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Glutamatergic dysfunction linked to energy and membrane lipid metabolism in frontal and anterior cingulate cortices of never treated first-episode schizophrenia patients

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ABSTRACT

Background: Glutamatergic dysfunction and altered membrane lipid and energy metabolism have been repeatedly demonstrated in the frontal/prefrontal and anterior cingulate cortex (ACC) in schizophrenia. Though having been already studied in animals, the presumed link between glutamatergic function and structural plasticity has not been investigated directly in the human brain yet. We measured glutamate (Glu), focal energy metabolism, and membrane phospholipid turnover to investigate main pathologies in those key brain regions of schizophrenia.

Methods: ¹H- and ³¹P-Chemical Shift Imaging (CSI) was combined in a single session to assess Glu and markers of energy (PCr, ATP) and membrane lipid (PME, PDE) metabolism in 31 neuroleptic-naïve first acute onset psychosis patients and 31 matched healthy controls. Multivariate analyses of covariance were used to assess disease effects on Glu and to investigate the impact of Glu alterations on phospholipid and energy metabolites.

Results: Glu levels of patients were increased in the frontal and prefrontal cortex bilaterally and in the ACC. Higher Glu was associated with increased left frontal/prefrontal PME and right frontal/prefrontal PDE in patients, which was not observed in healthy controls. In contrast, higher Glu levels were associated with lower PCr or ATP values in the frontal/prefrontal cortex bilaterally and in the right ACC of controls. This was not observed in the right ACC and left frontal/prefrontal cortex of patients.

Conclusion: Frontal glutamatergic hyperactivity is disconnected from physiologically regulated energy metabolism and is associated with increased membrane breakdown in right and increased membrane restoration in left frontal and prefrontal cortical regions. As indicated by previous findings, this pathology is likely dynamic during the course of first acute illness and possibly associated with negative symptoms and cognitive impairment. Our findings underline the importance of further research on neuroprotective treatment options during the early acute or even better for the ultra-high risk state of psychotic illness.

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1. Introduction

In this work, we follow two lines of evidence concerning the pathogenesis of schizophrenia. First, we consider the n-methyl-d-aspartate (NMDA) glutamate receptor dysfunction hypothesis, which pertains to biological correlates of hypofrontality (Coyle, 2004; Kantrowitz and

Javitt, 2012; Olney et al., 1999). Secondly, we consider the membrane lipid hypothesis (Horrobin et al., 1994) that assumes deficient generation or excessive breakdown of membrane phospholipids as a causative pathology in schizophrenia, and that is based on evidence of disturbed membrane lipid turnover in specific brain regions of schizophrenic patients. Both hypotheses have been related to disturbances of neurodevelopment, neuronal and synaptic plasticity as well as to hyperexcitation, excitotoxicity and oxidative stress (Farooqui et al., 2007; Horrobin, 1998; Keshavan, 1999; Michaelis, 1998).

Glutamate (Glu) is the excitatory neurotransmitter of pyramidal cells and is present in about 40% of the brain's synapses. It plays a crucial

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role in cortical projections to diverse subcortical regions (hippocampus, amygdala, basal ganglia), in inter-cortical projections and in thalamo-cortical tracts, such as the glutamatergic connections between thalamus and medial prefrontal and anterior cingulate cortices. In addition, corticofugal glutamatergic neurons control monoaminergic neurons and provide direct activation of dopaminergic, noradrenergic and serotonergic neurons. Furthermore, they inhibit monoaminergic neurons via activation of intermediary GABA-ergic inter-neurons enabling highly sensitive regulation of monoamines (Vogt, 1993).

In animal studies, the different regulation states of the glutamate/dopamine system were found to be important for early neuro-development and synaptic plasticity during adolescence/early adulthood (Bondi et al., 2012). Accordingly, regulation deficits were identified to predispose disturbed synaptic plasticity in functionally important brain regions leading to the manifestation of symptoms summarized as “hypofrontality” in humans (Grace, 1993; Yin et al., 2012). Excess dopaminergic activity can enhance release of Glu, which in turn may cause excitotoxic cell or dendrite damage (Finlay and Zigmond, 1997; Reid et al., 1997) with crucial consequences in the adolescent/young adult brain.

