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32 Abstract

33 Neuroimaging studies in schizophrenia have linked elevated glutamate metabolite levels to non-remission
34 following antipsychotic treatment, and also indicate that antipsychotics can reduce glutamate metabolite
35 levels. However, the relationship between symptomatic reduction and change in glutamate during initial
36 antipsychotic treatment is unclear. Here we report proton magnetic resonance spectroscopy (1H-MRS)
37 measurements of Glx and glutamate in the anterior cingulate cortex (ACC) and thalamus in patients with
38 first episode psychosis (n=23) at clinical presentation, and after 6 weeks and 9 months of treatment with
39 antipsychotic medication. At 9 months, patients were classified into Remission (n=12) and Non-Remission
40 (n=11) subgroups. Healthy volunteers (n=15) were scanned at the same three time-points. In the thalamus,
41 Glx varied over time according to remission status (P=0.020). This reflected an increase in Glx between 6
42 weeks and 9 months in the Non-Remission subgroup that was not evident in the Remission subgroup
43 (P=0.031). In addition, the change in Glx in the thalamus over the 9 months of treatment was positively
44 correlated with the change in the severity of Positive and Negative Syndrome Scale (PANSS) positive, total
45 and general symptoms (P<0.05). There were no significant effects of group or time on glutamate
46 metabolites in the ACC, and no differences between either patient subgroup and healthy volunteers. These
47 data suggest that the nature of the response to antipsychotic medication may be related to the pattern of
48 changes in glutamatergic metabolite levels over the course of treatment. Specifically, longitudinal
49 reductions in thalamic Glx levels following antipsychotic treatment are associated with symptomatic
50 improvement.

51 Introduction

52 In around one third of patients with schizophrenia, treatment with antipsychotic medication is ineffective¹⁻
53 ³, but the underlying neurobiological mechanisms of treatment response are not well understood.

54 Schizophrenia is associated with disruptions in brain glutamatergic neurotransmission^{4,5}, and recent
55 neuroimaging studies have indicated that the nature of the antipsychotic response may be related to brain
56 glutamate levels⁶⁻¹¹. In patients with first episode psychosis prior to treatment, elevated glutamate in the
57 anterior cingulate cortex (ACC) have been associated with a lower likelihood of reaching remission after 4
58 weeks of amisulpride⁸. Similarly, in established schizophrenia, higher levels of Glx (the combined signal
59 from glutamate plus glutamine) in the medial frontal cortex have been associated with a poor response
60 after restarting antipsychotic medication¹⁰. Elevated ACC glutamatergic metabolites have also been
61 reported in first episode patients who had failed to achieve remission following antipsychotic treatment⁷,
62 in patients who were treatment resistant^{6,9} and in patients resistant to clozapine¹². Elevated glutamate
63 metabolites in treatment-resistant schizophrenia have also been described in the caudate nucleus¹¹.

64

65 While brain glutamate metabolite levels have thus been related to antipsychotic response⁸, levels of these
66 metabolites may be reduced by antipsychotic medication¹³. In patients with first episode psychosis,
67 longitudinal reductions in glutamate in the ACC and left thalamus have been observed over 4 weeks of
68 antipsychotic treatment⁸, and longitudinal reductions in glutamine and Glx in the left thalamus have been
69 reported after 30 and 80 months of treatment^{14,15}. Glutamate reductions have also been reported in the
70 frontal cortex, following 4 and 6 months of antipsychotic treatment¹⁶⁻¹⁸, and in the striatum following 4
71 weeks of antipsychotic treatment^{18,19}. However, reductions in glutamatergic metabolites in the thalamus²⁰
72 or ACC^{20,21} have not been detected by other studies. Studies in patients with chronic schizophrenia have
73 produced mixed findings, some reporting reductions in glutamate levels following antipsychotic treatment
74 in the frontal²² and temporal cortex²³, but others finding no change (in frontal cortex²³⁻²⁵, temporal cortex²⁵
75 and thalamus^{23,25}).

