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Functional magnetic resonance spectroscopy in patients with schizophrenia and bipolar affective disorder: Glutamate dynamics in the anterior cingulate cortex during a working memory task

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Abstract
The glutamate system is implicated in the pathophysiology of schizophrenia and mood disorders. Using functional magnetic resonance spectroscopy (^{1}H-fMRS), it is possible to monitor glutamate dynamically in activated brain areas and may give a closer estimate of glutamatergic neurotransmission than standard magnetic resonance spectroscopy. 14 patients with schizophrenia, 15 patients with bipolar disorder II (BPII) and 14 healthy volunteers underwent a 15 min N-back task in a 48s block design during ^{1}H-fMRS acquisition. Data from the first, second and third 16s group of 8 spectra for each block were analysed to measure levels of glutamate and Glx (glutamate + glutamine), scaled to total creatine (TCr), across averaged 0-back and 2-back conditions. A 6 × 3 repeated-measures analysis of variance (rmANOVA) demonstrated a

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significant main effect of time for Glx/TCr ($P = 0.022$). There was a significant increase in Glu/TCr ($P = 0.004$) and Glx/TCr ($P < 0.001$) between the final spectra of the 0-back and first spectra of the 2-back condition in the healthy control group only. 2×2 rmANOVA revealed a significant time by group interaction for Glx/TCr ($P = 0.019$) across the 0-back condition, with levels reducing in healthy controls and increasing in the schizophrenia group. While healthy volunteers showed significant increases in glutamatergic measures between task conditions, the lack of such a response in patients with schizophrenia and BPII may reflect deficits in glutamatergic neurotransmission. Abnormal increases during periods of relatively low executive load, without the same dynamic modulation as healthy volunteers with increasing task difficulty, further suggests underlying abnormalities of glutamatergic neurotransmission in schizophrenia.

1. Introduction

There has been considerable interest in the role of glutamatergic neurotransmission in the pathophysiology of schizophrenia (Moghaddam and Javitt, 2012). It is proposed N-methyl-D-aspartate receptor (NMDAR) hypofunction leads to excess cortical glutamate release (Lisman et al., 2008; Olney et al., 1999) with downstream effects on dopamine and other neurotransmitter systems (Howes et al., 2015; Stone et al., 2007). Determining the nature of glutamatergic abnormalities in schizophrenia may prove useful in understanding symptomatology and further developing novel treatment strategies (Stone, 2011). Glutamatergic abnormalities are not specific to schizophrenia however, and there has been particular interest in this system with respect to the pathophysiology (Sanacora et al., 2012) and treatment of mood disorders (Zarate et al., 2010).

Proton magnetic resonance spectroscopy (^{1}H-MRS) has been central to the investigation of glutamatergic dysfunction in schizophrenia, revealing abnormal levels of glutamate and its metabolite glutamine across various cortical and subcortical brain regions in patients compared with controls (Marsman et al., 2013; Merritt et al., 2016). Alterations in medial prefrontal glutamate levels have also been reported in mood disorders with reduced levels generally reported in major depressive disorder (MDD) (Yuksel and Ongur, 2010). In patients with bipolar affective disorder, it has been suggested that medial prefrontal glutamate levels may be elevated, although this finding has not been consistently seen (Jun et al., 2014; Taylor, 2014; Yuksel and Ongur, 2010).

A significant limitation of ^{1}H-MRS studies is that measurements are determined over a single static time period. Functional ^{1}H-MRS (^{1}H-fMRS) is a technique where a series of MRS acquisitions are acquired upon functional brain activation. It is being increasingly applied to investigate dynamic changes in brain metabolites (Duarte et al., 2012) and may give a closer estimate of glutamatergic neurotransmission than standard magnetic resonance spectroscopy (Jelen et al., 2018; Mullins, 2018).

^{1}H-fMRS studies have demonstrated increases in glutamatergic measures in the occipital cortex following visual stimulation (Bednarik et al., 2015; Ip et al., 2017; Lin et al., 2012; Mangia et al., 2007; Prichard et al., 1991; Schaller et al., 2013), the motor cortex using a finger-tapping (Schaller et al., 2014) and hand-clenching paradigm (Chen et al., 2017), and the anterior cingulate cortex (ACC) using painful stimuli (Cleve et al., 2015; Mullins et al., 2005) in healthy controls. This technique has been further applied in healthy controls to demonstrate increases in glutamate in the dorsolateral prefrontal cortex (dLPFC), during a working memory task (Woodcock et al., 2018), and the ACC using the Stroop Task as a cognitive paradigm (Kuhn et al., 2016; Taylor et al., 2015b). Taylor et al. (2015a) undertook a subsequent ^{1}H-fMRS study of the ACC using the Stroop task in patients with schizophrenia and MDD compared with healthy volunteers. While the healthy control group again showed significant increases in glutamate concentrations during the Stroop Task, this was not seen in the schizophrenia or MDD group.

The purpose of this study was to examine dynamic glutamatergic levels in the ACC during performance of the N-back task in patients with schizophrenia and bipolar II disorder (BPII), compared with healthy controls, using ^{1}H-fMRS at 3 Tesla. The N-back task is a working memory task with variable levels of complexity, allowing the investigation of different degrees of cognitive load and has been shown to robustly activate the ACC, dysfunction of which is consistently implicated in schizophrenia (Adams and David, 2007) and bipolar disorder (Maletic and Raison, 2014). It was hypothesized that there would be increases in glutamatergic measures in the more cognitively demanding 2-back condition compared with the 0-back condition but that the schizophrenia group would show smaller increases than the healthy control and BPII groups.

2. Experimental procedures

2.1. Participants

This study was reviewed and approved by the London Harrow Research Ethics Committee and all participants provided written informed consent.15 participants with a diagnosis of schizophrenia were recruited through the Psychosis Clinical Academic Group, South London and Maudsley NHS Foundation Trust together with 15 BPII participants from the Improving Access to Psychological Therapies services. 14 healthy controls were recruited from local and online advertisements.

All participants were right handed and aged between 22 and 57 years old. Volunteers with major medical illnesses, other psychiatric disorders, magnetic resonance imaging (MRI) contraindications or current substance abuse were

Table 1 Participant demographics.

