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Beyond static measures: A review of functional magnetic resonance spectroscopy and its potential to investigate dynamic glutamatergic abnormalities in schizophrenia

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Abstract

Abnormalities of the glutamate system are increasingly implicated in schizophrenia but their exact nature remains unknown. Proton magnetic resonance spectroscopy (¹H-MRS), while fundamental in revealing glutamatergic alterations in schizophrenia, has, until recently, been significantly limited and thought to only provide static measures. Functional magnetic resonance spectroscopy (fMRS), which uses sequential scans for dynamic measurement of a range of brain metabolites in activated brain areas, has lately been applied to a variety of task or stimulus conditions, producing interesting insights into neurometabolite responses to neural activation. Here, we summarise the existing ¹H-MRS studies of brain glutamate in schizophrenia. We then present a comprehensive review of research studies that have utilised fMRS, and lastly consider how fMRS methods might further the understanding of glutamatergic abnormalities in schizophrenia.

1 Introduction

A growing body of evidence supports the role of glutamatergic abnormalities in the pathophysiology of schizophrenia. It has been hypothesised that dysfunctional N-methyl-D-aspartate receptors (NMDARs) on GABAergic interneurons leads to excess cortical glutamate release (Lisman et al., 2008; Olney et al., 1999) and downstream effects on dopamine and other neurotransmitter systems in patients with schizophrenia (Howes et al., 2015; Stone et al., 2007). Supporting this theory, the NMDAR antagonists phencyclidine (PCP) and ketamine induce positive psychotic symptoms including hallucinations, thought disorder and delusions, together with negative symptoms such as apathy, emotional withdrawal and cognitive impairment (Javitt, 2007; Krystal et al., 2005). Other evidence from genetic studies has identified a number of genes associated with schizophrenia that converge on the NMDAR signalling pathways (Harrison and Weinberger, 2005). Despite theoretical advances, the exact nature of glutamate alterations in schizophrenia remains unknown.

Proton Magnetic Resonance Spectroscopy (^1H -MRS) is a non-invasive technique which has been used to measure certain aspects of glutamatergic transmission *in vivo*. ^1H -MRS studies have been carried out measuring concentrations of glutamate, its metabolite glutamine, or their combination (Glx), in different predefined brain regions in individuals at high risk of psychosis (De la Fuente-Sandoval et al., 2011), patients with first-episode psychosis (Th  berge et al., 2002) and chronic schizophrenia (Bustillo et al., 2014). Although ^1H -MRS studies have been crucial in supporting the hypothesis of glutamatergic dysfunction in schizophrenia, a significant limitation is that glutamate concentrations are determined over a single static time period, preventing an understanding of potential aberrations in dynamic glutamate responses.

Functional magnetic resonance spectroscopy (fMRS), is a technique which is increasingly being applied to investigate dynamic changes in brain chemistry (Duarte et al., 2012). Instead of recording a single averaged spectrum, as is the case with ^1H -MRS, fMRS detects multiple spectra over time, so neurochemical changes resulting from functional activation can be studied. fMRS studies have contributed to further understanding of brain energy metabolism (Shulman et al., 2009), visual activation (Lin et al., 2012; Prichard et al., 1991), pain processing (Cleve et al., 2015; Gussew et al., 2010; Gutzeit et al., 2013; Mullins et al.,

2005), panic disorder (Dager et al., 1999) and motor cortex activation (Schaller et al., 2014), amongst others. Recently, cognitive studies utilising fMRS have been able to detect task-related neurochemical changes for a number of paradigms like working memory (Michels et al., 2012), object recognition (Apšvalka et al., 2015; Lally et al., 2014) and the Stroop paradigm (Lally et al., 2014; Taylor et al., 2015b; Kuhn et al., 2016). Most recently, the technique has been used in patients with schizophrenia and mood disorder, investigating the glutamatergic response to functional activation using the Stroop paradigm (Taylor et al., 2015a). Despite increasing applications of this technique, there remains an ongoing debate over the exact size and interpretation of neurochemical changes measured by fMRS. However, carefully considered future applications of fMRS in schizophrenia should provide critical information into the nature of defects in the glutamatergic system.

In this review, we summarise brain glutamate findings from existing ^1H -MRS studies in patients with schizophrenia and consider the limitations of this technique. We then comprehensively review fMRS studies that have examined glutamatergic metabolites and consider how fMRS methods may be utilised to further our understanding of glutamatergic neurotransmission in schizophrenia. Lastly, we evaluate the strengths and weaknesses of fMRS as a technique before considering its potential in future research.

2 Static ^1H -MRS: Glutamatergic Findings in Schizophrenia

Glutamate and glutamine levels are thought to be maintained in equilibrium through the glutamate/glutamine cycle (Shen et al., 1999). In this process, glutamine is synthesised in astrocytes from glutamate which has been removed from the synaptic cleft following its release. To complete the cycle, neurons convert glutamine back to glutamate via glutaminase (**Figure 1**), with up to 80-90% of the glutamate involved in neurotransmission converted to glutamine (Rothman et al., 2003; Kanamori et al., 2002; Mason et al., 1995).