Combining the notions of glutamatergic dysfunction, dysregulation of brain activity, and impaired membrane phospholipid turnover, we hypothesize that disease related glutamatergic dysfunction is linked to altered energy and membrane metabolism. However, so far it has been difficult to demonstrate this association in the human brain. Therefore, we investigated this link by means of combined proton and phosphorous MR spectroscopic ($^1\text{H}/^{31}\text{P}$ -MRS) measurements.

In vivo proton spectroscopy (^1H -MRS) allows quantification of several brain metabolites, including Glu. Glu is synthesized in axon terminals of glutamatergic neurons, either from α -ketoglutarate or from glutamine (Gln). It has been estimated that 80% of stimulus-released Glu is derived from Gln (Erecinska and Silver, 1990; Ross, 1991). A dynamic balance exists between Glu and Gln to maintain appropriate levels of Glu (Erecinska and Silver, 1990; Williamson et al., 1996).

A recent meta-analysis of ^1H -MRS studies that focused on the Glu/Gln complex revealed decreased medial frontal Glu and increased Gln in first-episode schizophrenia patients (FEP) (Marsman et al., 2013). Reviewing findings in never-medicated and medicated patients separately, drug-naïve patients were reported with no changes of glutamatergic levels (measured by Glx, Gln) in the dorsolateral prefrontal cortex, but elevated glutamatergic levels in the medial prefrontal and anterior cingulate cortex (ACC) (Poels et al., 2014). Single studies reported also increased glutamatergic levels in medial temporal lobe structures and the hippocampus (Glx) (Kraguljac et al., 2013) as well as in the associative striatum and cerebellum (Glx and Glu) (de la Fuente-Sandoval et al., 2013). Findings of glutamatergic levels in the thalamus are still heterogeneous with some studies showing an increase in never treated patients (Aoyama et al., 2011; Theberge et al., 2002, 2007). There is also some uncertainty on the interpretation of glutamatergic alterations. While some authors interpreted increased (medial frontal) Gln as an expression of a Glu deficit due to disturbed Glu/Gln cycling (high Gln associated with low Glu (Bartha et al., 1997)), others ascribed the increased Gln level (e.g., in the ACC) to an increased glutamatergic activity (Theberge et al., 2002).

^{31}P -MRS, on the other hand, makes it possible to assess phospholipid metabolites (phosphomonoesters, PME, precursors in phospholipid synthesis and membrane restoration; phosphodiester, PDE, metabolites of phospholipid degradation and indicator of focal membrane damage) and high-energy phosphates (adenosine triphosphate, ATP, main energy transferring compound; phosphocreatine, PCr, interim storage of excess energy). In schizophrenia research, the main findings of ^{31}P -MRS studies include phospholipid alterations in prefrontal, anterior cingulate cortices, thalamus, insular cortex, basal ganglia (e.g., caudate nucleus) and anterior cerebellum (Gangadhar et al., 2004; Jayakumar et al., 2003; Jensen et al., 2004; Smesny et al., 2007; Volz et al., 2000). In never treated patients, the most consistent findings were observed in prefrontal and frontal

brain regions, namely decrease of PME and/or increase in PDE as well as decrease in ATP and/or increase in PCr (Keshavan et al., 2000). These findings were interpreted as expression of focally increased neuronal/synaptic membrane damage accompanied by deficits in maintaining or re-establishing physiological membrane texture and energy supply (Smesny et al., 2007).

In this study, combined $^1\text{H}/^{31}\text{P}$ -MRS was performed in cohorts of never treated FEP and matched healthy controls (HC) to address the following hypotheses:

1. Glu is dysregulated in the frontal/prefrontal and anterior cingulate cortex indicating glutamatergic dysfunction. If verified, this finding serves as the precondition to investigate the following main hypothesis.
2. In the frontal/prefrontal and anterior cingulate cortex of patients altered glutamatergic function is associated with focally altered PDE and PME and/or altered PCr and ATP.

