Glutamatergic hypo-function in the left superior and middle temporal gyri in early schizophrenia: a data-driven three-dimensional proton spectroscopic imaging study

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INTRODUCTION
The N-methyl-D-aspartate receptor (NMDAR) hypo-function model of schizophrenia originated from pharmacological studies documenting emergence of psychotic-like symptoms in healthy volunteers exposed to the NMDA-blocker ketamine [1]. Though NMDA-blockers reduce the post-synaptic effect of glutamate in single neurons, in the rat, systemic ketamine increases extra-cellular glutamate in frontal cortex [1]. This paradoxical increment in glutamate release with ketamine, has been postulated to result from higher sensitivity of NMDA receptors in GABAergic interneurons than in pyramidal neurons, resulting in disinhibition of pyramidal neurons and increase in presynaptic glutamate release in cortical fields [1]. However, in a chronic condition like schizophrenia, persistently increased glutamate release could result in dendritic and axonal damage, with subsequent reductions in glutamate concentrations as well as in tissue volumes. Consistent with this view, longitudinal MRI studies early in the illness, have documented brain tissue reductions [2].

The 1H-MRS schizophrenia glutamate literature remains unclear. Three meta-analyses have examined measurements of glutamate and glutamine, all with the single-voxel approach. One reported increased medial frontal glutamine and reduced glutamate regardless of stage of illness [3]. Another reported increased glutamate, glutamine and glutamate + glutamine (Glx), in the “basal ganglia” regardless of age or medication [4]. Finally, in never-medicated schizophrenia, no abnormalities were found [5]. However, in schizophrenia, a disease with subtle but broad gray and white matter involvement and progressive structural changes [2], proton MR spectroscopic imaging (1H-MRSI) enables measurement in much larger brain regions, thereby reducing the bias of voxel selection intrinsic to single-voxel studies.

Here we report the novel use of 3-dimensional 1H-MRSI with a short echo-time (TE) to measure Glx and other metabolites of interest early in schizophrenia. Consistent with the NMDAR hypofunction model and supported by reports of elevated Glx in dAC by us [6] and by others in the dorsal striatum [7], we hypothesized increased Glx in schizophrenia in these structures.

MATERIALS AND METHODS
Subjects
Schizophrenia patients (SP) were recruited from the University of New Mexico (UNM) Hospitals. Inclusion criteria were: (1) DSM-5...
Neuropsychological and clinical assessments
SPs and HCs completed the Measurement-and-Treatment-Research-to-Improve-Cognition-in-Schizophrenia (MATRICS) [8] battery. Patients were assessed with the Positive-and-Negative-Syndrome-Scale (PANSS) [9].

Magnetic resonance studies
Acquisition. Subjects underwent an MR study at 3T using a TIM Trio scanner with a 32 channel head-coil. High resolution T1-weighted imaging was carried out using a 5-echo multi-echo MPRAGE [TE = 1.64, 3.5, 5.36, 7.22, 9.08 ms, TR (repetition time) = 2530 ms, TI (inversion time) = 1200 ms, 7° flip angle, number of excitations = 1, slice thickness = 1 mm, FOV (field of view) = 256 mm, resolution = 256 × 256].

Whole-brain 1H-MRSI was acquired using the EPSI sequence which uses echo-planar acquisition after spin-echo excitation [TE = 17.6 ms, TR = 1531 ms, TR(HO) = 511 ms, non-selective lipid inversion nulling with TI = 198 ms, FOV = 280 × 280 × 180 mm, 50 × 50 × 18 k-space samples, echo-train length = 1000 points, bandwidth = 2500 Hz, reduced k-space sampling (acceleration factor = 0.7), and acquisition time = 17 min] [10]. EPSI included a water reference measurement that was interleaved with the metabolite signal acquisition.

General analytical approach
Although we collected whole-brain EPSI data, not all data met spectral quality criteria. Hence, a systematic approach was followed to compromise between the maximum amount of data per subject, maintaining spectral quality and minimizing false-positive rates (FPR). Because 1H-MRSI metabolites vary greatly by brain tissue composition [11], for statistical analyses we selected AFNI since it allowed us covariation of gray/gray-plus-white matter at the voxel level [12]. However, to use this feature all subjects must provide data for each voxel analyzed. Hence, with this approach, imputation of some missing data was necessary, as in other neuroimaging modalities [13]. Furthermore, inclusion of some suboptimal spectral data in voxels mainly surrounded by best spectra, was also implemented (see Supplementary Table 1 for spectral quality metrics). This is in part justified by the relatively high smoothing of these data such that each spectral value is affected by the surrounding voxels’ values. We mitigated the FPR in the statistical levels 1 and 2 analyses described below. The processing pipeline is summarized in Fig. 1.