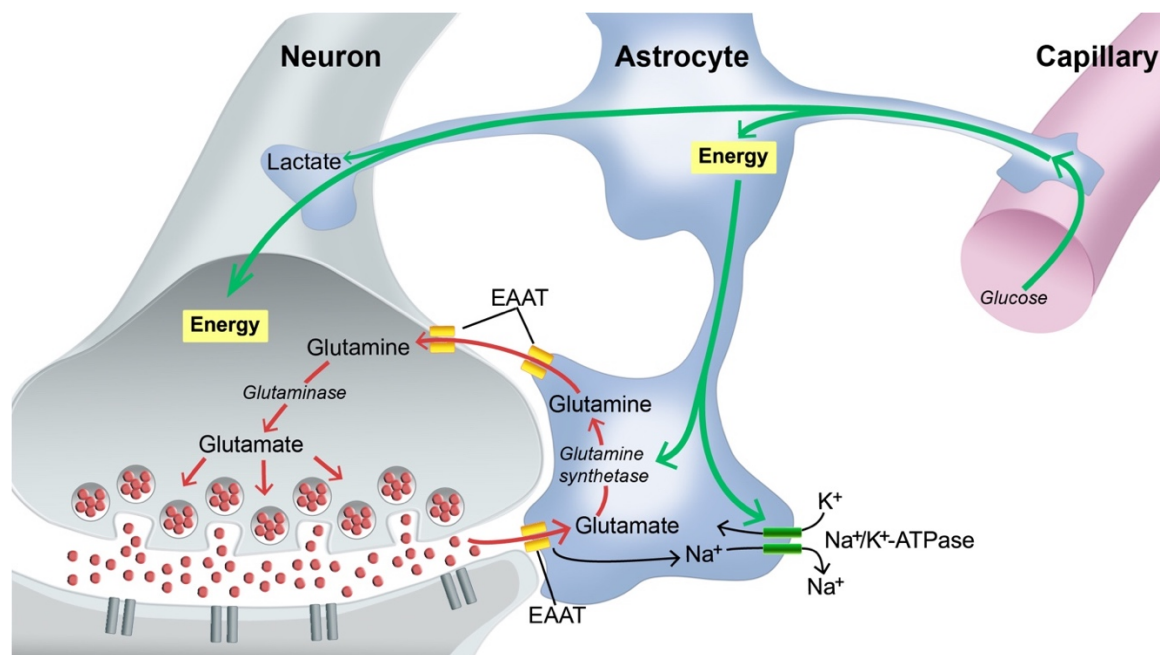


Figure 1: Glutamate/ glutamine cycle. Glutamate removed from synaptic cleft by excitatory amino acid transporters (EAATs) into astrocytes and converted into glutamine by enzyme glutamine synthetase. Glutamine is then transported out of astrocytes into nerve terminals and converted back to glutamate via glutaminase. (Figure after that presented by (Huettel et al., 2014))

There have been over 50 ^1H -MRS studies investigating glutamatergic metabolites in schizophrenia to date (Merritt et al., 2016). With higher magnetic field strengths of 3 Tesla (T) or greater, the reliability of determining glutamate and glutamine concentrations from their overlapping resonances has increased (Snyder and Wilman, 2010; Wijtenburg and Knight-Scott, 2011; Mullins et al., 2008), due to the increased frequency separation and less strong coupling (Mason et al., 1994). ^1H -MRS studies have either quantified glutamate and glutamine levels separately, or as the combined measure Glx, which is necessary at lower field strengths. The main findings in schizophrenia are summarised below by brain region.

Medial Prefrontal Cortex and Anterior Cingulate Cortex

Numerous studies have utilised ^1H -MRS to investigate glutamatergic metabolites in medial frontal lobe areas. Several studies have reported elevated levels of glutamine, in unmedicated patients with first-episode psychosis, in regions including the medial prefrontal cortex (mPFC) (Bartha, 1997) and the pregenual ACC (Théberge et al., 2002; Théberge et al., 2007). Other ^1H -MRS work, in unmedicated patients with first-episode psychosis, has examined prefrontal regions including the dorsal ACC, reporting no difference in glutamate levels (Tibbo et al., 2013). In individuals at high risk of psychosis, other studies have found evidence of increased glutamine (Stone et al., 2009) and increased Glx levels (Tibbo et al., 2004). However, in studies of chronic medicated patients, the majority have found either no difference (Ohrmann et al., 2005; Reid et al., 2010; Wood et al., 2007) or reduced levels of Glx (Ohrmann et al., 2005; Rowland et al., 2013), and reduced glutamate levels when compared to healthy controls (Tayoshi et al., 2009; Théberge et al., 2003). In the largest schizophrenia ^1H -MRS study to date, Wijtenburg et al. (2017) reported lower glutamate and elevated glutamine/glutamate levels in the ACC of adults with schizophrenia.

Medial Temporal Lobe and Hippocampus

A number of ^1H -MRS studies have investigated glutamatergic levels in regions that include the medial temporal lobe and hippocampus. There have been inconsistencies with reports of increased glutamate or Glx in chronic medicated patients (Chang et al., 2007; Van Elst et al., 2005), while other studies have not demonstrated differences in these regions, when comparing this population with healthy control subjects (Hutcheson et al., 2012; Kraguljac et al., 2012). In contrast to any elevations seen in chronic patients, studies investigating high risk groups have found no significant differences in glutamate or glutamine in the hippocampus (Lutkenhoff et al., 2010; Stone et al., 2009), with one study reporting reduced hippocampal glutamate levels (Bloemen et al., 2011). Similarly, in first-episode psychosis patient groups, no differences in Glx in these regions have been demonstrated (Galińska et al., 2009; Hasan et al., 2014).

Thalamus

In individuals at high risk of psychosis, studies have shown there to be reduced thalamic glutamate levels (Stone et al., 2009) and that lower levels are associated with poor clinical outcomes in those at ultra-high risk (UHR) of psychosis (Egerton et al., 2014). In those patients with first-episode psychosis, or chronic schizophrenia, a number of studies have

reported increased levels of glutamine in the thalamus (Aoyama et al., 2011; Théberge et al., 2002; Théberge et al., 2003). These results have not necessarily been consistent however, and other studies measuring Glx in the thalamus have not found significant differences in first-episode (Galińska et al., 2009) or chronic patients (Szulc et al., 2011), when compared with healthy controls.

Basal Ganglia

Work by De la Fuente-Sandoval et al. (2011; 2013b) has demonstrated increased glutamate and Glx in the dorsal caudate nucleus in both UHR and first-episode psychosis groups. In one of these studies, a group of antipsychotic-naïve first-episode psychosis patients was followed up after 4-weeks of treatment with risperidone and significant reductions in glutamate and Glx were demonstrated (De la Fuente-Sandoval et al., 2013b). This team also followed up the UHR group after 2 years and demonstrated that individuals who transitioned to psychosis had significantly higher levels of glutamate in the dorsal caudate nucleus compared with the non-transition and healthy control groups (De la Fuente-Sandoval et al., 2013a). Those studies involving chronic medicated patients have not demonstrated differences in glutamate, glutamine or Glx in the basal ganglia (Block et al., 2000; Tayoshi et al., 2009) or specifically Glx in the putamen (Yamasue et al., 2003) compared with healthy controls.

