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Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: A multicentre ¹H-MRS study (OPTiMiSE).

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Abstract

Conventional antipsychotic medication is ineffective in around a third of patients with schizophrenia, and the nature of the therapeutic response is unpredictable. We investigated whether response to antipsychotics is related to brain glutamate levels prior to treatment. Proton magnetic resonance spectroscopy was used to measure glutamate levels (Glu/Cr) in the anterior cingulate cortex (ACC) and in the thalamus in antipsychotic-naïve or minimally-medicated patients with first episode psychosis (FEP, n = 71) and healthy volunteers (n = 60), at three sites. Following scanning, patients were treated with amisulpride for 4 weeks (n = 65), then ¹H-MRS was repeated (n = 46). Remission status was defined in terms of Positive and Negative Syndrome Scale for Schizophrenia (PANSS) scores. Higher levels of Glu/Cr in the ACC were associated with more severe symptoms at presentation and a lower likelihood of being in remission at 4 weeks (P < 0.05). There were longitudinal reductions in Glu/Cr in both the ACC and thalamus over the treatment period (P < 0.05), but these changes were not associated with the therapeutic response. There were no differences in baseline Glu/Cr between patients and controls. These results extend previous evidence linking higher levels of ACC glutamate with a poor antipsychotic response by showing that the association is evident before the initiation of treatment.

Introduction

Treatment with conventional antipsychotic medications is ineffective in around a third of patients with schizophrenia.¹ Non-response is associated with a sustained illness burden, and high health, personal and societal costs.² Understanding the underlying biological mechanisms of therapeutic response is fundamental to the development of new treatments that may be useful when conventional medications are ineffective.

Research using positron emission tomography indicates that psychosis is associated with elevated striatal dopamine function.⁵ However this is more evident among those patients who respond to antipsychotic treatment than in those who do not.³⁻⁶ Schizophrenia has also been linked to hypofunction of N-methyl-D-aspartate (NMDA) glutamate receptors and increased frontal cortical glutamate release.⁷⁻⁹ Data from MR spectroscopy (¹H-MRS) studies suggest that a poor antipsychotic response may be related to increased glutamate levels in the anterior cingulate cortex (ACC). This has been reported in patients who failed to achieve remission following a first episode of psychosis compared to those in remission,¹⁰ and in treatment-resistant patients with chronic schizophrenia relative to treatment-responsive patients.^{11,12}

These ¹H-MRS findings raise the possibility that higher ACC glutamate levels may predict a poor response to antipsychotics. However, because glutamate levels were measured in patients who had already been unwell for some time, it is possible that the findings were secondary to unremitted psychotic symptoms or to previous treatment. Some longitudinal ¹H-MRS studies in schizophrenia have reported reductions in glutamatergic metabolites in the frontal cortex, thalamus, temporal cortex and striatum during antipsychotic treatment,¹³⁻¹⁶ but other studies have found no such changes.¹⁷⁻¹⁹ The mixed findings from these investigations may be related to the patient samples being small, clinically heterogeneous, and having been treated with a variety of different antipsychotics.²⁰ In rodents, antipsychotic compounds can reduce NMDA antagonist-stimulated frontal glutamate levels, although this is not seen under all experimental conditions,²¹⁻²⁷ which may relate to the receptor pharmacology of the antipsychotic and the level of basal glutamatergic tone.

The primary aim of the present study was to examine whether response to antipsychotic medication is related to ACC glutamate levels prior to treatment, while minimizing the potentially confounding factors discussed above. We also examined the left thalamus, as glutamate metabolites in this region have been linked with clinical outcomes in individuals at clinical high risk of psychosis.²⁸ The second objective was to test the hypothesis that antipsychotic treatment was associated with a longitudinal reduction in glutamate levels. Amisulpride was selected as the antipsychotic medication because it is relatively selective for D_{2/3} receptors,^{29, 30} so any therapeutic response is attributable to dopamine D_{2/3} blockade. Furthermore, in a large trial of different antipsychotics in first episode patients, amisulpride was associated with a high rate of symptomatic remission (approximately 40% after 4 weeks)³¹. Finally, amisulpride is widely available across Europe at low cost.

METHODS

This study was conducted as part of the Optimisation of Treatment and Management of Schizophrenia in Europe (OPTiMiSE) clinical trial (www.optimisetrialeu.com; EudraCT-Number: 2010-020185-19; clinicaltrials.gov identifier: NCT01248195). Participants in the ¹H-MRS substudy were assessed at three study sites (King's College London, UK; University Medical Centre Utrecht, The Netherlands; Mental Health Centre, Glostrup, Denmark). All sites had local ethical and regulatory approval. Participation required provision of written informed consent.

