

Anterior Cingulate Glutamate Levels Related to Clinical Status Following Treatment in First-Episode Schizophrenia

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Many patients with schizophrenia show a limited symptomatic response to treatment with dopaminergic antipsychotics. This may reflect the additional involvement of non-dopaminergic neurochemical dysfunction in the pathophysiology of the disorder. We tested the hypothesis that brain glutamate levels would differ between patients with first-episode psychosis who were symptomatic compared with those with minimal symptoms following antipsychotic treatment. Proton magnetic resonance spectroscopy (1H-MRS) spectra were acquired at 3 Tesla in the anterior cingulate cortex and left thalamus in 15 patients with first-episode psychosis in symptomatic remission, and 17 patients with first-episode psychosis who were still symptomatic following at least one course of antipsychotic treatment. Metabolite levels were estimated in ratio to creatine (Cr) using LCModel. Levels of glutamate/Cr in the anterior cingulate cortex were significantly higher in patients who were still symptomatic than in those in remission ($T(30) = 3.02$; $P = 0.005$). Across the entire sample, higher levels of glutamate/Cr in the anterior cingulate cortex were associated with a greater severity of negative symptoms ($r = 0.42$; $P = 0.017$) and a lower level of global functioning ($r = -0.47$; $P = 0.007$). These findings suggest that clinical status following antipsychotic treatment in schizophrenia is linked to glutamate dysfunction. Treatment with compounds acting on the glutamatergic system might therefore be beneficial in patients who respond poorly to dopaminergic antipsychotics.

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INTRODUCTION

In the treatment of schizophrenia with antipsychotic drugs, on average around 60% occupancy of brain D2 dopamine receptors is required to produce a therapeutic response (Kapur *et al*, 2000). However, a substantial proportion of patients still show a poor response even when D2 occupancy is at this level (Pilowsky *et al*, 1993). This may reflect the importance of non-dopaminergic neurochemical dysfunction in the pathophysiology of schizophrenia. Increasing evidence from pharmacological, neuropharmacological, and genetic studies suggests that the primary deficit in schizophrenia may be a developmentally driven abnormality in the glutamate system (see (Egerton *et al*, 2012; Stone *et al*, 2007)). Several clinical trials have demonstrated the efficacy

of adjunctive glutamatergic therapies in treating symptoms that have not responded to antipsychotic medication (Javitt, 2004). The greater efficacy of clozapine compared with other antipsychotics, and its effectiveness in otherwise treatment-resistant patients, has been linked to its glutamatergic actions (Javitt *et al*, 2005). It has thus been suggested that patients who are still symptomatic following antipsychotic medication may exhibit persistent glutamatergic abnormalities, and may therefore benefit from treatments that act on the glutamate system (Stone *et al*, 2010).

Proton magnetic resonance imaging (1H-MRS) studies at field strengths of 3 Tesla or above in antipsychotic naive or minimally medicated first-episode psychosis patients have identified increases in the glutamate metabolite glutamine (Gln) or the Gln/glutamate ratio in the anterior cingulate cortex (Bustillo *et al*, 2010; Theberge *et al*, 2002), increases in Gln in the thalamus (Theberge *et al*, 2002), and increases in glutamate in the associative striatum (de la Fuente-Sandoval *et al*, 2011). This suggests that brain glutamatergic activity is increased in the early stages of the disorder, when patients are usually experiencing acute psychotic symptoms. 1H-MRS studies of the glutamate system in medicated

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patients with chronic schizophrenia have produced less consistent findings (Jessen *et al*, 2011; Reid *et al*, 2011; Shirayama *et al*, 2010; Tayoshi *et al*, 2009; Theberge *et al*, 2003; Wood *et al*, 2007). A recent meta-analysis indicated that although frontal glutamatergic activity may be elevated in untreated patients in the early phase of schizophrenia, reductions in frontal glutamate and Gln are evident in older patients at later stages of the disorder (Marsman *et al*, 2011). This apparent difference between the early and later stages of the disorder is not simply an effect of cumulative antipsychotic exposure, as prolonged treatment (for up to 80 months) is not associated with a change in glutamate metabolites in the anterior cingulate cortex (Aoyama *et al*, 2011; Bustillo *et al*, 2010; Theberge *et al*, 2007).