2.1 Evaluation of Static ^1H -MRS Technique and Findings

Static measurements of ^1H -MRS have been crucial in supporting the theory of glutamate dysfunction in schizophrenia. Unfortunately, many of these studies are difficult to compare when considering the various potential factors that could influence the results. First, the nature of findings varies depending on stage of illness (Bustillo et al., 2014; De la Fuente-Sandoval et al., 2011; Théberge et al., 2002) and treatment effects (Poels et al., 2014). Second, studies are performed on scanners of varying field strength (1.5 T- 7 T), using different data acquisition and post-processing methods (Wijtenburg et al., 2015). The resonance frequencies of glutamate and glutamine significantly overlap at 1.5 T and at these lower field strengths it is necessary to combine these measures as Glx. Finally, differing voxel sizes and placements are used for a variety of different brain regions.

Despite these limitations in comparing ^1H -MRS studies, there appear to be consistent differences in glutamatergic metabolites across several cortical and subcortical regions in patients with schizophrenia compared with controls, as emphasised by two recent meta-analyses. The first showed reduced glutamate and increased glutamine in medial frontal cortex regions (including the ACC and mPFC) of patients compared with healthy controls with meta-regressions of age on effect size that showed progressive decreases with age of glutamate and glutamine (Marsman et al., 2013). A more recent meta-analysis has reported higher glutamate and Glx in basal ganglia, higher glutamine in thalamus and higher Glx in the medial temporal lobe (Merritt et al., 2016). These elevations in glutamatergic metabolites are consistent with human studies of NMDA hypofunction which have demonstrated increased concentrations of glutamate (Stone et al., 2012) and glutamine (Rowland et al., 2005) in the ACC following ketamine administration to healthy controls.

It is interesting to note that of the significant findings in the meta-analyses, the majority of brain regions showed an increase in glutamine or Glx instead of glutamate. Elevations in glutamine may also drive the changes seen in Glx. Together, these measures could represent an increase in glutamatergic activity as after glutamate is released into the synapse it is quickly removed and converted back to glutamine via glutaminase for recycling (Danbolt, 2001). This could also potentially result from reduced glutaminase activity, slowing the conversion of glutamine to glutamate, or be directly related to NMDA hypofunction which has been shown to increase glutamine synthetase activity, thus leading to elevated glutamine (Rodrigo and Felipo, 2007).

2.2 Limitations of Static ^1H -MRS Technique

One of the main limitations of static ^1H -MRS is that it cannot provide information regarding which cell type a particular metabolite is in or how much of the metabolite is devoted to a specific function (Wijtenburg et al., 2015). It provides a whole tissue measure within the selected ^1H -MRS voxel, rather than distinguishing between intrasynaptic, extrasynaptic or intracellular compartments. Therefore, the glutamate signal does not simply reflect glutamate neurotransmission, instead it has been suggested to represent multiple functions and may instead be an index of cortical excitability (Stagg et al., 2011). It has also been suggested that not all glutamate is visible using ^1H -MRS and that there may be a significant

amount of invisible glutamate that likely contributes to the neurotransmitter pool (Kauppinen et al., 1994). However, as the majority of visible glutamine is involved in glutamate/glutamine neurotransmission cycle (Rothman et al., 2003; Kanamori et al., 2002; Mason et al., 1995), glutamine has been suggested to be used as an indirect measure of glutamatergic neurotransmission (Théberge et al., 2002).

The other main limitation of studies using static ^1H -MRS is that all were acquired in a resting condition at only a single time point. This assumes that metabolite concentrations being measured are not dynamic. It is now clear that concentrations of certain metabolites will change when tissue within a region of interest is stimulated (Lally et al., 2014; Prichard et al., 1991) and as such fMRS is being increasingly applied to investigate dynamic changes in metabolites involved in cerebral functioning. If there are abnormalities in glutamatergic neurotransmission in schizophrenia, fMRS as a technique has the potential to be a more sensitive measure of this than static ^1H -MRS alone.

3 Functional Magnetic Resonance Spectroscopy

Here, the basic principles of fMRS will be described before a review of how this technique has been developed and applied in a variety of human brain studies, with a specific focus on glutamatergic findings, including the novel application to study schizophrenia.

3.1 General Principles of fMRS

3.1.1 Studied nuclei and metabolites

Functional MRS is similar to static MRS in that it can be used to investigate different metabolites containing nuclei such as hydrogen (^1H) and carbon (^{13}C) that possess net nuclear spin. It is this inherent property that makes such nuclei magnetic resonance sensitive. ^1H is the most sensitive nucleus and ^1H -fMRS can be used to measure concentrations and dynamics of a number of metabolites that have important roles in neurotransmission, neuromodulation and brain energy metabolism (Rothman et al., 2003). These include aspartate, gamma-aminobutyric acid (GABA), lactate, glucose, together with glutamate and glutamine.

^{13}C -fMRS, commonly known as ^{13}C MRS, is a highly specialised technique in which substrates such as glucose labelled with the ^{13}C isotope are administered to subjects and then fMRS methods are used to measure the rates at which the isotope is incorporated into different

metabolites (Rothman et al., 2003). This has proved especially useful in the investigation of brain metabolism pathways and metabolic fluxes. Although ^{13}C MRS utilises fMRS methodology, it is a much more complex and costly technique than ^1H -fMRS. The remainder of this review will focus on those studies employing ^1H -fMRS to measure glutamatergic dynamics.

3.1.2 ^1H -fMRS technique and resolutions

The simplest definition of ^1H -fMRS is a series of MRS acquisitions taken during evoked responses caused by changed stimuli (e.g. visual stimuli or pain), task performance or by pharmacological challenge, in an attempt to measure metabolite dynamics. While the acquisitions in static ^1H -MRS are time-averaged to present a single value for the entire duration of the acquisition and which is considered a static measure of trait aspects of metabolism, ^1H -fMRS records spectra at different time points during the scanning session, with each being considered a measure of the metabolic state at that particular point in time.

