Increased Glutamine in Patients Undergoing Long-term Treatment for Schizophrenia
A Proton Magnetic Resonance Spectroscopy Study at 3 T

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**IMPORTANCE** The N-methyl-D-aspartic acid receptor hypofunction model of schizophrenia predicts a paradoxical increase in synaptic glutamate release. In vivo measurement of glutamatergic neurotransmission in humans is challenging, but glutamine, the principal metabolite of synaptic glutamate, can be quantified with proton magnetic resonance spectroscopy (1H-MRS). Although a few studies have measured glutamate, glutamine, and glutamine to glutamate ratio, it is not clear which of these 1H-MRS indices of glutamatergic neurotransmission is altered in schizophrenia.

**OBJECTIVE** To examine glutamine, glutamate, and glutamine to glutamate ratio in the dorsal anterior cingulate, as well as their relationships with symptoms and cognition in schizophrenia.

**DESIGN, SETTING, AND PARTICIPANTS** Cross-sectional design using 3-T 1H-MRS in participants recruited from university-based psychiatric outpatient clinics who underwent neuroimaging at an affiliated research facility. Participants were 84 patients with a DSM-IV-TR diagnosis of schizophrenia and 81 psychiatrically healthy volunteers, matched in age, sex, ethnicity, and occupational level to the head of household of family of origin.

**MAIN OUTCOMES AND MEASURES** Glutamine, glutamate, and glutamine to glutamate ratio. Also symptoms and cognition.

**RESULTS** Glutamine was increased in the schizophrenia group (P = .01) as well as the glutamine to glutamate ratio (P = .007) but not glutamate (P = .89). Glutamine levels were positively correlated with severity of psychotic symptoms (P = .02). Choline was also increased in schizophrenia (P = .002).

**CONCLUSIONS AND RELEVANCE** Elevated glutamine, which was directly related to psychotic symptoms, is consistent with increased glutamatergic synaptic release in schizophrenia, as predicted by the N-methyl-D-aspartic acid receptor hypofunction model. Further understanding the underlying mechanism of glutamatergic dysfunction in schizophrenia may lead to new pharmacological strategies to treat psychosis.
The N-methyl-D-aspartic acid receptor (NMDAR) model of schizophrenia postulates that hypofunction of these receptors in γ-aminobutyric acid interneurons leads to disinhibition of pyramidal neurons and a paradoxical increase in presynaptic glutamate release across multiple cortical fields. The most direct evidence for this model comes from pharmacological studies with NMDAR blocking agents. Awake rats treated with short-term ketamine have elevations of extracellular prefrontal glutamate. Likewise, anesthetized rats exhibited increased prefrontal glutamate, the principal metabolite of synaptic glutamate, but reduced glutamate with short-term phencyclidine administration. Consistently, healthy humans exposed to short-term subanesthetic dosages of ketamine develop many of the positive, negative, and cognitive deficits present in schizophrenia, as well as elevations of prefrontal glutamine. However, although heuristically useful, this acute pharmacological model has intrinsic limitations to understand a chronic illness like schizophrenia.

The measurement of glutamate neurotransmission in vivo is challenging in humans. To our knowledge, only 1 study has measured NMDAR occupancy in schizophrenia. However, a few proton magnetic resonance spectroscopy studies (1H-MRS), usually at 3 and 4 T, have assessed glutamine. Glutamate released in the synapse is reuptaked into the glia, converted to glutamine, transferred to the presynaptic terminal, and recycled into vesicular glutamate. However, the status of glutamine concentration, probably the best available measure of in vivo synaptic glutamatergic neurotransmission in humans in schizophrenia, is far from clear.

In the present investigation, we measured glutamine and glutamate, in the largest sample of patients with schizophrenia to date, to our knowledge, from the dorsal anterior cingulate region where we previously documented glutamate elevations in the context of acute NMDAR blockade. We used a sequence optimized for separation of glutamine and glutamate and documented the quality of spectral fitting. Consistent with the NMDAR hypofunction model, we hypothesized increased glutamine in the schizophrenia group. Because the meta-analysis reported greater reduction in glutamine and glutamate with age in schizophrenia, we also tested for this effect.