76

77 If there are longitudinal reductions in glutamate levels with antipsychotic treatment, we hypothesised that
78 these may be related to symptomatic improvement. The present study aimed to test this by examining the
79 relationship between glutamate metabolites in the ACC and thalamus and remission status at three
80 timepoints over the first 9 months of antipsychotic treatment in patients with first episode psychosis. The
81 present dataset is an extension to our previous study, which investigated the relationship between
82 glutamate and treatment response over 4 weeks, reporting that elevated glutamate in the ACC at first
83 presentation predicted poor antipsychotic response⁸. Here we extend to a longer follow-up period of 9
84 months in a subset of this cohort. This duration of treatment corresponds to that in our previous cross-
85 sectional study, which found that after 9 months of treatment, first episode patients who had not achieved
86 remission had higher ACC glutamate levels than those in remission⁷. To aid interpretation of our findings,
87 we also assessed a sample of healthy volunteers over the same time period.

88

89 **Results**

90 At 9 months, 12 patients met Remission criteria and 11 patients met criteria for Non-Remission. There
91 were no significant differences in demographic variables between the Remission and Non-Remission
92 subgroups, in substance use (Supplementary Table 1) or in duration of or adherence to antipsychotic
93 medication at any timepoint (Table 1 and Supplementary Notes). At the time of the baseline scan, 4
94 patients were medication naïve, and all but one of the remaining patients were receiving amisulpride. At
95 the 6 weeks and 9 month timepoint the Remission and Non-Remission groups were taking a similar set of
96 antipsychotic drugs, and did not differ in chlorpromazine equivalent dose (Table 1). Please see Table 1 and
97 Supplementary Notes for group differences in PANSS scores at each timepoint.

98

99 Variables relating to 1H-MRS data quality are provided in Supplementary Table 2. For one patient Glx and
100 glutamate data from the thalamus were below 20% CRLB, reducing the sample to n=22, and for one
101 healthy volunteer Glx data from the thalamus were below 20% CRLB, reducing the sample to n=14. There
102 were no significant group differences for spectra quality (Supplementary Table 2) or voxel tissue content
103 (Table 2).

104

105 For both Glx and Glutamate in the ACC, there were no significant main or interaction effects of remission
106 status or time (Figure 1, Supplementary Table 3). This was also the case when time to follow-up was
107 included as a covariate, and when analysis was restricted to patients who were adherent to antipsychotic
108 medication at least 75% of the time. There were no significant relationships between the longitudinal
109 percentage change in ACC glutamatergic metabolites and the percentage change in symptoms over time
110 (Supplementary Table 4). There was also no significant difference in ACC glutamate metabolite levels over
111 time in healthy volunteers compared to the overall patient sample (Figure 2).

112

113 Glx levels in the left thalamus showed a significant interaction between remission status and time
114 ($F(2,40)=4.337$, $P=0.020$, repeated measures ANOVA, Figure 1). The main effects of remission status
115 ($F(1,20)=0.121$, $P=0.731$) and time ($F(2,40)=2.541$, $P=0.091$) were non-significant. At 9 months Glx levels in
116 the thalamus were significantly higher in the Non-Remission compared to Remission group ($F(1,20)=5.244$,
117 $P=0.033$, Cohen's $d=0.98$, one-way ANOVA). This was related to a significant effect of time in the Non-
118 Remission group ($F(2,20)=6.183$, $P=0.008$, repeated measures ANOVA), which reflected an increase in Glx
119 concentration between 6 weeks and 9 months ($P=0.031$, Cohen's $d=1.24$, Bonferroni-corrected pairwise
120 comparisons). Within the Remission subgroup, Glx levels did not vary significantly over time
121 ($F(2,20)=1.849$, $P=0.183$, repeated measures ANOVA). Similar results were obtained when the analysis was
122 restricted to patients who reported being medication adherent at least 75% of the time (Supplementary
123 Figure 2). There were no significant differences in thalamic Glx levels over time in the healthy volunteer
124 group compared to the overall patient sample (Figure 2, Supplementary Table 3).