For optimal temporal resolution, spectral acquisition times need to be short enough to allow dynamic measures of metabolite concentrations. To permit the required level of temporal resolution, ^1H -fMRS techniques require high magnetic field strengths of at least 1.5 T, but as with most MRS studies, higher field is always advantageous. With higher field strengths, improvements in sequences and signal detection systems, temporal resolution has increased from 6 minutes in one of the first studies (Prichard et al., 1991), to 5 seconds in a more recent ^1H -fMRS study (Gussew et al., 2010). In addition to increasing the signal to noise ratio (SNR) and the increase in temporal resolution that results, a higher field strength also improves spectral resolution, allowing a greater number of metabolites to be accurately detected. At a field strength of 7 T, the sensitivity threshold to detect concentration changes is approximately $0.2 \mu\text{mol/g}$ for most quantified metabolites (Mangia et al., 2006). A final advantage of higher field strengths is less strong coupling, which is particularly important for quantifying glutamate and glutamine signals separately (Mason et al., 1994). With these improvements in signal and detection, measurement of glutamatergic metabolite dynamics is becoming more and more feasible.

The spatial resolution of ^1H -fMRS is limited by SNR and as such relatively large voxel sizes are required (typically $\sim 20 \times 20 \times 20\text{mm}^3$). The brain areas that can be investigated by ^1H -

fMRS are therefore limited, which is especially true for regions of interest such as certain brainstem nuclei that are too small ($<1\text{cm}^3$). Other factors that limit the application of ^1H -fMRS to certain brain regions include proximity to CSF or bone tissue, which leads to potential inhomogeneity within the voxel and distortion of the spectra (Richards, 2001). A final limitation of the large voxel size is that it can lead to the acquisition of signal from a region that includes brain tissue that is not specifically activated by the stimulus or task, potentially resulting in a drop in the overall size of metabolite changes detected.

3.2 Applications of ^1H -fMRS

3.2.1 Visual studies

The first ^1H -fMRS study in 1991, reported transient elevations of lactate in the occipital cortex during a period of prolonged visual stimulation (Prichard et al., 1991). ^1H -fMRS studies of this region have since been able to detect small (2-4%), but significant, increases in glutamate concentration following visual stimulation (Mangia et al., 2007; Lin et al., 2012; Schaller et al., 2013; Bednarik et al., 2015), with one study reporting a non-significant reduction (Mekle et al., 2017). Most recently, combined fMRI-MRS measurements have been used to acquire simultaneous glutamate and BOLD-fMRI signals from the occipital cortex at 7 T using a visual paradigm (Betina Ip et al., 2017). Together with a significant increase in glutamate, a significant correlation between glutamate and BOLD- fMRI time courses ($r = 0.381$, $p = 0.031$) was demonstrated, strengthening the link between glutamate and functional activity in the human brain.

3.2.2 Motor studies

Schaller et al. (2014) investigated glutamate changes in the motor cortex upon functional activation using a finger-tapping paradigm. They reported small (2%), significant, increases in glutamate during 5-min blocks of motor stimulation. A further ^1H -fMRS study of the motor cortex reported a significant increase in the Glx to total creatine ratio (11%), during a 10-min period of hand-clenching (Chen et al., 2017).

3.2.3 Pain studies

Several studies have utilised ^1H -fMRS as a non-invasive tool to study the brain's response to pain. Mullins et al. (2005) demonstrated a dynamic increase in glutamate (9.3% from baseline) in the ACC when comparing averaged MRS spectra acquired during a 10-min block

of rest, with spectra from a 10-min block of cold-pressure painful stimulus. Gussew et al. (2010) utilised time-resolved ^1H -fMRS to estimate dynamic glutamate changes in the anterior insular cortex, linking data acquisition to the time course of cyclic acute heat pain stimulation, at a temporal resolution of 5s. Again, significant increases in glutamate (18% from baseline) were detected during the pain stimulus. Another time-resolved ^1H -fMRS study has since demonstrated significant increases in Glx (21.5% from baseline) and significant reductions in GABA (15.1%) in the ACC during acute pain stimulus (Cleve et al., 2015). Finally, work from Gutzeit et al. (2011) has shown significant increases in glutamine and Glx in the left insular cortex, together with significant increases in glutamate, glutamine and Glx in the bilateral insula during electrically induced dental pain stimuli (Gutzeit et al., 2013).

3.2.4 Cognitive studies

^1H -fMRS has been increasingly used to examine neurochemistry during cognition, with alterations in lactate (Urrila et al., 2003; Urrila et al., 2004), GABA (Floyer-Lea et al., 2006; Michels et al., 2012) and glutamate measures reported in a range of brain regions using different cognitive tasks. Here, we again focus on studies reporting glutamatergic changes.

Lally et al. (2014) utilised ^1H -fMRS during a repetition-priming paradigm, demonstrating significantly higher glutamate levels for abstract compared to real world object stimuli in the lateral occipital cortex (LOC). Similarly, Apšvalka et al. (2015) used event-related ^1H -fMRS at 3 T in a repetition suppression task, demonstrating a 12% increase in glutamate levels after novel visual stimuli presentations in the LOC. This was followed by an average 11% decrease in glutamate, comparing repeated to novel presentations. Using a block design mental imagery task (imagining swimming), Huang et al. (2015) showed a significant increase in Glx in the mPFC in a group of competitive swimmers.

An exploratory study by Taylor et al. (2015b) investigated dynamic glutamate levels in the ACC using a Stroop task as the activation paradigm at 7 T. Spectra were acquired every 3s during a 4-min rest period, 4-min task condition and 4-min recovery period. The task period was associated with a significant increase in glutamate of $2.6 \pm 1.0\%$ ($0.24 \pm 0.09 \mu\text{mol/g}$), followed by a trend toward returning to baseline in the recovery period (all statistics were performed on the 4-min, 80 averaged spectra). A further study has again demonstrated an

increase in glutamate during Stroop task performance that recovered during rest, whereas glutamine was shown to increase during the task and continued to increase after the task (Kuhn et al., 2016).

Most recently, ^1H -fMRS has been used to investigate the dynamics of hippocampal glutamate during paired-associate learning and memory in healthy subjects (Stanley et al., 2017). Glutamate increased across all subjects during both encoding (5.2%) and retrieval (4.2%), with a significant difference in the temporal dynamics during these two processes. Interestingly, fast learners demonstrated a significant increase in glutamate early in learning, whereas this increase was only observed later in slow learners.