2. Methods

2.1. Subjects

We investigated 31 FEP (16 males/15 females) and 31 HC (16 males/15 females), all right-handed and matched for age and gender (Table 1). All patients suffered their first schizophrenic episode, paranoid-hallucinatory sub-type, according to DSM-IV criteria for schizophrenia. All diagnoses were independently established by two board certified psychiatrists (S.Sm., H.S.) and confirmed by standardized structured clinical interviews (SCID-IV) (Wittchen et al., 1997). Psychopathology

Table 1

Group demographics and psychopathological measures. All patients were recruited between 2009 and 2013, either at the time of first hospital admission or when seeking help in the outpatient psychosis prevention and early intervention clinic. Due to this clinical setting, patients were included shortly before or when just meeting the DSM-IV time criterion (see also duration of untreated psychosis DUP) and clinical criteria for a schizophrenic episode. At the time of measurement 28 patients fulfilled the criteria of paranoid-hallucinatory schizophrenia (incl. time criterion) and 3 patients the DSM-IV criteria of schizophreniform disorder. Regular follow-up interviews confirmed meanwhile diagnosis of paranoid-hallucinatory schizophrenia also in the latter three patients. Additionally to interview questions about substance use in all participants, FEPs additionally underwent a breath alcohol concentration test and a screening blood and urine test for illegal substances (among others amphetamines, cocaine, morphine and its derivatives). None of these screening tests in FEP revealed a positive result at the time of hospital admission.

	First episode patients (FEP)	Healthy controls (HC)
N	31	31
Gender (male)	16 (52%)	15 (48%)
Age (years) mean (\pm SD)	25.97 (\pm 4.95)	25.42 (\pm 5.18)
<i>Alcohol</i>		
Less than weekly	6 (19.4%)	20 (64.5%)
1–6 drinks/week	23 (74.2%)	10 (32.3%)
Daily	2 (6.4%)	1 (3.2%)
<i>Marijuana</i>		
No	10 (32.3%)	18 (58.1%)
Less or equal 2 g/week	18 (58.1%)	13 (41.9%)
More than 2 g/week	3 (9.7%)	0 (0.0%)
<i>Psychiatric medication</i>		
Antipsychotic medication	Naïve	
Antidepressant	0	0
Benzodiazepine (sporadic)	6 (19.4%)	0
Duration of untreated psychosis (DUP)	5.2 \pm 1.7 month	
<i>Psychopathology</i>		
PANSS total score	58.7 (\pm 7.7)	
PANSS positive subscale	31.8 (\pm 3.5)	
PANSS negative subscale	27.5 (\pm 9.2)	
PANSS global subscale	41.7 (\pm 8.1)	

Data are mean (\pm standard deviation, SD) or n (%). PANSS denotes Positive and Negative Syndrome Scale.

was assessed using PANSS scores (Kay et al., 1987). All patients were neuroleptic-naïve.

Healthy volunteers were recruited by newspaper advertisement. They were examined using a semi-structured interview to assess current mental status and to exclude personal or first-degree family history of psychiatric disorders or history of neurological or major medical conditions, possibly affecting brain metabolism. None of the volunteers suffered from concurrent substance dependence or had a history of substance dependence or abuse. The study was approved by the Ethics Committee of the Jena University Hospital. All subjects gave written informed consent to participate in the study.

2.2. Experimental setting, scan parameters and post-processing of data

All measurements were performed at the same time of day following the examination protocol in Table 2 (TA ≈ 85 min). The table also contains the data processing steps to quantify proton and phosphorous metabolic compounds.

The planning procedure to identically position the ^1H - and ^{31}P -CSI (Chemical Shift Imaging) slice is displayed in Fig. 1a–b. A region of interest was selected in the frontal part of the CSI slab including 8 voxels assigned to the frontal and prefrontal as well as the anterior cingulate cortex (Fig. 1c). Region specific metabolite measures were determined for ^1H compounds (expressed as absolute concentrations in mmol/l and corrected for the individual GM, WM and CSF fractions in spectroscopic voxels (Gussew et al., 2012)) and as total phosphorous normalized intensities for ^{31}P compounds (see Fig. 1d–e, respectively).