Group	SCZ	BPII	HC	*P*
n	15	15	14	
Age	40.1 ± 10.0	38.6 ± 10.6	33.8 ± 10.5	0.248
M/F	11/4	6/9	7/7	0.171
MADRS	9.0 ± 8.6	9.20 ± 12.0	2.1 ± 2.6	0.059
YMRS	3.5 ± 4.4	4.7 ± 3.6	1.3 ± 2.4	**0.046**
SAPS	29.0 ± 23.5	3.8 ± 3.5	0.8 ± 1.8	**<0.001**
SANS	13.8 ± 9.0	3.9 ± 3.9	0.1 ± 0.5	**<0.001**

Values shown are means ± standard deviations. Abbreviations: **M/F**, male/female; **MADRS**, Montgomery-Asberg Depression Scale; **YMRS**, Young Mania Rating scale; **SAPS**, Scale for Assessment of Positive Symptoms; **SANS**, Scale for Assessment of Negative Symptoms. **P**- One-way ANOVA test (alpha = 0.05, two tailed) or Chi-squared for binary data, bold values indicate statistical significance.

excluded from the study. Healthy volunteers with a family history of psychiatric disorder in a first degree relative were also excluded.

Diagnosis was established by a psychiatrist and trained research assistant with the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). All participants were assessed with the Scale for the Assessment of Positive/Negative Symptoms (SAPS/SANS) (Andreasen, 1984a, 1984b), Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979) and Young Mania Rating Scale (YMRS) (Young et al., 1978). Thirteen schizophrenia patients were receiving regular antipsychotic medication (four taking olanzapine; three taking risperidone; two taking aripiprazole; three taking clozapine; one taking haloperidol) and two were not currently medicated. Five BPII patients were receiving antidepressants (three taking citalopram; one taking sertraline; one taking fluoxetine) and the remaining ten were not taking psychotropic medication. The healthy controls were medication-naïve. Demographic information including age, sex and clinical rating scores are shown in Table 1.

2.2. N-back task paradigm

Participants performed the N-back task for 15 min while ^{1}H-fMRS spectra were acquired (Fig. 1). This consisted of 18 blocks lasting 48s each, rotating through alternating conditions (0-back, 1-back, 2-back, 3-back i.e., $n = 0$, 1, 2 or 3). As sequential letters were presented individually, subjects were asked to respond "Yes" as quickly and accurately as possible on a two-button keypad, each time a new letter was presented that was the same as one presented "*n*" items before. For the 0-back condition this involved correctly identifying the letter "X" whenever it was displayed. Both accuracy and response times were recorded for analysis.

2.3. ^{1}H-fMRS data acquisition and analysis

All measurements were acquired on a GE Discovery MR750 3.0T scanner with a body-transmit coil and 12-channel Head/Neck/Spine receiver coil. ^{1}H-fMRS voxels were $3 \times 2 \times 2$ cm (12 cm^3) in size. In each participant the voxel was not rotated, but was aligned with its inferior edge on the superior surface of the corpus callosum, with its centre positioned 7 mm posterior to the genu of the corpus callosum in the sagittal plane and in the midline of the brain, as defined by the interhemispheric fissure in the transverse plane (Fig. 2), using a high resolution structural 3D MP-RAGE sequence ($T_R = 7312$ ms, $T_E = 3016$ ms, $T_I = 400$ ms, FOV = 270 mm, flip-angle (α) = 11°, matrix size = 256 × 256 mm^2, slice thickness = 1.2 mm, 196 slices) in the sagittal plane for localization of the spectroscopy.

^{1}H-fMRS spectra were acquired individually throughout the N-back task using Point RESolved Spectroscopy (PRESS), with CHEmical Selective suppression for water suppression and outer volume suppression (OVS) with Very Selective Suppression (VSS) pulses ($T_R = 2000$ ms, $T_E = 105$ ms, NEX = 8). A modified version of GE's PROBE (proton brain examination) sequence was used to commence the N-back task and send a trigger pulse at the start of every T_R. Shimming was optimized with an auto-prescan performed twice before each scan. For each participant 16 water-unsuppressed and 432 water-suppressed spectra were collected; 3 spectra (each an average of an eight-step phase cycle) acquired every 48s during each of the 18 blocks of N-back task.

Spectral analysis was performed using the TARQUIN software package, version 4.3.6 (Wilson et al., 2011). The TARQUIN algorithm performed a fully automated fit to the data using a predefined basis set comprising of the following components: alanine; aspartate; creatine (Cr); gamma-aminobutyric acid; glucose; glutamine; glutathione; glutamate (Glu); glycerophosphorylcholine; myo-inositol; lactate; lipid peaks at 0.9, 1.3a, 1.3b, and 2.0 ppm; macromolecules at 0.9, 1.2, 1.4, and 2.0 ppm; N-acetyl-aspartate (NAA); N-acetyl-aspartateglutamate; phosphorylcholine; phosphocreatine (PCr); scyllo-inositol; and taurine.

Spectra acquired from the 0-back and 2-back task conditions (8 blocks of each) were averaged together and measures of Glu and Glx (Glu + glutamine), scaled to total creatine (TCr − Cr + PCr), were recorded allowing the mean differences in concentrations between task conditions to be calculated. Additionally, averaged measures of the first, second and third spectra from each of the cumulative 0-back and 2-back conditions were recorded to investigate metabolite level changes across blocks. The 1-back and 3-back conditions (1 block each) were not analysed as these were included purely as a distractor between the 0-back and 2-back conditions.

Several ^{1}H-fMRS studies have reported 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; Mangia et al., 2007). This effect can be addressed by referencing to another peak that may have experienced the same effect (Lally et al., 2014), which was achieved through the use of TCr scaling in this study. We excluded any metabolite measures with a Cramer-Rao minimum variance bounds (CRMVB) of greater than 20%, a signal-to-noise ratio (SNR) > 5, a full-width half-maximum (FWHM) of < 0.50 ppm or fit quality (Q) of < 2.5. One participant in the schizophrenia group had a gold tooth causing interference and poor spectral quality so was excluded from all analyses.

