Progressive changes in glutamate concentration in early stages of schizophrenia: A longitudinal 7-Tesla MRS study

(Running Title: Longitudinal glutamate in schizophrenia at 7T)

Peter Jeon1,2, Roberto Limongi3, Sabrina Ford4, Michael Mackinley5, Kara Dempster6, Jean Théberge1,2,7,8, Lena Palaniyappan1,3,4

Affiliations:

1. Department of Medical Biophysics, Western University, London, Canada
2. Lawson Health Research Institute, Imaging Division, London, Canada
3. Robarts Research Institute, Western University, London, Canada
4. Department of Psychiatry, Western University, London, Canada
5. Department of Neuroscience, Western University, London, Canada
6. Department of Psychiatry, Dalhousie University, Halifax, Canada
7. St. Joseph’s Health Care, Diagnostic Imaging, London, Canada
8. Department of Medical Imaging, Western University, London, Canada

Correspondence: Lena Palaniyappan, Robarts Research Institute, 1151 Richmond Street N., Room 3208, UWO, London, Ontario, Canada, N6A 5B7

Telephone: (519) 931-5777 (ext. 24398)

Email: lpalaniy@uwo.ca

Abstract Word Count: 233/250
Total Word Count: 3757/4000

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
Abstract

Progressive reduction in glutamatergic transmission has been proposed as an important component of the illness trajectory of schizophrenia. Despite its popularity, to date, this notion has not been convincingly tested in patients in early stages schizophrenia. In a longitudinal 7T magnetic resonance spectroscopy (1H-MRS), we quantified glutamate at the dorsal anterior cingulate cortex in 21 participants with a median lifetime antipsychotic exposure of less than 3 days and followed them up after 6 months of treatment. Healthy controls were also scanned at two time points. While patients had significantly lower overall glutamate levels than healthy controls (F(1,27) = 5.23, p = 0.03), we did not observe a progressive change of glutamate concentration in patients (F(1,18) = 0.47, p = 0.50), and the group by time interaction was not significant (F(1,27) = 0.86, p = 0.36). On average, patients with early psychosis receiving treatment showed a 0.02 mM/year increase, while healthy controls showed a 0.06 mM/year reduction of MRS glutamate levels. Bayesian analysis of our observations does not support early, post-onset glutamate loss in schizophrenia. Interestingly, it provides evidence in favour of a lack of progressive glutamate change in our schizophrenia sample – indicating that the glutamatergic level at the onset of illness was the best predictor of the levels 6 months after treatment. A more nuanced view of glutamatergic physiology, linked to early cortical maturation, may be required to understand glutamatergic dynamics in schizophrenia.

Keywords: first-episode schizophrenia, magnetic resonance spectroscopy, Bayesian
Introduction

Glutamatergic disruption is implicated in the wide range of symptoms observed in schizophrenia \(^{1-3}\). Specifically, disinhibition of the excitatory glutamatergic outputs of the prefrontal cortex is thought to disrupt dopaminergic signaling in the striatum \(^4,5\) resulting in acute psychotic symptoms. However, sustained disinhibition of prefrontal glutamatergic neurons might lead to excitotoxic damage with subsequent reduction in glutamate, with greater reductions occurring in patients with more severe forms of schizophrenia \(^6\). Magnetic resonance spectroscopy (MRS) studies in schizophrenia report higher glutamate levels in younger patients at early stages while lower glutamate levels in older patients at later stages of illness, when compared to healthy controls \(^7\).

A progressive pathology defined by gray matter changes \(^8-10\), ventricular enlargement \(^11-15\), and network-level dysconnectivity \(^16,17\) is thought to be the basis of the longitudinal trajectory of schizophrenia. It is posited that glutamatergic dendritic spine reduction triggered by early excitotoxic processes lies at the centre of such morphological changes \(^18,19\). The progressive pathology of schizophrenia is likely limited to certain hubs of the brain \(^16\), with the anterior cingulate cortex (ACC) being a prominent region where both structural, functional \(^20\) and neurochemical deficits \(^21-24\) have been consistently demonstrated in schizophrenia. Nevertheless, longitudinal MRS studies investigating progressive glutamate changes in the ACC are limited.

Using 1.5T in patients at various stages of schizophrenia, Choe et al. \(^25\) reported a notable reduction in prefrontal Glx (glutamate + glutamine) signal 1-6 months after treatment. Théberge et al. \(^10\) and Bustillo et al. \(^26\) demonstrated static glutamate levels in the ACC using 4T. Merritt et
al. 27 also failed to see a progressive reduction in Glx using 3T in schizophrenia. When using 7T MRS, with superior specificity for glutamate quantification 28, a cross-sectional association of decreasing ACC glutamate with increasing age was observed in schizophrenia 29 at an accelerated rate compared to healthy young adults 30. Nevertheless, to date, longitudinal 7T MRS studies have not been reported in schizophrenia.

In this study, we tested if (1) glutamatergic deficit indexed by ACC MRS measure of glutamate is present in early stages of psychosis, (2) whether this deficit progressively worsens in the first 6 months of treatment, and (3) if patients show an exaggerated longitudinal decline compared to healthy controls. To our knowledge, this is the first longitudinal report of 7T MRS in schizophrenia.

