OXFORD

RESEARCH ARTICLE

Glutamatergic Neurometabolites in Clozapine-Responsive and -Resistant Schizophrenia

Meghan Elizabeth Goldstein, BPharm, PhD; Valerie Margaret Anderson, PhD; Avinesh Pillai, MSc; Robert R. Kydd, MBChB, PhD; Bruce R. Russell, BPharm, PhD

Department of Psychiatry, University of California, San Diego, CA (Dr Goldstein); School of Pharmacy and Centre for Brain Research, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand (Drs Goldstein, Anderson, and Russell and Mr Pillai); Centre for Brain Research and Department of Psychological Medicine, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand (Dr Kydd).

Correspondence: Bruce Russell School of Pharmacy, BPharm, PhD, Faculty of Medical and Health Sciences University of Auckland, Private Bag 92019, Auckland, New Zealand (b.russell@auckland.ac.nz).

Abstract

Background: According to the current schizophrenia treatment guidelines, 3 levels of responsiveness to antipsychotic medication exist: those who respond to first-line antipsychotics, those with treatment-resistant schizophrenia who respond to clozapine, and those with clozapine-resistant or ultra-treatment resistant schizophrenia. Proton magnetic resonance spectroscopy studies indicate that antipsychotic medication decreases glutamate or total glutamate + glutamine in the brains of patients with schizophrenia and may represent a biomarker of treatment response; however, the 3 levels of treatment responsiveness have not been evaluated.

Methods: Proton magnetic resonance spectroscopy spectra were acquired at 3 Tesla from patients taking a second generation non-clozapine antipsychotic (first-line responders), patients with treatment-resistant schizophrenia taking clozapine, patients with ultra-treatment resistant schizophrenia taking a combination of antipsychotics, and healthy comparison subjects.

Results: Group differences in cerebrospinal fluid-corrected total glutamate + glutamine levels scaled to creatine were detected in the dorsolateral prefrontal cortex [df(3,48); F = 3.07, P = .04, partial η^2 = 0.16] and the putamen [df(3,32); F = 2.93, P = .05, partial η^2 = 0.22]. The first-line responder group had higher dorsolateral prefrontal cortex total glutamate + glutamine levels scaled to creatine than those with ultra-treatment resistant schizophrenia [mean difference = 0.25, standard error = 0.09, P = .04, family-wise error-corrected]. Those with treatment-resistant schizophrenia had higher total glutamate + glutamine levels scaled to creatine in the putamen than the first-line responders (mean difference = 0.31, standard error = 0.12, P = .05, family-wise error-corrected) and those with ultra-treatment-resistant schizophrenia (mean difference = 0.39, standard error = 0.12, P = .02, family-wise error-corrected).

Conclusions: Total glutamate + glutamine levels scaled to creatine in the putamen may represent a marker of response to clozapine. Future studies should investigate glutamatergic anomalies prior to clozapine initiation and following successful treatment.

Keywords: schizophrenia, magnetic resonance imaging, clozapine, glutamate, antipsychotic

Introduction

According to current schizophrenia treatment guidelines, 3 levels of responsiveness to antipsychotic medication exist: those who respond to first-line antipsychotics, those with

treatment-resistant schizophrenia (TRS) who respond to clozapine, and those with clozapine-resistant or ultra-treatment resistant schizophrenia (UTRS). TRS occurs in approximately

1

one-third of patients with schizophrenia (Lieberman, 1998; Conley and Kelly, 2001). While clozapine is the gold-standard antipsychotic for the management of TRS, anywhere from 40% to 70% of patients experience residual symptoms despite an adequate trial of clozapine (Buckley et al., 2001; Chakos et al., 2001; Mouaffak et al., 2006). The residual symptoms experienced by patients with TRS and UTRS have devastating effects on the individuals, their families, and the wider community.

While current medications for schizophrenia work primarily by blocking D_2-type dopamine receptors (Kapur et al., 2000; McIlwain et al., 2011), recent theories suggest that abnormalities in glutamatergic neurotransmission may underlie the deteriorating course of schizophrenia, ultimately leading to dopaminergic dysregulation (Olney and Farber, 1995; Sharp et al., 2001). The N-methyl D-aspartate (NMDA) receptor model suggests that hypofunctioning of these receptors in gamma-aminobutyric acid (GABA) interneurons causes a paradoxical, compensatory increase in presynaptic glutamate (Glu) release while GABA synthesis and release is downregulated (Lisman et al., 2008). An important consequence of the failure of this negative feedback loop is increased dopamine levels in the mesolimbic and mesocortical dopamine pathways leading to positive and negative/cognitive symptoms, respectively (Laruelle et al., 2003; Sesack et al., 2003).

Proton magnetic resonance spectroscopy (^{1}H-MRS) allows the in vivo assessment of NMDA receptor functioning through measurement of glutamatergic compounds: Glu (NMDA receptor agonist), glutamine (Gln; precursor to Glu), and total Glu + Gln (Glx). In both healthy individuals and patients with schizophrenia, glutamatergic compounds decline with age (Kaiser et al., 2005), albeit at a faster rate in patients (Marsman et al., 2013). Several pathophysiological phenomena could account for this, including altered synaptic activity, changed Glu receptor functioning, abnormal Gln-Glu cycling, or dysfunctional Glu transport.

Since the meta-analysis by Marsman et al. (2013), several recent studies and 1 systematic review indicate that medication status is a key determinant of Glu levels in schizophrenia; specifically, Glu or Glx is elevated in unmedicated patients, at any stage of illness (Kegeles et al., 2012; Kraguljac et al., 2012a; de la Fuente-Sandoval et al., 2013; Kraguljac et al., 2013; Poels et al., 2014). Kegeles et al. (2012) reported 30% elevations of GABA and Glx in the medial prefrontal cortex of unmedicated patients with schizophrenia but not the dorsolateral prefrontal cortex (DLPFC) relative to controls. In a sample of medicated patients, Kraguljac et al. (2012a) found no differences in Glx levels in the anterior cingulate cortex (ACC) or the hippocampus relative to controls. Conversely, elevated hippocampal Glx was observed in unmedicated patients vs controls (Kraguljac et al., 2013). A decrease in initially elevated striatal Glx was reported such that patients had similar Glx levels to controls following 4 weeks of treatment with risperidone (de la Fuente-Sandoval et al., 2013). Several other studies reviewed in Poels et al. (2014) reported findings congruent with these studies, particularly in the medial prefrontal cortex and basal ganglia structures, though findings in the DLPFC were not consistent.

It has been suggested that ^{1}H-MRS may enable clinicians to noninvasively assess response to antipsychotic medication by measuring glutamatergic compounds. A small number of studies have investigated the relationship between Glu/Gln/Glx levels and treatment response as opposed to medication effects of antipsychotics. Patients in the sample described by de la Fuente Sandoval et al. (2013) experienced significant symptom improvement that coincided with normalized striatal Glx with

risperidone treatment. A small study of patients treated with non-clozapine antipsychotics reported increased striatal DA synthesis capacity (assessed using positron emission tomography) and normal ACC Glu in responders while the TRS group displayed normal striatal DA synthesis capacity and elevated ACC Glu relative to healthy volunteers (Demjaha et al., 2014). Similarly, Egerton et al. (2012) reported higher Glu in the ACC of first-episode patients who remained symptomatic after at least 1 course of antipsychotic medication compared to those whose symptoms remitted. Following 40 days of individualized antipsychotic treatment, Szulc et al. (2011, 2013) found no changes in DLPFC Glx, but when the patient group was divided into responders and nonresponders, the group of responders had lower Glx levels at baseline.