Participants

Inclusion required that patients were within the first two years of onset of the first psychotic episode, with previous antipsychotic exposure of less than 15 days in the last year. There was no wash-out period before baseline imaging. Inclusion also required that participants were 18 to 40 years old, and had a diagnosis of schizophrenia, schizophreniform or schizoaffective disorder, as defined by DSM-IV criteria in conjunction with the Mini International Neuropsychiatric Interview.³² Female participants were required to be using a reliable method of contraception. Exclusion criteria included contraindications to the study medications or to MRI scanning, pregnancy, undergoing compulsory treatment or being under legal custody,

or being unable to provide written informed consent. Healthy volunteers (n = 60) were 18-40 years old, and had no history of psychiatric illness or MRI contraindications.

Assessment and antipsychotic treatment schedule

In the patient group, symptoms and level of functioning were assessed at baseline and 4 weeks using the Positive and Negative Syndrome Scale for Schizophrenia (PANSS)³³ and Personal and Social Performance (PSP) scale³⁴ respectively. The PANSS inter-rater reliability score was 0.82 (Supplementary Methods). Illicit substance use was determined by self-report.

After baseline ¹H-MRS, patients received open-label amisulpride, with individual dosing between 200-800mg, as determined by the clinical judgement of the treating physician, and to a target dose of 400mg/day based on the EUFEST study.³¹ The 4-week period corresponds to the period over which the greatest degree of symptomatic improvement is observed.^{35,36} The protocol prohibited the use of other antipsychotics, but other classes of concomitant medication were permitted and recorded. PANSS scores after amisulpride administration were used to determine whether or not patients had reached symptomatic remission, defined according to the criteria of Andreasen,³⁷ with exclusion of the 6-month observation period (Supplementary Methods). At the end of the 4-week treatment period, the ¹H-MRS acquisition was repeated.

¹H-MRS

¹H-MRS data were acquired at 3 Tesla. There was no restriction on the time of day for scanning, or for matching the time of day for baseline and follow-up scan acquisition. ¹H-MRS voxels were positioned in the ACC (20 x 20 x 20mm) and in the left thalamus (15 right-left x 20 x 20mm) (Supplementary Figure 1). Spectra were acquired using Point RESolved Spectroscopy (TE = 30msec; TR = 3000msec; 96 averages). Spectra were analyzed using LC Model version 6.3-1L.³⁸ The predefined primary measure was total voxel glutamate scaled to creatine (Glu/Cr). Voxel tissue content was extracted using Gannet 2.0 (<http://www.gabamrs.com/>). A detailed

description of the multisite MRI data acquisition and quality control is provided in the Supplementary Methods.

Statistical analysis

Binary logistic regression determined the association between Glu/Cr and outcome (Remission versus Non-Remission). Linear regression examined relationships between Glu/Cr and symptom scores, where the minimum possible PANSS scores were subtracted when calculating the PANSS percentage change.³⁹ ¹H-MRS metabolite estimates were group-mean centred by site for the regression models. Repeated measures general linear model examined the effect of amisulpride administration and remission status on voxel Glu/Cr levels. Between-group comparisons of Glu/Cr levels were performed using univariate ANOVA, including site as a fixed factor. All analyses were performed in SPSS (version 23, IBM), with a significance level of 0.05 for primary analyses involving Glu/Cr.

RESULTS

Patient characteristics

Seventy-two patients completed baseline ¹H-MRS acquisition. One patient was excluded from all analyses due to a protocol violation (previous antipsychotic medication lasting >15 days). Forty-five (63%) patients were antipsychotic-naïve; the remaining 26 had received antipsychotics for a median of 9 days in the period immediately prior to inclusion (Table 1).

After scanning, 65 patients were treated with amisulpride (duration of treatment 30.6 ± 7.6 days), while 6 withdrew from the study. The amisulpride dose at the end of treatment was 347.7 ± 138.2 mg/day. At this point, 41 patients (63%) met remission criteria and 24 (37%) did not. Compared to the Remission group, the Non-Remission group was younger, more symptomatic at baseline, and was taking higher doses of amisulpride at the end of treatment (Table 1).

¹H-MRS data quality

Glu/Cr values in patients were normally distributed (ACC: df = 70; Shapiro-Wilk = 0.98; P = 0.22; Thalamus: df = 62; Shapiro-Wilk = 0.98; P = 0.51). Spectral quality measures are available in Supplementary Tables 1 and 2. Data failing quality control were excluded from analyses. Spectral quality did not differ between the Remission and Non-Remission patient groups and there were no significant group differences in voxel tissue content. Site effects were apparent across several variables (Supplementary Tables 1 and 2).