Methods

Participants

We recruited 21 first-episode schizophrenia (FES) volunteers with inclusion criteria of lifetime antipsychotic exposure being less than 14 days along with 10 healthy control volunteers, group-matched for age, gender, and parental socio-economic status. Patient volunteers were recruited from the referrals received by the PEPP (Prevention and Early Intervention for Psychosis Program) at London Health Sciences Center. All patients had established consensus diagnosis after 6 months of first-episode schizophrenia by 3 psychiatrists (LP, KD, and primary treatment provider at PEPP) based on the DSM-5 criteria 31. Participants whose 6-month diagnoses were bipolar or major depressive disorder with psychoses as well as suspected drug-induced psychoses were excluded from the study. Healthy control volunteers had no personal history of mental
illness and no family history of psychotic disorder. All participants were screened to exclude significant head injury, major medical illness, or MRI contraindications and provided written, informed consent according to the guidelines of the Human Research Ethics Board for Health Sciences at Western University, London, Ontario.

**MRS Acquisition and Analysis**

MRS measurements were acquired using a Siemens MAGNETOM 7T head-only MRI scanner (Siemens, Erlangen, Germany) and a site-built head coil (8-channel transmit, 32-channel receive) at the Centre for Functional and Metabolic Mapping of Western University (London, Ontario). A two-dimensional sagittal anatomical image (37 slices, TR = 8000 ms, TE = 70 ms, flip-angle (α) = 120°, thickness = 3.5 mm, field of view = 240×191 mm) was used as reference to prescribe a 2.0 x 2.0 x 2.0 cm (8 cm³) ¹H-MRS voxel on the bilateral dorsal ACC (Figure 1). Voxel positioning was set by having the posterior end of the voxel coinciding with the precentral gyrus and the caudal face of the voxel coinciding with the most caudal positioning that was not part of the corpus callosum. Voxel angle was set to be tangential to the corpus callosum. A semi-LASER ¹H-MRS sequence (TR = 7500 ms, TE = 100 ms, bandwidth = 6000 Hz, N = 2048) was used to acquire 32 channel-combined, VAPOR ³² water-suppressed spectra as well as a water-unsuppressed spectrum to be used for spectral editing and quantification. During scan, participants were asked to rest by fixing their gaze on a white cross on a 50% gray background.

Using the tools outlined in Near et al. ³³, the 32 spectra were phase and frequency corrected before being averaged into a single spectrum to be used for all subsequent analyses. QUECC ³⁴ and HSVD ³⁵ were applied to the spectrum for lineshape deconvolution and removal of residual
water signal, respectively. Spectral fitting was done using fitMAN 36, a time-domain fitting algorithm that uses a non-linear, iterative Levenberg-Marquardt minimization algorithm to echo time-specific prior knowledge templates. The metabolite fitting template included 17 brain metabolites: alanine, aspartate, choline, creatine, γ-aminobutyric acid (GABA), glucose, glutamate, glutamine, glutathione, glycine, lactate, myo-inositol, N-acetyl aspartate, N-acetyl aspartyl glutamate, phosphorylethanolamine, scyllo-inositol, and taurine. No significant macromolecule contribution was expected due to the long echo time and hence was omitted from the metabolite template. Metabolite quantification was then performed using Barstool 37 with corrections made for tissue-specific (gray matter, white matter, CSF) T₁ and T₂ relaxations through partial volume segmentation calculations of voxels mapped onto T₁-weighted images acquired using a 0.75 mm isotropic MP2RAGE sequence (TR = 6000 ms, TI₁ = 800 ms, TI₂ = 2700 ms, flip-angle 1 (α₁) = 4°, flip-angle 2 (α₂) = 5°, FOV = 350 mm × 263 mm × 350 mm, Tₐcq = 9 min 38 s, iPAT PE = 3 and 6/8 partial k-space). All spectral fit underwent visual quality inspection as well as Cramer-Rao lower bounds (CRLB) assessment for each metabolite.

**Clinical Assessments**

Symptom severity at baseline was measured using PANSS-8 38 scale, on the same day of the first scan. We also quantified the overall social and occupational functioning at the time of first presentation using SOFAS 39, administered on the same day of the scanning. We assessed Duration of Untreated Psychosis (DUP) based on multiple sources of information provided by the patient, the referring sources, and caregivers as well as by reviewing clinical charts. We used the first emergence of positive psychotic symptoms as the starting point for calculating the DUP, in line with prior work in this regard 40.
To determine cannabis use in the past six months, the Cannabis Abuse Screening Test (CAST) was used. The CAST is a six-item Likert-scale self-report questionnaire which asks the participant about cannabis use and how it effects their daily activities and relationships. Scores range from six to 30, with higher scores indicating more cannabis use. To determine alcohol use in the past six months, the Alcohol Use Disorders Identification Test (AUDIT-C) was used. The AUDIT-C is a three-item Likert-scale self-report questionnaire which asks the participant about alcohol use frequency and quantity. Scores range from 0 to 12, with higher scores indicating more alcohol use. Alcohol users and nonusers were classified by AUDIT-C scores of four or more and less than four, respectively. Lastly, nicotine use in the past six months was determined by the single item Fagerström Test for Nicotine Dependence and smoking index. The Fagerström test indicates time to the first cigarette after waking, and the smoking index is calculated by the number of years regularly smoking × the number of cigarettes per day, divided by 20, to determine packs per year. A lower Fagerström test value indicates more nicotine dependence, and a higher smoking index indicates more nicotine use. The 10-item Drug Abuse Screening Test (DAST-10) was also employed for substances other than cannabis, alcohol and nicotine, though our cohort did not endorse any such use.