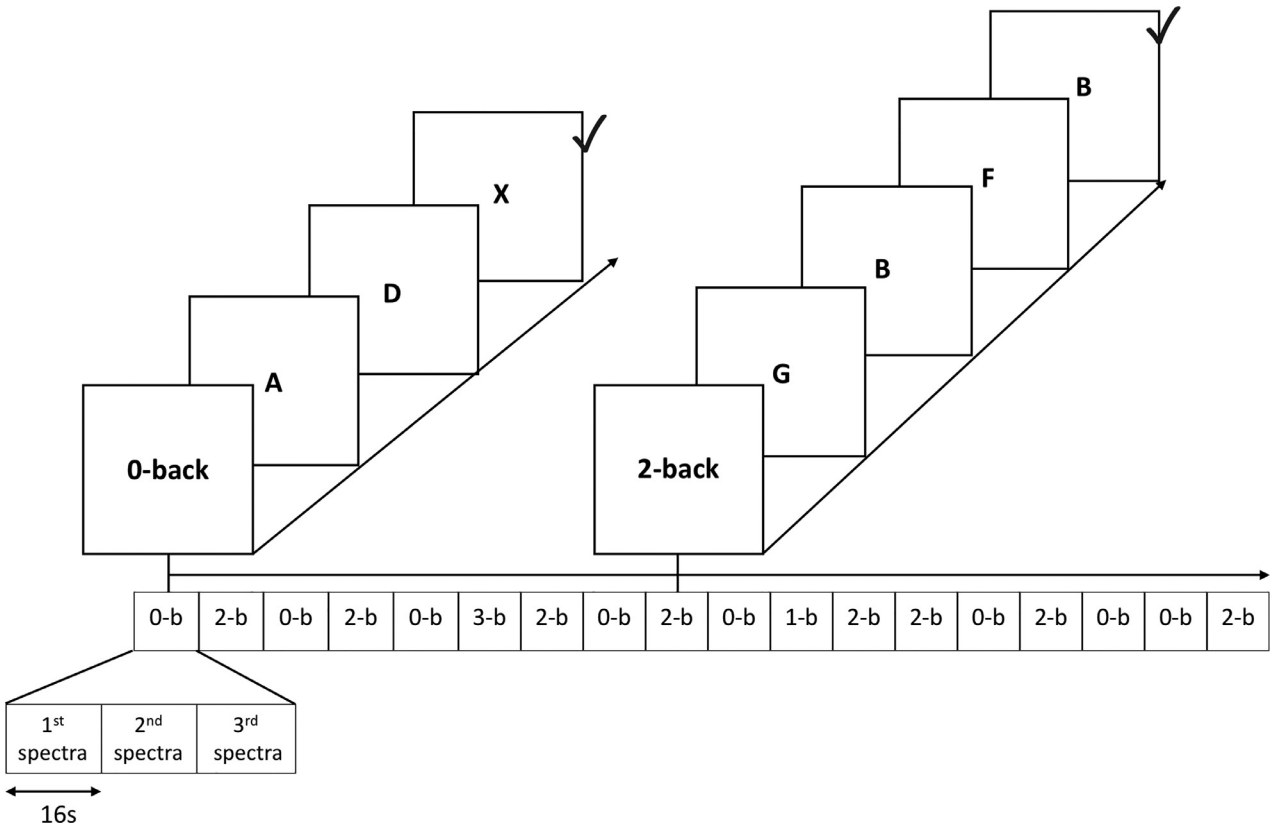

Fig. 1 N-back task paradigm depiction.
The alternating N-back conditions each lasted 48s each, with 18 blocks in total (**0-b**; 0-back; **1-b;** 1back, **2-b**; 2-back; **3-b**; 3-back). During each 48s block three spectra were acquired (One every 16s, NEX = 8). Instructions were presented at the beginning of each block for 3000 ms, 18 sequential letters were presented for 1000 ms with 1500 ms between subsequent letters and participants were allowed 1900 ms to record their response.

To explore relative ^{1}H-fMRS voxel tissue composition, the structural images were segmented into grey matter, white matter and cerebrospinal fluid (CSF) using Statistical Parametric Mapping 8 (SPM8; Wellcome Department of Imaging Neurosciences, University College London, United Kingdom) with the coordinates of each voxel were mapped onto the segmented structural image using in-house software.

2.4. Statistics

All statistical analyses were performed with SPSS version 22.0 (SPSS, Chicago, Illinois) using two-tailed tests. Shapiro-Wilk tests were used to ensure assumptions of normality. *A priori* hypotheses were evaluated using paired *t*-tests to compare mean metabolite changes for the three groups, together with all participants combined, between the total averaged 0-back and 2-back task conditions. To accommodate for multiple comparisons, a Bonferroni-correction was applied (alpha = 0.05/4). A 6 × 3 repeated-measures analysis of variance design (rmANOVA) (alpha = 0.05) using glutamatergic measures across the first, second and third spectra from each of the cumulative 0-back and 2-back conditions was used to determine significant variations over time and across the three groups.

In addition, several *post-hoc* analyses were performed. Bonferroni-corrected pairwise comparisons of glutamatergic measures between each time point were used to determine between which time points any significant changes occurred in the rmANOVA. Further paired *t*-tests were used to compare mean metabolite changes for each group between those time points where such significant changes occurred (alpha = 0.05/4). An exploratory 2 × 2 rmANOVA was performed (alpha = 0.05), comparing glutamatergic measures at the beginning and end of the 0-back condition in the healthy control versus schizophrenia group. To confirm any changes detected in glutamate or Glx corrected for TCr, separate analyses were carried out using correction for total N-acetylaspartate (TNAA). Finally, Pearson correlation coefficients were used to examine any relationship between changes in Glu/TCr or Glx/TCr levels between 0-back and 2-back conditions and 1) age, 2) change in response accuracy, 3) change in response times and 4) clinical rating scores for each group (uncorrected, alpha = 0.05).

3. Results

3.1. Metabolite level differences

Concentrations of Glu/TCr and Glx/TCr in the total averaged 0-back and 2-back conditions were not found to be significantly different in any of the separate or combined groups (Table 2). The 6 × 3 rmANOVA (alpha = 0.05) yielded