Some of the differences in the findings in studies of glutamate in schizophrenia may be related to differences in the symptom profiles of the patients studied, as glutamate metabolite levels may vary with the level of negative symptoms (Reid *et al*, 2011), cognitive deficits (Bustillo *et al*, 2011; Shirayama *et al*, 2010), and global functioning (Aoyama *et al*, 2011; Tibbo *et al*, 2004; Van Elst *et al*, 2005). In the present study, we tested the hypothesis that glutamate metabolite levels in the anterior cingulate cortex and thalamus would be higher in first-episode psychosis patients who were symptomatic compared with those with minimal symptoms following treatment.

PATIENTS AND METHODS

The study was approved by the Joint South London and Maudsley and the Institute of Psychiatry Research Ethics Committee. All subjects gave written informed consent to participate. Thirty-two patients who had presented with a first episode of schizophrenia (as defined by DSM-IV criteria), and who had completed at least one course of treatment with antipsychotic medication were recruited from the South London and Maudsley NHS Foundation Trust. Only patients who had presented within the past 2 years, and who had experienced a single episode of psychosis were included. The mean \pm SD time since first presentation was 10.6 ± 7.1 months. Exclusion criteria included a history of head injury, concomitant medical or neurological illness, drug or alcohol dependence (except nicotine), presence of metallic implants contraindicated at 3 Tesla, and pregnancy. Current and previous use of nicotine, cannabis, alcohol and antipsychotic medication, and family history of psychiatric illness were determined through self-report data. Symptoms at the time of imaging were assessed using the Positive and Negative Syndrome Scale (PANSS; Kay *et al*, 1987) and global functioning was assessed using the global assessment of functioning scale (Hall, 1995). Symptom severity scores were used to allocate patients to group (symptomatic remission *vs* no remission) according to established criteria (Andreasen *et al*, 2005). Symptomatic remission was defined as having scores of mild or less on the all eight of the following PANSS positive (P), negative (N), or general (G) syndrome scale items: Delusions (P1); Conceptual Disorganization (P2); Hallucinatory behavior (P3); Blunted affect (N1); Social withdrawal (N4); Lack of spontaneity (N6); Mannerisms/posturing (G5), and Unusual thought content (G9) (Andreasen *et al*,

2005). No remission was defined as having scores of moderate or more on the same items.

All scans were acquired on a General Electric (Milwaukee, Wisconsin) 3 Tesla HDx magnetic resonance system as detailed previously (Stone *et al*, 2009). An initial localizer scan was followed by acquisition of structural images, including an axial 2D T2-weighted fast spin echo scan and an axial fast fluid-attenuated inversion recovery scan. 1H-MRS voxels were prescribed in the anterior cingulate cortex and left thalamus. The anterior cingulate cortex voxel was prescribed from the midline sagittal localizer, with the centre of the $20 \times 20 \times 20$ mm voxel placed 13 mm above the genu of corpus callosum perpendicular to the AC-PC line (Figure 1a). A second (15 (right-left) $\times 20 \times 20$ mm) voxel was placed in centre of the left thalamus to include the maximum amount of grey matter (Figure 1b). ^1H -MRS spectra (PRESS—Point RESolved Spectroscopy; TE = 30 msec; Tr = 3000 msec; 96 averages) were acquired using the standard GE PROBE (proton brain examination) sequence, which uses a standardized chemically selective suppression water suppression routine. Shimming was optimized, with auto-prescan performed twice before each scan.

Data Analysis

Spectra were analyzed using LCModel version 6.1-4 F (Provencher, 1993), using a standard basis set of 16 metabolites (L-alanine, aspartate, creatine, phosphocreatine, GABA, glucose, Gln, glutamate, glycerophosphocholine, glycine, myo-inositol, L-lactate, N-acetylaspartate, N-acetylaspartylglutamate, phosphocholine, and taurine), acquired with the same field strength (3 Tesla), localization sequence (PRESS), and echo time (30 msec). Model metabolites and concentrations used in the basis set are fully detailed in the LCModel manual (<http://s-provencher.com/pages/lcm-manual.shtml>). Poorly fitted metabolite peaks (Cramer-Rao minimum variance bounds of $> 20\%$ as reported by LCModel) were excluded from further analysis. As information on voxel cerebrospinal fluid content was not available for correction of water-scaled metabolite concentration estimates, the ratio of the reported metabolite concentration to that of the creatine (Cr) plus phosphocreatine resonance was used for analysis.