**Statistical Analyses**

All frequentist statistical tests were computed using IBM SPSS Statistics version 26. Group demographic differences were calculated using t tests and chi-square tests for continuous and dichotomous variables, respectively. Repeated measure ANOVA was used to assess group × time interaction (primary hypothesis), as well as group effect and time effect, with parameter
estimates examined to test individual group effects. As age and gender are known modifiers of glutamate levels, they were entered as covariates in the ANOVA model. Lastly, Pearson correlation was used to explore the correlation of annualized, baseline-adjusted glutamate change to DUP, SOFAS, as well as symptom severity at first presentation, measured using PANSS-8 total score at baseline in patients. Correlations between defined daily dose (DDD) and annualized, baseline-adjusted glutamate change and follow-up glutamate concentrations were also examined.

To investigate relationships of annualized glutamate with cannabis and nicotine, Pearson correlations were used. Glutamate change was analyzed with total CAST to determine relationships with cannabis, along with smoking index and Fagerström scores to determine nicotine use. To determine alcohol use, a *t*-test was used to compare glutamate change values between alcohol users and nonusers.

We performed a (Bayesian) hierarchical generalization of an analysis of covariance (Bayesian ANCOVA) to evaluate whether there is a between-group difference of the effect of time on the follow-up measurement of glutamate concentration. We decided to use a Bayesian approach as an alternative to traditional frequentist test because it allows us to weigh the evidence in support of our main hypothesis relative to the evidence in support of the null hypothesis. We achieved this via model comparison and Bayes factors (BF) 46,47.

We fit one saturated ANCOVA model and all possible reduced models comprising follow-up glutamate concentration ([Glu]_{follow-up}) as a dependent variable, Group as factor, and baseline glutamate concentration ([Glu]_{baseline}) and Interval (days) as covariates. We compared the
evidence supporting this (saturated) model with the evidence supporting the reduced models (including the null model). We relied on the largest BF$_{10}$ relative to the null model to select the winning model. Note that we included [Glu]$_{\text{baseline}}$ as a covariate of no interest. Therefore, our main hypothesis was represented by the triple-interaction model (Group $\times$ [Glu]$_{\text{baseline}}$ $\times$ Interval).

In all models, the posterior distributions over parameters were estimated using the ‘generaltestBF’ function in the “R Bayes Factor” package. In this data set, the small number of subjects in each group might cause the posterior distribution to be strongly influenced by the prior distribution. Therefore, we used informed “wide” priors scaled to the observed data (r-scale for each effect = 0.5). We report the mean and standard deviation for each estimate obtained from the relevant posterior distribution (10,000 samples) along with the 95% highest density interval (HDI).

Results

Demographic Data

Demographic and clinical data of subjects are shown in Table 1. Our patient sample had a mean DUP of 29.38 weeks (SD = 26.65 weeks) and a mean antipsychotic duration of 2.95 days (SD = 3.11 days) prior to the first scan session. Patient and healthy control SOFAS scores were significantly different ($t$(29) = 12.466, $p < 0.001$). The time in between baseline and follow-up (FUP) scan was 5.93 months (SD = 1.25) for patients and 7.25 months (SD = 1.90) for healthy controls.

CRLB values indicating the quality of glutamate measurement was quantified for both groups. For HC glutamate CRLB were 3.41% (SD = 1.27%) and 3.55 % (SD = 0.89%) for baseline and
FUP, respectively. For FES glutamate quantification, CRLB values were 3.52% (SD = 1.20%) and 3.96% (SD = 1.12%) for baseline and FUP, respectively. Thus the 2 groups had acceptable qualitative metrics for glutamate estimation at both time points.

**Longitudinal Glutamate**

Paired t tests revealed no significant differences between FES (M = 6.51 mM, SD = 0.64 mM) and HC (M = 7.25 mM, SD = 1.34 mM) unadjusted baseline glutamate concentration (t(29) = 1.66, p = 0.13, Cohen’s d = 0.70) as well as unadjusted FUP glutamate concentration (M = 6.49 mM, SD = 1.29 mM; M = 6.86 mM, SD = 0.73 mM; t(29) = 1.02, p = 0.32, Cohen’s d = 0.35). Repeated measures ANOVA revealed a group effect (F(1,27) = 5.23, p = 0.03, Cohen’s d = 0.90) between FES (M = 6.43 mM, SD = 0.84 mM) and HC (M = 7.20 mM, SD = 0.86 mM) but no effect on time (F(1,27) = 1.21, p = 0.28) or group × time interaction (F(1,27) = 0.86, p = 0.36).

Parameter estimates revealed that at baseline, FES had lower glutamate than healthy controls (t(29) = 2.83, p = 0.009, Cohen’s d = 1.11), but this difference was not present at follow-up (t(29) = 1.20, p = 0.24, Cohen’s d = 0.41). A simple contrast of time in each group revealed no significant effect in both the healthy control group (F(1,7) = 0.25, p = 0.63, Cohen’s d = 0.41) and in patients (F(1,18) = 0.47, p = 0.50, Cohen’s d = 0.003) (Figure 1).

