Supplementary Online Content


**eMethods.** Supplemental Methods

**eFigure 1.** Full Model

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This supplementary material has been provided by the authors to give readers additional information about their work.
Participant and study details
SSD participants were either schizophrenia (n=40) or schizoaffective disorder (n=5) as determined with the Structured Clinical Interview for DSM-IV-TR, Patient Version. Controls had no past or present Axis I psychiatric disorder as determined with the Structured Clinical Interview for DSM-IV-TR, Non-Patient Version. Age and sex were frequency matched between patients and controls. We also made attempt to frequency-match the smoking status between patients and controls. The patient group was a mixture of early and chronic schizophrenia with an average length of illness of 14.7 (12.1) years. Four patients were not on antipsychotics and the rest were on antipsychotic medications, including 3 on typical, 34 on atypical, and 4 on a combination of atypical and typical antipsychotics. Of the atypical antipsychotics, 17 were on clozapine, 3 on risperidone, 8 on olanzapine, and 10 on quetiapine. Patients on daily use of benzodiazepines or nonbenzodiazepine GABAergic hypnotics were excluded.

The GABA data from 68% of the participants in a previous study reporting an age effect on GABA\(^1\) overlapped with the current sample. Imaging, electrophysiology, and cognitive testing/clinical assessments were conducted within a 2-week window on separate days, although scheduling outside of the window was not exclusionary. The order of testing varied based on laboratory and participant availability. There were no significant differences in overall time intervals among different tasks between patients and controls (p=0.51). Patients with a change of medication during the study were excluded.

ERP Recording and Analysis
Subjects sat in a semi-reclining chair inside a sound-attenuated chamber. Subjects were presented with 1000 auditory stimuli, of which 800 (80%) were standard tones presented at 75 dB, 60-msec, 1000 Hz; 200 (20%) were duration-deviant tones at 75 dB, 150-msec, 1000 Hz. All tones had a 5 msec rise/fall time, with a stimulus onset asynchrony of 300 msec. Subjects were asked to ignore the tones while viewing a silent movie. A nose electrode served as reference. Electrode impedance was kept below 5 kΩ. FZ was used for MMN measurement because this location typically shows the largest patient-control differences on MMN\(^2\,^3\). Eyeblink artifacts were minimized using a VEOG-based eyeblink spatial filter routine implemented in Neuroscan software. Records were then filtered at 0.1–
30 Hz\(^3\) in 24db/octave, epoched, baseline-corrected, threshold-filtered at ±75 μV for artifact rejection, followed by visual inspection to exclude any overt artifacts from muscle contraction. Over 90% of standard and deviant trials were retained after these procedures in each subject and there were no statistical significant differences in the number of trials retained between schizophrenia patients and normal controls for either standard or deviant trials (all p's > 0.05). Standard and deviant trials were averaged separately, followed by a subtraction of the two averaged waveforms. MMN was scored by peak detection within a 100–225 msec post-stimulus window by an automatic algorithm followed by visual inspection to verify correct placement of each marker for peak detection. Scoring was blinded to clinical and MRS information.

**MRS Acquisition and Analysis**

MR scanning was conducted on a 3T Siemens Tim Trio equipped with a 32-channel head coil. Head position was fixed with foam padding to minimize movement. Anatomical T1-weighted images were acquired for spectroscopic voxel placement with a ‘MP-RAGE’ sequence. The spectroscopic voxel was 4.0 X 3.0 X 2.0 cm\(^3\) prescribed on the midsagittal slice and positioned parallel to the genu of the corpus callosum and scalp with the midline of the voxel corresponding to the middle of the genu of the corpus callosum. The center of this voxel was approximately positioned below the electrode FZ and the voxel covered the medial frontal and anterior cingulate cortex. Automated shimming followed by manual shimming was conducted to achieve approximately 12hz water linewidth.