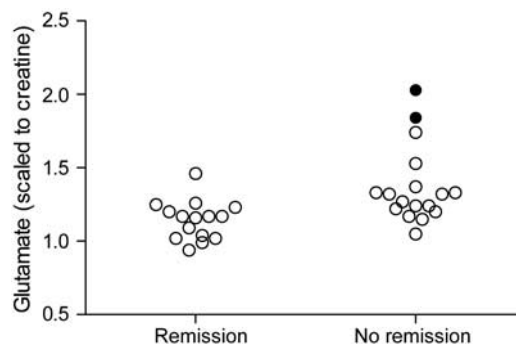


Figure 1 Level of glutamate (scaled to creatine) in the anterior cingulate cortex. Glutamate levels were significantly higher in non-remitted ($n = 17$) than remitted ($n = 15$) schizophrenia patients ($T(30) = 3.02$; $P = 0.005$). This difference remained significant $T(28) = 2.79$; $P = 0.009$ after removal of the two outlying values as identified by Cook's D and indicated by the filled symbols.

Statistical analysis was performed in SPSS version 15.0 (Chicago, Illinois). Group differences in demographic and clinical variables, spectral quality and glutamate/Cr ratios were explored using Fisher's exact test or independent-samples *t*-tests as appropriate. Levene's test was used to check for equality of variance across groups. Statistical significance was defined as $P < 0.05$. Relationships between glutamate/Cr ratio and symptom scores were explored using Pearson's product moment correlation, correcting for the number of correlations performed (threshold $P = 0.05/4$ comparisons = 0.013). Potential influences of age, medication status, or outlying values (as indicated by Cook's *D*) on significant findings were subsequently determined using partial correlation or exclusion of specific cases.

RESULTS

Clinical and Demographic Characteristics

Clinical and demographic variables are presented in Table 1. Fifteen patients met the criteria for symptomatic remission (mean \pm SD age = 30 ± 6 years, 11 male, 14 right handed), while seventeen were still symptomatic ('no remission'; mean \pm SD age = 26 ± 7 years, 13 male, 17 right handed). There were no significant group differences in age, gender, handedness, or current tobacco, alcohol or cannabis use, or history of amphetamine, cocaine, ecstasy or ketamine use. In the symptomatic remission group, one patient was receiving amisulpride, one aripiprazole, five olanzapine, three risperidone, one quetiapine, and four were no longer receiving any antipsychotic medication. In the symptomatic non-remission group, one patient was receiving amisulpride, seven aripiprazole, three olanzapine, three risperidone, two

quetiapine, and one was no longer taking any antipsychotic medication. There was no significant difference in the type of antipsychotic medication between groups. As expected, symptom severity on all PANSS subscales and the level of global functioning were significantly worse in the no remission compared with the remission group (Table 1).

Quality of 1H-MRS Spectra

Representative spectra for the anterior cingulate cortex and left thalamus voxels are provided in Supplemental Figure S1 available online. Serial water-scaled phantom data did not provide any evidence of step changes or scanner drift over the course of the study. Spectral quality was assessed as the NAA signal to noise ratios (S/N, defined as the ratio of the peak height at 2.01 ppm minus baseline to twice the root mean square of the residuals of the fit, as reported by LCModel) and linewidths as reported by LCModel. Spectra were of good quality in the anterior cingulate cortex and thalamus and there were no significant group differences in spectral quality in either region (anterior cingulate cortex: S/N mean \pm SD remission = 22.3 ± 4.2 ; no remission 20.0 ± 5.0 ; $t_{30} = 1.42$; $P = 0.17$; linewidth \pm SD remission = 0.039 ± 0.007 ppm; no remission 0.038 ± 0.005 ppm; $P = 0.49$; or in the left thalamus (S/N mean \pm SD remission = 19.1 ± 2.7 ; no remission 18.5 ± 3.5 ; $t_{29} = 0.58$; $P = 0.56$; linewidth \pm SD remission = 0.056 ± 0.018 ppm; no remission 0.047 ± 0.007 ppm; $t_{29} = 1.78$; $P = 0.09$). The mean \pm SD% CRLB for each metabolite in each voxel for remitted and non-remitted patients are presented in Supplemental Table 1 available online. There were no significant group differences in metabolite %CRLBs (P values range from 0.07 to 0.95).