Annualized glutamate concentration values were not significantly different between the two groups (t(29) = -0.813, p = 0.423) and indicated a 0.02 mM/year (SD = 0.33 mM) increase in patients and a 0.06 mM/year (SD = 0.19) reduction in healthy controls, with the difference amounting to a small to moderate sized effect (Cohen’s d = 0.26). Lastly, the time interval
between scans in months was not related to glutamate concentration differences (FUP – baseline) in either group (HC: \( r = -0.23, p = 0.52 \); FES: \( r = -0.41, p = 0.06 \)).

**Glutamate versus Clinical Measures**

There was no significant correlation between annualized glutamate concentration changes and DUP (\( r = -0.07, p = 0.77 \)), SOFAS (\( r = -0.09, p = 0.70 \)), or PANSS-8 total (\( r = 0.25, p = 0.28 \)). We also did not see any correlation between baseline (unadjusted) glutamate concentration and DUP (\( r = 0.03, p = 0.91 \)), SOFAS (\( r = -0.38, p = 0.09 \)), or PANSS-8 total (\( r = 0.31, p = 0.17 \)).

Across all participants, there was no significant difference in annualized glutamate change between alcohol users (\( n = 23 \)) and nonusers (\( n = 6 \)) (\( t(27) = 1.89, p = 0.07 \)). No significant difference was found between alcohol users and nonusers when FES was considered (\( t(17) = 1.37, p = 0.19 \)) separately. No significant difference was found in annualized glutamate change between smokers and non-smokers among the FES patients (\( t(20) = -0.72, p = 0.63 \)). Across all participants, there was no significant correlation between total CAST scores (\( n = 28 \)) and annualized glutamate change (\( r = -0.08, p = 0.75 \)). Lastly, there was no significant correlation between DDD at follow-up (\( n = 21 \)) and follow-up glutamate concentration (\( r = -0.16, p = 0.50 \)) as well as between DDD at follow-up and annualized glutamate change (\( r = -0.16, p = 0.48 \)).

**Bayesian Statistical Analysis**

After controlling for baseline values, the \([\text{Glu}]_{\text{follow-up}}\) in the FEP group was the same as in the HC group. The Bayesian ANCOVA model revealed that the triple interaction (Group ×
[Glu]_{\text{baseline}} \times \text{Interval}) did not perform better than the null model (Table 2). This means that, in practice, the longitudinal change in glutamate concentration of both groups are alike.

Table 2 also shows that a model including only the [Glu]_{\text{baseline}}, a model including both [Glu]_{\text{baseline}} and Interval, and a model including its interaction outperformed the null model (BF\text{10} > 3). However, the more complex models underperformed the “[Glu]_{\text{baseline}}” model (the best model). This suggests that, with moderate evidence (10 > BF_{10} > 3) \text{47}, [Glu]_{\text{baseline}} is the best predictor of [Glu]_{\text{follow-up}} regardless of both follow-up measurement time and Group. Table 3 shows the relevant parameter estimates along with the 95\% interval of most credible values.

**Discussion**

We report longitudinal 7T-MRS glutamate measurements in FES patients who were medication-naïve at baseline. Although baseline glutamate concentrations were lower in FES compared to HC, follow-up measurements revealed no difference in glutamate concentration between the two groups. Our current work supports the lower glutamate concentration observed in FES compared to HC at baseline as reported in current literature \text{49}. Annualized glutamate concentration changes also showed no difference between FES and HC. Bayesian statistical approach also provided evidence in favour of a lack of progressive glutamate change in our FES sample, indicating that the glutamatergic level at the onset of illness was the best predictor of the levels 6 months after treatment. Taken together, our results indicate that early in the illness, patients with schizophrenia already show abnormalities in ACC glutamate, and do not show evidence for a progressive deterioration in glutamatergic status.
Prior longitudinal studies at lower field strengths have been equivocal on the issue of progressive glutamatergic reduction in schizophrenia. Synthesizing this longitudinal literature, Egerton et al. noted an almost even split between studies that reported significant glutamate decrease in at least one brain region and studies reported no glutamate reduction, with at least 3 further studies showing no longitudinal glutamate reduction in the ACC after 6 weeks, 4 months, and 9 months. Our findings are more aligned with these recent studies as well as the lack of progressive ACC glutamate change reported by Bustillo et al. [at 1, 6, 12 months] and Théberge et al. [10 months] at 4T. In contrast, Egerton et al. observed a reduction in glutamate to creatine ratios at 3T in the ACC after 4 weeks of antipsychotic treatment, though 2 of the 3 sites in this multi-site study did not show the same effect. In summary, our current study supports the extant literature on the lack of progressive glutamate changes in the ACC during the early course of treatment in schizophrenia.

Our study has a number of strengths including the use of 7T scanner, and recruiting highly symptomatic, mostly drug-naïve individuals. Several limitations should also be considered when interpreting the results. We chose a single voxel (dorsal ACC) for the current 6-month follow-up study. We cannot exclude the possibility of progressive glutamate changes in different brain regions (as shown by Théberge et al. and Goto et al. in thalamus and basal ganglia) or in ACC over a longer time scale with multiple time points. Additionally, though glutamine is sometimes considered to reflect the synaptic pool of glutamate due to its glial localisation, our acquisition was not optimised for quantifying glutamine; we had high CRLB values (23.70 ± 15.93% and 26.83 ± 12.06% for baseline and follow-up, respectively) for glutamine.
quantification. As a result, the glutamate concentration reported here must be interpreted as reflective of largely neuronal origin \(^{55,56}\).

