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Effects of acute N-acetylcysteine challenge on cortical glutathione and glutamate in schizophrenia: A pilot *in vivo* proton magnetic resonance spectroscopy study

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ABSTRACT

Findings from in vivo brain proton magnetic resonance spectroscopy (^{1}H MRS) and preclinical studies have suggested region- and medication status-dependent increases in glutamate (Glu) levels and deficiencies in glutathione (GSH) levels in schizophrenia. N-acetylcysteine (NAC), a GSH synthesis precursor, has demonstrated modest clinical benefit in schizophrenia. The objective of this study was to examine the effects of acute administration of NAC on GSH and Glu levels measured with ^{1}H MRS in 19 patients with schizophrenia and 20 healthy control subjects. Levels of GSH were acquired in dorsal anterior cingulate cortex (dACC), and those of Glu in dACC and medial prefrontal cortex (mPFC), at baseline and 60 min following acute oral administration of 2400 mg of NAC. No differences in the levels of GSH or Glu were found at baseline or following NAC administration between patients with schizophrenia and control subjects in either of the targeted brain regions. Future studies measuring GSH levels in brain regions previously found to exhibit glutamatergic abnormalities or using genetic polymorphisms, while controlling for the age and medication status of the cohorts, are warranted to better identify groups of patients more likely to respond to NAC and its mode of action and mechanisms.

1. Introduction

Glutathione (GSH; γ-L-glutamyl-L-cysteinylglycine), a non-protein thiol and the primary antioxidant in living tissue, protects the brain against redox imbalance and associated oxidative stress (Baker et al., 2008; Dean et al., 2011). Disturbances of GSH metabolism have been linked to schizophrenia (SCZ). Initial attention was drawn to the GSH system by postmortem findings of GSH deficits in the prefrontal cortex (PFC) of patients with SCZ (Gawryluk et al., 2011), and in cerebrospinal fluid and PFC of patients with SCZ in vivo using proton magnetic resonance spectroscopy (^{1}H MRS) (Do et al., 2000; Gawryluk et al., 2011). Experimentally-induced GSH deficits in rodents are associated with impairments in long-term potentiation (LTP) and N-methyl-D-aspartate receptor (NMDAR) dysfunction (Steullet et al., 2006), and may also contribute to cognitive deficits, suggesting a role of GSH depletion in cognitive impairments in SCZ (Cruz-Aguado et al., 2001; Dean et al., 2009). These findings suggest that raising brain GSH levels may be therapeutically beneficial in SCZ.

Due to limits on the transport of GSH across the blood-brain barrier (Kannan et al., 1990), its brain levels cannot be efficiently raised by dietary supplementation (Zeevalk et al., 2007,2008). Cysteine, a primary amino acid, is rate limiting for GSH synthesis. However, cysteine is highly toxic and is, thus, unsuitable for direct supplementation as a GSH synthesis precursor. Therefore, N-acetylcysteine (NAC), a prodrug for cysteine, has been proposed and investigated as a more viable approach for stimulating the in situ synthesis and elevations of brain GSH (Atkuri et al., 2007; Baker et al., 2008). Importantly, NAC has a relatively high bioavailability, is safely used for a number of medical

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conditions (e.g., acetaminophen toxicity, HIV infection, and pulmonary diseases) and is established to provide therapeutic benefit through various mechanisms with no clinically significant adverse effects at oral doses up to 8 g/day (Atkuri et al., 2007). Furthermore, preclinical evidence suggests that restoring GSH levels with NAC may be therapeutic in SCZ. Mice with a chronic deficit in GSH induced by knocking out the gene for the glutamate-cysteine ligase modulatory subunit (GCLM) demonstrated elevations in anterior cortical glutamate and glutamine levels that were similar to those that have been observed in unmedicated patients or early SCZ (das Neves Duarte et al., 2011). Treating these mice with NAC normalized glutamate and glutamine levels (das Neves Duarte et al., 2011). A primary molecular target of NAC in the brain is the cystine-glutamate antiporter, which maintains extrasynaptic glutamate levels by exchanging cystine (a sulfur-linked cysteine dipeptide) with glutamate (Awad et al., 2000; Baker et al., 2002; Benquet et al., 2002; Jabaudon et al., 1999; Lipton et al., 2002; Sucher and Lipton, 1991; Varga et al., 1997). In a rodent model, NAC treatment prevented the acute PCP-induced increase of extracellular glutamate, reversed social deficits and working memory impairments that occurred after PCP exposure, and was dependent on cystine-glutamate exchange for these effects (Baker et al., 2008).