125

126 Glutamate levels in the left thalamus showed a significant effect of time ($F(2,40)=7.306$, $P=0.002$, repeated
127 measures ANOVA), while the main effects of remission status ($F(1,20)=0.036$, $P=0.852$) and the remission
128 status x time interaction were not significant ($F(2,40)=1.310$, $P=0.281$, Figure 1). The effect of time
129 reflected a significant decrease in thalamic glutamate across both patient subgroups between baseline and
130 6 weeks ($P=0.005$, Bonferroni-corrected pairwise comparisons), and a significant increase between 6 weeks

131 and 9 months ($P=0.010$, Cohen's $d=-0.67$). The results remained the same when the analysis was restricted
132 to patients who were adherent to antipsychotic medication at least 75% of the time. When the entire
133 patient sample was compared to the healthy volunteer sample, the effect of time on glutamate in the
134 thalamus was apparent across all subjects (healthy volunteers and patients) ($F(2,70)=3.753$, $P=0.028$,
135 repeated measures ANOVA), and was related to a significant decrease in glutamate between baseline and
136 6 weeks ($P=0.045$, Cohen's $d=-0.52$, Bonferroni-corrected pairwise comparisons, Figure 2). No significant
137 effect of diagnostic group, and no interaction were found (Supplementary Table 3).

138

139 There was a positive correlation between the percentage change in Glx levels in the thalamus and the
140 percentage change in PANSS positive score between baseline and 9 months ($r=.512$, $P=0.015$, Pearson's
141 bivariate correlation): the greater the longitudinal reduction in thalamic Glx, the greater the improvement
142 in positive symptoms over the course of treatment (decrease in PANSS positive score). This correlation
143 remained significant when one outlying value identified using Cook's D was excluded ($r=.493$, $P=0.023$,
144 Figure 3). Secondary analyses found positive correlations between the percentage change in Glx in the
145 thalamus and the percentage change in PANSS general ($r=.446$, $P=0.037$) and PANSS total ($r=.501$, $P=0.018$)
146 scores, but not the PANSS negative score ($r=-.053$, $P=0.815$, Figure 3) or PSP score ($r=-.135$, $P=0.550$).
147 Relationships remained significant when partial correlations were conducted to control for time to follow-
148 up.

149

150 In contrast, there were no significant relationships between the percentage change in glutamate in the
151 thalamus and percentage symptom change (Supplementary Table 4).

152

153 Repeated measures MANOVA analyses assessed metabolite changes over time for N-acetyl-aspartate,
154 creatine, myo-inositol, and choline (Supplementary Table 5). There were no significant main effects of
155 group, time or interaction in remission versus non-remission groups in the ACC or left thalamus, or in
156 patients versus healthy volunteers in the ACC. In the thalamus, there was a significant interaction between

157 group (patient vs healthy volunteer) and time ($F(2,70)=3.520, P=0.010$). Post-hoc tests did not find
158 significant effects when groups and timepoints were analysed separately.

159

160 Discussion

161 This study investigated the relationship between brain glutamatergic metabolites and the response to
162 antipsychotic medication over the first 9 months of treatment for psychosis. The main finding was that Glx
163 in the thalamus increased over time in Non-Remitters, such that after 9 months Glx levels were higher in
164 patients who were not in remission than in those who were. Furthermore, symptomatic improvement over
165 the course of treatment was associated with a longitudinal reduction in thalamic Glx levels. These results
166 extend our previous observations over shorter periods of treatment⁸ to indicate that longer-term
167 symptomatic response may be linked to the level of glutamatergic metabolites.

168

169 In a recent longitudinal study over 4 weeks of antipsychotic treatment (containing an overlapping sample
170 of the participants to the current study) we also found that glutamate levels decreased over time in the
171 thalamus, but there was no significant relationship between the reduction over this timeframe and
172 symptomatic improvement⁸. In this extended study, the treatment period was 9 months, which suggests
173 that longitudinal differences in relation to symptomatic response may emerge after longer periods of
174 treatment. This 9 month period is comparable to the time since presentation in an earlier cross sectional
175 study in first episode psychosis, in which we also observed numerically but non-significantly higher
176 thalamic Glx in the Non-Remission compared to Remission group⁷.