Reconstruction
EPSI reconstruction was carried out using the MIDAS package as previously described [14]. Processing included corrections for $B_0$ shifts, generation of white-matter, gray-matter, and CSF tissue segmentation maps using FSL FAST, lipid $k$-space extrapolation and linear registration of the T1-weighted MR images to the EPSI images. Data was acquired using a spatial oversampling approach [15] that obtains data with a relatively small nominal voxel volume of 0.31 cm$^3$ which following application of $B_0$ correction and convolution smoothing resulted in an effective voxel volume of 1.55 cm$^3$. Following mild spectral smoothing of 2 Hz (which has minimal effect on quantification accuracy [16]), spectra were analyzed using a time-frequency parametric modeling method [17] that incorporated a-priori information on the resonance peaks and a Gaussian line-shape model. Metabolite maps were interpolated to 64 × 64 × 32 points (voxel size 4.375 × 4.375 × 5.625 mm). Correction for frequency shifts due to $B_0$ inhomogeneity was done using two steps. First, the spectra were frequency and phase corrected using a standard eddy-current correction procedure with the time-domain phase correction function obtained from the water reference EPSI. Second, following spectral analysis the final data were corrected for the phase and frequency offset values obtained from the fitting.

Registration
EPSI water reference measurement was spatially registered to the MPRAGE T1-weighted image with MIDAS-MSREG [14].

Spectral fitting
The fitted metabolite values from the corrected spectra were estimated using MIDAS-FITT2 program [14] for N-acetyl-aspartate (NAA), total creatine (creatinine and phosphocreatine, denoted t-Cr), total choline (choline, glycerol-phospho-choline, and phos-phocholine, denoted t-Chol), myo-inositol, lactate and glutamate plus glutamine (denoted Glx; see Fig. 1 for example of fitted spectrum). However, due to sensitivity to noise at the voxel level lactate was not analyzed.

The amplitude of macromolecular resonances were considered negligible due to use of lipid inversion nulling during the acquisition. Still, any residual signal was part of the spectral baseline that was characterized using a wavelet smoothing approach incorporated into the spectral model [10]. Individual metabolite maps were signal normalized and corrected for any bias-field variations using the signal from the water reference measurement. This water signal was corrected for tissue water density based on the fractional tissue volume obtained from the down-sampled tissue segmentation maps and converted to 100% water equivalent based on the assumption of tissue water content in gray-matter, white-matter, and CSF of 79%, 70%, and 98%, respectively [11]. Results were not corrected for relaxation rates and represent institutional units.

Warping
The individual metabolite maps were exported from MIDAS and warped using SPM12’s Normalize function into MNI space. In this process the output voxel size is set to the spectroscopic voxel size (4.375 × 4.375 × 5.625 mm). We did not set to a usual smaller grid (e.g.: 1 × 1 × 1 mm) in order to minimize interpolation. Two warping steps were done as per https://www.fil.ion.ucl.ac.uk/spm/doc/spm8_manual.pdf. First, using the individual subject gray matter map in spectroscopic imaging space, a linear 12-parameter affine transformation was done to account for differences in position. Then a second non-linear warping was done to account for small anatomical differences. These warped maps were then visually inspected to insure a quality alignment using SPM12’s Check Reg function.

Quality filtering
To correct for partial volume effects, the metabolite maps are divided by 1-$f_{CSF}$, where $f_{CSF}$ is the fraction of CSF in each voxel. To ensure quality of the metabolite values from spectra, the metabolite maps were filtered to include spectra that met three criteria: overall linewidths between 2 and 12 Hz (inclusive); Cramér–Rao lower bound (CRLB) for spectral fitting of each metabolite of ≤20%; and $f_{CSF}$ ≤ 0.3 [18] (these were labeled best spectra). Spectra that failed to meet these qualifications were split into two groups, poor and intermediate spectra. Intermediate spectra had: linewidhts greater than 1 and less than or equal to 16; CRLB between 1 and 99, inclusively; $f_{CSF}$ ≤ 0.3; and at least 18 of the 26 nearest neighboring voxels (directly touching the voxel) were best spectra. Not all 26 surrounding voxels were required to be best spectra, because when intermediate spectra bordered CSF...
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Fig. 1  Summary of data processing steps (two rightmost columns, include software used), with corresponding descriptions (to the left) and representative illustrations. NAA is N-acetyl-aspartate, Glx is glutamate plus glutamine, t-Cr is creatine plus phosphocreatine and t-Cho is choline, glycerol-phospho-choline, plus phos-phocholine.