Table 1 Demographic and Clinical Characteristics

	Remission, N = 15	No Remission, N = 17	Statistic
Age, mean \pm SD, years	30 \pm 6	25 \pm 7	$T(30) = 1.80$; $P = 0.08$
Gender (male/female)	11/4	13/4	Fisher's $P = 1.00$
Handedness (right/left)	14/1	17/0	Fisher's $P = 0.47$
Family history (yes/no/uk)	2/8/5	2/10/5	Fisher's $P = 1.00$
Education, mean \pm SD, years	13.3 \pm 1.9	12.9 \pm 1.7	$T(30) = 0.61$; $P = 0.55$
Current tobacco use (yes/no)	12/3	9/8	Fisher's $P = 0.15$
Current alcohol use (yes/no)	9/6	12/5	Fisher's $P = 0.71$
Current cannabis use (yes/no)	4/11	5/12	Fisher's $P = 1.00$
Amphetamine use, ever (yes/no)	4/11	4/13	Fisher's $P = 1.00$
Cocaine use, ever (yes/no)	3/12	5/12	Fisher's $P = 0.69$
Ecstasy use ever (yes/no)	5/10	6/11	Fisher's $P = 1.00$
Ketamine use ever (yes/no)	1/14	4/13	Fisher's $P = 0.34$
Current antipsychotic medication Am/Ar/Ol/Ri/Qu/N	1/1/5/3/1/4	1/7/3/3/2/1	Fisher's $P = 0.18$
Time since presentation, mean \pm SD, months	12.3 \pm 7.4	9.1 \pm 6.6	$T(30) = 1.27$; $P = 0.21$
PANSS-positive, mean \pm SD	10.5 \pm 2.8	16.5 \pm 4.4	$T(30) = 4.54$; $P < 0.001$
PANSS-negative, mean \pm SD	9.7 \pm 2.6	17.1 \pm 5.5	$T(30) = 4.77$; $P < 0.001$
PANSS-general, mean \pm SD	21.1 \pm 4.8	27.9 \pm 7.3	$T(30) = 3.08$; $P = 0.004$
PANSS-total, mean \pm SD	41.1 \pm 8.8	60.8 \pm 12.0	$T(30) = 5.25$; $P < 0.001$
GAF score, mean \pm SD	64.4 \pm 10.8	45.6 \pm 8.3	$T(30) = 5.55$; $P < 0.001$

Abbreviations: Am, amisulpride; Ar, aripiprazole; N, none; Ol, olanzapine; Qu, quetiapine; Ri, risperidone; uk, unknown.

Metabolite Levels in Remitted vs Non Remitted Patients

The non-remitted patients had significantly higher levels of glutamate/Cr in the anterior cingulate cortex than those in remission (Figure 1; $T(30) = 3.02$; $P = 0.005$). There was a tendency towards higher variance in glutamate/Cr estimates in the no remission compared with the remission group (Levene's test: $F = 3.54$; $P = 0.07$), and the group difference in glutamate/Cr remained significant accounting for inhomogeneity of variance ($T(24.3) = 3.14$; $P = 0.004$) and after removal of two outlying values (as identified by Cook's D and shown in Figure 1; $T(28) = 2.79$; $P = 0.009$). The higher levels of anterior cingulate glutamate/Cr in non-remitted patients compared with remitted patients also remained significant after exclusion of the five patients no longer taking antipsychotics ($T(25) = 2.36$; $P = 0.026$), and when analyzed as water-scaled glutamate levels ($T(30) = 2.23$; $P = 0.03$). Across all patients, there was a significant decline in anterior cingulate cortex glutamate/Cr with increasing age ($r = -0.425$; $P = 0.015$). However, the group difference in glutamate/Cr remained significant after controlling for age effects ($F(1,31) = 5.774$; $P = 0.023$). There were no significant effects of current tobacco (mean \pm SD, n : non-smokers 1.29 ± 0.27 , 11; smokers 1.25 ± 0.23 , 21; $T(30) = 0.45$; $P = 0.67$), current alcohol use (mean \pm SD, n : non-alcohol drinkers 1.24 ± 0.24 , 11; alcohol drinkers 1.28 ± 0.24 , 21; $T(30) = 0.44$; $P = 0.66$), current cannabis use (mean \pm s.d., n : non-cannabis users 1.27 ± 0.26 , 9; cannabis users 1.26 ± 0.24 , 23; $T(30) = 0.11$; $P = 0.91$), or history of ever using other recreational drugs (amphetamine: $T(30) = 0.87$; $P = 0.39$; cocaine: $T(30) = 0.51$; $P = 0.61$; ecstasy: $T(30) = 0.39$; $P = 0.70$; ketamine: $T(30) = 0.33$; $P = 0.74$) on anterior cingulate glutamate/Cr levels, and no significant relationship between time since presentation and anterior cingulate glutamate/Cr levels ($n = 32$; $r = -0.05$; $P = 0.77$). Although there was no significant group difference in glutamate plus Gln (Glx)/Cr ($T(30) = 1.24$; $P = 0.223$), levels of Glx/Cr were higher in non-remitted than remitted patients following the removal of three outlying values as identified with Cook's D (Figure 2, $T(27) = 2.20$; $P = 0.036$). There were no significant group differences in the other metabolites quantifiable in the anterior cingulate cortex