Overall nonresponse to antipsychotic medication is associated with elevated Glu or Glx in various brain regions; however, schizophrenia patients treated with second-generation antipsychotics (SGAs) have not been compared to those with TRS taking clozapine, and no ^{1}H-MRS study to date has included a group with UTRS. N-acetyl aspartate (NAA) and choline (Cho) have been evaluated in clozapine-treated patients, but glutamatergic neurometabolites have not been reported (Bustillo et al., 2001; Ertugrul et al., 2009). We recruited 3 groups of patients with established schizophrenia: those taking a non-clozapine SGA (antipsychotic-responsive, first-line responders), those taking clozapine monotherapy (clozapine-responsive; TRS), and a group that failed to show a clear response to clozapine monotherapy who were taking a combination of 2 antipsychotics (clozapine-resistant; UTRS). We hypothesised that levels of glutamatergic compounds would be elevated in those with TRS or UTRS compared with first-line responders who in turn would have similar levels to healthy volunteers.

Methods

Participants

This study was approved by the Regional Ethics Committee. Patients who met the DSM-IV criteria for schizophrenia were identified by their treating clinician either from a community mental health centre or a forensic psychiatric inpatient unit. Subsequent to obtaining written informed consent, data regarding duration of illness and medication history were verified from clinical notes and patient interviews. The treatment groups comprised those taking: (1) a second-generation (atypical) non-clozapine antipsychotic (first-line responders); (2) treatment-resistant patients taking clozapine monotherapy (TRS group); (3) a combination of antipsychotics (clozapine-resistant; UTRS) having failed a trial of clozapine monotherapy. Clozapine could be one of the antipsychotics used in combination, or the combination could be 2 alternative antipsychotics as long as a past failure with clozapine monotherapy could be confirmed with the clinical notes as opposed to clozapine cessation or down-titration secondary to side effects. All participants were stabilized on their prescribed antipsychotic medication for at least 6 weeks. Treatment group classification was based on medication history and current medication at the time of the study prescribed in accordance with criteria for TRS using published algorithms (NICE, 2002; APA, 2004; Royal Australian New Zealand College of Psychiatrists, 2005). To control for state effects related to symptom severity, patients were required to be at most mildly ill, determined using the Clinical Global Improvement scale scores (CGI). A healthy comparison group with no history of mental or neurological illness was recruited by advertising

in the community. Age, sex, years of education, and ethnicity were matched on a group basis. All participants were between the ages of 18 and 45 years. Exclusion criteria included a history of traumatic brain injury (loss of consciousness for more than 3 minutes), neurological illness, significant physiological comorbidity, or contraindication to magnetic resonance imaging.

Symptom severity was assessed using the Positive and Negative Symptom Scale (PANSS); the scores were converted to CGI scores based on severity but not improvement (Kay et al., 1987). All PANSS interviews were conducted by the same trained investigator. ProScreen Cups (US Diagnostics Inc., PSCupA-6MBAU) were used to screen urine samples collected at each study session for amphetamines, cocaine, benzodiazepines, tetrahydrocannabinol (THC), or opiates. Standardized premorbid IQ scores were derived from the "spot the real word" test within the IntegNeuro neurocognitive computerized test battery (Brain Resource Company, Sydney, Australia) (Baddeley et al., 1993; Gordon, 2003, 2005). Chlorpromazine equivalents (CPZEs) were calculated to compare daily antipsychotic dose (Andreasen et al., 2010), except for amisulpride, which in the absence of a power formula was calculated using expert consensus for dosing (Gardner et al., 2010). Sample size was based on previous studies showing a relationship between antipsychotic treatment and spectroscopic measures in schizophrenia (Bustillo et al., 2008; Ertugrul et al., 2009).

Image Acquisition

Imaging was performed using a 3T Siemens Magnetom Skyra (Siemens). A 32-channel head coil was used for the majority of scans; where the participant could not fit comfortably into this coil, a 20-channel head coil was used (n = 4). A standard T1-weighted magnetic resonance scan was acquired: magnetization prepared rapid gradient echo; repetition time, 1900 ms; echo time, 2.39 ms; inversion time, 900 ms; flip angle, 9 deg; voxel size $0.9 \times 0.9 \times 0.8$ mm. ^{1}H-MRS data were acquired by single voxel spectroscopy using a point resolved spin echo sequence 80 averages; repetition time, 2000 ms; echo time, 30 ms. Using this method, 3 mutually orthogonal slices were stimulated successively, and the voxel of interest is the result of the intersection

of these slices. Three voxels of interest were acquired: (1) a 2-cm^3 voxel placed in the left DLPFC; (2) a 2-cm^3 voxel placed in the ACC; and (3) a $1.5 \times 1.5 \times 3.5$-cm voxel centered on the left putamen (Figure 1).

^{1}H-MRS Data Processing and Quantification

Spectra were analyzed using LCModel (Provencher, 2001). To ensure high-quality data, data sets that met 1 or more of the following criteria were excluded: (1) obvious movement artefact on imaging or spectroscopy; (2) metabolite concentration uncertainties that exceeded a Cramer-Rao Lower Bound of 20%, as provided by LCModel; (3) spectra with a LCModel full-width half maximum that exceeded 0.1 ppm.

Assessment of Voxel Tissue Homogeneity

The proportion of grey matter, white matter, and cerebrospinal fluid (CSF) in each voxel was determined by segmenting participants' structural scan using FMRIB's Automated Segmentation Tool. Neurometabolite ratios expressed as a ratio to creatine containing compounds (creatine and phosphocreatine) were corrected for CSF fraction within the voxel based on the assumption that CSF has NAA, Cr (Creatine), Cho, Glu, and Glx levels of zero. This technique has been employed in other studies with good test-retest reliability (Brooks et al., 1999; Mullins et al., 2003). If voxel tissue type differed significantly between the groups, tissue type was entered as a covariate in the statistical analysis, since differences in neurometabolite concentrations in grey and white matter have been reported (Hetherington et al., 1994; Schuff et al., 2001).