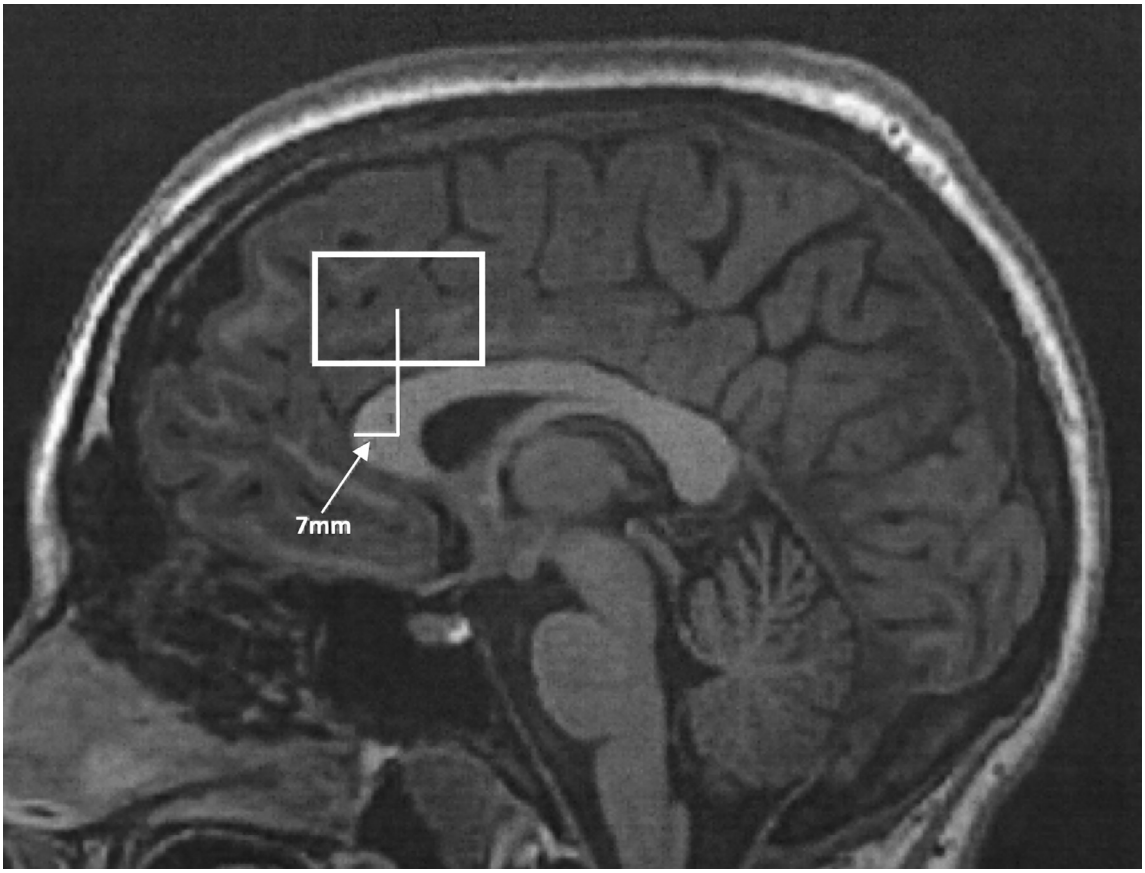

Fig. 2 ^{1}H-fMRS voxel placement.
Sagittal cross-section depicting ^{1}H-fMRS voxel placement, located in the bilateral ACC.

Table 2 Pairwise comparisons for 0-back and 2-back conditions for mean Glu/TCr and Glx/TCr concentrations.

	0-b	2-b	P	0-b3	2-b1	*P*
HC						
[Glu/TCr]	0.90 ± 0.13	0.92 ± 0.11	0.630	0.86 ± 0.10	0.97 ± 0.07	**0.004**
[Glx/TCr]	1.05 ± 0.15	1.08 ± 0.11	0.540	1.01 ± 0.10	1.16 ± 0.12	**<0.001**
SCZ						
[Glu/TCr]	0.89 ± 0.14	0.89 ± 0.08	0.964	0.98 ± 0.13	0.94 ± 0.06	0.346
[Glx/TCr]	1.07 ± 0.17	1.09 ± 0.15	0.604	1.11 ± 0.22	1.14 ± 0.15	0.467
BPII						
[Glu/TCr]	0.91 ± 0.12	0.92 ± 0.11	0.854	0.88 ± 0.08	0.94 ± 0.15	0.295
[Glx/TCr]	1.04 ± 0.14	1.06 ± 0.11	0.527	1.04 ± 0.11	1.11 ± 0.17	0.246
TOTAL						
[Glu/TCr]	0.90 ± 0.13	0.91 ± 0.10	0.667	0.90 ± 0.11	0.95 ± 0.10	0.073
[Glx/TCr]	1.05 ± 0.15	1.08 ± 0.12	0.292	1.05 ± 0.15	1.14 ± 0.14	**0.004**

Values shown are means ± standard deviations. Abbreviations: **[Glu/TCr]**, The concentration of glutamate scaled to total creatine in IU (Institutional Units); **[Glx/TCr],** The concentration of Glx (glutamate + glutamine) scaled to total creatine in IU; **HC**, Healthy controls; **SCZ**, Schizophrenia; **BPII**, Bipolar disorder II; **TOTAL**, All groups combined; **0-b**, 0-back total average; **2-b**, 2-back total average; **0-b3**, Averaged 3rd spectra of 0-back; **2-b1**, Averaged 1st spectra of 2-back; **P**, *P*-value of paired *t*-test (alpha = 0.05/4 (Bonferroni corrected); two-tailed), bold values indicate statistical significance.

a significant main effect of time for Glx/TCr ($P = 0.022$) but not for Glu/TCr ($P = 0.673$). There were no significant time by group interactions for Glu/TCr ($P = 0.296$) or Glx/TCr ($P = 0.610$) and no significant main effects of group for either Glu/TCr ($P = 0.689$) or Glx/TCr ($P = 0.919$). Post-hoc tests using the Bonferroni correction revealed Glx/TCr levels significantly increased ($P = 0.038$) between the end of the 0-back condition (averaged third spectra of 0-back) and the beginning of the 2-back condition (averaged first spectra of 2-back). Further analyses revealed a significant increase in both Glu/TCr ($P = 0.004$) and Glx/TCr ($P < 0.001$) in the healthy control group between these two time points. The individual schizophrenia and BPII groups did not show any significant changes in Glu/TCr or Glx/TCr for this compar-