177

178 The results of the present study are broadly consistent with a previous report in patients with
179 schizophrenia showing that higher social and occupational functioning scores 80 months after diagnosis
180 are associated with a greater degree of thalamic Glx reduction over those 80 months¹⁴. Together these
181 findings suggest that thalamic Glx levels may be more related to symptomatic outcome after a period of
182 several months, rather than the initial period of treatment. A recent study observed a trend for an increase
183 in thalamic Glx levels over 5 years in first episode psychosis patients, although the relationship with

184 treatment response was not investigated³⁴. At 9 months, there were no group differences in substance use
185 or spectral quality. However, there was a numerically higher percentage of cannabis users in the non-
186 remission group, with a higher frequency of use. It is possible that cannabis use or other unknown external
187 factors may have contributed to the observed increase in thalamic Glx in non-remitters³⁵.

188

189 Thalamocortical dysconnectivity is thought to be a key pathophysiological feature of schizophrenia³⁶, and
190 may be mediated by alterations in thalamic glutamatergic transmission^{37,38}. Human neuroimaging studies
191 have demonstrated that antipsychotic administration can modify thalamic activity and metabolism³⁹⁻⁴², but
192 there are fewer data on the role of the thalamus and its cortical connectivity in mediating clinical outcome,
193 with some studies⁴³⁻⁴⁵ but not others^{42,46} suggesting an association. This could be explored in future work
194 combining serial 1H-MRS glutamate and functional connectivity measurements in relation to early and
195 longer-term clinical outcomes.

196

197 Contrary to our expectations, we did not detect any significant relationships between remission status and
198 glutamatergic metabolite levels in the ACC. This is inconsistent with most previous reports linking
199 antipsychotic non-response to elevated glutamate in the ACC^{6-10,12}, although one other study did not
200 detect differences in ACC glutamate in relation to response¹¹. In our recent study that involved a larger
201 sample (n=46) overlapping with the present cohort⁸, non-remission at 4 weeks was associated with
202 elevated ACC glutamate prior to treatment with amisulpride. The lack of significant difference in the
203 current smaller sample (n=23) may reflect limitations of sample size. In addition, compared to our larger
204 study⁸, a greater proportion of participants in the present study had received antipsychotics prior to
205 baseline, which may have affected ACC glutamate metabolite levels¹³.

206

207 In line with data from previous studies^{5,8,21}, we did not detect significant differences in thalamic or ACC Glx
208 or glutamate concentrations between the total patient sample and healthy volunteers. Although a recent
209 meta-analysis of the literature suggests that there may be differences between first episode patients and
210 healthy volunteers in glutamine levels in the thalamus and ACC⁵, the acquisition parameters we used at 3

211 Tesla did not allow reliable quantification of glutamine. While Glx in the thalamus increased over time in
212 Non-Remitters, this effect did not reach significance for the glutamate signal alone. This may relate to
213 differences in the Glx versus glutamate measurement, or could indicate that glutamine is contributing to
214 this effect.

215

216 A strength of this study is the relatively long follow-up period of 9 months in comparison to most previous
217 studies^{16,19,22,23,25,47} which, together with scanning early in treatment permitted investigation of the
218 relationships between brain glutamate and short and longer term outcome under antipsychotic treatment.
219 A further strength is sample homogeneity, through the inclusion of participants in their first episode of
220 psychosis who had received minimal prior antipsychotic medication.