Statistical Analysis

Demographic and clinical variables were analyzed using ANOVA, Fisher's exact, and independent samples t tests as appropriate. The Shapiro-Wilkes test was performed to test the normality of CSF-corrected neurometabolite data sets; assumptions of normality were not violated for each outcome. CSF-corrected neurometabolite ratios that were greater than 2 SDs above or below
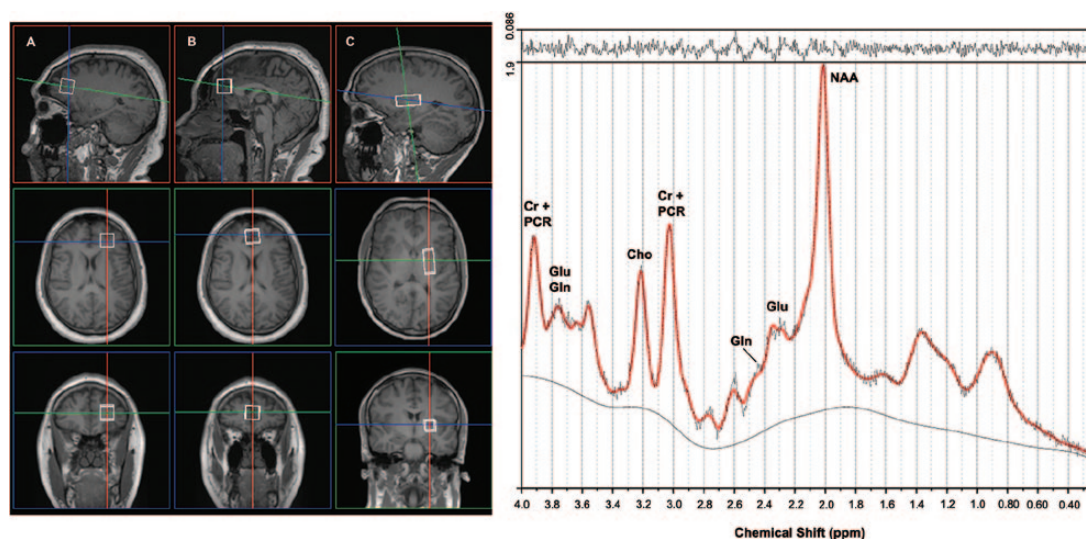

Figure 1. Top: Voxel location in the left dorsolateral prefrontal cortex (DLPFC). (A) Anterior cingulate cortex (ACC) (B), and left putamen (C). Bottom: Example of one fitted spectra (red line). Peak areas for N-acetyl aspartate (NAA), glutamate (Glu), glutamine (Gln), creatine (Cr), and choline (Cho) are labelled. Top irregular line is the residual signal after fitting. The baseline used for fitting with LCModel is indicated by the lower continuous line.

the group mean were classified as outliers and excluded from the analyses. Other neurometabolite values from the same case were not excluded as outliers if they were within 2 standard deviations of the mean.

CSF-corrected neurometabolite ratios were analyzed with ANCOVA and the following covariates: age, years of education, smoking status, and a positive test for THC on the day of testing. A previous meta-analysis reported that glutamatergic metabolites decreased at a faster rate with age in patients with schizophrenia compared with healthy controls (Marsman et al., 2013). Although there was a significant group difference in duration of illness, age was included rather than duration of illness as a covariate in order to allow omnibus comparisons between patients and controls. In the patient groups, duration of illness was correlated with age (n = 42, Pearson's R^2 = 0.52, P < .001, 2-tailed), eliminating the need to include both as covariates in the analyses. Years of education and smoking status differed significantly between the groups (Table 1), and a total of 5 participants tested positive for THC at the time of imaging. The proportion of voxel grey matter significantly differed between the groups in the ACC only and was added as covariate in analyses of this region. Additional analyses with daily antipsychotic dose expressed in CPZEs were performed in the patient groups.

Group means are reported as estimated marginalized means and standard errors. Planned posthoc comparisons were performed with ANOVA, and the Tukey method was used to correct for multiple comparisons. Informed 2-tailed regression correlations (Pearson) were performed where significant group differences in metabolites were identified to investigate associations between metabolites and PANSS and CGI symptom scores or daily antipsychotic dose in CPZE. All analyses were performed using the SPSS version 19.0 software and statistical significance was taken at P<.05.

Results

Demographics

A total of 156 people with schizophrenia and 30 healthy comparison subjects were approached to participate in this study. Due to strict exclusion criteria, only 58 people (16 healthy comparison subjects and 42 patients) were included in the final analysis (Table 1). There was a difference in years of education between groups [df (3,54), F = 2.9, P = .04]; posthoc tests showed that those with TRS had fewer years of education than healthy comparison subjects [mean difference (MD) = 2.69 years, P = .03]. There

Table 1. Demographic Data of Study Participants by Treatment Group

Characteristic	First-Line Responders (n = 15)	TRS (n = 16)	UTRS (n = 11)	Healthy Comparison Subjects (n = 16)	Test Statistic (FWE-Corrected)
Gender (male/female)	12/3	12/4	9/2	13/3	df (3, 54); χ^2 = 0.43, P = 1.00 (FET)
Age (mean years, SD)	30.7 (7.2)	33.7 (8.6)	35.0 (7.3)	34.1 (7.9)	df (3,54); F = 0.81; P = .50
Education (mean years, SD)	12.4 (3.0)	11.1 (2.8)*	12.4 (2.2)	13.8 (2.1)*	**df (3,54); F = 2.88; P = .04**
Standardized premorbid IQ (mean, SD)	-1.059 (1.137)	-0.673 (1.039)	-1.118 (1.138)	-0.326 (0.823)	df (3,54); F = 1.85; P = .15
Duration of illness (mean years, SD)	7.4 (5.3)*	13.0 (7.0)*	11.3 (5.4)	-	**df (2,39); F = 3.44; P = .04**
Duration of untreated psychosis (mean months, SD)	13.3 (15.9)	7.4 (7.2)	21.1 (21.5)	-	df (2,39); F = 2.67; P = .08
Psychiatric ratings					
PANSS total score (mean, SD)	59.9 (11.1)	59.7 (15.0)	62.4 (12.5)	-	df (2,39); F = 0.16; P = .86
PANSS positive subscale score (mean, SD)	13.4 (5.3)	11.4 (5.5)	14.0 (5.8)	-	df (2,39); F = 0.88; P = .42
PANSS negative subscale score (mean, SD)	17.1 (5.8)	19.7 (6.7)	20.0 (6.9)	-	df (2,39); F = 0.88; P = .42
PANSS general subscale score (mean, SD)	29.5 (5.9)	28.6 (6.6)	28.4 (4.2)	-	df (2,39); F = 0.14; P = .87
Dose at time of scan (chlorpromazine equivalents)	426.5 (205.8)	446.0 (242.3)	855.4 (369.6)**	-	**df (2,39); F = 9.80; P < .001**
Clozapine dose (mean mg/d)	-	385.9 (181.0)	433.3 (154.1)	-	t (19.1) = 0.69, P = .50
Smoking status (% smokers)	87%	56%	73%	31%	**df(3,54); χ^2 = 10.64, P = .01 (FET)**
Positive test for THC	3	1	1	0	
Head coil used (32 channel/20 channel)	13/2	15/1	10/1	16/0	
Concomitant psychotropic medication					
Benztropine	2	1	2	-	
Citalopram	1	0	1	-	
Clomipramine	0	0	1	-	
Fluoxetine	0	1	0	-	
Lamotrigine	0	1	0	-	
Lithium	0	0	1	-	
Lorazepam	0	1	0	-	
Nicotine replacement therapy (patches)	2	0	1	0	
Nortriptyline	0	0	1	-	
Paroxetine	0	0	2	-	
Sodium valproate	2	3	2	-	
Zopiclone	1	0	0	-	

Abbreviations: FET, Fisher's exact test; FWE, family-wise error corrected.

were no differences between treatment groups in gender, age, premorbid IQ scores, duration of untreated psychosis, PANSS total scores or subscales, or CGI scores. There was a higher percentage of smokers in patient groups (87%, 56%, and 73%) compared with healthy comparison subjects (31%); df(3,54); χ^2 = 10.64, P = .01 (Fisher's exact test). There was a difference in duration of illness between treatment groups [df(2,39), F = 3.4, P = .04] and the dose of antipsychotic at the time of the study [df(2,39), F = 9.8, P < .001]. Posthoc tests showed that first-line responders had a shorter duration of illness than those with TRS (MD = 5.58 years, P = .04), and those with UTRS were receiving higher doses of antipsychotic than both first-line responders (MD = 428.9 CPZE, P = .001) and those with TRS (MD = 409.4 CPZE, P = .001).