NAC has also been evaluated for its ability to ameliorate symptoms in patients with SCZ or psychosis (Berk et al., 2008; Conus et al., 2018; Gray et al., 2017; Lavoie et al., 2008; Rapado-Castro et al., 2017; Sepehrmanesh et al., 2018; Skvarc et al., 2017). In an exploratory clinical trial, Berk et al., (2008) randomized 140 medicated patients with SCZ and residual symptomatology to either placebo or 1 g b.i.d. NAC for 24 weeks. Patients who received NAC showed greater improvements on the PANSS total, general, and negative subscales, as well as on the CGI-S scale (effects sizes 0.43–0.57). Conus et al. (2018) randomized 63 subjects with early psychosis to either 2700 mg of NAC or placebo as add-on treatment for 6 months and observed no changes in symptoms, but did observe some improvements in neurocognitive measures, as well as increases in medial PFC (mPFC) GSH in the NAC group with lower levels of mPFC GSH at baseline. Similar to other NMDA modulating compounds (Kantrowitz, 2018), NAC has also been shown to improve mismatch negativity (MMN) impairments (Lavoie et al., 2008) in an 11-patient, randomized, double-blind, crossover study of NAC (2000 mg/day) and placebo for 60 days each. Further, levels of GSH have been reported to be abnormally low in prefrontal cortex in SCZ (Do et al., 2000; Kumar et al., 2018) and in first-episode psychosis (Wang et al., 2019), although other studies in SCZ (Matsuzawa et al., 2008; Terpstra et al., 2005) or in individuals at clinical high risk for psychosis (Da Silva et al., 2018) failed to find any GSH deficits.

The first step in the in situ synthesis of GSH involves a GCL-catalyzed reaction of cysteine and glutamate to form γ-glutamylcysteine, which potentially associates the glutamatergic and GSH systems. Levels of PFC glutamate (Glu), glutamine (Gln), or their combination (Glx) have been found to be abnormal in SCZ. ^{1}H MRS studies of the glutamate system in first episode, medication-naïve patients (Bartha et al., 1997; Theberge et al., 2002; Theberge et al., 2007), unmedicated patients (Kegeles et al., 2012), or unmedicated subjects at high risk for SCZ (de la Fuente-Sandoval et al., 2015; Merritt et al., 2016; Tibbo et al., 2004), have generally found elevations of glutamatergic compounds in the mPFC. Studies of medicated patients have shown variable glutamatergic levels (Poels et al., 2014). In a recent study, elevated baseline levels of mPFC and striatal Glx were found to decrease and normalize following 4 weeks of antipsychotic treatment in first-episode psychosis patients (de la Fuente-Sandoval et al., 2018), consistent with a medication effect on Glx levels.

These clinical MRS studies and preclinical literature suggest that treatment with NAC is likely to raise GSH levels and lower Glu levels in the PFC, but only in participants with abnormally low GSH levels at baseline (Conus et al., 2018). Therefore, we examined the effect of NAC challenge on PFC Glu and GSH in medication-free patients with SCZ and healthy control subjects with the hypothesis of normalization of mean Glu levels and elevations of mean GSH levels in patients, and no effects in control subjects.

2. Methods

2.1. Participants

Subjects enrolled in this study were medically healthy, psychiatrically stable (i.e., not an acute safety risk, able to tolerate a medication free interval), non-substance using adult (ages 18–59) patients with SCZ or schizoaffective disorder. Diagnoses were confirmed with the Structured Clinical Interview for DSM-IV Axis I Disorders (First et al., 1996) or Diagnostic Interview for Genetic Studies (Nurnberger et al., 1994). Patients with SCZ, who were recruited from the Lieber Schizophrenia Research Clinic at Columbia University and the New York State Psychiatric Institute (NYSPI), were antipsychotic-free for at least 21 days prior to the neuroimaging scans. Matched healthy subjects with no psychiatric or substance use history, who had never taken psychiatric medications and were non-substance-using, were also recruited to serve as the normal control group. All participants provided written informed consent after receiving oral and written descriptions of the study procedures and aims. The study was approved by the Institutional Review Board of the New York State Psychiatric Institute.

2.2. Study design and intervention

After a screening visit, all subjects who met the inclusion criteria were asked to return for their MRS scans, during which they participated in one baseline MRS scan, followed by a single-dose NAC challenge, and then one hour later by a second identical MRS scan.

A previous clinical trial in patients with SCZ using 2000 mg/day for 24 weeks found that no adverse effects occurred more frequently in patients taking NAC than those taking placebo (Berk et al., 2008). Much higher doses of NAC (e.g., 140 mg/kg) are regularly given for acetaminophen poisoning, prevention of contrast-induced nephropathy, or for viscid mucous secretions in a variety of pulmonary conditions. One study administered 150 mg/kg IV of NAC as a constant infusion over 60 min and demonstrated a Tmax of 0–15 min after the end of the infusion for blood GSH, and 30–50 min after the end of the infusion for brain GSH (Holmay et al., 2013). A single challenge dose of 2400 mg NAC po was administered in a clinical population exhibiting elevated Glu with resulting normalization of Glu levels detected by MRS beginning one hour after dose administration (Schmaal et al., 2012). Therefore, we administered a single dose of 2400 mg po and scanned from 60–120 min after NAC administration.

2.3. Magnetic resonance spectroscopy

All neuroimaging studies were conducted on a research-dedicated General Electric 3.0 T EXCITE MR system at NYSPI. Structural MRI consisting of a series of high-resolution scans of standardized axial, coronal and sagittal T1-, T2-, and spin density-weighted scans, with slices appropriately obliqued for prescribing the ^{1}H MRS voxel, was conducted. In addition, a T1-weighted spoiled gradient-recalled echo (SPGR) volumetric scan (TR/TE 30/8 ms, flip angle 45°, field of view 24 cm, 256 × 256 matrix, 124 coronal slices and a slice thickness of 1.0 mm) was acquired for brain tissue segmentation.