221

222 One limitation of the study was that the majority of participants were not antipsychotic naïve at baseline
223 and may have already experienced initial symptomatic improvement. Even short-term antipsychotic
224 exposure may reduce glutamate levels⁸ and may have reduced our ability to detect subsequent reductions.
225 The 52% response rate in the present sample is slightly lower than that reported in the literature in first
226 episode psychosis (approximately 60%^{26,48-50}), which may be accounted for by prior medication exposure,
227 or by the longer follow-up time period. Other limitations include controlling for the potential effects of
228 medication adherence, which was estimated through self-report and clinical notes. Although the findings
229 remained significant when the analysis was restricted to participants who reported being medication
230 adherent at least 75% of the time, inclusion of more accurate measures of adherence, such as
231 antipsychotic plasma levels, would have been helpful. The majority of patients initially received the same
232 antipsychotic medication, amisulpride, which is a relatively selective D2 dopamine receptor antagonist⁵¹.
233 However, subsequently there was more variation in the particular antipsychotic medications used.
234 Differences in the pharmacological profile of antipsychotics could have differential effects on glutamatergic
235 neurotransmission⁵², which may have increased variability over the observation period. Nevertheless, at
236 the 9 month timepoint, the Remission and Non-Remission groups were taking a similar set of antipsychotic
237 drugs, the levels of medication adherence were comparable, and a similar proportion of patients were no

238 longer taking medication. The data also showed a reduction in thalamic glutamate across all participants
239 between baseline and 6 weeks, which may reflect a methodological factor impacting on the measurement.
240 This indicates the utility of including a healthy volunteer or other non-intervention group for interpretation
241 of longitudinal studies. Lastly, this study used adapted Andreasen's criteria for remission, consistent with
242 previous studies^{7,26}. Therefore our study did not account for fluctuations in symptoms or remission status
243 that may have occurred over the 9 month period, which would require regular symptom monitoring.

244

245 In summary, the findings of the present study extend the literature linking ACC glutamate to antipsychotic
246 response⁶⁻¹⁰ by indicating that response to antipsychotic medication over the first 9 months of treatment
247 may be related to longitudinal changes in glutamatergic metabolites in the thalamus. The association
248 between elevated thalamic Glx levels and Non-Remission is consistent with the notion that brain glutamate
249 transmission is a potential therapeutic target for novel treatments for psychosis.

250

251 **Methods**

252 The study included participants recruited in two studies: OPTiMiSE (Optimisation of Treatment and
253 Management of Schizophrenia in Europe; www.optimisetrialeu.com; EudraCT-Number: 2010-020185-19;
254 clinicaltrials.gov identifier: NCT01248195²⁶) (n=34, using the London sample from the OPTiMiSE study⁸),
255 and TRFEP (The neurobiological determinants of treatment response in psychosis²⁷) (n=6, with an
256 additional n=3 taking part in both studies). Both studies were granted ethical approval by the South
257 London and Maudsley NHS Trust Ethics Committee, and all participants provided written informed
258 consent. Patients were recruited from early intervention community teams and wards. We aimed to recruit
259 24 participants to detect a change in Glx levels with antipsychotic treatment, according to power
260 calculations reported in a recent meta-analysis¹³. Of 43 patients who agreed to participate in the study, a
261 total of n=23 patients completed all 3 scans (n=14 from the OPTiMiSE study, n=6 from the TRFEP study,
262 and n=3 taking part in both studies) (Supplemental Figure 1). In the patient group, inclusion required
263 presentation with a first episode of psychosis within the past 2 years, aged between 18-40, and a diagnosis
264 of a psychotic disorder according to ICD 10 criteria or DSM-IV criteria. Inclusion required previous

265 antipsychotic exposure of <15 days (OPTiMiSE study), or no exposure to antipsychotic medication within
266 the past 6 weeks (TRFEP study). Exclusion criteria included being unable to provide written informed
267 consent, being coercively treated or being under legal custody. Healthy volunteers (n=36) were recruited
268 through online advertisements, with n=15 completing all three MRI sessions. Healthy volunteers were 18-
269 40 years old with no history of psychiatric illness. All subjects had no history of head injury or
270 contraindications to MRI scanning.

271

272 In the patient sample, symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS)²⁸,
273 and functioning was assessed using the Personal and Social Performance (PSP) scale at each MRI scan visit.
274 Medication adherence and illicit drug use was determined using clinical notes and self-report of dates
275 when medication was taken. Chlorpromazine Equivalent Doses were calculated²⁹. The primary clinical
276 outcome measure was remission at 9 months, based upon adapted Andreasen criteria³⁰, consistent with
277 our larger study²⁶, and previous cross-sectional study in first episode psychosis⁷.