Antipsychotics prescribed in the first-line responder group included olanzapine (n = 6), risperidone (n = 5), aripiprazole (n = 3), and amisulpride (n = 1). The combination of antipsychotics in those with UTRS were clozapine and amisulpride (n = 3), clozapine and aripiprazole (n = 4), clozapine and risperidone (n = 1), and clozapine and quetiapine (n = 1). There was no significant difference in daily clozapine dose between the group with TRS and the 9 participants with UTRS who were receiving clozapine. There were 2 individuals who failed clozapine monotherapy due to an inadequate response whose current antipsychotics did not include clozapine: aripiprazole and quetiapine (n = 2). Five participants tested positive for THC but no other recreational drugs at the time of imaging: first-line responders (n = 3), TRS (n = 1), UTRS (n = 1).

^{1}H-MRS Findings

DLPFC

There was a significant group difference in Glx/Cr [df(3,48); F = 3.07, P = .04, partial η^2 = 0.16] (Table 2), but no group differences were detected for NAA/Cr, Glu/Cr, or Cho/Cr. When CPZE was included as an additional covariate in the ANCOVA, the group difference remained significant [df(3,47); F = 2.77, P = .05, partial η^2 = 0.15]. Posthoc tests revealed that the first-line responder group had higher levels of Glx/Cr than those in the UTRS group (MD = 0.25, SE = 0.09, P = .04, FWE-corrected) (Figure 2).

When Glx/Cr in the patient groups was corrected for age, years of education, smoking status, and THC positive on day of testing, there was no significant correlation with daily antipsychotic dose in CPZE [df(5,35); F = 1.469, P = .23, R^2 = 0.17], total PANSS scores [df(5,35); F = 0.87, P = .51, R^2 = 0.11], positive PANSS scores [df(5,35); F = 0.90, P = .49, R^2 = 0.11], negative PANSS scores [df(5,35); F = 0.95, P = .46, R^2 = 0.12], or PANSS general scores [df(5,35); F = 1.06, P = .40, R^2 = 0.13].

ACC

Since there was a significant group difference in the proportion of GM within the ACC voxel [df(3,46); F = 4.68, P = .01], proportion of GM was included in the ANCOVA as an additional covariate. No significant group differences in NAA/Cr, Glu/Cr, Glx/Cr, or Cho/Cr were detected in this region (Table 2).

Putamen

A significant group difference in Glx/Cr was detected in the putamen [df(3,33); F = 3.14, P = .04, partial η^2 = 0.22] (Table 2). No group differences in other neurometabolites were observed. When CPZE was included as an additional covariate in the ANCOVA, the group difference remained significant [df(3,32); F = 2.93, P = .05, partial η^2 = 0.22]. Posthoc tests indicated that the group with TRS had significantly higher Glx/Cr than the first-line responders (MD = 0.31, SE = 0.12, P = .05, FWE-corrected) and those with UTRS (MD = 0.39, SE = 0.12, P = .02, FWE-corrected). The group with TRS appeared to have higher Glx/Cr than the healthy comparison group, but this result did not reach significance (MD = 0.30, SE = 0.12, P = .07, FWE-corrected) (Figure 3).

Table 2. CSF-Corrected Neurometabolite Ratios in the Left Dorsolateral Prefrontal Cortex, Anterior Cingulate Cortex, and Left Putamen

	First-Line Responders	TRS	UTRS	Healthy Comparison Subjects	Test Statistic	With CPZE as a Covariate
DLPFC	n = 15	n = 16	n = 11	n = 16	n = 58	n = 58
NAA/Cr	1.46 (0.07)	1.51 (0.06)	1.60 (0.07)	1.60 (0.07)	df (3,50); F = 1.05, P = .38	df (3,49); F = 1.04, P = .38
Glu/Cr	1.23 (0.05)	1.17 (0.05)	1.08 (0.06)	1.12 (0.05)[a]	df (3,49); F = 1.77, P = .17	df (3,48); F = 1.51, P = .22
Glx/Cr	1.50 (0.06)[b]	1.37 (0.06)	1.24 (0.07)	1.30 (0.06)[a]	**df (3,48); F = 3.07, P = .04, partial η^2 = 0.16**	**df (3,47); F = 2.77, P = .05, partial η^2 = 0.15**
Cho/Cr	0.34 (0.01)	0.35 (0.01)	0.37 (0.01)	0.33 (0.01)	df (3,50); F = 1.62, P = .20	df (3,49); F = 0.17, P = .92
ACC	n = 14	n = 14	n = 9	n = 13	n = 50[c]	n = 50[c]
NAA/Cr	1.63 (0.08)	1.58 (0.08)	1.72 (0.10)	1.73 (0.09)	df (3,41); F = 0.78, P = .51	df (3,40); F = 1.03, P = .39
Glu/Cr	1.92 (0.11)	1.78 (0.11)	1.91 (0.13)	1.96 (0.12)	df (3,41); F = 0.49, P = .69	df (3,40); F = 0.46, P = .71
Glx/Cr	2.31 (0.16)	2.12 (0.16)	2.28 (0.19)	2.40 (0.17)	df (3,41); F = 0.46, P = .71	df (3,40); F = 0.56, P = .65
Cho/Cr	0.36 (0.01)	0.37 (0.01)	0.38 (0.02)[a]	0.34 (0.01)	df (3,40); F = 1.15, P = .34	df (3,39); F = 0.36, P = .78
Putamen	n = 12	n = 8	n = 9	n = 13	n = 42	n = 42
NAA/Cr	1.06 (0.19)	1.02 (0.24)	1.22 (0.16)	1.13 (0.17)	df (3,34); F = 2.12, P = .12	df (3,33); F = 2.65, P = .07
Glu/Cr	1.12 (0.13)	1.23 (0.19)	1.08 (0.17)	1.16 (0.13)[a]	df (3,33); F = 1.12, P = .36	df (3,32); F = 0.74, P = .54
Glx/Cr	1.53 (0.08)	1.83 (0.10)	1.43 (0.10)	1.48 (0.09)[a]	**df (3,33); F = 3.14, P = .04, partial η^2 = 0.22**	**df (3,32); F = 2.93, P = .05, partial η^2 = 0.22**
Cho/Cr	0.26 (0.01)	0.25 (0.01)	0.27 (0.01)	0.27 (0.01)	df (3,34); F = 0.44, P = .73	df (3,33); F = 0.50, P = .69

ACC, anterior cingulate cortex, Cho, choline, CPZE, chlorpromazine equivalents, Cr, creatine, CSF, cerebrospinal fluid, DLPFC, dorsolateral prefrontal cortex, Glu, glutamate, Glx, total glutamate + glutamine, NAA, N-acetyl aspartate, TRS, treatment-resistant schizophrenia, UTRS, ultra-treatment-resistant schizophrenia.