Levels of GSH and Glu were obtained from a 4.5 cm × 3.0 cm × 1.5 cm-voxel in the dorsal anterior cingulate cortex (dACC) parallel and superior to the dorsal anterior surface of the corpus callosum and centered on the interhemispheric fissure (Fig. 1B). In addition, Glu levels were acquired from a 2.5 cm × 3.0 cm × 2.5 cm-voxel in the medial PFC (mPFC), prescribed to contain portions of Brodmann areas 24, 32 and 10, including the pregenual anterior cingulate cortex (Fig. 2A).

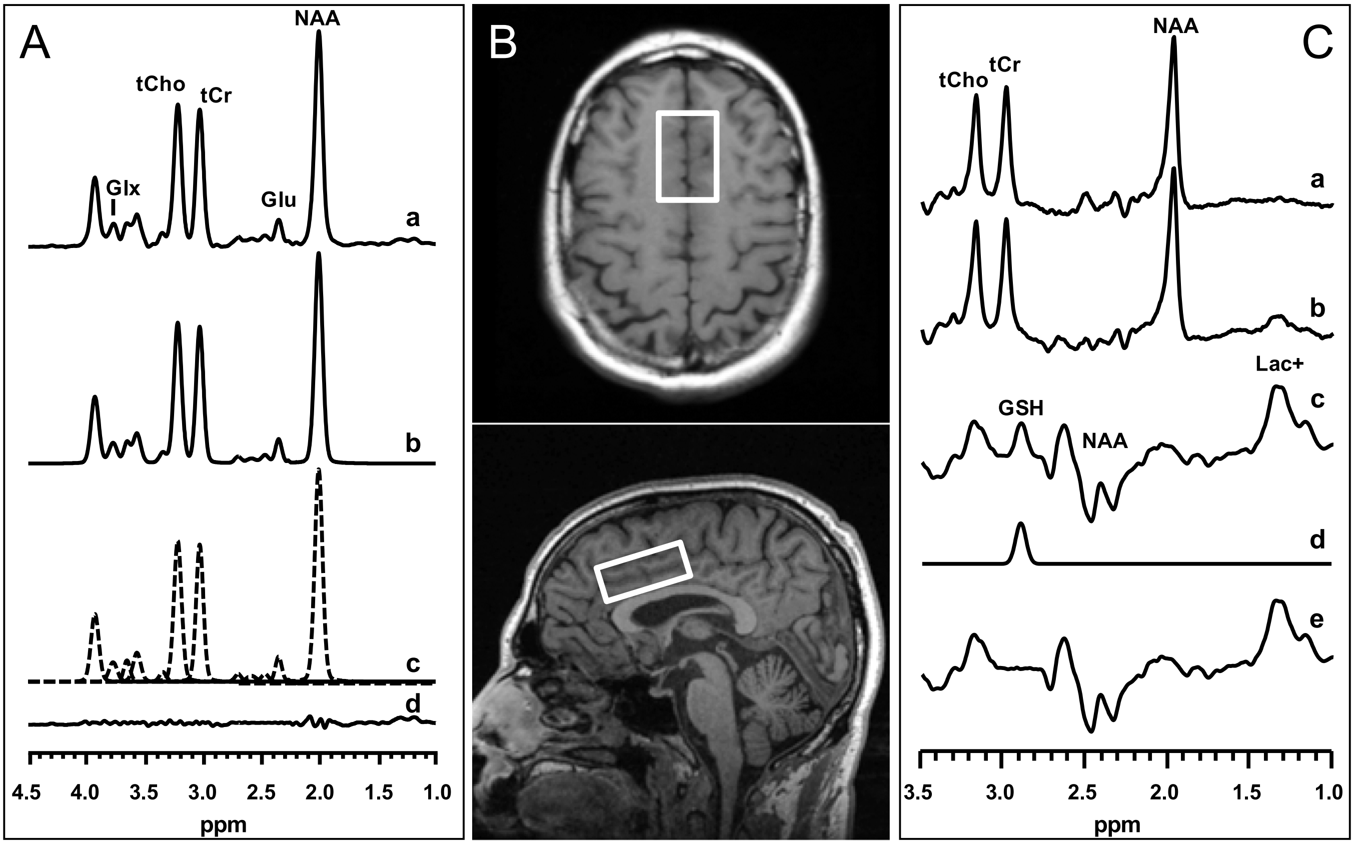

Fig. 1. Dorsal ACC glutamate and glutathione acquisitions
[A] Sample CT-PRESS MRS data from a dACC voxel, depicting (a) an experimental spectrum showing a clearly resolved C-4 glutamate (Glu) resonance at 2.35 ppm, as well as the resonances for N-acetyl-L-aspartate (NAA), total creatine (tCr), total choline (tCho) and combined resonances of C-2 glutamate and C-2 glutamine (Glx); (b) model fitting of spectrum (a) to obtain the metabolite peak areas of interest; (c) individual components of the model-fitted spectrum (a); (d) residuals of the difference between spectra (a) and (b). [B] Oblique axial (top) and midsagittal (bottom) images showing the dACC voxel size and location. The voxel is located superior and parallel to the superior, anterior surface of the corpus callosum, centered on the interhemispheric fissure. Dimensions are 4.5 cm (anterior-posterior) x 3.0 cm (left-right) x 1.5 cm (superior-inferior), and volume is 20.25 cc. [C] Demonstration of dorsal anterior cingulate cortex glutathione (GSH) detection with J-edited ^{1}H MRS and TE/TR 68/1500 ms: (a) and (b), single-voxel subspectra acquired with the editing pulses on and off; (c), difference between spectra (a) and (b) showing the edited brain GSH resonance at 2.98 ppm; (d), selective model fitting of the GSH resonance in spectrum (c) to obtain the GSH peak area; (e), residual of the difference between spectra (c) and (d). Also detected are the resonances for NAA, tCho, tCr, and lactate (Lac) contaminated with residual lipid signals (hence Lac +).