**Methods**

**Participants**

 Patients were recruited from the University of New Mexico Hospitals and the Albuquerque Veterans Administration Medical Center. Inclusion criteria were (1) DSM-IV-TR schizophrenia diagnosis made through consensus by 2 research psychiatrists using the Structured Clinical Interview for DSM-IV-TR, Patient Version and review of psychiatric records and family informants and (2) if treated, clinically stable receiving the same antipsychotic medications for more than 4 weeks. Exclusion criteria were diagnosis of neurological disorder or substance use disorder (except for nicotine). Healthy controls were excluded if they had (1) any DSM-IV-TR axis I disorder (Structured Clinical Interview for DSM-IV-TR Non-Patient Version); (2) first-degree relatives with any psychotic disorder; or (3) history of neurological disorder. The study was approved by the University of New Mexico institutional review board. Participants gave written informed consent and were reimbursed for their participation.

**Magnetic Resonance Studies**

**Acquisition**

 Scanning was performed on a Siemens 3-T Tim Trio (VB-17; 12-channel head coil). An axial T2 image was acquired for 1H-MRS voxel prescription. T1-weighted anatomical images were obtained with 3-dimensional magnetization-prepared rapid acquisition with gradient echo for voxel tissue segmentation (repetition time, 1500 milliseconds; echo time, 3.87 milliseconds; inversion time, 700 milliseconds; flip angle, 10°; field of view = 256 × 256 mm; 1-mm-thick slice). Conventional 1H-MRS spectra were acquired using the standard PRESS sequence provided with the scanner (svs_sc), with an echo time of 40 milliseconds and repetition time of 1.5 seconds. The water-suppressed scan included 192 averages with 3-pulse chemical shift selective water suppression, while a non-water-suppressed scan included 16 averages.

**Voxel Location**

The voxel for all spectroscopy scans was prescribed to include mostly gray matter in the anterior cingulate using sagittal and reformatted coronal images. The voxel was 2 × 2 × 3 cm³ and positioned parallel to and above the corpus callosum, starting from the genu of the corpus callosum and extending 3 cm posteriorly (Figure 1A, representative voxel placement). The magnetic field homogeneity in the voxel was adjusted using the system’s automated shimming routine.

**Spectral Fitting**

Localized spectra were quantified using LCModel fitting (version 6.13; Figure 1B, representative fitted spectra). Simulated basis sets for sequence parameters included the following metabolites: alanine, aspartate, creatine, phosphocreatine, γ-aminobutyric acid, glutamine, glutamate, glycerocephosphocholine, phosphocholine, myo-inositol, lactate, N-acetylaspartate, N-acetyl-aspartylglutamate, scyllioinositol, and guanidine. The following sums were also reported by the fitting program: creatine + phosphocreatine (total creatine), glycerocephosphocholine + phosphocholine (choline), and N-acetylaspartate + N-acetyl-aspartylglutamate (N-acetylaspartate compounds [NAAc]). Lipids (0.9 ppm [Lip09], 2 resonances at 1.3 ppm [Lip13a and Lip13b], and 2 ppm [Lip 20]) and macromolecules (0.9 ppm [MM09], 1.2 ppm [MM12], 1.4 ppm [MM14], 1.7 ppm [MM17], and 2.0 ppm [MM20]) were simulated using the default settings of LCModel, which include soft constraints for peak position and line width and prior probabilities of the ratios of macromolecule and lipid peaks. Spectra were fitted in the spectral range between 0.4 and 4.2 ppm in reference to the non-water-suppressed data using “water scaling.”

 We automatically selected individual metabolite spectra with goodness of fit, as measured by the Cramér-Rao lower bound (CRLB), of 20 or less. However, for glutamine, we re-
laxed this conventional criterion to 30 or less, striving for a balance between reasonable fits and keeping most of the data. 12,13

Partial Volume Correction
The results from LCModel for the metabolites of interest were corrected for partial volume (using segmented T1 images) and relaxation effects, as outlined previously14 (eAppendix in Supplement).

Neuropsychological and Clinical Assessments
Patients and controls completed the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS),15 a battery of tests designed to assess cognition in schizophrenia. Patients were assessed for psychopathology with the Positive and Negative Syndrome Scale.16 They were also rated with the Simpson-Angus Scale,17 the Barnes Akathisia Rating Scale,18 and the Abnormal Involuntary Movement Scale.19 Neuropsychological and clinical assessments were completed within 1 week of the scan acquisition.