Baseline glutamate levels and prediction of remission

Baseline ACC Glu/Cr was associated with the likelihood of remission following treatment ($\chi^2 = 4.61$; P = 0.03, Nagelkerke $r^2 = 0.10$; B = 3.63; SE = 1.76; Wald = 4.25; P = 0.04; Exp(B) = 37.88), The regression model correctly classified 69% of cases (90% Remitters and 30% Non-Remitters). Due to baseline group differences, age and PANSS total score were subsequently added to the regression model. This increased the predictive accuracy to 75% (85% Remitters, 57% Non-Remitters) ($\chi^2 = 15.64$; P = 0.001, Nagelkerke $r^2 = 0.30$). Secondary analyses including only antipsychotic-naïve patients also found that ACC Glu/Cr levels predicted remission status ($\chi^2 = 4.73$; P = 0.03, Nagelkerke $r^2 = 0.15$; 66% overall accuracy). Glu/Cr in the thalamus at baseline was not associated with remission status ($\chi^2 = 0.09$; P = 0.76, Nagelkerke $r^2 = 0.002$). None of the other metabolites in either the ACC or thalamus at baseline, including creatine, were associated with remission status (Supplement Table 2).

In the ACC, Glu/Cr was significantly higher in the Non-Remission than Remission group ($F_{1,63} = 5.99$; P = 0.02; $\eta_p^2 = 0.09$; Table 2; Figure 1). This difference remained significant when only antipsychotic-naïve patients were included in the analysis ($F_{1,41} = 5.21$; P = 0.03), and when the number of days to follow-up was covaried for ($F_{1,61} = 4.63$; P = 0.04).

Relationships between glutamate levels and clinical measures

At baseline, higher levels of ACC Glu/Cr were associated with greater severity of overall symptomatology (PANSS total: n = 69; $r^2 = 0.08$; $\beta = 0.28$; t = 2.38; P = 0.02;

PANSS general: $n = 69$; $r^2 = 0.09$; $\beta = 0.31$; $t = 2.66$; $P = 0.01$, Figure 2) and lower level of functioning (PSP: $n = 63$; $r^2 = 0.12$; $\beta = -0.34$; $t = -2.84$; $P = 0.006$; Supplement Figure 2). These findings were not significant when limited to antipsychotic-naïve patients. Similar relationships were evident between baseline ACC Glu/Cr and symptom severity (PANSS total: $n = 65$; $r^2 = 0.09$; $\beta = 0.30$; $t = 2.49$; $P = 0.02$; PANSS positive: $n = 65$; $r^2 = 0.09$; $\beta = 0.30$; $t = 2.46$; $P = 0.02$; PANSS general: $n = 65$; $r^2 = 0.07$; $\beta = 0.27$; $t = 2.20$; $P = 0.03$, Figure 2) and functioning (PSP: $n = 54$; $r^2 = 0.20$; $\beta = -0.44$; $t = -3.58$; $P < 0.001$, Supplement Figure 2) at 4 weeks across the whole sample, and in only patients who were antipsychotic-naïve at baseline (PANSS positive: $n = 39$; $r^2 = 0.16$; $P = 0.01$; PSP: $n = 36$; $r^2 = 0.15$; $P = 0.02$). There were no significant associations between baseline ACC Glu/Cr and the subsequent longitudinal percentage change in PANSS or PSP scores in the whole sample ($P > 0.05$), but when analysis was restricted to antipsychotic-naïve patients, baseline ACC Glu/Cr correlated with percentage reduction in total PANSS score ($n = 39$; $r^2 = 0.13$; $\beta = -0.36$; $t = -2.31$; $P = 0.03$, Supplement Figure 3).

In the thalamus, baseline Glu/Cr was not significantly associated with symptom severity or functioning, at either baseline or follow-up ($P > 0.05$). Baseline Glu/Cr in the thalamus was associated with the longitudinal percentage change in both the PANSS total ($n = 65$; $r^2 = 0.09$; $\beta = -0.30$; $t = -2.34$; $P = 0.02$) and the PANSS general ($n = 65$; $r^2 = 0.08$; $\beta = -0.29$; $t = -2.19$; $P = 0.03$) score over the treatment period (Supplement Figure 4). However no significant relationships were detected in only the antipsychotic-naïve subsample.

Effect of amisulpride on glutamate levels

Forty-six patients completed $^1\text{H-MRS}$ at 4 weeks (33 ± 5 days). In the ACC, Glu/Cr levels were significantly lower at 4 weeks than at baseline in both the total patient sample (Table 2; Figure 4; main effect of time: $F_{1,44} = 5.67$; $P = 0.02$; $\eta_p^2 = 0.11$; mean reduction = 3.6%), and in the antipsychotic-naïve subgroup ($F_{1,29} = 6.68$; $P = 0.02$). ACC Glu/Cr was lower over both time-points in the remission group ($n = 13$; $F_{1,44} = 6.22$; $P = 0.02$). The time*remission status interaction was non-significant.