| Preprocessing Steps | Data Filtering Steps | Analysis Steps |
|---------------------|---------------------|----------------|
| **T₁ (MPRAGE)**  
{³H-MRSI (EPSI)} | **Acquisition** | **Statistics 1st level**  
(AFNI: 3dttest++ with ClustSim option) |
| **Processing of spectra (EPSI) data** | **Reconstruction**  
(MIDAS) | **Clustering of t-tests by adjacent 1st nearest neighbors in matching directionality with a p-value of ≤0.001** |
| **Water reference {³H-MRSI} to T₁** | **Registration**  
(MIDAS MSREG) | **Statistics 2nd level**  
(AFNI: 3dclust with examination of ClustSim output) |
| **{³H-MRSI**} | **Fitting**  
(MIDAS FITT2) | **Comparison of clusters and CCLAV voxel thresholds to determine significance level**  
(CCLAV ≤ 0.05) |
| Metabolite and T₁ maps to group space utilizing MNI GM template | **Warping**  
(SPM12) | **—** |

**Best spectra:**  
2 ≤ LW ≤ 12 and  
CRLB ≤ 20 and  
Fₐ₇ ≤ 0.3  
**Intermediate spectra:**  
1 ≤ LW ≤ 16 and  
0 ≤ CRLB < 100 and  
Fₐ₇ ≤ 0.3 and Not Best spectra  
**Surrounded by best spectra voxels (≥18/26 neighbors)**  
**Poor spectra:**  
Not Best or Intermediate spectra  

**Metabolite Group Mask of best or intermediate voxels present in ≥ 75% of subjects in each diagnostic group**  
**Group Mask Creation**  
(Matlab)  
**Metabolite maps by 10 mm**  
**Smoothing**  
(SPM12)  
**Imputed voxels in Metabolite Group Mask**  
**Poor spectra replaced with diagnostic group mean for voxel**  
**Imputation**  
(Matlab)  

**Voxels in brain: 11206**  
**Intermediate spectra:**  
395  
**Poor spectra:**  
2478  
**Best spectra:**  
8333  
**Intermediate (283⁰) and Best (623⁰) spectra in ≥75% of subjects**  
**Imputed spectra:**  
479  
**Metabolite Group Mask:**  
6994  

*Average number of voxels in spectroscopic space, per subject for Glx in the schizophrenia group.  

*Number of voxels in spectroscopic space in the Glx Metabolite Group Mask.
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spaces, about one third of neighboring voxels were expected to have poor spectra due to \( f_{SP} > 0.3 \). Intermediate spectra were considered to have some potential useful spectral information and hence their metabolite values were kept. All others were considered poor spectra and their metabolite values were removed.

Metabolite group mask
This mask was composed only of the best and intermediate spectra voxels that were present in at least 75% of subjects in each diagnostic group. We chose this 75% threshold through analyzing the distribution of voxel counts relative to possible thresholds for each metabolite in the data. As seen in Supplementary Fig. 2, both Glx and myo-inositol were the metabolites with the lowest median (75%) for the percentage of subjects with best or intermediate spectra in the metabolite group mask. This is expected since these are the two metabolites harder to fit. Hence, the 75% threshold was selected for the five group masks. Missing subject values in the metabolite group masks (Supplementary Fig. 3) were later imputed as described below.

Smoothing
These metabolite group masks were then smoothed using a spatially stationary Gaussian filter with a kernel width of 10 mm in SPM12 to minimize potential spatial warping errors [11]. The effective smoothing of the spectroscopic data in the X-Y plane is 15.8 mm and in the Z-plane is 19.6 mm.