Metabolite levels were assessed using univariate ANCOVA, within-group factor CSF-corrected metabolite level, between-group factor treatment group, and the following covariates: age, years of education, smoking status, and THC positive on day of testing.

Note: [a]One spectrum was removed; outlier value.

[b] One spectrum was rejected because of a Cramer-Rao lower bound exceeding 20%.

[c] Proportion of grey matter added as an additional covariate in analyses of the ACC.
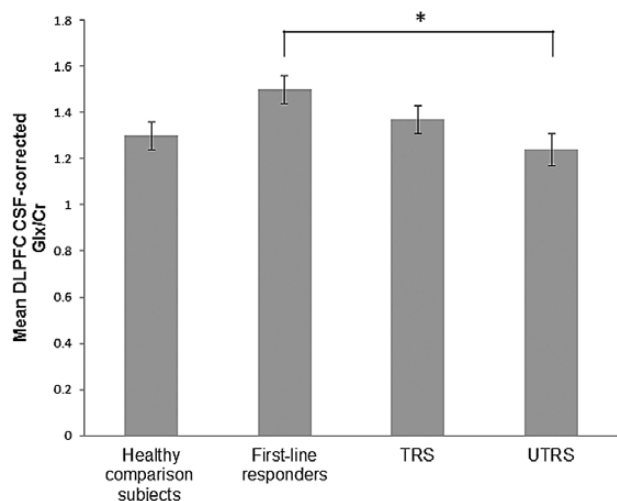
Figure 2. Cerebrospinal fluid-corrected dorsolateral prefrontal cortex (DLPFC) glutamate + glutamine levels scaled to creatine (Glx/Cr) in first-line treatment responders, patients with treatment-resistant schizophrenia (TRS), patients with ultra-treatment-resistant schizophrenia (UTRS), and healthy comparison subjects; group difference [df(3,48); F = 3.07, P = .04, partial η^2 = 0.16]. Data are estimated marginalized means covaried for age, years of education, smoking status, and tetrahydrocannabinol (THC) positive test result on day of scanning. Error bars represent the standard error. Posthoc tests revealed that the first-line responder group had higher levels of Glx/Cr than those in the UTRS group (mean difference [MD] = 0.25, standard error = 0.09, P = .04, Tukey correction for multiple comparisons).
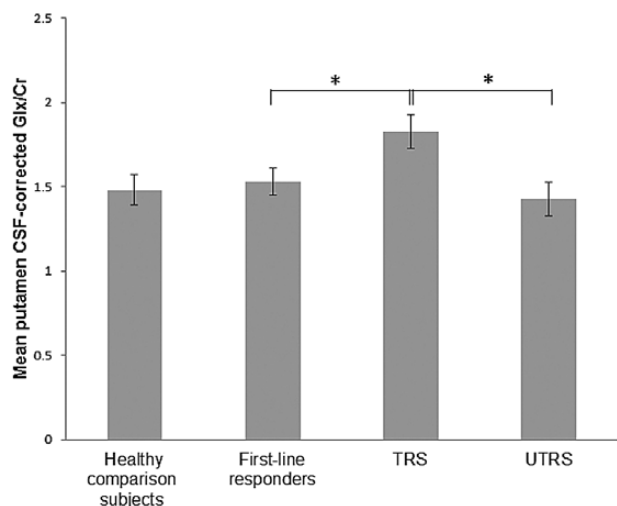

Figure 3. Cerebrospinal fluid-corrected putamen glutamate + glutamine levels scaled to creatine (Glx/Cr) in first-line treatment responders, patients with treatment-resistant schizophrenia (TRS), patients with ultra-treatment-resistant schizophrenia (UTRS), and healthy comparison subjects; group difference [df(3,33); F = 3.14, P = .04, partial η^2 = 0.22]. Data are estimated marginalized means covaried for age, years of education, smoking status, and tetrahydrocannabinol (THC) positive test result on day of scanning. Error bars represent the standard error. Posthoc tests indicated that the group with TRS had significantly higher Glx/Cr than the first-line responders (MD = 0.31, SE = 0.12, P = .05, Tukey correction for multiple comparisons) and those with UTRS (MD = 0.39, SE = 0.12, P = .02, Tukey correction for multiple comparisons). The group with TRS appeared to have higher Glx/Cr than the healthy comparison group, but this result did not reach significance (MD = 0.30, SE = 0.12, P = .07, Tukey correction for multiple comparisons).

When Glx/Cr in the patient groups was corrected for age, years of education, smoking status, and THC positive on day of testing, there was no significant correlation with daily antipsychotic dose in CPZE [df(5,23); F = 1.03, P = .43, R^2 = 0.18], total PANSS scores [df(5,23); F = 0.96, P = .46, R^2 = 0.17), positive PANSS scores [df(5,23); F = 0.76, P = .59, R^2 = 0.14), negative PANSS scores [df(5,23); F = 0.62, P = .69, R^2 = 0.12), or general PANSS scores [df(5,23); F = 0.70, P = .63, R^2 = 0.13).

Discussion

This is the first report, to our knowledge, of Glx and Glu in a group of patients with clozapine-responsive TRS, and the first study to report ^{1}H-MRS measurements in a group of patients with clozapine-resistant UTRS. In this sample of patients with 3 distinct levels of treatment response, we found regional differences in Glx levels, but none of the treatment groups had significantly different Glx levels to healthy volunteers. All patients were similarly responsive to their respective antipsychotic treatment as evidenced by PANSS scores; therefore, the differences detected are more likely to reflect trait rather than state features. The inclusion of daily antipsychotic dose expressed as CPZEs as a covariate did not affect the main group effect. Posthoc tests revealed that the first-line responder group had higher Glx in the DLPFC compared with those with UTRS, while the TRS group had higher Glx in the putamen compared with both the first-line responders and those with UTRS.

The lack of differences in Glu or Glx levels between the patient groups and healthy volunteers in the present study is in line with several recent reports, which found elevated Glu or Glx only in unmedicated patient groups relative to controls (Kegeles et al., 2012; Kraguljac et al., 2012a; de la Fuente-Sandoval et al., 2013; Poels et al., 2014). Increased Glx in the DLPFC of first-line responders compared with those with UTRS is an unexpected finding, since response to antipsychotic medication is associated with decreased Glx in frontal brain regions such as the ACC (Szulc et al., 2011, 2013; Egerton et al., 2012; Demjaha et al., 2014). Studies examining the DLPFC in the same group of patients before and after antipsychotic medication report decreased Gln, decreased Glx, or no change in Glx (Stanley et al., 1996; Szulc et al., 2011; Goto et al., 2012). This is the first study to compare patients taking SGAs with those with TRS or UTRS; because the present study is cross-sectional, we cannot determine whether the prescribed medication normalized elevated baseline glutamatergic abnormalities. Elevated DLPFC Glx in the first-line responder group may be due to the effect of THC; 3 participants in this group tested positive for THC on the day of scanning, while only 1 participant in TRS group and 1 in the UTRS tested positive for THC. Nevertheless, a THC-positive test was included as a covariate in the analysis, and studies of habitual marijuana users demonstrate decreased Glu and GABA in the ACC (Prescot et al., 2011, 2013). The UTRS group was exposed to significantly higher doses of antipsychotic medication, which may in part account for these differences. Though no correlation was found between antipsychotic dose in CPZE and Glx in the DLPFC and the results remained significant despite the inclusion of CPZE as a covariate, there are limitations of this approach. The total daily dose of antipsychotics in CPZE was calculated for the UTRS group by adding the CPZEs of each antipsychotic agent, and these values may not be simply additive (Andreasen et al., 2010).