In the thalamus, Glu/Cr was also lower at follow-up than at baseline (Table 2; Figure 4; main effect of time: $F_{1,35} = 4.68$; $P = 0.04$; $\eta_p^2 = 0.12$; mean reduction = 2.6%), but the main effect of remission status and the time*remission status interaction were non-significant. The effect of time was not significant in only antipsychotic-naïve patients.

In both the ACC and the thalamus, the longitudinal percentage change in Glu/Cr was not significantly associated with the percentage change in symptom severity over the treatment period, or with amisulpride end dose.

Glutamate levels in patients compared to healthy volunteers

Compared to the patient group, the healthy volunteer group did not differ in gender ($P = 0.83$), but were older (26.8 ± 5.4 vs. 24.7 ± 5.4 years, $T(129) = 2.41$; $P = 0.02$). There were no significant differences in Glu/Cr in the ACC or thalamus, or in any other metabolite, in patients compared to healthy volunteers ($P > 0.05$, Supplement Table 2), including when age was included in the model, or when analysis was restricted to antipsychotic-naïve patients. In healthy volunteers, neither Glu/Cr in the ACC nor thalamus changed significantly over time (Supplement Table 2; ACC: $F_{1,38} = 0.18$; $P = 0.68$; Thalamus: $F_{1,36} = 0.76$; $P = 0.39$).

DISCUSSION

We used $^1\text{H-MRS}$ to examine the relationship between ACC glutamate levels and the response to amisulpride in antipsychotic-naïve or minimally-medicated patients with first episode psychosis. As hypothesised, we found that higher pre-treatment levels of Glu/Cr in the ACC were associated with a lower likelihood of symptomatic remission.

An association between higher ACC Glu/Cr levels and poor response to treatment is consistent with data from previous cross-sectional studies in patients who had already been treated with antipsychotic medication. These also reported that non-responders had higher ACC glutamate levels than responders.^{10, 11} Our findings are also in line with data from a prospective study in chronic schizophrenia which indicated that patients with higher frontal Glx (glutamate plus glutamine) levels were more likely to

show a poor response to a subsequent switch in antipsychotic medication.⁴⁰ The present study extends these findings by providing the first evidence that the association between ACC glutamate levels and antipsychotic response is present at the onset of psychosis and predates antipsychotic treatment. Thus, it does not appear to be an effect of chronic illness or of antipsychotic medication.

Although we analysed the data in terms of categorical treatment outcomes (remission/non-remission), across the total patient sample the distribution of ACC Glu/Cr levels was normal (unimodal). This suggests that the relationship between glutamate levels and treatment response in psychosis may be a graded, as opposed to a categorical one. Indeed, a continuous relationship was evident when we examined the association between ACC Glu/Cr levels and clinical measures; at both baseline and after treatment, ACC Glu/Cr was positively correlated with symptom severity and negatively correlated with level of functioning. In patients with first episode psychosis, greater illness severity and a low level of functioning are associated with relatively poor clinical outcomes.^{41, 42} While these clinical measures and brain glutamate levels might be related to outcomes via distinct mechanisms, a more parsimonious interpretation is that illness severity, a low level of functioning and a poor response to treatment are all manifestations of central glutamate dysfunction.

Although currently available antipsychotics vary in their affinity for different receptor subtypes, antagonism at D_{2/3} dopamine receptors is fundamental to their therapeutic effect.⁴³ Studies using PET and SPECT suggest that striatal dopamine dysfunction is more markedly elevated in patients who respond to antipsychotic treatment than in poor responders.^{4, 5 6} Independent work suggests that there are differences in functional connectivity between the striatum and the ACC in responders and non-responders,⁴⁴ and that the strength of functional and structural connectivity between striatal and medial frontal regions (including the ACC) changes in association with an antipsychotic response.⁴⁵⁻⁴⁹ Our findings add to this literature by raising the possibility that these differences in connectivity may be related to glutamate function in the ACC. This hypothesis could be tested by combining ¹H-MRS glutamate imaging with measures of functional connectivity in patients studied over the course of antipsychotic treatment.

Our second major finding was that 4 weeks of amisulpride was associated with small (~3%) but significant reductions in glutamate in both the ACC and the thalamus, independent of the therapeutic response. These observations are in line with previous ¹H-MRS studies, which have reported longitudinal reductions in glutamatergic metabolites in the frontal cortex, thalamus, temporal cortex and striatum during antipsychotic treatment,¹³⁻¹⁶ although not all have found significant changes.¹⁷⁻¹⁹ They are also consistent with evidence that medial frontal Glx is higher in non-medicated than in medicated patients.⁵⁰ The lack of significant relationship between reductions in glutamate and reductions in symptoms over amisulpride treatment is consistent with most^{14, 15, 17, 19} but not all^{16, 51} previous studies. Although higher glutamate levels at baseline are associated with higher symptoms severity and a poor antipsychotic response, the small reductions in glutamate that occur with antipsychotic treatment may not translate into symptomatic improvement. Nevertheless, as antipsychotics can alter brain glutamate levels, medication status and treatment history need to be clearly defined in ¹H-MRS studies in psychosis.

