Findings in Chronic Participants with Schizophrenia at 3T or higher

Stage of Illness	Region	Authors	Scanner & Method	Antipsychotic Medication Use	Participants (SZ/HC)	SZ Mean Age (yrs ± std)	Results (vs. HC)
Chronic	anterior cingulate & medial lateral part of left frontal lobe	Jessen et al. (2013)	3T PRESS (TE=140ms)	Yes	20/20	34.5±10.2	↑ NAAAG in SZ (trend level) in anterior cingulate; ↑ NAAAG/NAA in SZ in anterior cingulate; no NAAAG diff. in left frontal lobe; negative correlation between NAAAG from the frontal lobe and negative symptoms
Chronic	anterior cingulate & centrum semiovale	Rowland et al. (2013)	3T MEGA-PRESS (TE=140ms)	Yes	21/20 ^a	YSZ: 30.2±6.6; OSZ: 51.1±4.0	No NAAAG diff. in anterior cingulate; ↑ NAAAG in younger SZ versus younger HC in centrum semiovale; ↓ NAAAG in older SZ versus older HC in centrum semiovale; positive correlation with NAAAG from centrum semiovale and negative symptom

↑ - increased, ↓ - decreased, diff. - difference, HC - healthy controls, ms - millisecond, N-acetylaspartylglutamate, OHC - older healthy controls, OSZ - older schizophrenia, std - standard deviation, TE - echo time, yrs - years, YHC - younger healthy controls, YSZ - younger schizophrenia

^aYSZ: 11; YHC: 10; OSZ: 10; OHC: 10