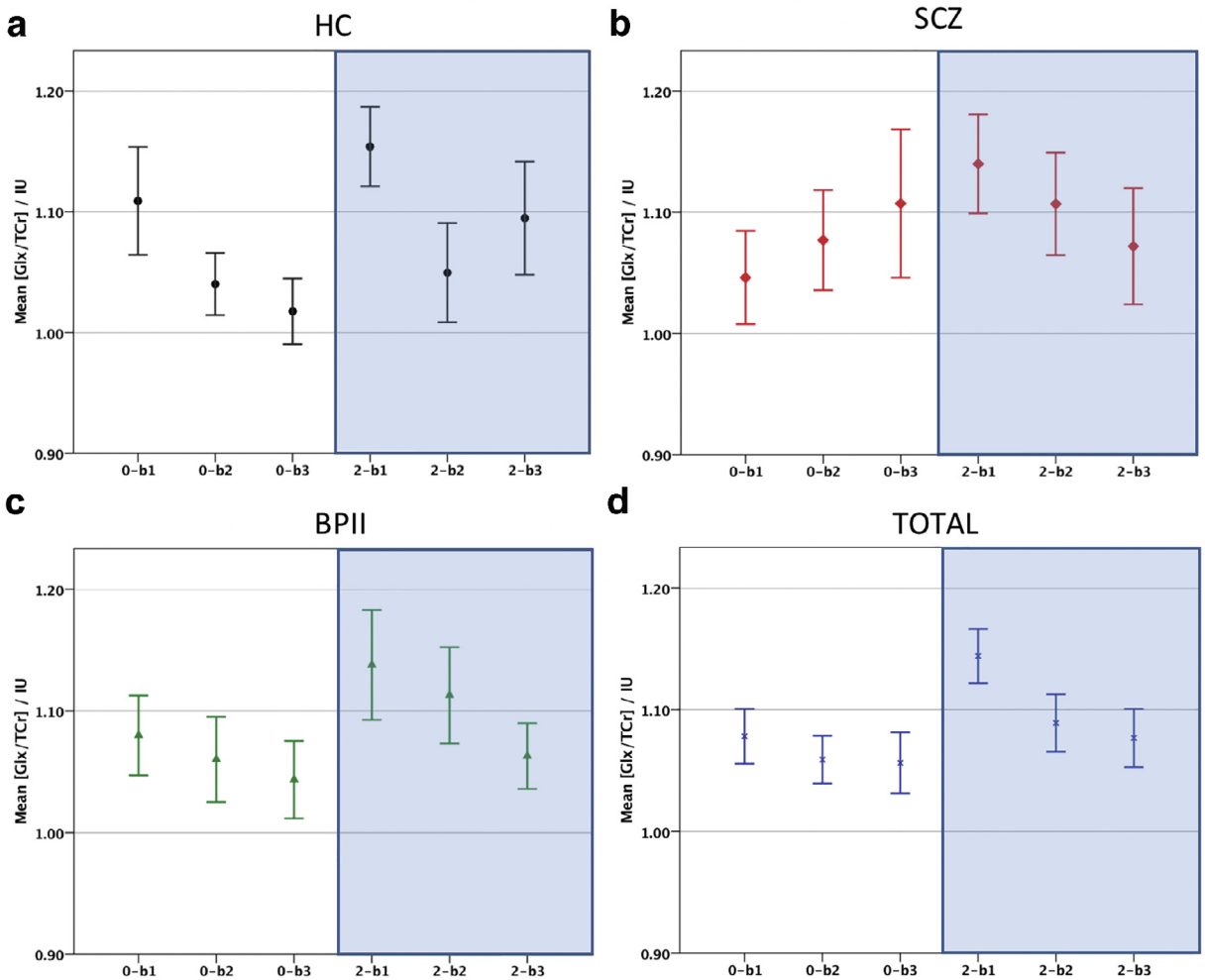

Fig. 3 Averaged time courses of Glx/TCr level estimates according to participant group.
(a) Healthy controls **(b)** schizophrenia **(c)** bipolar disorder II and **(d)** all groups combined. Unshaded area indicates 0-back and shaded area indicates 2-back task condition. Each time point represents 16s period average. **0-b1**, averaged 1st spectra of 0-back; **0-b2**, averaged 2nd spectra of 0-back; **0-b3**, averaged 3rd spectra of 0-back; **2-b1**, averaged 1st spectra of 2-back; **2-b2**, averaged 2nd spectra of 2-back; **2-b3**, averaged 3rd spectra of 2-back. **IU**, Institutional Units. Error bars represent standard error of the mean.

ison. Averaged time courses of Glx/TCr levels for the first, second and third spectra across the 0-back and 2-back conditions are shown in Fig. 3, according to participant group.

Across the 0-back condition healthy control and BPII groups showed a general reduction in Glx/TCr while patients with schizophrenia showed an increase (Fig. 4). Exploratory analyses with 2×2 rmANOVA (alpha $= 0.05$) yielded a significant time by group interaction ($P = 0.019$) for Glx/TCr across the 0-back condition in healthy control versus schizophrenia group, with no significant main effects of time ($P = 0.616$) or group ($P = 0.832$).

The *post-hoc* analyses using correction with TNAA mirrored the main findings obtained for correction with TCr. 6×3 rmANOVA revealed a significant main effect of time for Glx/TNAA ($P = 0.039$) but not Glu/TNAA ($P = 0.413$). Concentrations of Glu/TNAA and Glx/TNAA in the total averaged 0-back and 2-back conditions were not significantly different in any of the separate or combined groups. For all groups combined there was a significant increase in Glx/TNAA ($P = 0.008$), but not Glu/TNAA ($P = 0.105$), between the averaged third spectra of 0-back and averaged first spectra of 2-back. A significant increase in Glu/TNAA ($P = 0.005$) and Glx/TNAA ($P = 0.006$), was seen between these two time points in the healthy control group but not in the individual schizophrenia and BPII groups.

3.2. Tissue segmentation

The mean ^{1}H-fMRS voxel tissue composition proportions are shown in Table 3 according to participant group. There was a significant difference in grey matter proportion between groups as determined by one-way ANOVA ($F(2,40) = 5.488$, $P = 0.008$). The schizophrenia groups had a significantly lower mean grey matter proportion compared with the healthy control group ($P = 0.006$) but not the BPII group. There was no significant difference in grey matter proportion between the healthy control and BPII groups. In terms of CSF and white matter proportions there were no significant differences between the groups.

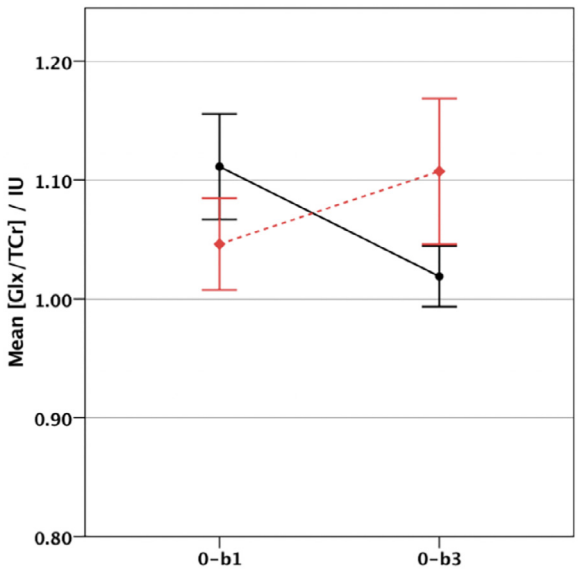

Fig. 4 Averaged levels of Glx/TCr across 0-back condition for schizophrenia and healthy control groups.
Glx/TCr in Institutional Units (IU) for healthy control (continuous line) and schizophrenia groups (broken line) across the 0-back condition. **0-b1**, averaged 1st spectra of 0-back; **0-b3**, averaged 3rd spectra of 0-back. Error bars represent standard error of the mean.

Table 3 Mean ^{1}H-fMRS voxel tissue segmentation proportions.

	Group			
	HC	SCZ	BPII	*P*
CSF	0.189 ± 0.038	0.233 ± 0.067	0.232 ± 0.051	0.054
GM	0.658 ± 0.035	0.596 ± 0.058	0.618 ± 0.052	**0.008**
WM	0.153 ± 0.033	0.171 ± 0.024	0.152 ± 0.017	0.083

Values shown are mean proportions ± standard deviations. Abbreviations: **CSF**, cerebrospinal fluid; **GM**, grey matter; **WM**, white matter; **P**, P-value of One-way ANOVA test (alpha = 0.05, two tailed), bold values indicate statistical significance.