As amisulpride has relatively selective affinity for D_{2/3} dopamine receptors,^{29, 30} we attribute the longitudinal changes in Glu/Cr following treatment with amisulpride to an effect of D_{2/3} blockade. This is consistent with data from preclinical models of psychosis, in which the administration of antipsychotics, including D_{2/3}-selective compounds, leads to decreases in stimulated glutamate efflux^{22, 24, 52} and glutamatergic pyramidal neuron activity.⁵³ This may occur because D_{2/3} antagonists block the dopaminergic inhibition of a subpopulation of cortical GABAergic neurons, increasing their inhibition of glutamatergic pyramidal neurons and decreasing glutamatergic activity.^{54, 55}

There was no significant difference in glutamate levels between patients and healthy volunteers in either the ACC or the thalamus. This is consistent with the majority of findings from previous ¹H-MRS studies in minimally-medicated or antipsychotic-naïve first episode patients^{14, 18, 56-60} and a recent meta-analysis⁶¹ although one study has reported greater ACC glutamate in patients than controls.⁶² Glutamine and the glutamine/glutamate ratio may be elevated in first episode psychosis,^{18, 57, 58} but we were unable to evaluate this as our acquisition parameters at 3 Tesla did not allow reliable quantification of glutamine.

Strengths of the present study include the use of antipsychotic-naïve or minimally-medicated patients with first episode psychosis, which increased the clinical homogeneity of the sample and minimized the potentially confounding effects of chronic illness and previous treatment. The standardisation of their subsequent treatment with a single, D_{2/3}-selective antipsychotic reduced the variance that could have resulted from using multiple different antipsychotic drugs, and allowed interpretation of the findings in terms of D_{2/3} antagonism. A further strength was the relatively large sample size compared to previous ¹H-MRS studies of the effects of antipsychotic treatment (see Egerton et al.,²⁰), achieved using a multi-centre design. To our knowledge, this is the first published multi-centre ¹H-MRS study in schizophrenia.

This study also had limitations. Multi-centre ¹H-MRS including MRI platforms from different manufacturers is not without additional technical challenges. For example, site effects were present throughout the study data, which may have decreased statistical power. These issues will be discussed in detail in a subsequent article focussing on multicentre ¹H-MRS methodology. The use of creatine-scaled values means that results may be influenced by differences or changes in voxel creatine levels. One study reported that creatine levels are altered in schizophrenia,⁶³ but no overall differences in creatine levels are reported in either meta-analysis,⁶⁴ or in the largest ¹H-MRS study in schizophrenia to date.⁶⁵ The glutamate measurements available from ¹H-MRS reflect the mean concentration of glutamate in a relatively large voxel, and it is not possible to specifically attribute findings to glutamatergic neurotransmission, or to glutamate levels in a particular cell type.⁶⁶ As the study required that patients were willing to participate in a clinical trial involving serial psychopathological assessments and neuroimaging sessions, and excluded patients receiving compulsory treatment, the sample may have included patients who were less severely unwell than in the general first episode population. Our analysis did not assess the possible influence of illicit substance use, concomitant medication or the time of day on ¹H-MRS measures, and, in patients who were not antipsychotic-naïve, there was no washout period before the baseline scan. Finally, the participants met a number of DSM-IV schizophrenia diagnostic subcategories, which may have

increased between-subject variability. However whether glutamatergic function varies across schizophrenia diagnostic subgroups is unknown.

The finding that high levels of ACC glutamate were associated with a poor response to antipsychotic treatment raises the question of whether ¹H-MRS glutamate measurements could be used to help predict treatment outcomes. The overall level of accuracy in predicting remission at 4 weeks was 69%, which increased to 75% when PANSS total score and age were included in the model. This is not sufficient for clinical decision-making or clinical trial stratification. Predictive accuracy may be improved by combining ¹H-MRS measurements with other biomarkers associated with antipsychotic response,⁶⁷ such as striatal dopamine function,^{4, 5} functional connectivity,⁴⁴ genetic factors,⁶⁸⁻⁷² inflammatory markers⁷³ and autoantibodies.^{74, 75}

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Conflict of Interest

GJB received honoraria for teaching from General Electric Healthcare during the course of this study, and acts as a consultant for IXICO. AE has received research funding from Roche and consultancy payment from Heptares Therapeutics. BG is the leader of a Lundbeck Foundation Center of Excellence for Clinical Intervention and Neuropsychiatric Schizophrenia Research (CINS), which is partially financed by an independent grant from the Lundbeck Foundation based on international review and partially financed by the Mental Health Services in the Capital Region of Denmark,

the University of Copenhagen, and other foundations. PM has received consultancy payment for Sunovion and Takeda. RPI has received honoraria as a speaker for Lundbeck. JMS has received honoraria from Roche and Janssen and consultancy payment from Takeda. The remaining authors declare no conflicts of interest.