To acquire GSH data, the standard J-edited spin echo difference MRS method (Geramita et al., 2011; Rothman et al., 1993; Shungu et al., 2016; Shungu et al., 2012) was implemented with TE/TR 68/1500 ms and a receive-only 8-channel phased-array head coil, as illustrated in Fig. 1C. Briefly, a pair of frequency-selective inversion pulses was inserted into the standard point-resolved spectroscopy (PRESS) method and applied on alternate scans at the frequency of the reduced form of glutathione (i.e., GSH) α-cysteinyl resonance at 4.56 ppm, while avoiding excitation of the oxidized form of glutathione (GSSG) α-cysteinyl resonance at 3.28 ppm (Nepravishta et al., 2012). At TE 68 ms, this resulted in two subspectra in which GSH, but not GSSG, was alternately inverted or not inverted. Subtracting these two subspectra yielded a ^{1}H MR spectrum consisting of only the edited GSH β-cysteinyl resonance at 2.98 ppm. Spectral data for this study were acquired in 15 min using 290 interleaved excitations (580 total) and TR 1500 ms with the editing pulses on or off.

Levels of Glu uncontaminated by glutamine or GABA were obtained using the constant-time PRESS (CT-PRESS) MRS technique (Dreher and Leibfritz, 1999; Mayer and Spielman, 2005), which consists of a standard PRESS method into which an additional 180° RF pulse is inserted and temporally shifted in a series of experiments, while the total sequence time remains constant. This yields a 2D data set in which chemical shift encoding achieved with the additional progressively time-shifted 180° RF pulse represents one time dimension (t1), while the second dimension (t2) is the usual time-domain signal acquisition. Performing 2D fast Fourier transformation with respect to the two time dimensions and projecting the resulting 2D frequency-domain spectrum onto the first frequency-domain axis (f1) yields a 1D spectrum in which all the multiplets appear as well-resolved homonuclear spin-decoupled singlets. In this study, an average TE (i.e., transverse relaxation time constant optimized for Glu detection) of 139 ms and 129 chemical shift encoding steps in increments of 0.8 ms in t1 dimension were used. Sample CT-PRESS spectra acquired in 6 min are shown in Figs. 1A (dACC) and 2B (mPFC). An unobstruted and uncontaminated Glu C-4 peak can be clearly seen at 2.35 ppm.

In secondary analyses, Glx levels were obtained from the combined resonances of glutamate and glutamine at 3.71 ppm in the CT-PRESS spectra to assess potential effects of glutamine, which contributes to the Glx peak, but whose reliability is limited when measured in isolation at 3T (Figs. 1A, 2B).

The total time to set up and then acquire the structural MRI and the three MRS scans was approximately 60 min. The same sequences were used to acquire edited GSH and Glu spectra before and one hour after NAC administration so that each study session lasted about 180 min, with the first and third hour spent in the scanner.