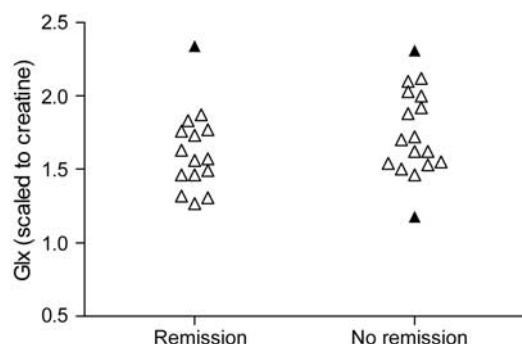


Figure 2 Level of Glx (glutamate plus glutamine, scaled to creatine) in the anterior cingulate cortex. There was no significant group difference in Glx including all cases ($T(30) = 1.24$; $P = 0.223$), but levels of Glx/Cr were significantly higher in non-remitted than remitted patients ($T(27) = 2.20$; $P = 0.036$) after removal of three outlying values as identified with Cook's D and indicated by the filled symbols.

spectra, including Cr (Table 2), and no significant group differences in any of the metabolites quantifiable in the left thalamus (Table 2).

Relationships with Symptoms

Across the entire sample, higher levels of glutamate/Cr in the anterior cingulate cortex were associated with a greater severity of negative symptoms (Figure 3a; $r = 0.42$; $P = 0.017$) and a lower level of global functioning (Figure 3b; $r = -0.47$; $P = 0.007$), but not severity of positive ($r = 0.232$; $P = 0.202$) or general ($r = 0.071$; $P = 0.698$) symptoms. The relationship between anterior cingulate cortex glutamate/Cr and global functioning survived correction for multiple comparisons (threshold $P = 0.05/4$ comparisons = 0.013). The relationships between anterior cingulate cortex glutamate/Cr and negative symptoms or global functioning remained significant after exclusion of the five patients no longer taking antipsychotic medication (PANSS negative: $r = 0.401$; $P = 0.038$; global functioning: $r = -0.436$; $P = 0.023$), and after controlling for age (PANSS negative: $r = 0.465$; $P = 0.008$; global functioning: $r = -0.447$; $P = 0.012$). Significant relationships between anterior cingulate Glx/Cr and PANSS negative symptom severity ($r = 0.384$; $P = 0.040$) and global functioning ($r = -0.404$; $P = 0.030$) were also evident after exclusion of three outlying values as identified with Cook's D. No significant relationships between symptoms and either glutamate or Glx levels in the left thalamus were detected.

Table 2 IH-MRS Metabolite Levels (mean \pm SD) in the Anterior Cingulate Cortex and Left Thalamus in First Episode Psychosis Patients Who were or were not in Symptomatic Remission Following Antipsychotic Treatment