Glx in the ACC was not elevated in those with TRS or UTRS as anticipated based on previous reports of elevated Glu and/or Glx in nonresponders to antipsychotic treatment (Egerton et al., 2012; Demjaha et al., 2014). There are several methodological differences that might account for this; for example, Demjaha et al. (2014) refer to their group as "treatment-resistant." However, these patients were not necessarily treated

with clozapine despite a long duration of illness. In our study, those with TRS were all stabilized on clozapine monotherapy, and their PANSS scores show no differences in symptom severity between the treatment groups. In contrast, the total PANSS scores between treatment groups reported by Demjaha et al. (2014) differed significantly (104.3 ± 10.6 vs 50.7 ± 5.8), which may reflect differences due in part to symptom severity. In addition, reporting metabolite ratios vs concentrations and implementing tissue volume correction of metabolite peaks may also account for group differences between the present study and treatment-resistant patients in previous reports.

The group with TRS taking clozapine had significantly higher Glx in the putamen than the first-line responders or those with UTRS. It has been suggested that glutamatergic anomalies may be more pronounced to DA-rich regions such as the striatum (de la Fuente-Sandoval et al., 2011). In the DLPFC and putamen, we found no correlations between Glx levels and antipsychotic dose (CPZEs) in line with other studies, suggesting that antipsychotics do not have a dose-dependent effect on glutamatergic metabolites (Szulc et al., 2005; Theberge et al., 2007; de la Fuente-Sandoval et al., 2009; Bustillo et al., 2010; Aoyama et al., 2011). Therefore, while antipsychotic treatment may not directly influence glutamatergic abnormalities, an increase in Glx or Glu in the putamen may be associated with the response to clozapine. While we cannot imply causality because the study is cross-sectional, our findings may support a proposed mechanism of action for clozapine's unique efficacy in TRS – increased glutamatergic neurotransmission via inhibition of glycine transporters or by interacting with the glycine site of the NMDA receptor (Javitt et al., 2004; Schwieler et al., 2008). NMDA receptors require both Glu and a coagonist at the glycine site to be fully active (Currá and Pallotta, 1996). While glycine site coagonists hold promise for those stabilized on non-clozapine antipsychotics (Heresco-Levy, 2005; Tsai, 1998, 2006), this is not the case for patients maintained on clozapine (Evins, 2000; Tsai, 1999). This may be due to full occupation of the glycine site associated with clozapine treatment, reflected by high Glx in the group with TRS vs the group with UTRS; however, there was no significant difference in clozapine dose between these groups.

Importantly, no differences were detected in Glu in any region, only differences in Glx, which may suggest that increased Gln is driving these group differences. A recent study reported increased Gln in patients undergoing long-term treatment for schizophrenia in the ACC unrelated to antipsychotic dose but did not include distinct treatment groups based on antipsychotic class (Bustillo et al., 2014). If increased Gln is the underlying cause of increased Glx in the present study, this may indicate a problem in the conversion from Gln to Glu, for instance, a reduction in presynaptic glutaminase that converts Gln to Glu resulting in the accumulation of Gln or a problem in the reuptake of Gln.

Other considerations are concomitant medications that were either not allowed (de la Fuente-Sandoval et al., 2013) or not reported in previous studies (Demjaha et al., 2012, 2014; Egerton et al., 2012). In the present sample, concomitant medications were mostly prescribed for those with UTRS. The most frequently co-prescribed medication was sodium valproate, which has been reported to increase Gln and therefore Glx in the parietal lobe; however, this was evenly prescribed across the treatment groups (Garcia et al., 2009). One participant in the UTRS group was taking lithium, which has been reported to decrease Glx in the basal ganglia (Shibuya-Tayoshi et al., 2008). Several participants were also prescribed antidepressants; there

is some evidence to suggest that antidepressants such as citalopram and nortriptylline reduce Glx and Gln (Block et al., 2009).

The main spectroscopic limitation for data acquisition was that water suppressed spectra were not collected as a reference for metabolite peaks; the levels are reported as a ratio to Cr. This approach is associated with high levels of accuracy and resilience to variations in SNR but is predicated upon the assumption that there is no group difference in Cr level (Kanowski et al., 2004). Despite previous reports of decreased Cr in patients with schizophrenia, a recent meta-analysis examining neurometabolites in schizophrenia reported that Cr did not appear to be significantly affected in the areas we studied (Kraguljac et al., 2012b). Fewer voxels were acquired in the ACC and putamen than the DLPFC, because we applied strict criteria to the quality of spectra; consequently, fewer spectra for those with UTRS were included in the final analysis. Others have failed to demonstrate glutamatergic laterality in patients with schizophrenia (Chang et al., 2007; Bustillo et al., 2011), we acknowledge that bilateral ^{1}H-MRS examination of these regions is required to generalize these findings.

This is the first study to report glutamatergic metabolite data in patients with schizophrenia based on the 3 levels of responsiveness to antipsychotic treatment observed in clinical practice. Glx in the putamen may represent a marker of response to clozapine treatment. Future studies should investigate Gln-Glu cycling and glutamatergic anomalies in patients prior to and following treatment with clozapine.

Acknowledgments

Statement of Interest

None.

References

Andreasen NC, Pressler M, Nopoulos P, Miller D, Ho B-C (2010) Antipsychotic dose equivalents and dose-years: a standardized method for comparing exposure to different drugs. Biol Psychiatry 67:255–262.

Aoyama N, Theberge J, Drost DJ, Manchanda R, Northcott S, Neufeld RWJ, Menon RS, Rajakumar N, Pavlosky WF, Densmore M, Schaefer B, Williamson PC (2011) Grey matter and social

functioning correlates of glutamatergic metabolite loss in schizophrenia. Br J Psychiatry 198:448–456.

APA (2004) Practice guideline for the treatment of patients with schizophrenia. 2nd ed. Arlington (VA): American Psychiatric Association.

Baddeley A, Emslie H, Nimmo-Smith I (1993) The Spot-the-Word test: a robust estimate of verbal intelligence based on lexical decision. Br J Clin Psychol 32:55–65.

Block W, Träber F, von Widdern O, Metten M, Schild H, Maier W, Zobel A, Jessen F (2009) Proton MR spectroscopy of the hippocampus at 3 T in patients with unipolar major depressive disorder: correlates and predictors of treatment response. Int J Neuropsychopharmacology 12:415–422.

Brooks WM, Friedman SD, Stidley CA (1999) Reproducibility of 1H-MRS in vivo. Magn Reson Med 41:193–197.

Buckley PF, Krowinski AC, Miller DD, Friedman L, Eaton Y, Tronetti M (2001) Clinical and biochemical correlates of 'high-dose' clozapine therapy for treatment -- refractory schizophrenia. Schizophr Res 49:225–227.