Statistical Analyses
The main dependent variable of interest was glutamine estimated concentrations in millimoles per kilogram of 1H-MRS-visible tissue water; however, to better contrast our data with the 1H-MRS literature, 2 other reported indices of glutamatergic metabolism were also analyzed: glutamate and the glutamine to glutamate ratio. Finally, we also present NAAc, myo-inositol, choline, and total creatine, other metabolites frequently reported in the schizophrenia literature. For glutamate, glutamine to glutamate ratio, myo-inositol, total creatine, and choline, we Bonferroni corrected to type I error α = 0.01 (0.05 ÷ 5). Because of previous supportive meta-analyses,9,20 we did not correct results for glutamine or NAAc.
Increased Glutamine in Schizophrenia

For each metabolite, the overall approach used analysis of covariance (ANCOVA) (SAS version 8; SAS Institute Inc), with participant group (schizophrenia vs control) as the grouping factor and age as the covariate (age has been found to relate with "H MRS–measured metabolites21). Differential relationships between metabolites and cognition by group were also examined with ANCOVA. If the interaction terms were non-significant, the ANCOVA models were run with only main effects. Relationships between metabolite concentrations and other participant characteristics were examined with multivariate regression.

Results

Demographic

Eighty-four patients with schizophrenia and 81 controls participated (eTable 1 in Supplement). There were no significant differences between the groups in age or socioeconomic status of the head of household of family of origin (all P’s between .47–.87). The schizophrenia group had a lower proportion of females (χ² = 7.2; P = .008) and a higher proportion of smokers (χ² = 5.4; P = .02). As expected, the schizophrenia group had worse personal socioeconomic status (t152 = 6.25; P ≤ .001).

Group Differences in Neurometabolites

Glutamine

For all metabolites, ANCOVAs had no significant group × age interactions (P’s between .07-.72). Twelve patients with schizophrenia and 5 controls had glutamine CRLB values greater than 30, leaving 72 and 76 participants, respectively, with analyzable data for this metabolite. Glutamine was increased in the schizophrenia group (F1,145 = 6.14; P = .01) (Figure 2), and it also increased with age (F1,145 = 15.5; P ≤ .001). The group effect remained after controlling for sex and smoking status (P = .03 and P = .02, respectively). The marginal interaction of group × age (F1,144 = 3.4; P = .07) (eFigure 1 in Supplement) suggests a larger increment in glutamine over time in patients with schizophrenia (0.07mM/y) compared with controls (0.05mM/y).

Other Glutamate-Related Measures

For glutamate, there were no effects of group (F1,162 = 0.2; P = .99) or age (F1,162 = 2.78; P = .10). However, the glutamine to glutamate ratio was increased in the schizophrenia group (F1,162 = 7.61; P = .007) (Figure 2) and it also increased with age (F1,162 = 13.1; P ≤ .001). The group effect remained after controlling for sex and smoking status (P = .02 and P = .01, respectively).

Other Metabolites

N-acetylaspartate compounds were lower in the schizophrenia group (F1,162 = 4.01; P = .05), with no effect of age (F1,162 = 0.19; P = .66) (eFigure 2 in Supplement). The group effect remained after controlling for sex (P = .04). However, NAAc reduction occurred only in the schizophrenic smokers (mean [SD], 15.74 [1.51]) compared with control smokers (mean [SD], 16.75 [1.11]). There were no differences between the schizophrenic nonsmokers and the control non-smokers (mean [SD], 16.16 [1.6] and 16.2 [0.9], respectively; P = .85).

Choline was increased in the schizophrenia group (F1,162 = 9.94; P = .002) and it also increased with age (F1,162 = 47.1; P < .001). The group effect remained after controlling for sex and smoking status (P = .01 and P = .003, respectively). Finally, myo-inositol and total creatine did not differ by group (F1,162 = 0.1; P = .76 and F1,162 = 0.17; P = .67, respectively) but both increased with age (F1,162 = 43.6; P < .001 and F1,162 = 36.43; P < .001).

Measures of spectral quality and voxel-tissue composition and their effect on metabolite group differences are pre
presented in eTable 2 in Supplement. The quality of fit (CRLB) did not differ between the groups for glutamine or choline but did for NAAc and glutamate.

**Clinical and Cognitive Metabolite Relationships**

In a multivariate analysis, glutamine was positively correlated with positive symptoms ($F_{1,61} = 6.07; P = .02$) (Figure 3), but not with negative symptoms ($F_{1,61} = 0.45; P = .51$) or antipsychotic dose$^{22}$ ($F_{1,61} = 0.11; P = .75$). The glutamine to glutamate ratio was marginally positively correlated with positive symptoms ($F_{1,61} = 3.17; P = .08$), but not with negative symptoms ($F_{1,61} = 0.43; P = .51$) or current antipsychotic dose ($F_{3,64} = 0.67; P = .42$). Neither choline nor NAAc were significantly related to either symptoms or antipsychotic dose (all $P$'s between .24 and .82).