3.3. Task performance

The mean accuracy and response times for the 0-back and 2-back conditions are shown in Table 4. In terms of accuracy, there was a significant difference between groups as determined by one-way ANOVA for the 0-back ($F(2,40) = 8.313$, $P < 0.001$) and 2-back conditions ($F(2,40) = 7.897$, $P = 0.001$). The schizophrenia group had significantly lower mean response accuracy for the 0-back condition compared with the healthy control ($P = 0.006$) and BPII groups ($P = 0.002$). There was no significant difference in accuracy for the 0-back condition between the healthy control and BPII groups. Likewise, for the 2-back condition, the schizophrenia group had significantly lower response accuracy compared with the healthy control ($P = 0.003$) and BPII groups ($P = 0.007$), but there was no statistically significant difference between the healthy control and BPII groups.

For the response times, there was a significant difference between groups for the 0-back ($F(2,40) = 6.364$, $P = 0.004$) and 2-back conditions ($F(2,40) = 3.764$, $P = 0.032$). The schizophrenia group took significantly longer to respond in the 0-back condition compared with the healthy control ($P = 0.012$) and BPII groups ($P = 0.010$), with no significant difference between the healthy control and BPII groups. In the 2-back condition the schizophrenia group again took significantly longer to respond compared with healthy control group ($P = 0.049$) but this did not reach significance when compared with the BPII group. There was no significant difference between healthy control and BPII group for response times in the 2-back condition.

3.4. Correlation analyses

Results from exploratory analyses examining bivariate correlations are shown in Tables 5 and 6. In terms of correlations with metabolite level changes between total averaged 0-back and 2-back conditions there was a significant moderate negative correlation between change in Glx/TCr and change in accuracy seen in the schizophrenia group and a significant weak positive correlation between change in Glx/TCr and change in response time for all groups combined.

For correlations with metabolite level changes, specifically between averaged third spectra of 0-back and averaged first spectra of 2-back, there was a significant strong negative correlation between change in Glu/TCr and change in response time in the healthy control group. In the BPII group there was a strong positive correlation between change in Glu/TCr and change in accuracy together with negative correlations between changes in Glu/TCr

Table 4 Mean accuracy and response times of each group for 0-back and 2-back conditions.

	Accuracy (% ± SD)				Response time (ms ± SD)			
	0-back	2-back	Difference	*P*	0-back	2-back	Difference	*P*
HC	94.0 ± 3.2	86.5 ± 8.5	−7.5 ± 6.9	**<0.001**	633 ± 99	766 ± 105	133 ± 107	**<0.001**
SCZ	84.7 ± 12.7	68.1 ± 18.5	−16.6 ± 11.0	**<0.001**	800 ± 221	945 ± 269	145 ± 143	**0.002**
BPII	95.2 ± 1.9	84.6 ± 11.7	−10.6 ± 10.4	**<0.001**	632 ± 68	789 ± 153	157 ± 114	**<0.001**

Accuracy measured in terms of mean percentage of correct responses (%) ± standard deviation (SD). Response time is mean response time in milliseconds (ms) ± standard deviation (SD). -values, paired *t*-tests for difference in accuracy and response times between 0-back and 2-back conditions (alpha = 0.05); two-tailed), bold values indicate statistical significance.

Table 5 Correlations with metabolite level changes between total averaged 0-back and 2-back condition.

	Age	*P*	Δ- Acc	*P*	Δ-RT	*P*	MADRS	*P*	YMRS	*P*	SAPS	*P*	SANS	P
HC														
Δ[Glu/TCr]	−0.091	0.758	0.025	0.933	0.275	0.341	0.150	0.609	0.265	0.360	0.253	0.383	0.242	0.404
Δ [Glx/TCr]	−0.083	0.779	0.029	0.921	0.325	0.256	0.251	0.387	0.261	0.368	0.302	0.295	0.252	0.385
SCZ														
Δ [Glu/TCr]	0.210	0.490	−0.513	0.073	0.326	0.276	−0.415	0.159	0.076	0.805	−0.474	0.102	0.054	0.862
Δ [Glx/TCr]	0.316	0.271	**−0.565**	**0.035**	0.463	0.096	−0.317	0.270	0.076	0.797	−0.202	0.488	0.015	0.961
BPII														
Δ [Glu/TCr]	0.138	0.637	−0.201	0.490	0.267	0.357	−0.274	0.343	−0.202	0.489	−0.117	0.690	−0.132	0.652
Δ [Glx/TCr]	0.115	0.683	−0.208	0.458	0.279	0.315	−0.109	0.698	−0.202	0.470	0.000	0.999	0.004	0.989
TOTAL														
Δ [Glu/TCr]	0.043	0.789	−0.174	0.276	0.267	0.091	−0.226	0.155	−0.006	0.971	−0.172	0.283	−0.053	0.743
Δ [Glx/TCr]	0.076	0.626	−0.207	0.183	**0.339**	**0.026**	−0.108	0.491	0.019	0.903	−0.079	0.615	−0.014	0.927

All correlation coefficients shown are Pearson r with accompanying *P* value (alpha = 0.05; two-tailed). Abbreviations: **Δ [Glu/TCr]** Change in average glutamate level, scaled to total creatine, between 0-back and 2-back condition; **Δ [Glx/TCr],** Change in average glutamine concentration, scaled to total creatine, between 0-back and 2-back condition; **Δ- Acc,** change in accuracy between 0-back and 2-back; **Δ-RT** change in response time between 0-back and 2-back condition; **MADRS**, Montgomery-Asberg Depression Scale; **YMRS**, Young Mania Rating scale; **SAPS**, Scale for Assessment of Positive Symptoms; **SANS**, Scale for Assessment of Negative Symptoms **HC**, Healthy controls; **SCZ**, Schizophrenia; **BPII**, Bipolar disorder II; **TOTAL**, All groups combined.

Table 6 Correlations with metabolite level changes from averaged final spectra of 0-back condition and averaged first spectra of 2-back condition.