Bustillo J, Chen H, Jones T, Lemke N, Abbott C, Qualls C, Canive J, Gasparovic C (2014) Increased glutamine in patients undergoing long-term treatment for schizophrenia: a proton magnetic resonance spectroscopy study at 3 T. JAMA psychiatry (Chicago, Ill).

Bustillo JR, Lauriello J, Rowland LM, Jung RE, Petropoulos H, Hart BL, Blanchard J, Keith SJ, Brooks WM (2001) Effects of chronic haloperidol and clozapine treatments on frontal and caudate neurochemistry in schizophrenia. Psychiatry Res 107:135–149.

Bustillo JR, Rowland LM, Jung R, Brooks WM, Qualls C, Hammond R, Hart B, Lauriello J (2008) Proton magnetic resonance spectroscopy during initial treatment with antipsychotic medication in schizophrenia. Neuropsychopharmacology 33:2456–2466.

Bustillo JR, Rowland LM, Mullins P, Jung R, Chen H, Qualls C, Hammond R, Brooks WM, Lauriello J (2010) 1H-MRS at 4 tesla in minimally treated early schizophrenia. Mol Psychiatry 15:629–636.

Bustillo JR, Chen H, Gasparovic C, Mullins P, Caprihan A, Qualls C, Apfeldorf W, Lauriello J, Posse S (2011) Glutamate as a marker of cognitive function in schizophrenia: a proton spectroscopic imaging study at 4 Tesla. Biol Psychiatry 69:19–27.

Chakos M, Lieberman J, Hoffman E, Bradford D, Sheitman B (2001) Effectiveness of second-generation antipsychotics in patients with treatment-resistant schizophrenia: a review and meta-analysis of randomized trials. Am J Psychiatry 158:518–526.

Chang L, Friedman J, Ernst T, Zhong K, Tsopelas ND, Davis K (2007) Brain metabolite abnormalities in the white matter of elderly schizophrenic subjects: implication for glial dysfunction. Biol Psychiatry 62:1396–1404.

Conley RR, Kelly DL (2001) Management of treatment resistance in schizophrenia. Biol Psychiatry 50:898–911.

Currá MC, Pallotta BS (1996) Single-channel evidence for glycine and NMDA requirement in NMDA receptor activation. Brain Res 740:27–40.

de la Fuente-Sandoval C, Favila R, Alvarado P, Leon-Ortiz P, Diaz-Galvis L, Amezcua C, Garcia-Munoz E, Graff-Guerrero A (2009) Glutamate increase in the associative striatum in schizophrenia: a longitudinal magnetic resonance spectroscopy preliminary study. Gac Med Mex 145:109–113.

de la Fuente-Sandoval C, Leon-Ortiz P, Favila R, Stephano S, Mamo D, Ramirez-Bermudez J, Graff-Guerrero A (2011) Higher levels of glutamate in the associative-striatum of subjects with prodromal symptoms of schizophrenia and patients with first-episode psychosis. Neuropsychopharmacology 36:1781–1791.

de la Fuente-Sandoval C, León-Ortiz P, Azcárraga M, Stephano S, Favila R, Díaz-Galvis L, Alvarado-Alanis P, Ramírez-Bermúdez J, Graff-Guerrero A (2013) Glutamate levels in the associative striatum before and after 4 weeks of antipsychotic treatment in first-episode psychosis: A longitudinal proton magnetic resonance spectroscopy study. JAMA Psychiatry 70:1057–1066.

Demjaha A, Murray RM, McGuire PK, Kapur S, Howes OD (2012) Dopamine synthesis capacity in patients with treatment-resistant schizophrenia. Am J Psychiatry 169:1203–1210.

Demjaha A, Egerton A, Murray RM, Kapur S, Howes OD, Stone JM, McGuire PK (2014) Antipsychotic treatment resistance in schizophrenia associated with elevated glutamate levels but normal dopamine function. Biol Psychiatry 75(5):e11–e13.

Egerton A, Brugger S, Raffin M, Barker GJ, Lythgoe DJ, McGuire PK, Stone JM (2012) Anterior cingulate glutamate levels related to clinical status following treatment in first–episode schizophrenia. Neuropsychopharmacology 37:2515–2521.

Ertugrul A, Volkan-Salanci B, Basar K, Karli Oguz K, Demir B, Ergun EL, Senturk S, Erbas B, Cila A, Ulug B (2009) The effect of clozapine on regional cerebral blood flow and brain metabolite ratios in schizophrenia: relationship with treatment response. Psychiatry Research: Neuroimaging 174:121–129.

Evins AE, Fitzgerald SM, Wine L, Rosselli R, Goff DC (2000) Placebo-controlled trial of glycine added to clozapine in schizophrenia. Am J Psychiatry 157(5):826–828. PMID: 10784481.

Garcia M, Huppertz HJ, Ziyeh S, Buechert M, Schumacher M, Mader I (2009) Valproate-induced metabolic changes in patients with epilepsy: assessment with 1H-MRS. Epilepsia 50:486–492.

Gardner DM, Murphy AL, O'Donnell H, Centorrino F, Baldessarini RJ (2010) International Consensus Study of Antipsychotic Dosing. Am J Psychiatry 167:686–693.

Gordon E (2003) Integrative neuroscience. Neuropsychopharmacology 28 Suppl 1:S2–8.

Gordon E, Cooper N, Rennie C, Hermens D, Williams LM (2005) Integrative neuroscience: the role of a standardized database. Clin EEG Neurosci 36:64–75.

Goto N, Yoshimura R, Kakeda S, Nishimura J, Moriya J, Hayashi K, Katsuki A, Hori H, Umene-Nakano W, Ikenouchi-Sugita A (2012) Six-month treatment with atypical antipsychotic drugs decreased frontal-lobe levels of glutamate plus glutamine in early-stage first-episode schizophrenia. Neuropsychiatr Dis Treat 8:119–122.

Heresco-Levy U, Javitt DC, Ebstein R, Vass A, Lichtenberg P, Bar G, Catinari S, Ermilov M (2005) D-serine efficacy as add-on pharmacotherapy to risperidone and olanzapine for treatment-refractory schizophrenia. Biol Psychiatry 57(6):577–585. PubMed PMID: 15780844.

Hetherington HP, Mason GF, Pan JW, Ponder SL, Vaughan JT, Twieg DB, Pohost GM (1994) Evaluation of cerebral gray and white matter metabolite differences by spectroscopic imaging at 4.1 T. Magn Reson Med 32:565–571.

Javitt DC, Duncan L, Balla A, Sershen H (2004) Inhibition of System A-mediated glycine transport in cortical synaptosomes by therapeutic concentrations of clozapine: implications for mechanisms of action. Mol Psychiatry 10:275–287.

Kaiser LG, Schuff N, Cashdollar N, Weiner MW (2005) Age-related glutamate and glutamine concentration changes in normal human brain: 1H MR spectroscopy study at 4 T. Neurobiol Aging 26:665–672.

Kanowski M, Kaufmann J, Braun J, Bernarding J, Tempelmann C (2004) Quantitation of simulated short echo time 1H human brain spectra by LCModel and AMARES. Magn Reson Med 51:904–912.