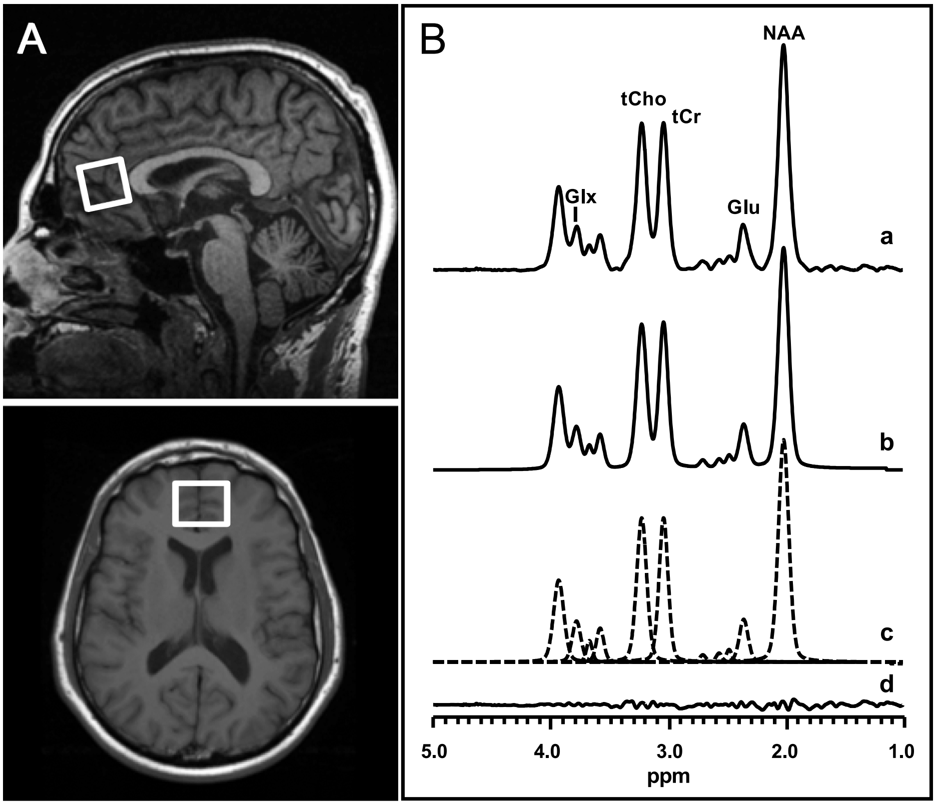

Fig. 2. Medial PFC glutamate acquisition
[A] Midsagittal (top) and oblique axial (bottom) images showing the medial PFC (mPFC) voxel size and location. The voxel is located anterior to the genu of the corpus callosum, oriented along the anterior-posterior commissure line, centered on the interhemispheric fissure. Dimensions are 2.5 cm (anterior-posterior) x 3 cm (left-right) x 2.5 cm (superior-inferior), and volume is 18.75 cc. [B] Sample CT-PRESS MRS data from a mPFC voxel, depicting (a) an experimental spectrum showing a clearly resolved C-4 Glu resonance at 2.35 ppm, as well as the resonances for NAA, tCr, tCho and Glx; (b) model fitting of spectrum (a) to obtain the metabolite peak areas of interest; (c) individual components of the model-fitted spectrum (a); (d) residuals of the difference between spectra (a) and (b).

2.4. ^{1}H MRS data processing and quantification

The 8-channel phased-array coil GSH data were combined into a single regular time-domain free-induction decay signal using the unsuppressed voxel tissue water signal from each receiver coil element to derive the required relative phased-array coil sensitivities (Shungu et al., 2016). The CT-PRESS data from which Glu levels were derived were processed as previously described (Mayer and Spielman, 2005). Using our spectral quality assessment criteria (Shungu et al., 2016), the areas of the individual spectral peaks, which are proportional to metabolite concentrations, were obtained by frequency-domain fitting of each resonance to a Gauss-Lorentz (i.e., pseudo-Voigt) lineshape function using the Levenberg-Marquardt nonlinear least-squares algorithm (Figs. 1A, C, and 2B). The levels of GSH, Glu and other metabolites were expressed as ratios of peak areas relative to that of the unsuppressed water signal (W) from the voxel, as previously described (Shungu et al., 2016).

To estimate the proportions of gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) contained in the voxels of interest, MEDx software (Medical Numerics, Germantown, MD) was used for voxel placement on the T1-weighted SPGR image, and SPM8 (http://www.fil.ion.ucl.ac.uk/spm) was used to generate the proportions of GM, WM, and CSF for each voxel. These were then compared between the groups and, in case of significant differences, included in the statistical model as covariates.

2.5. Statistical analysis

Group differences were evaluated using Student's *t*-test for continuous variables and, for categorical variables, Pearson χ^2 tests, or Fisher's exact test where warranted by low cell counts. Differences in metabolites before and after NAC challenge were calculated as (post NAC—pre NAC)/pre NAC. General linear model (GLM) was used to examine age effects on neurochemicals at baseline and on NAC effects. $p < 0.05$ was used to determine significance.

3. Results

3.1. Sample characteristics and demographics

Of the 45 subjects who consented to participate in this study, three (two patients, one control) withdrew consent before any study procedures. Of the 42 individuals who underwent MRI scanning (21 patients, 21 controls), two withdrew because of anxiety in the scanner prior to receiving NAC (one patient and one control), and data for one patient who completed the study were excluded for failure to meet the quality criteria (Shungu et al., 2016). Therefore, 19 patients and 20 control subjects completed procedures and were included in the analyses. The demographics are reported in Table 1. There were no demographic differences between groups. Five of the 19 patients were antipsychotic-naïve.

3.2. ^{1}H MRS measures

We first examined group differences at baseline in mPFC Glu and dACC GSH and Glu. At baseline, there were no significant group differences in any of these metabolites (Table 2). Further, there were no significant changes in mPFC Glu or dACC GSH and Glu after NAC

Table 1
Clinical and demographic factors.

	Patients (n = 19)	Control subjects (N = 20)	p-value
Age*	38.2 (14.5)	37.6 (11.3)	0.9
Sex	5F, 14M	6F, 14M	0.8
Race	12AA, 4C, 1As, 2 Mixed	12AA, 4C, 1As, 3 Mixed	0.98
Ethnicity	3H, 16NH	5H, 15NH	0.48
Smoking status	4S, 15NS	1S, 19NS	0.13
PANSS*	68 (22)	—	—

F, female; M, male; AA, African American; C, Caucasian; As, Asian; H, Hispanic; NH, non-Hispanic; S, smoker; NS, nonsmoker.
* Mean (standard deviation).

Table 2
Glu and GSH metabolite results*.

^{1}H MRS measure	Patients	Controls	p-value	Effect Size
Baseline mPFC Glu/W	0.099 (0.013)	0.100 (0.013)	0.88	0.08
Baseline dACC GSH/W	0.002 (0.0006)	0.002 (0.0004)	0.43	< 0.01
Baseline dACC Glu/W	0.127 (0.015)	0.128 (0.014)	0.74	0.07
Δ mPFC Glu/W**	0.009 (0.078)	-0.007 (0.077)	0.55	0.21
Δ dACC GSH/W	0.013 (0.232)	0.096 (0.214)	0.28	0.37
Δ dACC Glu/W	0.019 (0.048)	-0.005 (0.078)	0.29	0.37

* Mean (standard deviation).
** **Δ** = (post NAC – pre NAC)/pre NAC.