278

279 MRI scans were conducted at baseline and repeated after a mean of 6 weeks and 9 months. All data were
280 acquired at 3-Tesla on a General Electric Healthcare (Chicago, USA) HDxt MR system. The same sequences
281 were acquired at each time-point. Whole brain sagittal T1-weighted images were acquired using a
282 modified ADNI GO protocol (See <http://adni.loni.usc.edu/methods/documents/mri-protocols/>) with an
283 echo time (TE) 2.848 ms; repetition time (TR) 6.984 ms; inversion time 400 ms; flip angle 11°, Field of view
284 260mm, slice thickness 1.2mm, matrix size 256x256mm. The structural images were reformatted to axial
285 orientation for 1H-MRS voxel positioning in the bilateral ACC and left thalamus. The centre of the ACC
286 voxel (20 x 20 x 20 mm) was positioned 16mm superior to the anterior portion of the genu of the corpus
287 callosum on the midline sagittal localiser, avoiding the corpus callosum. The voxel in the left thalamus (15 x
288 20 x 20 mm) was also positioned from the axial image, using the coronal and sagittal localisers to minimise
289 cerebrospinal fluid (CSF) content in the voxel (voxel placement and example spectra previously published⁸).

290

291 1H-MRS spectra were acquired using PRESS (Point RESolved Spectroscopy), at TE = 30 msec; TR = 3000
292 msec; 96 averages; bandwidth / sample frequency = +/- 2500Hz; number of complex points = 4096. Data
293 were acquired using the standard GE PROBE (PROton Brain Examination) sequence, which includes
294 acquisition of unsuppressed water reference spectra (16 averages). The target water line-widths after
295 shimming were < 7Hz in the ACC and < 10Hz in the left thalamus. For follow-up scans, radiographers
296 referred to the baseline scan voxel position to reduce variability in voxel placement.

297
298 Spectra were analyzed using LC Model version 6.3-01^{31,32} using a standard LC Model basis set acquired using
299 PRESS at 3-Tesla and a TE of 30msec containing 16 metabolites. Poorly fitted metabolite peaks (Cramer–
300 Rao lower variance bounds (CRLB) >20% as reported by LCModel) were excluded from further analysis. All
301 metabolite values are reported in institutional units.

302
303 To correct metabolite concentration estimates for voxel CSF content, T1-weighted images were segmented
304 into grey matter, white matter and CSF images using Statistical Parametric Mapping 8, version 6313 (SPM8;
305 Wellcome Department of Imaging Neurosciences, University College London, UK). Voxel coordinates were
306 obtained from spectra file headers using General Electric’s spectroscopy processing tool SAGE and mapped
307 against the T1-weighted structural images using in-house software, to calculate the percentage tissue
308 content of the individual 1H-MRS voxels. Metabolite values were then corrected using the following
309 equation³²:

310
$$\text{Uncorrected metabolite} \times (\text{wm} + 1.21 \times \text{gm} + 1.55 \times \text{csf}) / (\text{gm} + \text{wm})$$

311 *gm = grey matter*

312 *wm =white matter*

313 *csf = cerebrospinal fluid*

314
315 Statistical analyses were performed using SPSS version 23 (SPSS inc. Chicago, Illinois, USA). For
316 demographic and clinical data, between group differences were assessed using Fisher’s Exact Test (2 tailed)

317 and independent samples Student's t-test. Equal variances were assumed unless Levene's test was
318 significant.