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Table 1.

Demographic and clinical characteristics of the sample. Site: London (L); Glostrup (G); Utrecht (U); DSM-IV diagnostic category: schizoaffective disorder (SA, 295.70); schizophrenia, undifferentiated type (SU, 295.90); schizophrenia, disorganised type (SD, 295.10); schizophrenia, paranoid type (SP, 295.30); schizophreniform disorder (SF, 295.40). Race: White (W) / Black (B) / Asian (A) / Other (O); Educational level: university finished / university unfinished / professional training finished / professional training unfinished / high school finished / high school unfinished / less

than high school; DP: Duration of psychosis prior to baseline assessment; AP naïve: number and percentage of sample antipsychotic naïve prior to study; AP Duration: For previously AP treated patients, the duration of treatment prior to inclusion; Substance use: cannabis (Ca); cocaine (Co); amphetamines (A); ecstasy (E); other (O). Concomitant medication: benzodiazepines (b); antidepressants (a); stimulants (s); mood stabilisers (m); PANSS positive and negative syndrome scale, PSP: personal and social performance scale. Percentage changes in PANSS scores include subtraction of minimum possible scores.

Table 2.

Voxel glutamate levels scaled to creatine (Glu/Cr) at baseline and after amisulpride administration. Values are expressed as mean \pm s.d. (n). Site: London (L); Glostrup (G); Utrecht (U).

Figure 1.

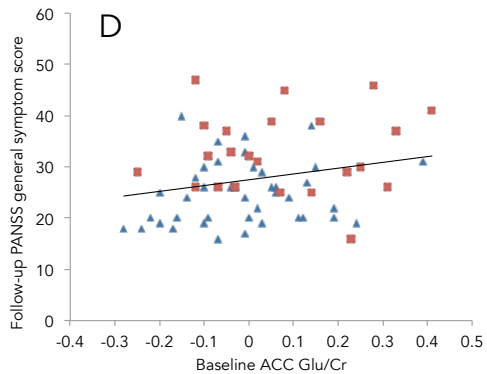
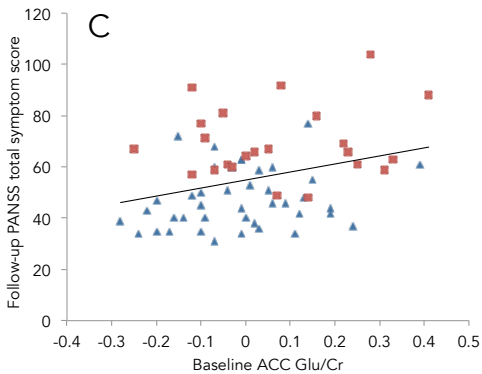
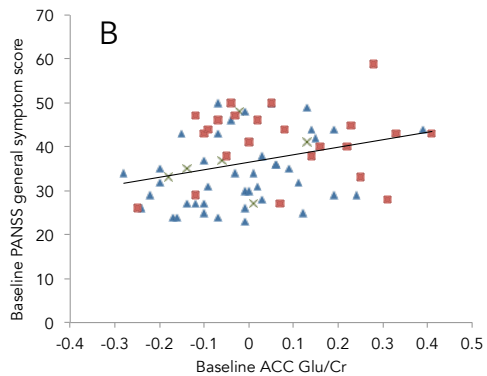
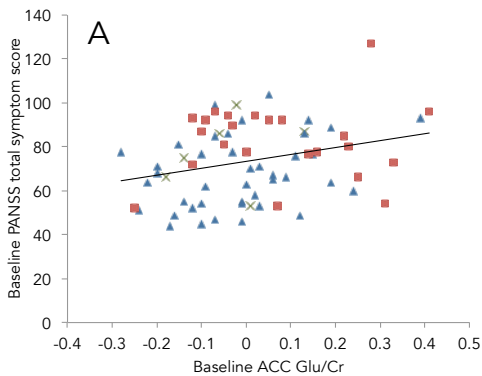
Baseline glutamate (Glu/Cr) levels in the anterior cingulate cortex (ACC) in the Remission (blue triangle) and No Remission (red square) groups. At baseline, ACC Glu/Cr was significantly higher in patients (n=23) who were not in remission after 4 weeks of treatment with amisulpride compared to those who were in remission (n=41) ($F_{1,63} = 5.99$; $P = 0.02$; $\eta_p^2 = 0.09$). The values represent individual data scaled to the site mean, with the group mean indicated by the grey bar.