Imputation
Because up to 25% of subjects in each diagnostic group did not have a particular voxel value in the metabolite group mask, the metabolite values for the poor spectra voxels were imputed using the diagnostic group (SP, HC) average concentration for that voxel. As the percentage of imputed voxels were clearly <10% (see Supplementary Table 1, row: Imputed voxels/Metabolite_Group_-Mask) on average across all metabolites and groups, the mean substitution imputation approach we selected, outperforms the removal of missing data [19]. This was done to insure we were reasonably preserving the maximum number of voxels possible for the analysis, without adding variability to the populations’ metabolite concentrations. Imputations only accounted for an average of between 2% and 7% of the total spectra in each metabolite group mask. We did not use AFNI’s 3dMEMA function, which can handle missing voxels, because it does not allow voxel level covariates (see: https://afni.nimh.nih.gov/MEMA*).

Statistical analyses, Level 1
The main analyses examined group differences separately for each of the five metabolites of interest with adjustments made on the voxel level for gray matter proportion \( \text{GM} \) and for age at the subject level, factors known to have large effects on metabolite’s variability [11]. AFNI’s 3dttest++ and 3dclustpackages were used which support subject and voxel-level covariates [12]. 3dtest++ implemented Student t-tests with an assumed equal variance for all voxels that fell within each metabolite group mask. Due to the significant smoothing of the metabolite data, we minimize FPR with permutation testing of 3dtest++’s ClustSim option [20]. ClustSim computed cluster-size thresholds using 10000 simulated noise-only t-tests for a more accurate spatial autocorrelation function of the noise. ClustSim then estimated the probability of false positive clustering given the sample and corrected the voxel clustering thresholds to account for this.

Statistical analyses, Level 2
The resulting maps were clustered using 3dclust following three criteria: (1) each voxel differed between the groups with \( p \)-value \( \leq 0.001 \); (2) group differences for each voxel in the cluster were in the same direction; and (3) voxels were nearest neighbors (faces touching). The corrected clustering thresholds from ClustSim were the number of voxels clustered in 3dclust to insure the cluster met a corrected cluster-level alpha-value (CCLAV) of \( 0.005 \).

Finally, Spearman’s Rho tested correlations between concentrations that differed between groups and clinical and cognitive assessments.

RESULTS
Demographics
Thirty-six SPs and 29 HCs participated (Table 1). Subject were similarly young (age/years, SP = 22.7 ± 3.9, HC = 23 ± 4.4, \( p = 0.78 \), with no significant differences in gender, race, cardiovascular risk, tobacco use and history of substance use disorders, except for cannabis. SP had greater diagnosis of past cannabis use \( (p = 0.01) \). SP also had worse personal \( (p < 0.001) \) and parental socio-economic status (SES; \( p = 0.002 \)) and lower MATRICS overall t-score \( (p = 0.001) \). Finally, 19 SP were naive to antipsychotic medications and 17 were treated. These subgroups did not differ in age of onset of psychosis, substance use histories, tobacco use, positive and negative symptoms severity, MATRICS overall t-score, age or the other demographic variables. The naive subgroup had a lower number of psychiatric hospitalizations than the treated SP \( (0.28 ± 0.5 \text{ vs } 1.3 ± 1.3, \text{ respectively, } p = 0.002) \).

Table 1. Demographic and clinical characteristics.

|                | Healthy controls \( n = 29 \) | Schizophrenia \( n = 36 \) | \( p \)-value |
|----------------|-------------------------------|-----------------------------|-------------|
| Age (years)    | 23 ± 4.4                      | 22.8 ± 3.9                  | 0.78        |
| Gender (male/female) | 17/12                      | 24/12                      | 0.5         |
| Race (Caucasian/black/native) | 26/1/2                      | 32/3/1                     | 0.5         |
| SES           | 4.1 ± 1.2                     | 5.9 ± 1.5                   | 0.001       |
| Familial SES  | 2.9 ± 1.6                     | 4.4 ± 1.8                   | 0.002       |
| Vascular risk score | 4.0 ± 0.2                   | 4.1 ± 0.2                   | 0.7         |
| MATRICS-overall T score | 48.4 ± 5.9                  | 34.0 ± 9.2                  | 0.001       |
| Smoker (yes/no) | 4/25                         | 8/28                        | 0.55        |
| Alcohol (yes/no) | 1/28                        | 3/33                        | 0.8         |
| Cannabis (yes/no) | 1/28                        | 18/18                      | 0.01        |
| Stimulant (yes/no) | 0/29                        | 2/34                        | 0.34        |
| Opioid (yes/no) | 0/29                         | 1/35                        | 0.5         |
| Sedative (yes/no) | 0/29                        | 0/36                        | 1.0         |
| Cocaine (yes/no) | 0/29                        | 2/34                        | 1.0         |
| Hallucinogens (yes/no) | 0/29                        | 2/34                        | 0.63        |
| Psychosis onset (years) | 20.5 ± 4.8                  | –                           | –           |
| Positive symptoms | 16.9 ± 5.0                  | –                           | –           |
| Negative symptoms | 15.7 ± 5.5                  | –                           | –           |
| Antipsychotic (yes/no) | 17/19                      | –                           | –           |
| Antipsychotic dose (mg) | 4.9 ± 7.1                    | –                           | –           |