Although we had sufficient power to detect if paired differences were present in the patient group, any interaction is likely underpowered. We observed a small glutamate decrease in healthy control (0.06 mM/year), comparable to the effect size observed in Birur et al. \(^{51}\) with 16 patients and 14 healthy controls. Bayesian statistical approaches are based on an expected prior distribution and not influenced by the central limit of sample size placed on conventional statistics. Bayesian approach also confirmed the evidence in favour of a lack of progressive glutamatergic changes in schizophrenia. For obvious ethical reasons, we lacked a patient group that remained untreated for 6 months to parse the effects of treatment from illness stage.

In summary, this longitudinal 7T MRS study of ACC found a stable glutamatergic deficit that does not progressively worsen in the early stages of schizophrenia. This supports the possibility of the putative excitotoxic processes predating the first presentation of psychosis, either in the prodromal stages or more distally during early development. Further, this also challenges the notion of a relentlessly progressive glutamatergic dysfunction in patients receiving treatment.

Acknowledgements

We thank Mr. Trevor Szekeres, Mr. Scott Charlton, Mr. Joseph Gati for their assistance in data acquisition and archiving. We thank all research team members of the NIMI lab and all the staff members of the PEPP London team for their assistance in patient recruitment and supporting
clinical care. We gratefully acknowledge the participants and their family members for their contributions. Requests for data should be addressed to Dr. Lena Palaniyappan lpalaniy@uwo.ca.

Funding

This study was funded by CIHR Foundation Grant (375104/2017) to LP; Schulich School of Medicine Clinical Investigator Fellowship to KD; AMOSO Opportunities fund to LP; BrainSCAN to RL; Parkwood Institute Studentship to MM; Canada Graduate Scholarship to KD. Data acquisition was supported by the Canada First Excellence Research Fund to BrainSCAN, Western University (Imaging Core); Innovation fund for Academic Medical Organization of Southwest Ontario; Bucke Family Fund, The Chrysalis Foundation and The Arcangelo Rea Family Foundation (London, Ontario).

Conflict of Interest

LP reports personal fees from Otsuka Canada, SPMM Course Limited, UK, Canadian Psychiatric Association; book royalties from Oxford University Press; investigator-initiated educational grants from Janssen Canada, Sunovion and Otsuka Canada outside the submitted work. All other authors report no relevant conflicts.

References

1. Coyle JT. Glutamate and schizophrenia: beyond the dopamine hypothesis. *Cell Mol Neurobiol*. 2006. doi:10.1007/s10571-006-9062-8

2. Merritt K, Egerton A, Kempton MJ, Taylor MJ, McGuire PK. Nature of Glutamate
3. Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry*. 1991. doi:10.1176/ajp.148.10.1301

4. McCutcheon RA, Krystal JH, Howes OD. Dopamine and glutamate in schizophrenia: biology, symptoms and treatment. *World Psychiatry*. 2020;19(1). doi:10.1002/wps.20693

5. Limongi R, Mackinley M, Dempster K, Khan AR, Gati JS, Palaniyappan L. Frontal – striatal connectivity and positive symptoms of schizophrenia: implications for the mechanistic basis of prefrontal rTMS. *Eur Arch Psychiatry Clin Neurosci*. 2020;(0123456789). doi:10.1007/s00406-020-01163-6

6. Kumar J, Liddle EB, Fernandes CC, et al. Glutathione and glutamate in schizophrenia: a 7T MRS study. *Mol Psychiatry*. 2018. doi:10.1038/s41380-018-0104-7

7. Marsman A, Van Den Heuvel MP, Klomp DWJ, Kahn RS, Luijten PR, Hulshoff Pol HE. Glutamate in schizophrenia: A focused review and meta-analysis of 1H-MRS studies. *Schizophr Bull*. 2013;39(1). doi:10.1093/schbul/sbr069

8. Gur RE, Cowell PE, Latshaw A, et al. Reduced dorsal and orbital prefrontal gray matter volumes in schizophrenia. *Arch Gen Psychiatry*. 2000;57(8). doi:10.1001/archpsyc.57.8.761

9. Zipursky RB, Lim KO, Sullivan E V., Brown BW, Pfefferbaum A. Widespread Cerebral Gray Matter Volume Deficits in Schizophrenia. *Arch Gen Psychiatry*. 1992;49(3). doi:10.1001/archpsyc.1992.01820030027004

10. Théberge J, Williamson KE, Aoyama N, et al. Longitudinal grey-matter and glutamatergic losses in first-episode schizophrenia. *Br J Psychiatry*. 2007;191(OCT.).
11. Weinberger DR, Torrey EF, Neophytides AN, Wyatt RJ. Lateral Cerebral Ventricular Enlargement in Chronic Schizophrenia. *Arch Gen Psychiatry*. 1979;36(7). doi:10.1001/archpsyc.1979.01780070013001

12. Weinberger DR, Bigelow LB, Kleinman JE, Klein ST, Rosenblatt JE, Wyatt RJ. Cerebral Ventricular Enlargement in Chronic Schizophrenia: An Association with Poor Response to Treatment. *Arch Gen Psychiatry*. 1980;37(1). doi:10.1001/archpsyc.1980.01780140013001

13. Andreasen NC, Smith MR, Jacoby CG, Dennert JW, Olsen SA. Ventricular enlargement in schizophrenia: Definition and prevalence. *Am J Psychiatry*. 1982;139(3). doi:10.1176/ajp.139.3.292