The MATRICS composite score was significantly lower in schizophrenic patients compared with controls ($t_{145} = 10.5; P = .001$) (Figure 4). We used ANCOVA to examine the relationship between each metabolite and MATRICS score across the 2 groups. Only glutamate had a different relationship with MATRICS score across the 2 groups ($F_{1,155} = 4.31; P = .04$; however, this did not survive Bonferroni correction). The schizophrenia group had a negative correlation trend ($r_{80} = -0.21; P = .07$), whereas the control group had no correlation ($r_{79} = 0.14; P = .19$).

**Discussion**

In the largest study to date, to our knowledge, we find increased levels of glutamine in the anterior dorsal cingulate cortex in patients undergoing long-term treatment for schizophrenia. Furthermore, glutamine was directly related to severity of psychotic symptoms. The glutamine to glutamate ratio, but not glutamate, was also increased and marginally related to psychosis. We also identified the often reported reduction of NAAc, a marker of neuronal viability, but only in the subgroup of schizophrenic smokers. Furthermore, an increase of choline compounds was also found. Finally, different relationships between cognition and glutamate were found among the groups, with a negative correlation in the patient group.

Only a few studies have measured glutamine in schizophrenia, all with the standard single-voxel $^1$H-MRS approach,
the majority at higher field strengths. A meta-analysis examined 8 studies of the anterior cingulate or the medial frontal region and reported increased glutamine (Cohen $d = 0.4$, comparable with $d = 0.30$ in the current study). However, this literature was limited by unclear quality of the spectral fits (except for $d^{12,13,27}$), lower field strength (1.5 T$^{29}$), inclusion of nonschizophrenic participants (“at risk for psychosis”$^{23}$), and small samples (from 9 up to 30, for schizophrenia groups$^9$). The present study confirms increased glutamine in schizophrenia and expands this finding in important ways.

The positive and specific relationship between glutamine and psychotic symptoms has not, to our knowledge, been previously reported. The effect size of this relationship was small and thus unlikely to be detected even in the largest previous study$^25$ (n = 30). Still, this finding is consistent with the NMDAR hypofunction. Ketamine increases glutamine in the anterior dorsal cingulate$^6$ and lamotrigine, a presynaptic glutamate inhibitor, prevents ketamine-induced psychosis. This region overlaps with more posterior cingulate cortex, involved in response preparation, and with more ventral areas, involved in reward.$^{32}$ Hence, it may be particularly important for salience detection, a cognitive process thought to underlie the formation of delusions and hallucinations.$^{28}$

Glutamine is the principal metabolite of synaptic glutamate and increased presynaptic release of glutamate could result in greater concentrations of glutamine. Consistently, somatosensory activation in rats resulted in increased glutamine and reduced glutamate assessed with $^1$H-MRS at 11.7 T.$^{10}$ In another high-field rodent study, short-term phencyclidine administration resulted in elevations in prefrontal glutamine and reductions in glutamate.$^4$ The stronger group effect in our study with the glutamine to glutamate ratio is consistent with the interpretation that this may be a more sensitive index of glutamatergic neurotransmission than glutamine.$^2$ A reduction in presynaptic glutaminase, the enzyme that converts glutamate to glutamine, with resultant accumulation of glutamine, cannot be excluded with the static metabolite measures we acquired. However, elevations of phosphate-activated glutaminase$^{30}$ and increased messenger RNA expression of the astrocytic glutamate transporter$^{38}$ were both detected in the prefrontal cortex of patients with schizophrenia, consistent with increased turnover of synaptic glutamate. Still, future studies of glutamate-glutamine cycling in schizophrenia with dynamic carbon 13-labeled MRS would be informative.

Although increased choline has been reported in some studies,$^{37}$ the meta-analysis found no evidence of differences, specifically in the anterior cingulate (10 studies, standard mean difference, 0.05 [95% CI, −0.15 to 0.24]). However, the largest sample of these studies was 43 (for the schizophrenia group). Hence, it is possible that the present study with homogeneous methods and larger samples could detect a real elevation of choline (our effect size was moderate, $d = 0.4$). Choline compounds are trimethylamines but are chemically heterogeneous and have a 2-fold greater concentration in glial compared with neuronal cells.$^{39}$ Consistently, the choline signal tends to be increased in neurodegenerative disorders with gliosis and/or increased membrane turnover.$^{34}$ In schizophrenia, there is clearly no histopathological evidence of gliosis.$^{35}$ Additionally, the effects of antipsychotic agents on glial cells have been inconsistent, with both increases (in cell density$^{16}$) and reductions (in cell numbers$^{37}$) reported in nonhuman primates undergoing long-term treatment. Furthermore, a 6-month exposure to halo-peridol failed to change $^1$H-MRS-measured choline in rodents.$^{38}$ We speculate that increased turnover of synaptic glutamate (identified as increased glutamine) may result in an adaptive increase in glial function with elevations in the choline signal (both groups had direct correlations between glutamine and choline [$F_{1,145} = 22.7; P < .001$] but did not differ [$F_{1,144} = 0.17; P = .68$]).