Table 3
Tissue segmentation results and spectral qualify assessment parameters.

Voxel/Tissue component (%)*	Patients	Controls	*p*-value
dACC gray matter	47.7 (3.2)	47.8 (4.2)	0.91
dACC white matter	39.4 (4.1)	39.8 (4.8)	0.75
dACC cerebrospinal fluid (CSF)	12.9 (3.8)	12.3 (3.7)	0.62
mPFC gray matter	52.5 (5.1)	52.7 (5.3)	0.90
mPFC white matter	30.2 (4.4)	31.0 (5.0)	0.62
mPFC cerebrospinal fluid (CSF)	17.3 (4.3)	16.3 (3.2)	0.43
SNR (NAA) dACC (CT-PRESS)	51.9 (6.2)	55.1 (6.1)	0.03
SNR (NAA) mPFC (CT-PRESS)	51.1 (5.6)	55.9 (5.8)	< 0.01
SNR (NAA) dACC (J-editing)	25.4 (3.5)	25.0 (2.3)	0.56
FWHM (Water) dACC	7.32 (0.89)	7.2 (0.88)	0.56
FWHM (Water) mPFC	11.3 (2.5)	11.1 (2.3)	0.83

* Mean (standard deviation).

challenge. Segmentation of volumetric MR images revealed no differences in tissue types between groups in either voxel (Table 3), and no effect of age on baseline or pre/post NAC metabolite levels. Secondary analyses of Glx showed similar results: baseline levels were not different between groups, and post-NAC levels showed no significant changes (Table 4). Additionally, Glu and Glx correlated significantly in both dACC ($r = 0.53$, $p = 0.02$) and mPFC ($r = 0.65$, $p = 0.006$) in control subjects, but only in dACC in patients ($r = 0.67$, $p = 0.002$; mPFC $r = 0.07$, $p = 0.8$). Results were unchanged when correcting for multiple comparisons. Finally, signal to noise ratios (SNR) estimated from the NAA peak in the CT-PRESS spectra were approximately 7% higher in control subjects than patients with SCZ for the dACC and mPFC.

4. Discussion

Using ^{1}H MRS this study measured the levels of dACC GSH and dACC and mPFC Glu, as well as the effects of a single dose of NAC on the two compounds in medication-free patients with SCZ in comparison to matched healthy control subjects. No differences in the levels of mPFC or dACC Glu or dACC GSH were observed between patients and control subjects at baseline or following NAC administration.

This study did not confirm our hypothesis of dACC GSH deficits in SCZ patients at baseline, a finding that is consistent with the totality of prior ^{1}H MRS measures of “frontal” or “prefrontal” GSH in SCZ, schizophreniform disorder, or schizoaffective disorder reported to date. This literature shows for these regions, irrespective of medication status of the cohorts, MRS technique, magnetic field strength or spectral quantification methods (Table 5), a highly consistent lack of GSH alteration. Our examination of the full prior literature of ^{1}H MRS of GSH in SCZ revealed 10 studies, 9 of which measured GSH in frontal or prefrontal regions (Brandt et al., 2016; Da Silva et al., 2018; Do et al., 2000; Kumar et al., 2018; Matsuzawa et al., 2008; Monin et al., 2015; Terpstra et al., 2005; Wang et al., 2019; Xin et al., 2016), while 1 study measured GSH in the medial temporal lobe (Wood et al., 2009) and, in addition to frontal or prefrontal regions, 2 studies also measured GSH in visual cortex and insula (Kumar et al., 2018), or in dorsolateral prefrontal cortex, orbitofrontal cortex and centrum semiovale (Wang et al., 2019). Of the 9 studies that measured GSH in frontal or prefrontal brain regions, 6 (Brandt et al., 2016; Da Silva et al., 2018; Matsuzawa et al., 2008; Monin et al., 2015; Terpstra et al., 2005; Xin et al., 2016) or 67% of the studies found no group differences, in agreement with the current study. By contrast, a significant group difference was reported by Do et al. (2000), who measured GSH levels at 1.5T in the prefrontal cortex of patients with schizophrenia or schizophreniform disorder using a multiple-quantum coherence filtering technique and found a 52% deficit compared to control subjects. This large GSH deficit has not been replicated, making it a potential outlier. More recently, Kumar et al. (2018) measured GSH levels at 7T using a short TE STEAM technique in patients with schizophrenia or schizoaffective disorder and found decreases in anterior cingulate cortex, but not in visual cortex nor insula. Also using a short TE STEAM technique at 7T, Wang et al. (2019) measured GSH levels in first-episode psychosis patients (including both affective and nonaffective psychosis) and reported decreases in ACC and thalamus, but not in dorsolateral prefrontal cortex, centrum semiovale or orbitofrontal cortex. However, it should be pointed out that because GSH spectral resonances are completely overlapped by those of more concentrated metabolites, even at 7T, the reliability of measuring brain GSH in vivo without a specialized “spectral editing” MRS technique has been questioned (Sanaei Nezhad et al., 2017). In this respect, of the MRS studies of GSH in SCZ that have been published, only three (Da Silva et al., 2018; Matsuzawa et al., 2008; Terpstra et al., 2005) were acquired using a spectral editing technique (J-difference editing or MEGA-PRESS) at 3T or 4T, similar to that used in the current study, and despite differences in medication status of the cohorts and a slight difference in magnetic field or voxel location, none of these studies, including our study, found significant differences compared to control subjects. Thus, a critical examination of the totality of the prior literature on ^{1}H MRS studies of GSH in SCZ agrees with the result of our study in suggesting the lack of a significant difference in “frontal” or “prefrontal” brain regions compared to control subjects.