3.2.5 Schizophrenia

The majority of ^1H -fMRS research to date has investigated dynamic metabolite level changes resulting from functional activation using a variety of stimuli in healthy volunteers. Taylor et al. (2015a) were the first group to apply the technique at 7 T to obtain dynamic measures of glutamate and glutamine in patient groups. Glutamatergic concentrations were measured in the ACC during a cognitive task in a group of 16 patients with schizophrenia, 16 with major depressive disorder (MDD) and 16 healthy controls. Probing the ACC has the potential to provide useful information in schizophrenia where glutamate and glutamine levels have been shown to be abnormal using static ^1H -MRS (Théberge et al., 2002; Théberge et al., 2003).

Taylor et al. (2015a) used the Stroop task, a measure of selective attention, that has been shown to robustly activate the ACC, although with some hypofunction in schizophrenia (Minzenberg et al., 2009). ^1H -fMRS spectra were acquired every 3s over a 20-min period, consisting of three 4-min blocks of rest incorporated with two 4-min blocks of the Stroop task. Although healthy controls demonstrated a significant glutamate response (3.2%) during the Stroop task, neither the schizophrenia or MDD group showed any significant changes in glutamate. The authors suggest that an increase in the number of processing steps involved in schizophrenia (Neufeld et al., 2010) may involve recruiting more brain areas than controls. This would mean a blunted response in the ACC and therefore explain the relatively smaller increase in glutamate. It should also be noted that baseline glutamate

was higher in the schizophrenia group and another explanation is that glutamate levels are closer to a glutamate ceiling, meaning upregulation is more difficult.

In contrast, the schizophrenia group did show significant increases in glutamine during the Stroop task which the healthy controls did not. This supports the hypothesis of NMDA receptor hypofunction in schizophrenia, consistent with the observation that ketamine administration leads to increased concentration of glutamine in the ACC in healthy controls (Rowland et al., 2005). Taylor et al. (2015a) conclude the increase in glutamine in the schizophrenia group may have resulted from NMDA receptor hypofunction when the ACC was challenged during the Stroop task.

Chiappelli et al. (2017) have since used ^1H -fMRS at 3 T to measure the glutamate response to stress in the dorsal ACC, using a somatic stress paradigm (painful heat stimuli), in a group of 23 patients with schizophrenia and 21 healthy controls. Spectra were acquired in five 5-min blocks, with two blocks before the painful stimuli, one during and two blocks immediately after. Repeated-measures ANOVA revealed that across groups, glutamate levels significantly decreased following exposure to painful heat stimuli [$F(\text{df}= 2) = 4.97, p= 0.009$], while ratio of glutamine to glutamate significantly increased [$F(\text{df}= 2) = 3.53, p= 0.035$]. However, patients with schizophrenia demonstrated an initial increase in glutamate levels in the heat stimuli block that was significantly different from controls. Interestingly, this acute glutamate response was positively correlated with childhood trauma ($r= 0.41, p= 0.050$) and inversely correlated with working memory ($r = -0.49, p= 0.023$). The authors suggest that further improvements in such methods to assess glutamate responses to could lead to novel approaches in understanding the interplay of stress and the glutamatergic system in the development of schizophrenia.

3.3 Evaluation of ^1H -fMRS Technique and Findings

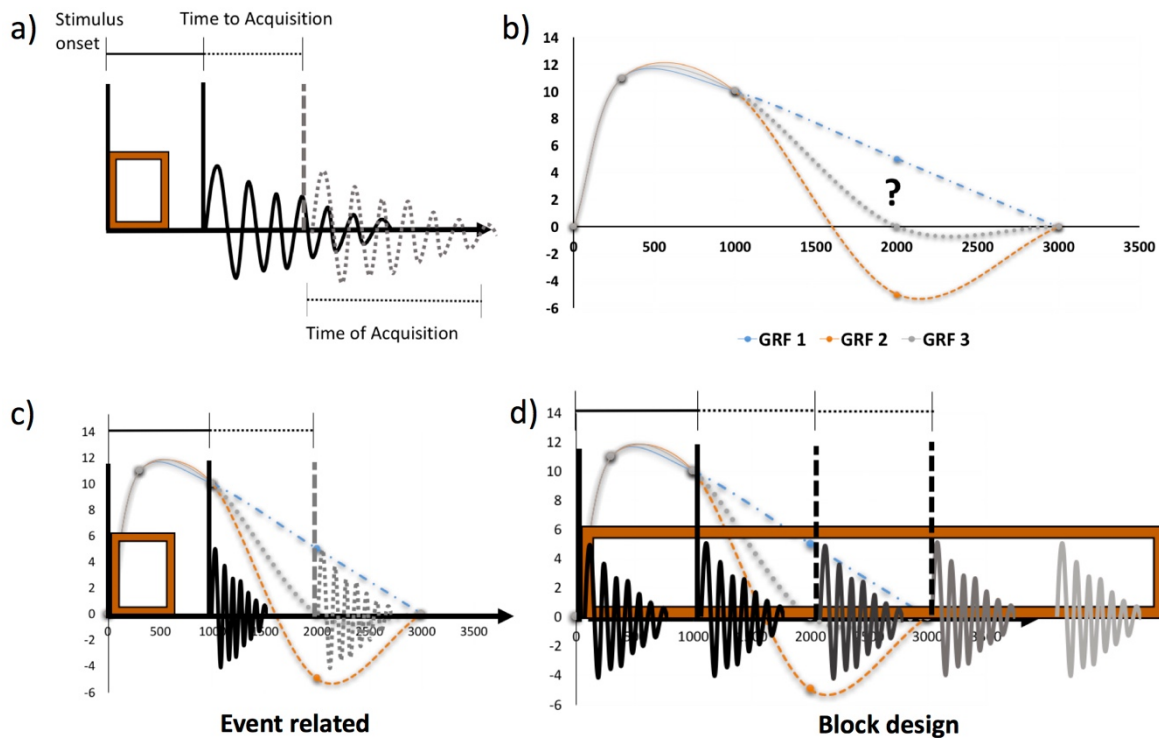
^1H -fMRS is a technique which has shown potential advantages over other functional and biochemical neuroimaging methods. Unlike positron emission tomography (PET) or single-photon emission computed tomography (SPECT) studies, ^1H -fMRS is non-invasive and participants are not exposed to any ionizing radiation. However, ^1H -fMRS is an experimental technique and the reliable measurement of metabolite dynamics requires sophisticated data acquisition, quantification and interpretation. ^1H -fMRS remains a contentious method,

with inconsistencies in the size of metabolite level changes reported and questions regarding the origin of the signal change.