\( * \)Socio-economic status.

\( ^{a} \)Vascular risk score, 0–4 (score of 1 each for cardiac illness, hypertension, dyslipidemia and diabetes).

\( ^{b} \)Antipsychotic dose, as olanzapine equivalents [47].
Neurometabolites differences

**Glutamate + glutamine.** One cluster (16 voxels) had lower Glx in SP vs HC's (CCLAV = 0.04; mainly in the left superior temporal gyrus-STG, middle temporal gyrus (MTG) and temporal white matter; Fig. 2a, Supplementary Fig. 1 and Supplementary Table 2). Both the antipsychotic-naïve (10/16 voxels; Fig. 2b) and the antipsychotic-treated SP's (8/16 voxels; Fig. 2c) had a similar area of reduced Glx when compared to the HC group; however, these contrasts (eg: antipsychotic-naïve vs, HC) did not meet CCLAV. Following co-variation for history of cannabis use or parental SES the cluster differences between SP vs HC's remained significant (CCLAV = 0.03 and 0.03, respectively).

**Total-Creatine**

There were two clusters, both with higher t-Cr in SP's vs HC's (Fig. 3a and Supplementary Fig. 1 and Supplementary Table 2). One involved 32 voxels mainly in the left STG, inferior parietal lobule (IPL), insula and superior longitudinal fasciculus (SLF). (CCLAV = 0.02). The second cluster involved 18 voxels mainly in the left middle temporal and angular gyri (CCLAV = 0.04). History of cannabis use or parental SES did not account for the clusters (CCLAV = 0.02 and 0.02, respectively for the first, and CCLAV = 0.02 and 0.04 respectively, for the second cluster). In the antipsychotic-treated subgroup, 32/32 voxels of the first cluster had higher t-Cr vs the HC group (Fig. 3b), while in the antipsychotic-naïve SP's only 1/32 voxels did (Fig. 3c). Also, for the second cluster, 14/18 voxels had increased t-Cr in the antipsychotic-treated group (Fig. 3b), but only 6/18 did in the antipsychotic-naïve SP's (Fig. 3c; none of these contrasts met CCLAV). In order to further explore the impact of antipsychotic exposure on t-Cr, independent contrasts between each SP subgroup and HC's were implemented. Antipsychotic-treated SP's had higher t-Cr than HC's in three clusters: (1) a larger left hemisphere cluster (100 voxels, CCLAV = 0.01; Supplementary Fig. 1 and Supplementary Table 2) involving left insula, postcentral gyrus, STG, IPL and SLF; (2) left and right cuneus (51 voxels, CCLAV = 0.01); and (3) left occipital (47 voxels, CCLAV = 0.01). No clusters were identified when comparing antipsychotic-naïve vs HC's.

**Other metabolites**

For NAAc, t-Cho and myo-inositol, there were no clusters that passed CCLAV in the contrasts between the SP vs HC groups. However, because of the large effects of medication exposure on t-Cr, we explored this variable for the other metabolites. In