14. Andreasen NC, Olsen SA, Dennert JW, Smith MR. Ventricular enlargement in schizophrenia: Relationship to positive and negative symptoms. *Am J Psychiatry*. 1982;139(3). doi:10.1176/ajp.139.3.297

15. Reveley AM, Reveley MA, Murray RM. Cerebral ventricular enlargement in non-genetic schizophrenia: A controlled twin study. *Br J Psychiatry*. 1984;144(1). doi:10.1192/bjp.144.1.89

16. Van Den Heuvel MP, Fornito A. Brain networks in schizophrenia. *Neuropsychol Rev*. 2014;24(1). doi:10.1007/s11065-014-9248-7

17. Limongi R, Jeon P, Mackinley M, et al. Glutamate and Dysconnection in the Salience Network: Neurochemical, Effective Connectivity, and Computational Evidence in Schizophrenia. *Biol Psychiatry*. 2020. doi:10.1016/j.biopsych.2020.01.021

18. Glausier JR, Lewis DA. Dendritic spine pathology in schizophrenia. *Neuroscience*. doi:10.1192/bjp.bp.106.033670
2013;251. doi:10.1016/j.neuroscience.2012.04.044

19. Plitman E, Nakajima S, de la Fuente-Sandoval C, et al. Glutamate-mediated excitotoxicity in schizophrenia: A review. *Eur Neuropsychopharmacol.* 2014;24(10). doi:10.1016/j.euroneuro.2014.07.015

20. Fornito A, Yung AR, Wood SJ, et al. Anatomic Abnormalities of the Anterior Cingulate Cortex Before Psychosis Onset: An MRI Study of Ultra-High-Risk Individuals. *Biol Psychiatry.* 2008;64(9). doi:10.1016/j.biopsych.2008.05.032

21. Gallinat J, Mcmahon K, Kühn S, Schubert F, Schaefer M. Cross-sectional study of glutamate in the anterior cingulate and hippocampus in schizophrenia. *Schizophr Bull.* 2016;42(2). doi:10.1093/schbul/sbv124

22. Reid MA, Salibi N, White DM, Gawne TJ, Denney TS, Lahti AC. 7T Proton Magnetic Resonance Spectroscopy of the Anterior Cingulate Cortex in First-Episode Schizophrenia. *Schizophr Bull.* 2019;45(1). doi:10.1093/schbul/sbx190

23. Poels EMP, Kegeles LS, Kantrowitz JT, et al. Glutamatergic abnormalities in schizophrenia: A review of proton MRS findings. *Schizophr Res.* 2014;152(2-3). doi:10.1016/j.schres.2013.12.013

24. Wang AM, Pradhan S, Coughlin JM, et al. Assessing Brain Metabolism with 7-T Proton Magnetic Resonance Spectroscopy in Patients with First-Episode Psychosis. *JAMA Psychiatry.* 2019;76(3). doi:10.1001/jamapsychiatry.2018.3637

25. Choe BY, Suh TS, Shinn KS, Lee CW, Lee C, Paik INHO. Observation of metabolic changes in chronic schizophrenia after neuroleptic treatment by in vivo hydrogen magnetic resonance spectroscopy. *Invest Radiol.* 1996;31(6). doi:10.1097/00004424-199606000-00006
26. Bustillo JR, Rowland LM, Mullins P, et al. 1H-MRS at 4 Tesla in minimally treated early schizophrenia. \textit{Mol Psychiatry}. 2010;15(6). doi:10.1038/mp.2009.121

27. Merritt K, Perez-Iglesias R, Sendt KV, et al. Remission from antipsychotic treatment in first episode psychosis related to longitudinal changes in brain glutamate. \textit{npj Schizophrenia}. 2019;5(1). doi:10.1038/s41537-019-0080-1

28. Henning A. Proton and multinuclear magnetic resonance spectroscopy in the human brain at ultra-high field strength: A review. \textit{Neuroimage}. 2018;168. doi:10.1016/j.neuroimage.2017.07.017

29. Brandt AS, Unschuld PG, Pradhan S, et al. Age-related changes in anterior cingulate cortex glutamate in schizophrenia: A 1H MRS Study at 7 Tesla. \textit{Schizophrenia Research}. 2016;172(1-3). doi:10.1016/j.schres.2016.02.017

30. Marsman A, Mandl RCW, van den Heuvel MP, et al. Glutamate changes in healthy young adulthood. \textit{Eur Neuropsychopharmacology}. 2013;23(11). doi:10.1016/j.euroneuro.2012.11.003

31. First MB, Williams JBW, Karg RS, Spitzer RL. Structured clinical interview for DSM-5 research version. \textit{Am Psychiatr Assoc Washington DC}. 2015.