In the ^1H -fMRS studies discussed in this review, there has been a wide range in the reported magnitude of glutamatergic metabolite level changes. Studies utilising a painful stimulus have demonstrated relatively large increases in glutamate (18%) and Glx (21.5%) in the anterior insular cortex (Gussew et al., 2010) and ACC (Cleve et al., 2015) respectively. Those studies using cognitive task paradigms have reported more moderate increases in glutamatergic measures upon functional activation. Increases in glutamate of 12% have been demonstrated in a repetition suppression task in the LOC (Apšvalka et al., 2015) and only 2.6% during a Stroop task in the ACC (Taylor et al., 2015b). A potential explanation for this is that cognitive tasks do not provide as robust a functional response as painful stimuli.

Another important point to consider is how quickly glutamate levels might change in response to stimuli. Studies that have used event-related ^1H -fMRS to detect glutamate changes upon brain activation suggest that changes occur reasonably rapidly (increasing within 3- 6 s) (Apšvalka et al., 2015; Cleve et al., 2015; Gussew et al., 2010; Lally et al., 2014) and that levels may also decrease as quickly (3- 9 s) when the stimulus is no longer present (Apšvalka et al., 2015; Lally et al., 2014). The magnitude of glutamate changes reported in these event-related ^1H -fMRS studies is generally larger than those using prolonged stimulation block experiments (Lin et al., 2012; Mangia et al., 2007) and it has been suggested that habituation, adaptation and homeostatic effects results in difficulty detecting rapid glutamatergic dynamics in such long blocks (Apšvalka et al., 2015). An additional benefit of event-related ^1H -fMRS, where signal acquisition is time locked to stimulation, is that it allows a glutamate response function (GRF) to be mapped. The importance of timing in ^1H -fMRS and proposed GRF models are explored further in **Box 1**.

Box 1: Importance of timing in functional magnetic resonance spectroscopy



a) Timing of MRS acquisition after stimulus is likely to be crucial with signal intensity varying according to time to acquisition. **b)** One hypothesised glutamate response function (GRF). The first two points are based on data sampled at 300 – 1000ms after stimulus (Lally et al., 2014; Apšvalka et al., 2015), while the final two points are proposed for three different models, GRF 1; a gradual decline to baseline, GRF 2; a rapid decline to baseline and GRF 3; a rapid decline below baseline before recovery. It is important to note the GRF will be dependent on the stimulus or condition that evokes it. The model presented here is conceptual and would need to be adapted for the particular GRF of each glutamate-stimulating paradigm under study. **c)** Event related design with acquisition time locked to stimulus onset. Characterising the GRF is important for interpretation as to receive highest signal, time to acquisition needs to coincide with maximum glutamatergic response. **d)** Block design with repeated acquisitions across stimulus block. Habituation, adaptation and homeostatic effects might lead to difficulty detecting rapid glutamatergic dynamics in long blocks where stimuli presentation is not time locked in some fashion to signal acquisition.

Given that MRS cannot differentiate between the extracellular and intracellular compartments, one critical question often leveled at those studies where large and rapid changes in glutamate occur is “Where does the glutamate come from/go to?”. Smaller increases, on the order of 2-5 % over 5 mins, can be explained by increased metabolic turnover. One theory, discussed by Mangia et al. (2007), is that sustained neuronal activation raises oxidative metabolism to a new steady state. Increases in glutamate concentration (together with similar reductions in aspartate) are consistent with increased flux through malate–aspartate shuttle (MAS) to maintain the cytosolic redox potential (McKenna et al., 2006). During functional activation of a brain region, the increase in

glucose oxidation rate has also been directly linked to increases in glutamate/glutamine cycling through the tricarboxylic acid cycle (TCA) (Rothman et al., 2003).

The cerebral metabolic rate of glucose utilisation (CMR_{glc}) in the human brain has been reported as approximately 0.26 $\mu\text{mol/g/min}$ (Hyder et al., 2016). In a scenario where all glucose is metabolized to glutamate, the maximum rate of glutamate production would be 0.52 $\mu\text{mol/g/min}$ (1 molecule of glucose generates two acetyl-CoA which enter the TCA cycle). If during brain activation, relative to resting state, a 20% increase in energy consumption is assumed, the maximum rate of glutamate production in this example would be 0.62 $\mu\text{mol/g/min}$. Approximating the typical glutamate concentration in cerebral cortex as 10.00 $\mu\text{mol/g/min}$, this would correspond to a maximum rise in glutamate concentration to 10.62 $\mu\text{mol/g/min}$, which is only a 6% increase.

What follows in this paragraph is a hypothetical explanation to describe those changes in the order of 12-18%, that are too large to be explained by increased metabolic turnover alone, especially when they occur within an order of seconds rather than minutes. It is important when comparing $^1\text{H-fMRS}$ studies to consider the exact methodology being used. Previous work has suggested that glutamate involved in neurotransmission, that is bound in pre-synaptic vesicles, is undetectable due to potential micro-environmental differences affecting its relaxation properties and/or resonant frequency (Kauppinen et al., 1994). Glutamate bound in pre-synaptic vesicle compartments, where metabolite movement is restricted, will have a faster T_2 relaxation rate compared to less restricted extracellular pools. In $^1\text{H-fMRS}$ studies that have used shorter echo times ($TE < 15\text{ms}$), the glutamate in pre-synaptic vesicles will contribute more to the total signal and as such these studies may not be as sensitive to changes in signal resulting from compartmental shifts (Lin et al., 2012; Mangia et al., 2007; Schaller et al., 2013). Those $^1\text{H-fMRS}$ studies that reported larger changes however utilise longer echo times ($TE > 30\text{ms}$) (Apšvalka et al., 2015; Cleve et al., 2015; Gussew et al., 2010; Lally et al., 2014), where T_2 relaxation has a greater effect on the signal detected (**See Table 1**). The speculative implication of this is that $^1\text{H-fMRS}$ is not detecting changes in overall glutamate concentration in the brain volume, but rather compartmental shifts due to neural activity, as it moves from pre-synaptic vesicles to more visible synaptic, extracellular and astrocytic pools. It should be noted that the TE cut-offs suggested above are again hypothetical and not based on empirical data.