In view of the absence of a group difference in GSH levels at baseline, the lack of a NAC effect on the levels of the antioxidant in this study is not surprising. This would be supported by the findings of (Conus et al., 2018) who reported increases of mPFC GSH in NAC-treated subjects compared to subjects treated with placebo, and were postulated as due to a *post hoc* finding of a greater baseline GSH deficit in the NAC group that likely potentiated the effect of NAC relative to placebo—a potential confound. It has been known for over four decades that in vivo synthesis of GSH is controlled by nonallosteric feedback inhibition of GCL (Richman and Meister, 1975), whereby GSH regulates

Table 4
Glx metabolite results*.

^{1}H MRS measure	Patients	Controls	*p*-value	Effect size (d)
Baseline mPFC Glx/W	0.0140 (0.0025)	0.0151 (0.00306)	0.29	0.39
Baseline dACC Glx/W	0.0144 (0.0020)	0.0157 (0.0026)	0.10	0.56
Δ mPFC Glx/W**	0.00697 (0.2022)	-0.0285 (0.264)	0.70	0.15
Δ dACC Glx/W	0.0738 (0.229)	-0.0293 (0.121)	0.10	0.56

* Mean (standard deviation).
** **Δ** = (post NAC – pre NAC)/pre NAC.

Table 5
Summary of ^{1}H MRS studies examining glutathione levels in psychosis.

Source	Patient population	Medication status	Magnet field strength	Voxel location	Finding	MRS technique and quantification method
Do et al. (2000)	9 Schizophrenia and 5 Schizophreniform Disorder (1 was first episode)	5 medicated, 5 drug naïve, 4 drug free	1.5T	Prefrontal Cortex	Decreased GSH	Double quantum coherence filter (DQC) technique; PRESS
Terpstra et al. (2005)	13 Schizophrenia or schizoaffective disorder	All were medicated	4T	Anterior Cingulate	No differences	Short echo time STEAM and MEGA PRESS; LC Model
Matsuzawa et al. (2008)	20 Schizophrenia	All were medicated	3T	Medial Frontal	No differences	MEGA PRESS
Wood et al. (2009)	30 First episode psychosis	13 were drug naive, 17 were medicated	3T	Medial Temporal	Elevated GSH	Short Echo PRESS; LC Model
Monin et al. (2015)	30 Early Psychosis	28 were medicated	3T	Medial Prefrontal Cortex	No differences	Short TE spin-echo full-intensity acquired localized single voxel spectroscopy; LC Model
Brandt et al. (2016)	27 Schizophrenia or schizoaffective disorder	All were medicated	7T	Anterior Cingulate	No differences	STEAM; LC Model
Xin et al. (2016)	25 Early psychosis	20 were medicated	3T	Medial Prefrontal Cortex	Elevated GSH in low risk groups for the glutamate-cysteine ligase catalytic gene (GCLC) regardless of group, no differences between control and patient groups	Short TE SPECIAL; LC Model
Da Silva et al. (2018)	30 clinical high risk	26 were drug naive	3T	Medial Prefrontal	No differences	MEGA PRESS; XSOS
Kumar et al. (2018)	28 with Schizophrenia or schizoaffective disorder	Most were medicated (details not included)	7T	Anterior Cingulate, Visual Cortex, Insula	Decreased GSH in anterior cingulate, no differences in visual cortex and insula	Short TE STEAM; LC Model
Wang et al. (2019	81 with First Episode Psychosis (including affective, substance-induced, and brief psychosis)	All inferred medicated (details not provided)	7T	Anterior Cingulate, Dorsolateral Prefrontal Cortex, Thalamus, Centrum Semiovale	Decreased GSH in ACC, no differences in DLPFC	Short TE STEAM; LC Model

its own synthesis: normal tissue GSH levels, as in the present study, inhibit GCL activity, thereby stopping GSH synthesis even if the supply of cysteine, the rate-limiting substrate, is adequate. Tissue GSH levels would have to be abnormally low for GCL to be disinhibited, and for administration of NAC to spur de novo GSH synthesis (Richman and Meister, 1975). In combination with this feedback inhibition, the coupling of the Glu and GSH pools via the role of Glu as precursor to GSH would lead to the expectation of a measurable response to NAC only under conditions of abnormal baseline levels of these two metabolites. Additionally, one dose of NAC may not be adequate to produce a measurable response even under conditions of low GSH and elevated Glu. Conus et al. (2018), who reported significant elevations of mPFC GSH in NAC-treated patients compared to placebo, administered NAC for 6 months. Berk et al. (2008) and Lavoie et al. (2008) administered NAC for six and two months, respectively, before they observed any effects on clinical ratings and EEG. However, a study in Parkinson and Gaucher diseases reported GSH elevations in response to a single intravenous dose of NAC (Holmay et al., 2013), likely due to the generally higher bioavailability of intravenously administered NAC.