	Remission Mean \pm SD, n	No Remission Mean \pm SD, n	Statistic
<i>Anterior cingulate cortex</i>			
Creatine	6.28 \pm 0.43, 15	6.05 \pm 1.00, 17	$T(30) = 0.81$; $P = 0.42$
Glutamate/Cr	1.14 \pm 0.13, 15	1.37 \pm 0.26, 17	$T(30) = 3.02$; $P = 0.005$
Glx/Cr	1.62 \pm 0.28, 15	1.75 \pm 0.30, 17	$T(30) = 1.25$; $P = 0.22$
NAA/Cr	1.25 \pm 0.12, 15	1.28 \pm 0.11, 17	$T(30) = 0.72$; $P = 0.48$
Choline/Cr	0.26 \pm 0.03, 15	0.27 \pm 0.04, 17	$T(30) = 0.79$; $P = 0.44$
mI/Cr	0.87 \pm 0.13, 15	0.88 \pm 0.12, 17	$T(30) = 0.18$; $P = 0.86$
<i>Left thalamus</i>			
Creatine	6.63 \pm 0.60, 14	6.92 \pm 0.74, 17	$T(29) = 1.19$; $P = 0.24$
Glutamate/Cr	0.92 \pm 0.16, 13	1.02 \pm 0.23, 16	$T(27) = 1.40$; $P = 0.17$
Glx/Cr	1.16 \pm 0.28, 13	1.28 \pm 0.29, 15	$T(26) = 1.10$; $P = 0.28$
NAA/Cr	1.70 \pm 0.20, 14	1.76 \pm 0.25, 17	$T(29) = 0.82$; $P = 0.42$
Choline/Cr	0.28 \pm 0.04, 14	0.28 \pm 0.02, 17	$T(29) = 0.50$; $P = 0.62$
mI/Cr	0.63 \pm 0.14, 14	0.58 \pm 0.11, 17	$T(29) = 1.06$; $P = 0.30$

Abbreviations: Choline, total choline plus phosphocholine. Cr, creatine plus phosphocreatine; Glx, glutamate plus glutamine; NAA, total N-acetyl aspartate plus N-acetyl aspartyl glutamic acid.

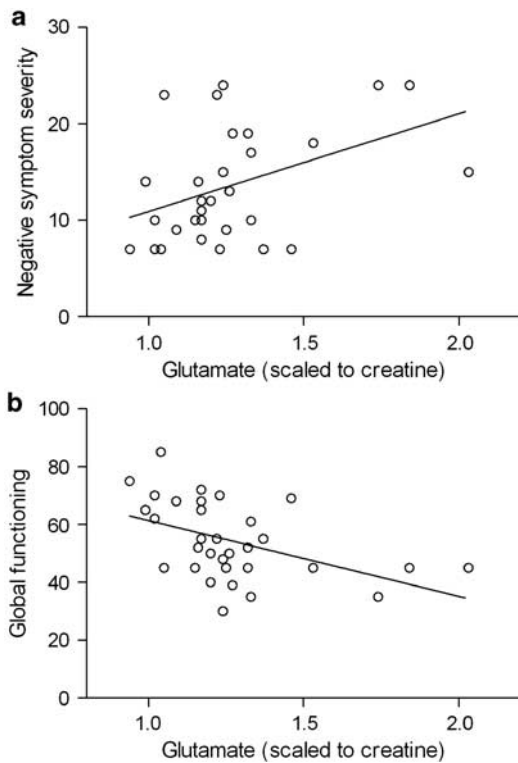


Figure 3 Higher levels of glutamate (scaled to creatine) in the anterior cingulate cortex were associated with (a) greater severity of negative symptoms (left; $r = 0.42$; $P = 0.017$), and with (b) lower levels of global functioning (right; $r = -0.47$; $P = 0.007$).

DISCUSSION

To our knowledge, this is the first evidence that anterior cingulate glutamate levels differ between patients with schizophrenia in remission following antipsychotic treatment and patients who are still symptomatic. Higher levels of glutamate and Glx in the anterior cingulate cortex in symptomatic compared with remitted patients are consistent with reports of elevated anterior cingulate glutamate turnover in non-medicated or minimally treated patients, who generally have high levels of psychotic symptoms (Bustillo *et al*, 2010; Theberge *et al*, 2002). It is also in line with evidence that frontal glutamate is reduced in chronic patients with a long history of antipsychotic treatment and who are generally less symptomatic (Marsman *et al*, 2011; Tayoshi *et al*, 2009; Theberge *et al*, 2003). Our finding is broadly consistent with the numerous reports of abnormalities in the anterior cingulate cortex in early psychosis. For example, structural abnormalities in this area are present at onset of psychosis and may be predictive of conversion to psychosis in those at high risk of developing the disorder (Borgwardt *et al*, 2007; Dazzan *et al*, 2011; Fornito *et al*, 2008; Pantelis *et al*, 2003; Rothlisberger *et al*, 2012; Fusar-Poli *et al*, 2011). Moreover, functional magnetic resonance imaging studies show that antipsychotic treatment may be particularly effective in normalizing activity in the anterior cingulate cortex ((Snitz *et al*, 2005) and see (Karch *et al*, 2012) for review). Our finding may suggest that this is linked to glutamatergic function.