One final consideration for ^1H -fMRS measures is the potential for the BOLD like effects on signal amplitude. Indeed, the unsuppressed water signal from MRS experiments has been used to measure BOLD related signal changes (Apšvalka et al., 2015; Hennig, 2012), and several studies report line width reductions in spectral measures as might be expected due to a decrease in local field homogeneity that accompanies the BOLD effect (Bednarik et al., 2015; Bednarik et al., 2017; Mangia et al., 2007), and so correct for these by introducing line broadening of “active” spectra in their ^1H -fMRS analysis. While the BOLD effect has been shown to have an impact in ^1H -fMRS studies, it would be expected to be a general effect, and so would affect all metabolites somewhat equally. This means if the BOLD effect was responsible for the increases in signal seen for glutamate (and lactate), a similar effect should be seen for all metabolites – a situation which doesn’t seem to occur for N-acetyl aspartate, creatine, choline and others. Similarly, if the BOLD effect was significant, it would reduce, or even prevent the occurrence of decreases in signal, as reported for GABA in certain paradigms (Chen et al., 2017; Cleve et al., 2015; Michels et al., 2012), and aspartate in others (Mangia et al., 2007). Awareness of the potential for BOLD related effects is also more likely to be important for block stimulation paradigms (especially ones > 1 min), where the slow haemodynamic response has time to develop. Event-related paradigms might be considered less susceptible to this effect, given that the metabolite signal is sampled 100-1000ms after stimulus onset, while the BOLD effect takes ~2 secs to start. Still, it is worth considering the potential for BOLD like changes to impact results by designing acquisition and analysis protocols to try and address and ameliorate this effect. This can be done by applying line broadening in pre-processing for the “active” spectra (Bednarik et al., 2015), by referencing to another metabolite peak that may have experienced the same BOLD effect (Lally et al., 2014), collecting interleaved metabolite and unsuppressed water spectra, and using the water spectra from the same stimulus to correct for BOLD related signal changes (Apšvalka et al., 2015), or using an event-related paradigms where the metabolite spectra are collected such that they are temporally distant from the expected peak of the BOLD signal.

4 Potential for Future Research

As ^1H -fMRS is still a developing technique there are a wide number of areas to consider for future work. It has only recently been applied to study glutamate dynamics in schizophrenia during the Stroop Task, as described by Taylor et al. (2015a). Future studies should utilise other cognitive tasks that activate the ACC, together with other brain regions, dysfunction of which are implicated in schizophrenia and other psychiatric disorders. One interesting avenue would be to employ a cognitive task with varying levels of complexity and cognitive load, to examine the glutamate response across different task conditions and whether this was impaired in schizophrenia. Alongside this, there is further work needed to understand the temporal dynamics of potential glutamatergic response functions (GRFs) and recovery processes, resulting from different glutamate-stimulating paradigms. This will require future studies to further employ specific event-related paradigms rather than block designs.

Future ^1H -fMRS studies in schizophrenia might explore how any abnormalities of glutamate dynamics early in the disease may help predict future functional outcome, or expand on static MRS work (Egerton et al., 2012) to better identify non-responders to dopamine-based antipsychotic medications. As such, glutamate ^1H -fMRS could aid in the stratification of participants in future trials to distinguish between dopamine-based antipsychotic responders and glutamate-based antipsychotic responders. Glutamate ^1H -fMRS may also have a prospective role in monitoring the response of patients on glutamate-based antipsychotics in clinical trials.

Apart from focusing on glutamate dynamics the application of ^1H -fMRS techniques to study GABA responses in psychiatric patient groups would also be of real value. Finally, it would be interesting to see ^1H -fMRS applied using pharmacological paradigms, for example to further understand glutamate/ glutamine dynamics following ketamine administration to healthy volunteers.

5 Conclusion

There is now a substantial body of evidence that schizophrenia is associated with altered levels of glutamatergic metabolites across different brain regions, the majority of which has come from static ^1H -MRS studies. As we move forward to further explore the nature and consequences of glutamatergic dysfunction in schizophrenia, ^1H -fMRS as a technique shows clear promise by allowing glutamate dynamics to be studied. Carefully planned and interpreted ^1H -fMRS studies that explore the glutamatergic response in schizophrenia will further our understanding of the temporal dynamics of any dysfunction, potentially providing new information on neuropathological mechanisms or even guiding treatment interventions.