319

320 The main 1H-MRS metabolites of interest were Glx and glutamate, corrected for voxel CSF content.
321 Repeated measures ANOVA assessed the effects of time, group and group*time on voxel Glx and
322 glutamate levels. A significant effect of time was followed up with Bonferroni-corrected pairwise
323 comparisons (to determine significant differences between timepoints). A significant effect of group was
324 followed up by one-way ANOVA tests (to determine group differences at separate timepoints). A
325 significant interaction was followed up with one-way ANOVA tests, and also a repeated measures ANOVA
326 in the remission and in the non-remission groups separately, with Bonferroni-corrected pairwise
327 comparisons (to determine significant differences between timepoints in each group separately). The
328 primary analysis compared the Remission and Non-Remission patient groups. Subsequent analyses
329 compared the healthy volunteer group to the total patient group. Relationships between the percentage
330 change in PANSS score (minus minimum possible scores)³³ or PSP score, and the percentage change in Glx
331 and glutamate over 9 months were assessed using Pearson's bivariate correlations (2 tailed). Outliers were
332 identified using Cook's distance estimates, excluding values higher than 4/n. Repeated measures MANOVA
333 assessed metabolite changes over time for other metabolites. The data that support the findings of this
334 study are available from the corresponding author upon reasonable request.

335 **Data availability statement**

336 The data that support the findings of this study are available from the corresponding author upon
337 reasonable request.

338

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346

347 **Conflicts of Interest Statement**

348 AE has received research funding from Roche and consultancy payment from Heptares Therapeutics. PM
349 has received consultancy payment for Sunovion and Takeda. RPI has received honoraria as a speaker for
350 Lundbeck. OH has received speaker bureau honoraria and charitable research funding from AstraZeneca,
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357 grants are the property of the Mental Health Services in the Capital Region of Denmark and administrated
358 by them. The remaining authors declare no conflicts of interest.

359

360 **Author Contributions**

361 All authors contributed extensively to the work presented in this paper. PM and AE have equal
362 contribution.

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493 **Figure legends**

494 **Figure 1 Glx (left) and Glutamate (right) at Baseline, 6 weeks and 9 months, in Remission and Non-Remission groups in A)**
495 **Anterior Cingulate Cortex and B) Left Thalamus** *Represents higher thalamic Glx levels in the Non-Remission group compared to
496 the Remission group at 9 months (P=0.033). Glx and glutamate values are CSF-corrected, presented as mean & within-subjects
497 standard deviation.

498 **Figure 2 Glx (left) and Glutamate (right) at Baseline, 6 weeks and 9 months, in First Episode Psychosis (FEP) patients and Healthy**
499 **Volunteers in A) Anterior Cingulate Cortex and B) Left Thalamus.**

500 **Figure 3 Correlations between change in PANSS score (-100% indicates full symptomatic improvement, whereas 0% denotes no**
501 **change in symptoms) and change in thalamic Glx levels over 9 months (negative values indicate reduction in thalamic Glx levels,**
502 **whereas positive values indicate increase in thalamic Glx).** A) Significant positive correlation between the percentage change in
503 Glx levels in the thalamus and the percentage change in PANSS positive score ($r=.493, P=0.023$), B) PANSS total ($r=.501, P=0.018$)
504 score and C) PANSS general score ($r=.446, P=0.037$), between baseline and 9 months. D) No significant correlation for the
505 percentage change in PANSS negative score.

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507 **Table 1 Subject demographics and clinical characteristics.** Mean and (Standard Deviation) presented. Remission and Non-
508 Remission groups were classified based on presentation at the 9 month timepoint. Significant differences between Healthy
509 Volunteers and FEP patients are denoted by *= $P<0.05$, ** = $P<0.01$, *** = $P<0.001$ in the FEP patient column; significant
510 differences between the Remission and Non-Remission group are denoted in the Remission group column. FEP; First episode
511 psychosis, PANSS; Positive And Negative Syndrome Scale, PSP; Personal and Social Performance scale; CPZ Equivalent Dose;
512 Chlorpromazine Equivalent Dose.

513 **Table 2 1H-MRS metabolite concentrations corrected for voxel cerebrospinal fluid (CSF) content, and 1H-MRS voxel % of white**
514 **matter, grey matter and CSF, at three timepoints.** Data are presented as mean (SD). Significant group differences are represented
515 by *= $P<0.05$. Glutamate (Glu), N-acetyl-aspartate (NAA), creatine (Cr), myo-inositol (ml), choline (Cho).

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