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**Fig. 2 Reduced glutamine plus glutamate (Glx) in schizophrenia.** Row a Reduced Glx (shown in red) in schizophrenia (SP) compared to healthy controls (HC) in one 16 voxel cluster (CCLAV = 0.04), mainly in the left superior temporal gyrus-STG and the middle temporal gyrus (MTG; see x, y, z, the MNI-based, cluster-center coordinates). Row b 10/16 voxels (shown in purple) have reduced Glx (p ≤ 0.001, at the voxel-level) in antipsychotic-naïve schizophrenia vs, healthy controls. Blue voxels also have reduced Glx but are not part of the original significant cluster, which is shown in red and purple. Row c 8/16 voxels (shown in purple) have reduced Glx (p ≤ 0.001) in antipsychotic-treated schizophrenia vs, healthy controls. Blue voxels also have reduced Glx but are not part of the original significant cluster, which is shown in red and purple.
antipsychotic-treated SP’s vs HC’s, t-Cho was increased mainly in left middle frontal gyrus (18 voxels, CCLAV = 0.04; Fig. 4a, Supplementary Fig. 1, and Supplementary Table 2). Finally in antipsychotic-naïve patients, NAA was reduced in mainly right medial, superior frontal gyri and middle frontal white matter (19 voxels, CCLAV = 0.05; Fig. 4b) and myo-inositol was reduced in the left cerebellum (34 voxels, CCLAV = 0.02; Fig. 4c). History of cannabis use or parental SES did not account for these cluster differences (for t-Cho, CCLAV = 0.03 and 0.04, respectively; for NAA, CCLAV = 0.05 and 0.04, respectively; for myo-inositol, CCLAV = 0.02 and 0.02, respectively).

Finally, the neurochemicals in the clusters affected in SP’s (Glx and t-Cr) did not correlate (Spearman’s Rho) with cognition (full sample) or symptoms (only in SP).

**DISCUSSION**

This is the first ¹H-MRS study to examine Glx as well as the other commonly measured neurometabolites in schizophrenia, with a data-driven 3-dimensional imaging approach that accounts for false positive rates. Contrary to our hypothesis, Glx was not increased in the dorsal anterior cingulate (due to suboptimal coverage, we could not test the dorsal striatum). Only one cluster differed between groups: the left STG/MTG had reduced Glx in SP’s, and this reduction was apparent in antipsychotic-naïve as well as in medicated patients, though the subgroup comparisons were not statistically significant. The only other neurometabolite affected was t-Cr, which was higher in SP’s than HC’s in two clusters in the left hemisphere (only in the antipsychotic-treated subgroup). When contrasting the medicated SP’s with HC’s, three much larger clusters became apparent: again, mainly in the left hemisphere, suggesting that antipsychotic exposure may largely account for the increments in t-Cr. Additionally in medicated SP’s, t-Cho was increased in a left frontal region. Finally, NAA and MINO were reduced only in antipsychotic-naïve SP’s vs HC’s. Given these novel findings, this study demonstrates the feasibility and utility of 3-dimensional EPSI to explore schizophrenia vs antipsychotic-related neurometabolic findings, in a manner that would not be possible with single-voxel ¹H-MRS.