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Authors	Year	Field strength	Population (n)	Brain region and voxel size	MRS technique and parameters	Stimulus or task	Experimental design	Glutamatergic metabolite findings
Mullins et al.	2005	4 T	Healthy (10)	ACC 20x20x20 mm ³	Single voxel STEAM (TR = 2000ms, TE = 20ms)	Pain: <i>Cold-pressor</i>	Block > 5-min	Glu ↑ 9.3 ± 6.1% (p<0.01) Glx ↑ 11.4 ± 8.7% (p <0.01)
Mangia et al.	2007	7 T	Healthy (12)	OCC 20x22x20 mm ³	Ultra-short echo time STEAM (TR = 5000ms, TE = 6ms, TM=32ms)	Visual: <i>Flickering checkerboard</i>	Block > 5-min	Glu ↑ 3.0 ± 1.0% (p<0.01)
Gutzeit et al.	2011	3 T	Healthy (14)	Insula 20x20x37.6 mm ³	PRESS (TR= 2000 ms, TE = 30 ms)	Pain: <i>Dental pain</i>	Block > 5-min	Gln ↑ 55.1% (p=0.005) Glx ↑ 16.4% (p<0.001)
Gussew et al.	2010	3 T	Healthy (6)	Insula 25x10x10 mm ³	PRESS (TR = 5000 ms, TE = 30 ms)	Pain: <i>Heat stimuli</i>	Event-related	Glu ↑ 18.1 ± 8.3% (p=0.003)
Gutzeit et al.	2013	3 T	Healthy (16)	Insula 20x20x24 mm ³	PRESS (TR= 2000 ms, TE =30ms)	Pain: <i>Dental pain</i>	Block > 5-min	Glu ↑ 9%, Gln ↑ 27% Glx ↑ 13.87% (p=0.001)
Lin et al.	2012	7 T	Healthy (10)	OCC 20x20x20 mm ³	STEAM (TR= 3000ms, TE= 15 ms, TM= 17 ms)	Visual: <i>Photic stimulation</i>	Block > 5-min	Glu ↑ 2.0 ± 1.0% (p=0.011) Gln ↓ 8.0 ± 5.0% (p=0.038)
Schaller et al.	2013	7 T	Healthy (6)	OCC 20x22x20 mm ³	SPECIAL sequence (TR = 5000ms, TE= 6ms)	Visual: Flashing checkerboard	Block > 5-min	Glu ↑ 4.0 ± 1.0% (p<0.001)
Cleve et al.	2014	3 T	Healthy (15)	ACC 36x20x12 mm ³	¹ H MEGA-PRESS (TR = 3000ms, TE= 68ms)	Pain: <i>Heat stimuli</i>	Event-related	Glx ↑ 21.5% (p<0.001)
Lally et al.	2014	3 T	Healthy (13)	LOC 20x20x20 mm ³	PRESS (TR = 3000ms, TE = 40 ms,)	Cognitive: <i>Object recognition, Abstract vs. object</i>	Event-related	Glu ↑ 11 ± 26 % (p=0.016)
Schaller et al.	2014	7 T	Healthy (11)	Motor cortex 17x20 x17 mm ³	SPECIAL sequence (TR= 7500ms, TE = 12ms)	Motor: <i>Finger/thumb tapping</i>	Block- 5-min	Glu ↑ 2.0 ± 1.0% (p<0.05)
Taylor et al.	2015	7 T	Healthy (7)	ACC 20x20x20 mm ³	Ultra-short echo time STEAM (TR= 3000ms, TE= 10ms, TM= 32ms)	Cognitive: <i>Stroop</i>	Block- 4-min	Glu ↑ 2.6 ± 1.0% (p=0.02)
Taylor et al.	2015	7 T	Healthy (16), MDD (16), SCZ (16)	ACC 20x20x20 mm ³	Ultra-short echo time STEAM (TR= 3000ms, TE= 10ms, TM= 32ms)	Cognitive: <i>Stroop</i>	Block- 4-min	Healthy: Glu ↑ 3.2% (p=0.006) SCZ: Gln ↑ 19 % (p=0.004)
Apšvalka et al.	2015	3 T	Healthy (13)	LOC 20x20x20 mm ³	PRESS (TR= 1500ms, TE= 105ms)	Cognitive: <i>Object recognition, Novel vs. repeated</i>	Event-related	Glu ↑ 12.0 % (p<0.05)
Huang et al.	2015	3 T	Healthy (41)	MPFC 20x15x30 mm ³	PRESS (TR= 3000ms, TE= 24ms)	Cognitive: <i>Mental imagery</i>	Block >5-min	Glx ↑ 2.1% (p<0.05)
Bednařík et al.	2015	7 T	Healthy (12)	OCC 20x20x20 mm ³	Semi-LASER sequence (TR= 5000ms, TE= 26ms)	Visual: <i>Flashing checkerboard</i>	Block >5-min	Glu ↑ 3.3% (P<0.0005)
Kühn et al.	2016	3 T	Healthy (18)	ACC 25x35x20 mm ³	SPECIAL sequence (TR= 3000ms, TE=8.5ms)	Cognitive: <i>Stroop</i>	Block > 5-min	Glu ↑ 4.1% (p=0.017) Gln ↑ 9.0% (p=0.002)

Mekle et al.	2016	7 T	Healthy (20)	OCC 20×20×20 mm ³	SPECIAL sequence (TR= 5000ms, TE= 6ms)	Visual: <i>Flashing checkboard</i>	Block/ steady state	Glu- no significant change
Betina Ip et al.	2017	7 T	Healthy (13)	OCC 20×20×20 mm ³	Semi-LASER sequence (TR= 4000ms, TE= 36 ms)	Visual: <i>Flashing checkboard</i>	Block- 1-min	Glu ↑ 1.92 ± 0.66% (p=0.011)
Chen et al.	2017	7 T	Healthy (13)	Motor cortex 15×20×30 mm ³	MEGA-sLASER) sequence (TR= 5000ms, TE= 72ms)	Motor: <i>Hand clenching</i>	Block > 5-min	Glx ↑ 11.0 ± 5.0 % (p<0.05)
Chiappelli et al.	2017	3 T	Healthy (21) SCZ (23)	dACC 40x20x15 mm ³	PR-STEAM (TR= 2000ms, TE= 6.5ms, TM= 10ms)	Pain: <i>Heat stimuli</i>	Block- 5-min	Across groups: Glu ↓ (p = 0.009) post-stimuli Gln/Glu ↑ (p=0.035) post-stimuli SCZ: Glu ↑ (p = 0.042) during stimuli (compared with controls)
Stanley et al.	2017	3 T	Healthy (16)	Hippocampus 17× 30×12 mm ³	PRESS (TR= 3375ms, TE= 23ms)	Cognitive: <i>Paired-associated learning and memory</i>	Block- 1-min	Encoding: Glu ↑ 5.2% (p<0.0001) Retrieval: Glu ↑ 4.2% (p=0.0002)

Table 1: List of ¹H-fMRS studies and glutamatergic metabolite findings, characterised by key parameters. The magnitude of glutamatergic metabolite changes are listed as percentage changes, where reported in each study. For some studies, the percentage changes were not clearly described and as such are calculated from the difference between active versus rest conditions. In these cases, standard deviations are not reported. Abbreviations: ACC- anterior cingulate cortex; dACC- dorsal anterior cingulate cortex; OCC- occipital cortex; LOC- lateral occipital cortex; MPFC- medial prefrontal cortex; TR- repetition time; TE- echo time; TM- mixing time; Glu- glutamate, Gln- glutamine, Glx- glutamate+glutamine.