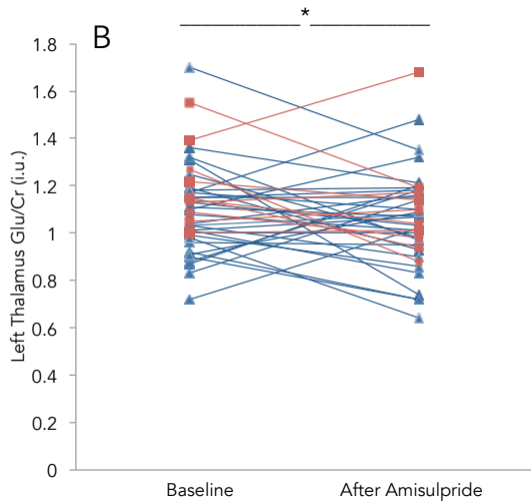
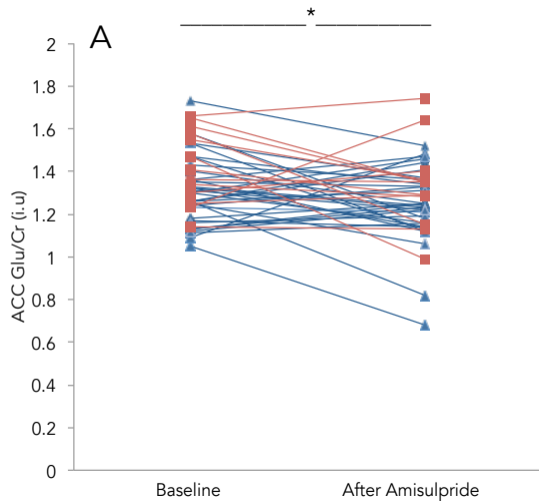
Figure 2.

Relationships between glutamate (Glu/Cr) levels in the anterior cingulate cortex (ACC) and symptom severity. **A:** ACC Glu/Cr and baseline positive and negative syndrome scale (PANSS) total score (n = 69; $r^2 = 0.08$; $\beta = 0.28$; $t = 2.38$; $P = 0.02$); **B:** ACC Glu/Cr and baseline PANSS general score (n = 69; $r^2 = 0.09$; $\beta = 0.31$; $t = 2.66$; $P = 0.01$); **C:** ACC Glu/Cr and follow-up PANSS total score (n = 65; $r^2 = 0.09$; $\beta = 0.30$; $t = 2.49$; $P = 0.02$); **D:** ACC Glu/Cr and follow-up PANSS general score (n = 65; $r^2 = 0.07$; $\beta = 0.27$; $t = 2.20$; $P = 0.03$). Glu/Cr measures are centred by site mean. Groups are indicated as Remission (blue triangle), Non-Remission (red square) and remission status unknown (green cross).

Figure 3.

In both the anterior cingulate cortex (ACC) and in the thalamus, Glu/Cr was significantly lower at the end of 4 weeks treatment with amisulpride than at baseline; (ACC: $F_{1,35} = 4.68$; $P = 0.04$; $\eta_p^2 = 0.12$; thalamus: $F_{1,44} = 5.67$; $P = 0.02$; $\eta_p^2 = 0.11$). In the ACC, but not in the thalamus, Glu/Cr was higher in the Non-Remission (red square) group than in the Remission group (blue triangle) at both baseline and at 4 weeks ($F_{1,44} = 6.22$; $P = 0.02$; $\eta_p^2 = 0.12$).