32. Tkáč I, Gruetter R. Methodology of 1H NMR spectroscopy of the human brain at very high magnetic fields. \textit{Appl Magn Reson}. 2005;29(1):139-157. doi:10.1007/BF03166960

33. Near J, Edden R, Evans CJ, Paquin R, Harris A, Jezzard P. Frequency and phase drift correction of magnetic resonance spectroscopy data by spectral registration in the time domain. \textit{Magn Reson Med}. 2015. doi:10.1002/mrm.25094

34. Bartha R, Drost DJ, Menon RS, Williamson PC. Spectroscopic lineshape correction by QUECC: Combined QUALITY deconvolution and eddy current correction. \textit{Magn Reson Med}. 2015.
35. van den Boogaart A, Ala-Korpela M, Jokisaari J, Griffiths JR. Time and frequency domain analysis of NMR data compared: An application to 1D 1H spectra of lipoproteins. *Magn Reson Med*. 1994;31(4). doi:10.1002/mrm.1910310402

36. Bartha R, Drost DJ, Williamson PC. Factors affecting the quantification of short echo in-vivo 1H MR spectra: Prior knowledge, peak elimination, and filtering. *NMR Biomed*. 1999;12(4):205-216. doi:10.1002/(SICI)1099-1492(199906)12:4<205::AID-NBM558>3.0.CO;2-1

37. Wong D. MRI Investigations of Metabolic and Structural Brain Changes in Alzheimer’s Disease and Vitamin D Deprivation. 2019.

38. Lin CH, Lin HS, Lin SC, Kuo CC, Wang FC, Huang YH. Early improvement in PANSS-30, PANSS-8, and PANSS-6 scores predicts ultimate response and remission during acute treatment of schizophrenia. *Acta Psychiatr Scand*. 2018;137(2). doi:10.1111/acps.12849

39. Rybarczyk B. Social and Occupational Functioning Assessment Scale (SOFAS). In: *Encyclopedia of Clinical Neuropsychology*. 2018. doi:10.1007/978-3-319-57111-9_428

40. Singh SP, Cooper JE, Fisher HL, et al. Determining the chronology and components of psychosis onset: The Nottingham Onset Schedule (NOS). *Schizophr Res*. 2005;80(1). doi:10.1016/j.schres.2005.04.019

41. Stentebjerg-Olesen M, Jeppesen P, Pagsberg AK, et al. Early nonresponse determined by the clinical global impressions scale predicts poorer outcomes in youth with schizophrenia spectrum disorders naturally treated with second-generation antipsychotics. *J Child Adolesc Psychopharmacol*. 2013;23(10). doi:10.1089/cap.2013.0007
42. Bush K, Kivlahan DR, McDonell MB, Fihn SD, Bradley KA. The AUDIT alcohol consumption questions (AUDIT-C): An effective brief screening test for problem drinking. *Arch Intern Med.* 1998;158(16). doi:10.1001/archinte.158.16.1789

43. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom K O. The Fagerström Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. *Br J Addict.* 1991;86(9). doi:10.1111/j.1360-0443.1991.tb01879.x

44. Skinner HA. The drug abuse screening test. *Addict Behav.* 1982;7(4). doi:10.1016/0306-4603(82)90005-3

45. IBM Corp. IBM SPSS Statistics for Windows, Version 26.0. 2019. 2019.

46. Kass RE, Raftery AE. Bayes factors. *J Am Stat Assoc.* 1995;90(430). doi:10.1080/01621459.1995.10476572

47. Keysers C, Gazzola V, Wagenmakers EJ. Using Bayes factor hypothesis testing in neuroscience to establish evidence of absence. *Nat Neurosci.* 2020;23(7). doi:10.1038/s41593-020-0660-4

48. Morey RD, Rouder JN. BayesFactor: Computation of Bayes Factors for Common Designs. *R Packag version 0912-42.* 2018.

49. Sydnor VJ, Roalf DR. A meta-analysis of ultra-high field glutamate, glutamine, GABA and glutathione 1HMRS in psychosis: Implications for studies of psychosis risk. *Schizophr Res.* 2020;(xxxx). doi:10.1016/j.schres.2020.06.028

50. Egerton A, Bhachu A, Merritt K, McQueen G, Szulc A, McGuire P. Effects of antipsychotic administration on brain glutamate in schizophrenia: A systematic review of longitudinal 1H-MRS studies. *Front Psychiatry.* 2017;8(APR). doi:10.3389/fpsyt.2017.00066
51. Birur B, Kraguljac NV, VerHoef L, et al. Neurometabolic correlates of 6 and 16 weeks of treatment with risperidone in medication-naive first-episode psychosis patients. *Transl Psychiatry*. 2020;10(1). doi:10.1038/s41398-020-0700-6

52. Cadena EJ, White DM, Kraguljac N V., et al. A Longitudinal Multimodal Neuroimaging Study to Examine Relationships Between Resting State Glutamate and Task Related BOLD Response in Schizophrenia. *Front Psychiatry*. 2018;9. doi:10.3389/fpsyt.2018.00632

53. Egerton A, Broberg B V., Van Haren N, et al. Response to initial antipsychotic treatment in first episode psychosis is related to anterior cingulate glutamate levels: a multicentre 1 H-MRS study (OPTiMiSE). *Mol Psychiatry*. 2018;23(11). doi:10.1038/s41380-018-0082-9

54. Goto N, Yoshimura R, Kakeda S, et al. Six-month treatment with atypical antipsychotic drugs decreased frontal-lobe levels of glutamate plus glutamine in early-stage first-episode schizophrenia. *Neuropsychiatr Dis Treat*. 2012;8. doi:10.2147/NDT.S25582

55. Bak LK, Schousboe A, Waagepetersen HS. The glutamate/GABA-glutamine cycle: Aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J Neurochem*. 2006;98(3). doi:10.1111/j.1471-4159.2006.03913.x