It is also likely that the lack of an effect of NAC administration on dACC glutathione levels is related to the fact that NAC is a direct antioxidant, rather than an indirect antioxidant, such as sulforaphane. Sulforaphane, an isocyothianate, has been shown to increase both peripheral and central levels of GSH (Sedlak et al., 2018).

Replicated findings of elevated mPFC Glu or Glx in our prior studies of medication-naïve subjects at clinical high-risk for psychosis (de la Fuente-Sandoval et al. 2015), of medication-naïve first-episode psychosis patients (de la Fuente-Sandoval et al., 2018) and in unmedicated patients with schizophrenia (Kegeles et al., 2012), led us to hypothesize similar elevations of mPFC Glu or Glx at baseline in this study. However, several studies reported Gln rather than Glu level increases (Bartha et al., 1997; Theberge et al., 2002; Theberge et al., 2007). A suggested explanation for the discrepant views was that it is glutamine, rather than glutamate, that is responsible for the elevations observed in glutamatergic compounds or Glx in the mPFC, and that glutamine or Glx better reflect hyperglutamatergic neurotransmission in SCZ than Glu, which may index primarily intracellular Glu levels rather than Glu release (Bartha et al., 1997; Theberge et al., 2002; Theberge et al., 2007). However, failure to find either "pure" Glu or Glx elevations, or a group difference in the mean level of either compound at baseline when measured simultaneously with CT-PRESS in this study, does not support this possibility. Rather, our failure to replicate prior reports of mPFC elevations of Glx or Gln at baseline (Bartha et al., 1997; Kegeles et al., 2012; Theberge et al., 2002; Theberge et al., 2007) could plausibly be due to the greater age of participants in the current study, as has been suggested (Marsman et al., 2013; Rowland et al., 2016). In further support of this age effect is that in our own prior studies conducted with MRS methodology and instrumentation that were identical to those used in the present study, we found baseline elevation of Glx in medication-naïve individuals at high risk for psychosis (mean age: 20.7 ± 4.1 years) (de la Fuente-Sandoval et al., 2015), in medication-naïve first-episode psychosis patients (mean age: 23.0 ± 6.1 years) (de la Fuente-Sandoval et al., 2018), and in a cohort of medication-free ($n = 7$) and medication-naïve ($n = 9$) SCZ patients (mean age: 32.0 ± 10.5 years) (Kegeles et al., 2012). By contrast, the patients in the present cohort were older (mean age: 38.2 ± 14.5 years), and only 5 of 19 patients were medication-naïve, while 14 subjects, although medication-free for at least 21 days prior to the scans, had been previously medicated. Therefore, the greater mean age of the patients, the longer duration of medication exposure, and the smaller proportion of medication-naïve SCZ patients in the present cohort, could potentially account for our failure to replicate our prior finding of elevations of mPFC Glx at baseline. Since glutathione may be a reservoir of brain glutamate (Sedlak et al., 2019), it is also possible that we did not observe differences in brain glutamate because we did not observe differences in brain glutathione (Koga et al., 2011). These same factors might also account for our failure of find a NAC effect on Glu or Glx in this study. It should, however, be noted that in a study of cocaine users, initially elevated Glu levels were reported to decrease in response to a single oral dose of NAC (Schmaal et al., 2012). Therefore, further studies controlling for age and medication exposure of the cohorts are warranted to fully establish the effects of NAC on brain levels of glutamatergic compounds in SCZ. Lastly, a potential confound in this study is that the SNR estimated from the NAA peak in the CT-PRESS spectra was approximately 7% higher in control subjects than patients with SCZ for the dACC and mPFC. However, the SNR overall was very high for both groups and is unlikely to account for the lack of Glu or Glx differences.

5. Conclusions

In summary, this study found no differences in mPFC or dACC Glu levels or in dACC GSH levels between patients with SCZ and healthy control subjects either at baseline or after one dose of NAC. Future studies measuring GSH levels in brain regions previously found to exhibit glutamatergic abnormalities or using genetic polymorphisms, while controlling for the age and medication status of the cohorts, are warranted to better identify groups of patients more likely to respond to NAC and its mode of action and mechanisms.

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Conflicts of interest and source of funding

Dr. Girgis receives research support from Otsuka, Allergan, and Genentech. Dr. Kantrowitz reports having received consulting payments from Krog & Partners Incorporated, Kinetix Group, Slingshot, Semantics MR LTD, Transperfect, BVF Partners and Cowen and Company. He has conducted clinical research supported by the NIMH, the Stanley Foundation, Taisho, Lundbeck, Boehringer Ingelheim, NeuroRX, Teva, Merck, and Lilly. He owns a small number of shares of common stock in GlaxoSmithKline. Dr. Javitt has received grants from Roche, personal fees from Sunovion, Lundbeck, Envivo, Forum, Takeda, Autifony, Pfizer, and Glytech, and other fees from Glytech and NeuroRx. He also has two patents issued for D-serine in movement disorders and reduction in D-serine nephrotoxicity and a patent for D-cycloserine in depression licensed to NeuroRx. Dr. Kegeles has received research support from Amgen. BioAdvantex provided NAC for this study. The project described was supported by 1R21MH099508 and a David Mahoney Neuroimaging Program Award from the DANA Foundation.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2019.03.018.

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