	Total patient group Baseline N=71; Follow-up N= 65.	Remission N = 41	No Remission N = 24	P value R vs. NR
Age: mean \pm s.d. years	24.7 \pm 5.4	26.2 \pm 5.9	22.3 \pm 3.9	P = 0.006
Gender (n) male/ female	48/23	30/11	13/11	P = 0.12
Site (n) L/G/U	26/31/14	17/16/8	5/13/6	P = 0.24
DSM-IV: SA/SU/SD/SP/SF	4/11/4/36/16	2/5/1/24/9	0/5/3/10/6	P = 0.21
Ethnicity (n) W/B/A/O	50/11/7/3	25/7/7/2	20/4/0/0	P = 0.10
Educational level	7/7/9/9/14/18/7	6/4/7/5/5/10/4	1/1/1/3/7/8/3	P = 0.35
DP: median (min - max) months	6 (1 - 24)	5 (1 - 24)	6 (1 - 24)	P = 0.35
AP naïve: n, (%)	45 (63%)	24 (59%)	18 (75%)	P = 0.11
Previously medicated: n, (%)	26 (37%)	17 (41%)	6 (25%)	P = 0.11
Previous AP: Am/O/AmO/Ar/AmAr/ QO	12/9/2/1/1/1	7/6/2/1/1/0	3/2/0/0/0/1	P = 0.50
Previous AP duration: median, (min-max) days	9 (3-14)	11 (3-14)	7 (4-8)	P = 0.24
Substance use ever Y/N	49/22	28/13	17/7	P = 0.83
Substance use ever type Ca/Co/A/E/O	45/18/14/14/15	26/10/8/8/7	5/6/4/4/7	-
Substance use during study Y/N	20/51	10/31	9/15	P = 0.22
Substance use during study Ca/Co/A/E/O	19/4/3/1/4	10/3/3/1/2	8/1/0/0/2	-
Days to clinical follow-up	30.6 \pm 7.6	32.1 \pm 5.4	28.9 \pm 9.1	P = 0.08
Amisulpride dose at follow-up (mg)	347.7 \pm 138.2	307.3 \pm 138.5	421.7 \pm 108.5	P = 0.001
Concomitant medication (n) b/a/s/m	9/14/1/1	6/9/1/0	3/5/0/1	-
<i>Symptoms and functioning at baseline: mean \pm s.d.</i>				
PANSS Positive	20.1 \pm 5.3	18.8 \pm 4.6	21.7 \pm 5.7	P = 0.030
PANSS Negative	17.1 \pm 6.5	15.2 \pm 6.5	19.8 \pm 6.0	P = 0.008
PANSS General	36.6 \pm 8.5	34.2 \pm 8.2	40.7 \pm 8.1	P = 0.002
PANSS Total	73.2 \pm 17.3	67.4 \pm 15.7	81.9 \pm 16.9	P = 0.001
PSP	51.3 \pm 12.2	52.2 \pm 12.1	50.8 \pm 13.1	P = 0.680
<i>Symptoms and functioning after amisulpride: mean \pm s.d.</i>				
PANSS Positive	12.9 \pm 4.7	10.8 \pm 2.8	17.1 \pm 4.9	P < 0.001
PANSS Negative	14.5 \pm 5.8	11.9 \pm 4.1	18.7 \pm 5.8	P < 0.001
PANSS General	27.7 \pm 7.7	24.8 \pm 6.1	33.6 \pm 7.1	P < 0.001
PANSS Total	55.3 \pm 16.2	47.5 \pm 11.5	69.4 \pm 14.0	P < 0.001
PSP	61.3 \pm 12.9	64.8 \pm 12.8	52.6 \pm 8.2	P = 0.001
<i>Percentage change in symptoms and functioning after amisulpride: mean \pm s.d.</i>				
PANSS Positive	53.1 \pm 32.3	65.9 \pm 27.7	27.9 \pm 25.2	P < 0.001
PANSS Negative	9.8 \pm 71.1	18.8 \pm 68.4	-5.3 \pm 76.0	P = 0.207
PANSS General	43.6 \pm 32.1	52.2 \pm 31.8	25.3 \pm 26.3	P = 0.001
PANSS Total	42.4 \pm 31.3	54.4 \pm 25.4	19.5 \pm 28.9	P < 0.001
PSP	-24.6 \pm 36.0	-31.8 \pm 37.7	-8.4 \pm 26.4	P = 0.029

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Site	Anterior Cingulate Cortex			Left Thalamus		
	Total Group	Patient Remission	No Remission	Total Group	Patient Remission	No Remission
Baseline						
L	1.33 ± 0.17 (26)	1.28 ± 0.14 (17)	1.57 ± 0.07 (5)	1.02 ± 0.13 (24)	1.00 ± 0.15 (16)	1.12 ± 0.22 (11)
G	1.37 ± 0.17 (31)	1.35 ± 0.16 (16)	1.38 ± 0.19 (13)	1.11 ± 0.19 (27)	1.12 ± 0.15 (14)	1.12 ± 0.05 (4)
U	1.26 ± 0.10 (13)	1.26 ± 0.11 (8)	1.26 ± 0.11 (5)	1.20 ± 0.21 (11)	1.23 ± 0.23 (8)	1.12 ± 0.14 (3)
After amisulpride						
L	1.23 ± 0.25 (18)	1.18 ± 0.22 (14)	1.31 ± 0.32 (4)	1.02 ± 0.16 (18)	0.98 ± 0.15 (14)	1.13 ± 0.18 (4)
G	1.32 ± 0.10 (21)	1.31 ± 0.12 (14)	1.33 ± 0.04 (7)	1.15 ± 0.20 (20)	1.12 ± 0.18 (14)	1.22 ± 0.24 (6)
U	1.25 ± 0.19 (7)	1.20 ± 0.09 (5)	1.39 ± 0.36 (2)	1.00 ± 0.23 (7)	1.03 ± 0.27 (5)	0.93 ± 0.07 (2)

Table 2.

Voxel glutamate levels scaled to creatine (Glu/Cr) at baseline and after amisulpride administration. Values are expressed as mean ± s.d. (n). Site: London (L); Glostrup (G); Utrecht (U).