	Age	*P*	Δ- Acc	*P*	Δ-RT	*P*	MADRS	*P*	YMRS	*P*	SAPS	*P*	SANS	*P*
HC														
Δ[Glu/TCr]	0.291	0.386	0.027	0.936	**−0.606**	**0.048**	−0.116	0.734	−0.142	0.677	0.006	0.987	−0.324	0.331
Δ [Glx/TCr]	0.073	0.811	0.101	0.744	−0.336	0.262	0.268	0.377	0.013	0.966	0.213	0.484	−0.125	0.683
SCZ														
Δ [Glu/TCr]	−0.703	0.052	−0.358	0.384	0.178	0.673	−0.607	0.111	0.323	0.435	−0.591	0.123	−0.190	0.652
Δ [Glx/TCr]	−0.063	0.837	−0.129	0.674	0.078	0.800	−0.523	0.067	0.240	0.431	−0.309	0.304	0.015	0.962
BPII														
Δ [Glu/TCr]	−0.303	0.395	**0.684**	**0.029**	−0.432	0.212	**−0.639**	**0.047**	0.273	0.445	0.145	0.690	−0.016	0.965
Δ [Glx/TCr]	−0.460	0.114	0.207	0.498	−0.497	0.084	**−0.585**	**0.036**	−0.189	0.536	0.302	0.315	0.332	0.268
TOTAL														
Δ [Glu/TCr]	−0.186	0.334	0.252	0.187	−0.219	0.254	**−0.632**	**<0.001**	0.098	0.615	**−0.492**	**0.007**	**−0.407**	**0.028**
Δ [Glx/TCr]	−0.229	0.161	0.133	0.420	−0.203	0.215	**−0.532**	**<0.001**	−0.071	0.669	−0.244	0.134	−0.128	0.438

All correlation coefficients shown are Pearson r with accompanying *P* value (alpha = 0.05; two-tailed). Abbreviations: **Δ [Glu/TCr]** Change in average glutamate level, scaled to total creatine, between averaged 3rd spectra of 0-back and averaged 1st Spectra of 2-back; **Δ [Glx/TCr],** Change in average glutamine concentration, scaled to total creatine, between averaged 3rd spectra of 0-back and averaged 1st Spectra of 2-back; **Δ- Acc,** change in accuracy between 0-back and 2-back; **Δ-RT** change in response time between 0-back and 2-back condition; **MADRS**, Montgomery-Asberg Depression Scale; **YMRS**, Young Mania Rating scale; **SAPS**, Scale for Assessment of Positive Symptoms; **SANS**, Scale for Assessment of Negative Symptoms **HC**, Healthy controls; **SCZ**, Schizophrenia; **BPII**, Bipolar disorder II; **TOTAL**, All groups combined.

and Glx/TCr, and MADRS score. Across groups there were highly significant negative correlations between changes in Glu/TCr and Glx/TCr, and MADRS score. For all groups combined there were also significant negative correlations between change in Glu/TCr and SAPS/SANS scores.

There were no significant correlations between age and any of the metabolite level change comparisons between 0-back and 2-back conditions in any of the separate or combined groups.

4. Discussion

A main effect of time for Glx/TCr was observed across the 0-back and 2-back conditions for all the groups. Although there were not any significant differences in Glu/TCr and Glx/TCr between total averaged 0-back and 2-back conditions in any of the groups, a significant increase in Glx/TCr was seen between the last spectrum of the 0-back condition and the first spectrum of the 2-back condition for all groups combined. The healthy control group specifically showed a significant increase in both Glu/TCr and Glx/TCr for this comparison, while the schizophrenia group showed no significant changes. Although the BPII group did not show significant changes for this comparison either, the averaged time-course pattern was closer to that of healthy controls and the lack of significance may be the result of insufficient power.

The absence of any significant changes in Glu/TCr or Glx/TCr in the schizophrenia group between the 0-back and 2-back conditions supports our hypothesis of an impaired glutamatergic response in patients with schizophrenia and is comparable to findings by Taylor et al. (2015a), where although increased glutamate levels were detected in the dorsal ACC during a Stroop task in healthy controls, no significant change in glutamate was observed in individuals with schizophrenia. These findings are supported by functional MRI studies that have demonstrated ACC deficits in schizophrenia in studies of executive function, including the N-back task (Minzenberg et al., 2009), with increases in other prefrontal cortex areas that may be compensatory in nature. As such, in schizophrenia, there may be a blunted activation state of the ACC with accompanying failure of glutamatergic modulation and impairments in task performance.

It was interesting to note that across the 0-back condition the schizophrenia group showed a general increase in glutamatergic measures, while the healthy control group showed a reduction, with a significant time by group crossover interaction for Glx/TCr. The schizophrenia group also had significantly lower accuracy and response times for this condition compared with the healthy control group. Taken together, these findings suggest during the 0-back condition, for what should be a period of relatively low executive load, the schizophrenia group found this condition cognitively challenging, with abnormal increases in glutamatergic measures. Furthermore, with increasing task difficulty they did not show the same dynamic modulation of glutamate levels as healthy volunteers.

Static ^{1}H-MRS studies of medial frontal lobe areas have reported increased glutamine (Stone et al., 2009) and Glx (Tibbo et al., 2004) in individuals at high risk of psychosis together with increased glutamine in unmedicated patients with first episode psychosis (Theberge et al., 2002; Theberge et al., 2007). In studies of chronic medicated patients however, studies have either found no difference (Reid et al., 2010; Wood et al., 2007) or reduced levels of Glx (Ohrmann et al., 2005; Rowland et al., 2013) and glutamate (Tayoshi et al., 2009; Theberge et al., 2003) compared with healthy controls, with one meta-analysis suggesting a progressive reduction with age in patients with schizophrenia (Marsman et al., 2013). It is conceivable that an excitotoxic effect may occur as a result from paradoxical increases in glutamatergic activity due to NMDAR hypofunction in the early stages of schizophrenia (Plitman et al., 2014). The longer-term result of this excitotoxicity would be a state of neurodegeneration with impaired glutamatergic modulation and is a potential explanation for the lack of any significant glutamatergic response between task conditions in the cohort of more chronic patients recruited in this study.

To our knowledge, there are no other ^{1}H-fMRS studies involving a BPII patient group to date. Only one ^{1}H-fMRS study has been performed in a group of patients with a mood disorder (MDD), where no significant increases in glutamate were detected during a Stroop Task (Taylor et al., 2015a) and is comparable with our finding of no significant glutamatergic response between the 0-back and 2-back conditions in a BPII group. Furthermore, in our study, depressive symptoms were negatively correlated with changes in Glu/TCr and Glx/TCr between the 0-back condition and 2-back condition (i.e. less depressed subjects had greater increases in glutamatergic concentrations). Together with alterations in glutamate metabolism, the role of inflammation in the pathophysiology in mood disorders is increasingly evident (Dantzer et al., 2008) and may provide explanation for the lack of a glutamatergic response in the BPII group in our study. One interesting hypothesis links the role of inflammation, glutamate and glia in mood disorders (Haroon et al., 2017). Inflammation can lead to increased release of glutamate into the extrasynaptic space by reducing the capacity of glial transporters to clear glutamate (McCullumsmith and Sanacora, 2015). This glutamate excess can activate extrasynaptic NMDARs, resulting in atrophy of dendritic spines, loss of synaptic integrity and neuronal loss (Hardingham and Bading, 2010). Furthermore, this extrasynaptic glutamate can stimulate presynaptic metabotropic glutamate receptors (mGluR2/3) with reductions in synaptic glutamate transmission (Duman, 2014).