We also found that higher levels of anterior cingulate glutamate or Glx were associated with both an increased severity of negative symptoms and a lower level of global functioning. A relationship between glutamate levels and global functioning is in accordance with similar findings in patients with chronic schizophrenia (Van Elst *et al*, 2005), while the association with negative symptoms is consistent with an inverse relationship between anterior cingulate glutamate levels and sensation seeking (Gallinat *et al*, 2007). However, negative symptom severity has also been linked to reduced anterior cingulate glutamate levels in stable, medicated schizophrenia patients (Reid *et al*, 2011). The reason for the difference in the direction of the correlation in this and the present study is unclear, but may reflect differences in the part of the anterior cingulate cortex from which spectra were acquired, or in the characteristics of the respective patient samples.

No differences in left thalamic metabolite concentrations were detected between remitted and non-remitted patients. Previous studies suggest that increased thalamic Gln levels in never-treated first-episode patients return to normal after 30, but not 10 months of antipsychotic treatment (Theberge *et al*, 2007), and that thalamic Glx levels decrease over 80 months of treatment (Aoyama *et al*, 2011). Given that in the present study the average length of time since presentation was 10 months, and both groups had similar treatment histories, the absence of group differences is unlikely to be related to an effect of antipsychotic treatment.

One limitation of the present study is that in the majority of spectra, we were unable to reliably quantify Gln concentrations. At field strengths of 3 Tesla or below, glutamate and Gln are difficult to separate due to overlapping resonances (Hancu, 2009). It is therefore possible that in some cases, glutamate values may have been contaminated by Gln. In this study, metabolite concentrations were reported in ratio to Cr. Compared with absolute quantitation, this approach is associated with high levels of accuracy, resilient to variation in signal-to-noise ratio (Kanowski *et al*, 2004). The limitation of this method is that it assumes there is no group difference in Cr concentration. However, our findings were significant both when assessed as the estimate of water-scaled glutamate concentration, and as the ratio of estimated glutamate concentration to Cr, and furthermore, there was no group difference in estimates of absolute Cr concentration. A further limitation is that glutamate levels are higher in grey than white matter (Pan *et al*, 1996; Pouwels and Frahm, 1998) and, as we did not assess voxel tissue content, the influence of individual differences in voxel percentage grey and white matter on glutamate levels cannot be determined.

Although there was no significant difference in medication type between patients who were or were not in remission, the sample comprised patients on a variety of different antipsychotics, and medication adherence was assessed through self-report. The absence of precise information on duration of antipsychotic treatment is a weakness, although at the recruitment site, all first-episode patients are prescribed antipsychotics, ideally for the first 2 years, and the duration of treatment is correlated with the time since first presentation. Antipsychotics may differentially impact on regional glutamatergic function (McLoughlin *et al*, 2009), and their potentially confounding effects could be better

assessed in a prospective design involving a standardized treatment protocol or monitoring of prescribing and adherence from presentation. As we did not exclude subjects on the basis of recreational drug use, or perform urine drugs screens at the time of imaging, we cannot exclude the possibility that drug use may have impacted on our findings. However, there were no significant group differences in self-reported recreational drug use or differences in anterior cingulate glutamate/Cr levels between subjects who did or did not report use of each drug. Finally, as our study was designed to compare patients who were symptomatic *vs* remitted following treatment, we did not include a healthy control group, therefore we could not assess whether the metabolite levels in the patients differed from those in healthy volunteers.

One interpretation of our findings is that within our sample of patients with schizophrenia, pre-existing differences in anterior cingulate glutamate levels predicted the subsequent response to antipsychotic treatment. However, we cannot exclude the possibility that the entire sample had elevated glutamate levels before treatment and that there was a longitudinal reduction in the patients who reached criteria for symptomatic remission, or that less severe symptoms were also associated with lower anterior cingulate glutamate levels before treatment. Prospective studies are required to address these issues. Nonetheless, our findings suggest that presence of residual symptoms in schizophrenia may be linked to glutamate dysfunction. Patients not in remission following initial antipsychotic treatment may therefore benefit from treatment with antipsychotics that have glutamatergic as well as dopaminergic actions (eg, clozapine), or other drugs that act on the glutamate system.

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DISCLOSURE

GJB received honoraria for teaching from General Electric during the course of this work. In the past 3 years, JS has received a non-restricted academic fellowship from Glaxo SmithKline, and honoraria from Roche, AstraZeneca, Behrenberg Bank, and Pfizer. The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)