We failed to replicate the report from the meta-analysis, suggesting that glutamine is increased in younger patients and reduced in older schizophrenia groups. Indeed, our data suggested the opposite effect, at a statistical trend level. An advantage of our approach is that partial volume correction for tissue differences was implemented. Because of the progressive gray and white matter reductions in schizophrenia,$^{39}$ accounting for partial volume effects with age is necessary. However, the 1 study that followed up patients with schizophrenia longitudinally (30 months) did detect glutamate reductions in the thalamus but not in the anterior cingulate.$^{40}$ Longer-term longitudinal $^1$H-MRS studies are necessary.

Many investigations have measured NAAc in schizophrenia/healthy control comparisons, mostly in single voxels at 1.5 T. These were summarized in the meta-analysis, with reductions in the hippocampus, thalamus, and frontal and temporal lobe reported in schizophrenia. For the anterior cingulate, there were no significant differences ($z = 0.71; P = .48$) but heterogeneity across studies was large. However, tobacco smoking was not considered in this meta-analysis or an earlier meta-analysis that also reported NAAc reduction in schizophrenia. One study reported reduced NAAc in nonpsychiatric smokers in the hippocampus, but not in the anterior cingulate. Hence, our findings suggest a specific effect of smoking in neuronal viability in schizophrenia. Alternatively, schizophrenia smokers may inhale deeper and extract more tobacco products than controls, resulting in neuronal damage/dysfunction and reduced NAAc.

Several limitations of this study should be acknowledged. First, separation of glutamine from glutamate, even at 3 T, is challenging. We implemented a PRESS sequence at an echo time of 40 milliseconds with documented advantage, but higher field strength would potentially increase spectral resolution. Additionally, in a more stringent analysis of participants with CLRB of 20 or less for glutamine, patients with schizophrenia (n = 49) had higher glutamine levels (mean [SD], 5.4 [2.8]) than controls (mean [SD], 4.2 [2.0]; n = 53; $F_{1,95} = 5.6; P = .01$). Likewise, the glutamine to glutamate ratio remained higher in the schizophrenia group ($F_{1,95} = 8.2; P = .005$). Also, in the patients, the relationship between glutamine and positive symptoms remained ($F_{1,37} = 6.2; P = .02$), adjusting for negative symptoms ($F_{1,37} = 0.5; P = .48$) and olanzapine equivalent dosage ($F_{1,37} = 0.2; P = .67$).
Second, spatial coverage was limited to a relatively large voxel including the dorsal anterior cingulate cortex, a region involved in schizophrenia and relevant to the NMDAR hypofunction model of the illness. Still, glutamine may be affected in other regions.

Third, the majority of the patients with schizophrenia were taking antipsychotic medications, a common confound in schizophrenia studies, and we did not measure blood levels. However, the results remained after accounting for antipsychotic dose and use of different psychotropic agents (eTable 3 in Supplement). Additionally, the one study in antipsychotic-naive patients also documented increased glutamine. Furthermore, long-term antipsychotic treatment in rats failed to affect glutamine in the anterior cingulate.

Fourth, the measures of spectral quality and fitting tended to be worse for the patients than for the controls and could have affected the results. However, the quality of fitting (CRLB) for glutamine and choline did not differ between the groups (eTable 2 in Supplement). Finally, the cross-sectional study design supports mainly descriptive, not causal interpretations.

Conclusions

In summary, we found increased glutamine in the anterior dorsal cingulate in patients undergoing long-term treatment for schizophrenia, which related to the severity of psychotic symptoms. This suggests that a basal increased release of presynaptic glutamate remains despite treatment with antipsychotic agents, consistent with a pathophysiological model of NMDAR hypofunction. The differential relationship between glutamatergic indices and clinical/cognitive manifestations of the illness suggest a dissociation of function for the glutamate pool: increased synaptic release (glutamine) with psychosis and increased glutamate metabolism (glutamate) with cognitive impairment. Ongoing longitudinal studies examining glutamine in the acute psychotic state and following antipsychotic treatment will further clarify the mechanism by which increased glutamatergic turnover contributes to the pathophysiology of psychosis. This line of translational investigation may identify subgroups of persistently symptomatic patients for whom drugs like metabotropic mGluR2-3 agonists may be particularly helpful.
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