Only two ¹H-MRS studies have investigated the STG in schizophrenia. One examined chronically-treated schizophrenia and bipolar-I (BP-I) patients with single voxels over STG bilaterally at 3T [21]. They found reduced glutamate in the left STG in BP-I but only marginal reductions in schizophrenia (p = 0.1). The other study used 2-dimensional ¹H-MRSI at 1.5T and failed to detect Glx differences in STG [22]. The STG has been implicated in the development of psychosis in structural MRI studies. Longitudinal STG volume reductions were reported in ultra-high-risk subjects who converted to psychosis, but not in non-converters [23]. Furthermore, first episode psychosis subjects also had progressive STG reductions which were more prominent in the left side and correlated with severity of delusions [23]. Functional and pharmacological studies have further implicated the STG in schizophrenia. Acute NMDAR hypofunction, a model for schizophrenia, reduces mismatch negativity (MMN), an evoked response which occurs in the...
process of comparing sound deviations, and that originates from STG [24]. MMN is impaired in schizophrenia, as well as in high-risk subjects who convert to psychosis [25]. Also, acute NMDAR hypofunction with ketamine induces schizophrenia-like symptoms and cognitive deficits in healthy volunteers [25]. Acute ketamine also increases glutamate in medial frontal cortex [26]. However, a 1 month exposure of low dose (2.58 mg/kg/day) phencyclidine resulted in medio-frontal glutamate reductions in rats [27]. Schizophrenia, even amongst most “first episode” patients, is a chronic illness, so acute ketamine exposure may not adequately model the persistent developmental effects of NMDAR hypofunction. In postmortem studies, increments in calcineurin, a protein activated by Ca\(^{++}\) inflow through NMDAR, were reported in STG across layers II–VI [28]. Also increased protein insolubility was found in STG and frontal cortex in schizophrenia [29]. Recently, reductions in synaptic levels of post-synaptic excitatory proteins were reported in primary auditory cortex of schizophrenia [30], consistent with synaptic glutamatergic hypofunction. Hence, this literature converges with our findings to support that left STG abnormalities with early hypoglumatergic dysfunction may be a trait characteristic of schizophrenia. The reduced Glx may be secondary to dendritic retraction (seen in post-mortem studies [31]), caused by a “cold” excitotoxic process due to bouts of increased synaptic glutamate. Alternatively, reduced Glx in left STG may represent the effects of a primary NMDAR hypofunction in a region critical for sound discrimination and language. Though some single-voxel studies have reported increased t-Cr [32, 33], these have not been replicated [34]. However, an \(^1\)H-MRSI study of the hippocampus revealed increased t-Cr in chronically-medicated schizophrenia [35]. We found increased t-Cr only in antipsychotic-treated patients. Antipsychotics are known to cause acute changes increasing subcortical and decreasing cortical metabolism [36, 37]. Furthermore, a reduction in the forward rate constant of creatine kinase, the enzyme that converts Cr to P-Cr, was reported in medicated schizophrenia [38]. Hence, it is possible that the increase in t-Cr represents an adaptation to the lowering cortical metabolic effects of antipsychotics, by increasing the total pool of creatine available for energetic transfer. Regarding t-Cho, alterations have not been consistently reported in single-voxel studies [34]. We speculate that increased t-Cho only in treated patients suggests an adaptive glial response to antipsychotics in the left medial frontal cortex. Investigations on inositol in schizophrenia have been sparse but a recent meta-analysis reported reductions in medial frontal regions in predominantly treated patients [39]. However, cerebellar or other regions were not examined. Our results suggest reduced cerebellar glial function in schizophrenia, but will require replication.

Fig. 4 Abnormalities in total choline (t-Cho), \(N\)-acetylaspartate (NAA) and myo-inositol (MINO) in schizophrenia. Row a Increased t-Cho (shown in pink) in antipsychotic-treated SP vs HC in one cluster (18 voxels, CCLAV = 0.04) in mainly the left middle frontal gyrus. Row b Reduced NAA (shown in green) in antipsychotic-naive SP vs HC in one cluster (19 voxels, CCLAV = 0.05) in mainly right medial, superior frontal gyri and middle frontal white matter. Row c Reduced myo-inositol (shown in light blue) in antipsychotic-naive SP vs HC in one cluster (34 voxels, CCLAV = 0.02) in mainly the left cerebellum.

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reduced in the largest study of medicated first episode psychosis patients at 7T [40]. These results were replicated in a smaller sample of treated early schizophrenia subjects also at 7 T [41]. Our findings of dAC NAA reduction only in the antipsychotic-naïve subgroup suggests a normalizing effect of treatment (only 3/19 voxels in the reduced cluster, were significantly lower (p < 0.001) in antipsychotic-treated SPs vs HC). It is also possible that the better signal-to-noise at 7T allows the detection of persistent NAA reductions in the dAC in treated patients [40, 41].

Although a few previous studies have used whole-brain 1H-MRSI in schizophrenia, we are not aware of any using a TE short enough to examine Glx. One reported lower NAA in broad white matter regions in chronically ill subjects [42]. Another found reduced NAA/t-Cr but examined only selected voxels overlapping with the hippocampus and the dorsolateral prefrontal cortex [43]. Neither of the previous whole-brain 1H-MRSI studies systematically examined all voxels acquired. To do so, normalization of all voxels in a common space and accounting for multiple comparisons are necessary, which are standard in other neuroimaging modalities (eg: MRI, DTI, fMRI). In the present study we demonstrate that these methods can be applied to 3-dimensional 1H-MRSI.

Our findings did not support the hypothesized Glx increase in dAC and in the dorsal striatum in schizophrenia. Unfortunately there were not enough voxels with good quality spectra encompassing the dorsal striatum to measure Glx (see mask Fig. 1). For the dAC, we did have adequate coverage. In this region two recent and much larger studies reported opposite changes in medicated samples: increased Glx in schizophrenia (SP’s, N = 104 [6]) and reduced glutamate in first episode psychosis (Psychoses, N = 81 [40]). The present study, which included young individuals with differences in antipsychotic exposure, may have been underpowered to detect dAC group differences.