56. Schousboe A, Scafidi S, Bak LK, Waagepetersen HS, McKenna MC. Glutamate Metabolism in the Brain Focusing on Astrocytes. In: ; 2014. doi:10.1007/978-3-319-08894-5_2

Figure Legends
Figure 1. (A) Axial, (B) coronal, and (C) sagittal views of MRS voxel (red square) in the dorsolateral anterior cingulate cortex (ACC) for glutamate measurement. (D) Estimated marginal means of glutamate concentration [mM] for healthy controls (HC, blue) and patients (FES, red) at baseline and follow-up scan sessions. Error bars indicate standard deviation. Asterisk denotes significant difference. (Note: y-axis values do not begin at 0 for graphical purposes).
| Characteristic                        | Patient group (N = 21) | Healthy controls (N = 10) | t/χ² | p     |
|---------------------------------------|------------------------|---------------------------|------|-------|
| Gender (male/female)                  | 16/5                   | 5/5                       | 2.13 | 0.145 |
| Marital status (Mar/S)                | 1/20                   | 1/9                       | 0.31 | 0.58  |
| Inpatient at baseline (Y/N)           | 11/10                  |                           |      |       |
| Family Hx (Y/N/DN)                    | 10/6/5                 |                           |      |       |
| AP exposure at baseline (M/SD; days)  | 2.95/3.11              |                           |      |       |
| Total DDD-days at baseline scan (M/SD)| 2.25/4.74              |                           |      |       |
| Total DDD-days at FUP scan (M/SD)     | 145.68/97.56           |                           |      |       |
| DUP (M/SD/median; weeks)              | 29.38/26.65/18         |                           |      |       |
| Ethnicity (Black/White/Other)         | 2/18/1                 | 0/5/5                     | 4.51 | 0.034 |
| Age (M/SD)                            | 22.33/5.29             | 21.60/3.37                | -0.47| 0.645 |
| SOFAS (M/SD)                          | 42.33/12.84            | 83.70/5.62                | 12.47| 0.000 |
| PANSS-8 total (M/SD)                  | 24.67/5.30             |                           |      |       |
| Smoker (yes/no)                       | 6/15                   | 0/10                      | 3.54 | 0.060 |
| Cannabis user (yes/no)                | 13/8                   | 0/10                      | 10.66| 0.001 |
| Time between scans (M/SD; months)     | 5.93/1.25              | 7.67/1.90                 | 2.63 | 0.021 |

P values for differences between groups were calculated using chi-square analyses for categorical variables and independent t tests for continuous variables.

Mar = married, S = single, Y = yes, N = no, Hx = history, DN = don’t know, AP = antipsychotic, M = mean, SD = standard deviation, DDD = defined daily dose, FUP = follow-up, DUP = duration untreated psychosis.

*White vs non-White comparison.*
Table 2. Bayesian model comparison.

| Model                  | BF<sub>10</sub> |
|------------------------|------------------|
| N                      | 1.000            |
| G                      | 0.465            |
| **B**                  | **4.080**        |
| G + B                  | 1.547            |
| G + B + G × B          | 1.732            |
| I                      | 0.351            |
| G + I                  | 0.201            |
| **B + I**              | **3.126**        |
| G + B + I              | 1.451            |
| G + B + G × B + I      | 1.828            |
| G + I + G × I          | 0.155            |
| G + B + I + G × I      | 0.905            |
| G + B + G × B + I + G × I | 1.929          |
| **B + I + B × I**      | **3.164**        |
| G + B + I + B × I      | 1.448            |
| G + B + G × B + I + B × I | 0.980          |
| G + B + I + G × I + B × I | 2.855          |
| G + B + G × B + I + G × I + B × I | 1.814  |
| G + B + G × B + I + G × I + B × I + G × B × I | 0.960 |

BF<sub>10</sub> is computed relative to null model.

N null, B baseline, I interval, G group.
Table 3. Model Averaged Posterior Summary

| Parameter                      | Mean  | SD    | Lower       | Upper       |
|--------------------------------|-------|-------|-------------|-------------|
| Intercept                      | 6.617 | 0.205 | 6.209       | 7.020       |
| FEP                            | -0.074| 0.178 | -0.434      | 0.270       |
| HC                             | 0.074 | 0.178 | -0.270      | 0.434       |
| **Baseline**                   | **0.511** | **0.247** | **0.048** | **1.007** |
| Interval                       | -0.004| 0.004 | -0.012      | 0.003       |
| FEP × [Glu]_{baseline}         | 0.149 | 0.225 | -0.288      | 0.595       |
| HC × [Glu]_{baseline}          | -0.149| 0.225 | -0.595      | 0.288       |
| FEP × Interval                 | -0.006| 0.004 | -0.014      | 0.001       |
| HC × Interval                  | 0.006 | 0.004 | -0.001      | 0.014       |
| [Glu]_{baseline} × Interval    | -0.005| 0.006 | -0.016      | 0.006       |
| FEP × Interval × [Glu]_{baseline} | -0.002| 0.005 | -0.013      | 0.009       |
| HC × Interval × [Glu]_{baseline} | 0.002 | 0.005 | -0.009      | 0.013       |
| \( \sigma^2 \)                 | 0.964 | 0.276 | 0.560       | 1.626       |

Only the Intercept, \([Glu]_{baseline}\), and \(\sigma^2\) parameters (bolded) were granted with \(PP > 0.95\).

\(\sigma^2\) posterior variance of the “deflection parameters”, \(PP\) posterior probability.