The Glu/TCr and Glx/TCr responses observed in the healthy control group (12.6% and 14.7%, respectively) are higher than those reported in other block-design ^{1}H-fMRS studies using the N-back (Woodcock et al., 2018), Stroop Task (Taylor et al., 2015a, 2015b) and other paradigms (Bednarik et al., 2015; Lin et al., 2012; Mangia et al., 2007; Schaller et al., 2013, 2014), where responses were in the range of 2–4%. The detected amplitude of changes in glutamatergic metabolites was more comparable with the range reported in event-related ^{1}H-fMRS studies (11–21.5%), where acquisition is time locked to stimulus onset (Apsvalka et al., 2015; Gussew et al., 2010; Lally et al., 2014). It is important to note for the initial planned comparison between total average 0-back and 2-back condition blocks there were no significant changes in glutamatergic metabolites in any

groups. A potential explanation for this is that during prolonged stimulation habituation, adaptation and homeostatic effects might lead to difficulty detecting rapid glutamatergic dynamics (Giove et al., 2003; Mullins, 2018). A relatively rapid change in glutamate within the dLPFC during an N-back task has been reported with levels significantly higher by 2.7% during the first 32s and non-significantly higher during the final 32s of a 64s 2-back task block, compared with a resting condition. Our study mirrors this finding with the significant increases in glutamatergic metabolites occurring in the first 16s of 2-back condition compared with preceding last 16s of 0-back condition. This early increase may indicate novelty of task onset and a potential explanation for the larger glutamatergic responses seen in our study is that the higher temporal resolution allowed more of an early glutamate response function to be detected.

An important issue to consider is what the changes detected by ^{1}H-fMRS signify. Increases in glutamatergic metabolites, in the order of 2–5% over a period of minutes, can be explained by increased metabolic turnover, via a number of pathways including increased glutamate/glutamine cycling through the tricarboxylic acid cycle (TCA) (Rothman et al., 2003), or increased flux through the malate-aspartate shuttle (MAS) (Mangia et al., 2007; McKenna et al., 2006). However, if increased glutamate/glutamine cycling would be causal for the increase in glutamate, the Glx level would not show such an increase due to a compensatory decrease in glutamine. It has been argued changes in the order of 12–18%, especially when occurring over a matter of seconds, are too large to be explained by increased metabolic turnover alone (Jelen et al., 2018). One emerging theory is that of compartmental shifts which proposes that during neural activity glutamate is released from presynaptic vesicles (where metabolite movement is restricted, with a faster T_2 relaxation rate) to more MRS visible synaptic, extracellular and astrocytic pools (where metabolite movement is less restricted and has a longer T_2) (Mullins, 2018). This would imply ^{1}H-fMRS is not detecting changes in overall glutamatergic concentrations in the specified brain volume, but rather compartmental shifts due to neural activity, as metabolites move from presynaptic vesicles to more visible synaptic, extracellular and astrocytic pools. Although speculative, the significant changes seen in the healthy control group in our study may represent a greater degree of variability and flexibility in glutamatergic neurotransmission, that is not seen in the schizophrenia or BPII groups.

4.1. Limitations

This study has a number of limitations. First, are the potential confounding effects of medication together with age and illness-stage of participants. Both antipsychotic and antidepressant medications are known to effect glutamatergic measures (de la Fuente-Sandoval et al., 2013; Sanacora et al., 2012) and as a result could have altered baseline and glutamate responses in the N-back task. Likewise, age and illness-stage were not specifically controlled for in groups, both of which are factors that have been reported to effect glutamatergic measures (Marsman et al., 2013; Ohrmann et al., 2005; Stone et al., 2009; Theberge et al., 2003). However, *post-hoc* correlation analyses did not reveal any significant relationship with age and any changes in glutamatergic measures between the 0-back and 2-back conditions in any of the separate or combined groups.

The acquired MRS signals were corrected for TCr but not for tissue type and T2 relaxation differences. Although this would not affect the relative changes in the measured metabolites within individuals, tissue segmentation analysis revealed significantly lower grey matter proportion in the schizophrenia group compared with healthy controls and as such differences in groups could potentially be influenced by differences in grey matter volume.

A further limitation was that the paradigm used in this study did not include a resting baseline condition. The number of errors seen in the schizophrenia group for the 0-back condition suggests this was challenging for some participants and glutamatergic measures may have already increased from a resting condition level, preventing changes between the 0-back and 2-back conditions from being detected. Recent work has noted the importance of the 'non-task-active' behavioural paradigm in ^{1}H-fMRS studies and suggests a continuous visual fixation cross exhibits less variability and lower levels of glutamate and therefore providing a better approximation of baseline state than other behavioural paradigms including eyes closed, flashing checkerboard or finger tapping (Lynn et al., 2018). An alternative explanation for the worse performance in the schizophrenia group that should also be considered is that of aberrant salience (Kapur, 2003), whereby these patients may be more likely to be inappropriately processing stimuli in their environment that would normally be considered irrelevant, and as a result might be less able/or willing to engage with and focus attention to the task in question.

Finally, another limitation of this study is the field strength of 3T. Although spectral resolution is considered reasonably high at 3T, our ^{1}H-fMRS technique was unable to reliably differentiate glutamate and glutamine. In addition, a number of spectra had to be excluded due to CRMVB values $>$ 20%, for the Glu/TCr rmANOVA analysis in particular, with subsequent reduction in power.

5. Conclusion

This ^{1}H-fMRS study has demonstrated significant dynamic changes in glutamatergic measures in the ACC during alternating conditions of the N-back task at 3 T in a healthy control group but not in schizophrenia or BPII groups. The lack of any significant dynamic changes in the schizophrenia and BPII groups may reflect a dysfunction of glutamatergic neurotransmission. Future ^{1}H-fMRS work at higher field strengths where both glutamate and glutamine can be reliably measured should investigate glutamatergic dynamics in schizophrenia and mood disorders, at various stages of illness progression in further detail. Such work might help in terms of stratifying patients to specific therapies, predicting functional outcome, or even be a potential aid in further developing novel treatment strategies that act to normalize impairments in glutamatergic neurotransmission in psychiatric disorders.

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Conflict of interest

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Contributors

JS designed the study and wrote the protocol. DL set up the ^{1}H-MRS acquisition sequences and the imaging methodology and provided support with data analysis. LJ, SK and CH managed the data collection. LJ managed the analysis and wrote the first draft of the manuscript. JS and AY had a role in overall supervision and final drafting of the report. All the authors contributed to and have approved the final manuscript.

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