Kapur S, Zipursky R, Jones C, Remington G, Houle S (2000) Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry 157:514–520.

Kay SR, Fiszbein A, Opler LA (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 13:261–276.

Kegeles LS, Mao X, Stanford AD, Girgis R, Ojeil N, Xu X, Gil R, Slifstein M, Abi-Dargham A, Lisanby SH, Shungu DC (2012) Elevated prefrontal cortex gamma-aminobutyric acid and glutamate-glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy. Arch Gen Psychiatry 69:449–459.

Kraguljac NV, Reid MA, White DM, den Hollander J, Lahti AC (2012a) Regional decoupling of N-acetyl-aspartate and glutamate in schizophrenia. Neuropsychopharmacology 37:2635–2642.

Kraguljac NV, Reid M, White D, Jones R, den Hollander J, Lowman D, Lahti AC (2012b) Neurometabolites in schizophrenia and bipolar disorder - a systematic review and meta-analysis. Psychiatry Res 203:111–125.

Kraguljac NV, White DM, Reid MA, Lahti AC (2013) Increased hippocampal glutamate and volumetric deficits in unmedicated patients with schizophrenia. JAMA Psychiatry 70:1294–1302.

Laruelle M, Kegeles LS, Abi-Dargham A (2003) Glutamate, dopamine, and schizophrenia from pathophysiology to treatment. Ann N Y Acad Sci 1003:138–158. PMID: 14684442.

Lieberman JA (1998) Pathophysiologic mechanisms in the pathogenesis and clinical course of schizophrenia. J Clin Psychiatry 60:9–12.

Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S, Grace AA (2008) Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. Trends Neurosci 31:234–242.

Marsman A, van den Heuvel MP, Klomp DWJ, Kahn RS, Luijten PR, Hulshoff Pol HE (2013) Glutamate in schizophrenia: a focused review and meta-analysis of (1)H-MRS studies. Schizophr Bull 39:120–129.

McIlwain ME, Harrison J, Wheeler AJ, Russell BR (2011) Pharmacotherapy for treatment-resistant schizophrenia. Neuropsychiatr Dis Treat 7:135–149.

Mouaffak F, Tranulis C, Gourevitch R, Poirier MF, Douki S, Olié JP, Lôo H, Gourion D (2006) Augmentation strategies of clozapine with antipsychotics in the treatment of ultraresistant schizophrenia. Clin Neuropharmacol 29:28–33.

Mullins PG, Rowland L, Bustillo J, Bedrick EJ, Lauriello J, Brooks WM (2003) Reproducibility of 1H-MRS measurements in schizophrenic patients. Magn Reson Med 50:704–707.

NICE (2002) Guidance on the use of newer (atypical) antipsychotic drugs for the treatment of schizophrenia. London: National Institute for Clinical Excellence.

Olney JW, Farber NB (1995) Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry 52:998–1007.

Poels EMP, Kegeles LS, Kantrowitz JT, Javitt DC, Lieberman JA, Abi-Dargham A, Girgis RR (2014) Glutamatergic abnormalities in schizophrenia: A review of proton MRS findings. Schizophr Res 152:325–332.

Prescot AP, Locatelli AE, Renshaw PF, Yurgelun-Todd DA (2011) Neurochemical alterations in adolescent chronic marijuana smokers: A proton MRS study. Neuroimage 57:69–75.

Prescot AP, Renshaw PF, Yurgelun-Todd DA (2013) γ-Amino butyric acid and glutamate abnormalities in adolescent chronic marijuana smokers. Drug Alcohol Depend 129:232–239.

Provencher SW (2001) Automatic quantitation of localized in vivo 1H spectra with LCModel. NMR Biomed 14:260–264.

Royal Australian New Zealand College of Psychiatrists CPGTft-ToSRD (2005) Royal Australian and New Zealand College of Psychiatrists clinical practice guidelines for the treatment of schizophrenia and related disorders. Aust N Z J Psychiatry 39:1–30.

Schuff N, Ezekiel F, Gamst AC, Amend DL, Capizzano AA, Maudsley AA, Weiner MW (2001) Region and tissue differences of metabolites in normally aged brain using multislice 1H magnetic resonance spectroscopic imaging. Magn Reson Med 45:899–907.

Schwieler L, Linderholm KR, Nilsson-Todd LK, Erhardt S, Engberg G (2008) Clozapine interacts with the glycine site of the NMDA receptor: Electrophysiological studies of dopamine neurons in the rat ventral tegmental area. Life Sci 83:170–175.

Sesack SR, Carr DB, Omelchenko N, Pinto A (2003) Anatomical substrates for glutamate-dopamine interactions: evidence for specificity of connections and extrasynaptic actions. Ann N Y Acad Sci 1003:36–52. PMID: 14684434.

Sharp FR, Tomitaka M, Bernaudin M, Tomitaka S (2001) Psychosis: pathological activation of limbic thalamocortical circuits by psychomimetics and schizophrenia? Trends Neurosci 24:330–334.

Shibuya-Tayoshi S, Tayoshi S, Sumitani S, Ueno S, Harada M, Ohmori T (2008) Lithium effects on brain glutamatergic and GABAergic systems of healthy volunteers as measured by proton magnetic resonance spectroscopy. Prog Neuropsychopharmacol Biol Psychiatry 32:249–256.

Stanley JA, Williamson PC, Drost DJ, Rylett RJ, Carr TJ, Malla A, Thompson RT (1996) An in vivo proton magnetic resonance spectroscopy study of schizophrenia patients. Schizophr Bull 22:597–609.

Szulc A, Galinska B, Tarasow E, Dzienis W, Kubas B, Konarzewska B, Walecki J, Alathiaki AS, Czernikiewicz A (2005) The effect of risperidone on metabolite measures in the frontal lobe, temporal lobe, and thalamus in schizophrenic patients. A proton magnetic resonance spectroscopy (1H MRS). Pharmacopsychiatry 38:214–219.

Szulc A, Galinska B, Tarasow E, Waszkiewicz N, Konarzewska B, Poplawska R, Bibulowicz D, Simonienko K, Walecki J (2011) Proton magnetic resonance spectroscopy study of brain metabolite changes after antipsychotic treatment. Pharmacopsychiatry 44:148–157.

Szulc A, Galinska-Skok B, Waszkiewicz N, Bibulowicz D, Konarzewska B, Tarasow E (2013) Proton magnetic resonance spectroscopy changes after antipsychotic treatment. Curr Med Chem 20:414–427.

Theberge J, Williamson KE, Aoyama N, Drost DJ, Manchanda R, Malla AK, Northcott S, Menon RS, Neufeld RWJ, Rajakumar N, Pavlosky W, Densmore M, Schaefer B, Williamson PC (2007) Longitudinal grey-matter and glutamatergic losses in first-episode schizophrenia. Br J Psychiatry 191:325–334.

Tsai G, Yang P, Chung LC, Lange N, Coyle JT (1998) D-serine added to antipsychotics for the treatment of schizophrenia. Biol Psychiatry 44(11):1081–1089. PubMed PMID: 9836012.

Tsai GE, Yang P, Chung LC, Tsai IC, Tsai CW, Coyle JT (1999) D-serine added to clozapine for the treatment of schizophrenia. Am J Psychiatry 156(11):1822–1825. PMID: 10553752.