For detection of glutamate, spectra were acquired with a phase rotation STEAM (PR-STEAM): TR/TM/TE = 2000/10/6.5-msec, VOI ~ 24-cm\(^3\), NEX=128, 2.5-kHz spectral width, 2048 complex points, and phases: \(\varphi_1=135^\circ\), \(\varphi_2=22.5^\circ\), \(\varphi_{13}=112.5^\circ\), \(\varphi_{ADC}=0^\circ\), which has been shown to be reproducible in healthy volunteers\(^4\) and participants with schizophrenia\(^5\). A water reference (NEX=16) was also acquired for phase and eddy current correction as well as quantification. A basis set of 19 metabolites was simulated using the GAVA software package (17): alanine (Ala), aspartate (Asp), creatine (Cr), \(\gamma\)-aminobutyric acid (GABA), glucose (Glc), glutamate (Glu), glutamine (Gln), glutathione (GSH), glycine (Gly), glycerophosphocholine (GPC), lactate (Lac), myo-Inositol (ml), N-acetylaspartate (NAA), N-acetylaspartyglutamate (NAAG), phosphocholine (PCh), phosphocreatine (PCr), phosphoroylethanolamine (PE), scyllo-Inositol (sl), and taurine.
(Tau). The basis set was imported into LCModel (6.3-0D) and used for quantification. Metabolite levels are reported in institutional units, and metabolites with Cramer Rao Lower Bounds (CRLBs) ≤ 20%, except for glutamine that was expanded to CRLB <30%, were included in statistical analyses. Expanding the criterion of CRLB for glutamine is an approach to allow for inclusion of the majority of data while maintaining reasonable quality fitting, and is documented in the literature. Spectral quality was good with signal-to-noise ratios of 36.5 (12.3) for controls and 33.0 (7.8) for patients and full-width-half-maximum (linewidth) of 0.035 (0.01) for controls and 0.037 (0.01) for patients. Metabolite fits were good as reflected by the following CRLBs for controls and patients, respectively:
glutamate- 4.9% (1.1%), 5.5% (1.4%); glutamine-17.6% (3.6%), 19.0% (4.7%); glutathione-7.9% (1.5), 8.8% (2.3%); n-acetyl aspartate- 2.7% (0.7%), 3.0% (0.8%); creatine- 2.7% (0.7%), 2.9% (0.8%); myoinositol- 3.6 (0.7%), 4.1% (1.1%), choline- 4.9% (1.3%), 5.3% (1.5%). Two participants with schizophrenia and 1 control did not have glutamine values due to fits being outside the acceptable range.

For detection of GABA, spectra were acquired from the same voxel using a macromolecule-suppressed MEGA-PRESS sequence: TR=2000, TE=68 ms, 20.36 msec length and 44 Hz bandwidth full width at half maximum (FWHM) editing pulses applied at 1.9 (ON) and 1.5 (OFF) ppm, and 256 averages (128 ON and 128 OFF); 86 water unsuppressed 16 averages. Water suppression was achieved using Siemens modified WET water suppression technique. GABA quantification was conducted with GANNET 2.0 toolkit, a Matlab program specifically developed for analysis of GABA MEGA-PRESS spectra. Individual spectra were frequency and phase corrected, and then “ON” and “OFF” subtracted resulting in the edited spectrum. The edited GABA peak was modeled as a single-Gaussian and values of GABA relative to water (modeled as a mixed Gaussian-Lorentzian) in institutional units were produced. The normalized fitting residual was calculated by dividing the standard deviation of the fitting residual by the amplitude of the fitted peak. Spectra were included if the normalized fitting residual of GABA was below 15%. The average normalized fitting residuals were 6.8% (1.8%) for controls and 7.3 (2.0%) for patients.

The spectroscopic voxel was segmented into gray, white, and CSF tissues using SPM8 and in house MATLAB code, and metabolite concentrations were corrected for the proportion of gray, white, and CSF tissue proportions. There were group differences in
the proportion of CSF such that controls had slightly more (1.1%) CSF than patients (p = 0.023), and white matter such that controls had slightly less (1.8%) white matter than patients (p = 0.018). Gray matter proportion was not significantly different between groups (p=0.29).

Structural Equation Modeling
The model with the best fit is presented in the main manuscript. Full and alternative models were tested. The full model is presented in Supplementary Figure 1. The full model did not fit well in either group and was rejected (schizophrenia group: RMSEA=0.162, AIC=28.0, chi-square: p <0.001; control group: RMSEA: 0.068, AIC=28.0, chi-square: p <0.001). The first alternative model with the direct path Gln/Glu to DST is presented in Supplementary Figure 2. Similar to the full model, this alternative model had a less preferred fit than the optimal model (schizophrenia group: RMSEA=0.162, AIC=29.7, chi-square: p=0.05; control group: RMSEA=0.068, AIC=26.9, chi square: p=0.3,) and was rejected. The second alternative model without the direct paths Gln/Glu to DST and GABA to DST is presented in Supplementary Figure 3. This model also showed a less preferred fit than the optimal model in both groups (schizophrenia group: RMSEA= 0.162, AIC=27.8, chi-square: p=0.37; control group: RMSEA=0.068, AIC=27.7, chi-square: p=0.37) and was rejected.

eReferences


eFigure 1. Full Model

A. Schizophrenia Group: Full Model

B. Control Group: Full Model
eFigure 2. Alternative Model

A. Schizophrenia Group: Alternative Model

B. Control Group: Alternative Model
eFigure 3. Alternative Model

A. Schizophrenia Group: Alternative Model

B. Control Group: Alternative Model