This study has several strengths. Volumetric EPSI allowed examination of most of the brain including numerous gray and white matter regions known to be involved in schizophrenia [44]. Voxels were automatically selected based on standard spectral quality criteria. We chose AFNI for analyses because it allows introduction of co-variates at the voxel level, a critical issue in 1H-MRSI analyses since the gray/white matter tissue effect is much larger than age or diagnostic group effects [6]. The sample studied included similar numbers of antipsychotic-naïve and treated young schizophrenia subjects. However, limitations must be acknowledged. First, the spectral resolution obtained at 3T did not allow reliable discrimination of glutamine from glutamate. Second, though whole-brain EPSI was acquired, brain areas with greater magnetic field inhomogeneity (eg: orbitofrontal regions, frontal and temporal poles and base of the brain, including dorsal striatum) did not yield consistent spectral quality and so could not be included in analyses. These are regions potentially important for schizophrenia. Although more severe, these challenges are not exclusive to three-dimensional 1H-MRSI. For example, other more standard neuro-imaging modalities in the Connectome Project, also found suboptimal brain coverage in some of these regions [45]. Clearly, single-voxel 1H-MRSI allows a scanning protocol to be tailored to a specific brain region, making data from these difficult regions more accessible. However, in schizophrenia structural studies detect involvement of a majority of the brain early in the illness [2]. Therefor spatial coverage in the several thousands of voxels offers some advantages over the usual single-voxel approaches. Third, our samples of 19 medication-naïve and 17 treated SP are relatively small, and this is particularly relevant to the likelihood of type-II error in the examination of the dAC. Medication status can clearly have an effect [46]. Fourth, we did not statistically correct for examining five metabolites. Finally, this was a cross-sectional study, so mechanistic interpretations are inherently limited.

In summary, a data-driven examination with three-dimensional EPSI in early schizophrenia revealed a reduction in glutamate metabolism (Glx) in the left STG/MTG regardless of antipsychotic status and a larger mostly left-sided area of increased t-Cr, only in medicated patients. Replication of these findings would support mechanistic postmortem studies and therapeutic neuromodulation studies of the left STG/MTG in schizophrenia. Also, the feasibility of our three-dimensional brain 1H-MRSI analysis approach is demonstrated in a psychiatric population. Finally, caution is warranted when referencing 1H-MRSI metabolite values to t-Cr in studies of treated schizophrenia populations.

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JRB received royalties from Up-to-date. JU reported no biomedical financial interests or potential conflicts of interest. GM reported no biomedical financial interests or potential conflicts of interest. TJ reported no biomedical financial interests or potential conflicts of interest. AAM reported no biomedical financial interests or potential conflicts of interest. MT was an employee of Lilly (1997–2008) and has received honoraria from or consulted for Abbott, AstraZeneca, Alkermes, Allergan, Bristol Myers Squibb, GlaxoSmithKline, Lilly, Johnson & Johnson, Otsuka, Merck, Gedeon Richter Plc, Sunovion, Forest, Roche, Elan, Lundbeck, Teva, Pamlab, Minerva, Neurocrine, Pfizer, Wyeth and Wiley Publishing; his spouse was a full time employee at Lilly (1998-2013). CG reported no biomedical financial interests or potential conflicts of interest. RL reported no biomedical financial interests or potential conflicts of interest. Supported by NIMH R01MH084898 to JRB and 1 P20 RR021938-01A1 and DHHS/NIH/NCRN 3 UL1 RR031977-02S2. The MIDAS software was supported by R01 EB016064. The authors declare no competing interests.

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**AUTHOR CONTRIBUTIONS**

JRB was involved in the design, acquisition, analysis, interpretation of data for the work, drafting, and revising of the paper. JU was involved in the analysis, interpretation of data for the work, drafting and revising of the paper. GM was involved in the analysis, interpretation of data for the work, drafting, and revising of the manuscript. TJ was involved in the analysis, interpretation of data for the work, drafting, and revising of the paper. AAM was involved in the interpretation of data for the work, drafting and revising of the paper. MT was involved in the interpretation of data for the work, drafting and revising of the paper. CG was involved in the interpretation of data for the work, drafting, and revising of the paper. RL was involved in the design, acquisition, analysis, interpretation of data for the work, drafting and revising of the paper.

**ADDITIONAL INFORMATION**

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