In case of ^1H -MRS we focussed our analyses on Glu which can be measured with high accuracy at 3 T, and which represents a direct expression of glutamatergic function. As a number of previous studies used Glx as a measure of the glutamatergic regulation state, we also provide the respective results as Supplementary material.

2.3. Statistical analyses

2.3.1. Analysis of glutamate values, gray matter fraction and psychopathology

Focussing on Glu as independent variable, we initially tested Glu for group differences in each spectroscopic voxel using T-tests. Results were Bonferroni-corrected for multiple comparisons ($n = 8$, $p = 0.00625$). To assess partial volume effects caused by possible differences of the gray matter (GM) fraction in voxels, we additionally performed univariate analysis of covariance (ANCOVA) including GM as independent variable, Glu as dependent variable and group (FEP vs. HC) as

between-subject factor. Here we focussed on significant group by GM interactions.

Correlation analysis was performed to test for associations between Glu and psychopathology in patients.

2.3.2. Analysis of associations between Glu alterations and metabolites of energy and membrane lipid metabolism

The effect of Glu alterations on energy and membrane phospholipid metabolism in particular brain regions was investigated by multivariate analysis of covariance (MANCOVA) incorporating an “interaction term”. To analyze associations between Glu and energy metabolism, both PCr and ATP were included as dependent variables (since PCr and ATP are presumably correlated), whereas group (FEP vs. HC) was included as between-subject variable, and Glu was used as covariate. Concerning associations between Glu and phospholipid metabolism, PME and PDE were both treated as dependent variables (like PCr and ATP). We focussed on the group by Glu interactions, i.e., whether and how the illness's impact on Glu affected the dependent variables PCr, ATP, PME, and PDE. The level of significance was set at $p \leq 0.05$. Taken into account that patients were untreated at the time of measurement and that the scan time of each session was > 1 h, the “n” of this population is comparably high among spectroscopy studies. Nevertheless, we estimated the *a priori* possibility to reveal significant results in a MANCOVA as low in this population. Therefore, and because our study is the first combined spectroscopy study in this field, we did not apodictically exclude results at trend level from further discussion. All statistical analyses were performed with IBM SPSS Statistics (Version 19).

3. Results

3.1. Demographics

FEPs were moderately ill according to the PANSS scores. Although alcohol and cannabis consumption was higher in FEP, none of them fulfilled the criteria of alcohol or drug abuse (Table 1).

3.2. Quality of MR spectra

All spectra in the predefined region-of-interest had sufficient SNR and narrow linewidth (^1H -MRS: SNR > 25 , linewidth < 7.5 Hz; ^{31}P -MRS: SNR > 15 , linewidth < 7.5 Hz). With Cramer–Rao–Lower-Bounds (CRLB) for PCr, PME and PDE being $< 10\%$, and $< 15\%$ for Glu, spectra quality was considered adequate to quantify metabolic intensities with sufficient accuracy.

Table 2

Experimental setup, scan parameters and post-processing of MRS and MRI data.

Experimental settings	MR scanner RF coil	3 T whole-body (Magnetom TIM Trio, Siemens Medical Solutions, Erlangen, Germany) Double-resonance transmit/receive volume head coil providing acquisition of $^1\text{H}/^{31}\text{P}$ -MRS data in the same session (Biomedical Rapid, Germany)
	T_1 -weighted, 3D brain MRI	MP-RAGE, 192 sagittal slices TR/TE/TI: 2300/3.03/900 ms; FOV _{AP} × HF: 25.6 × 25.6 cm ² , 256 × 256 matrix
	2D ^1H -CSI	PRESS based, TE/TR: 30/2000 ms, Vol: 15 × 9 × 1.5 cm ³ , FoV _{AP} × LR: 24 × 24 cm ² , 16 × 16 phase encoding steps, nominal voxel size: 15 × 15 × 15 mm ³ ; manual shim; with and without water suppression; TA = 15 min
	3D ^{31}P -CSI	FID sequence with slice selective rf excitation and 8 × 8 × 8 phase encoding steps; TR: 3 s; FoV _{AP} × LR × HF: 24 × 24 × 24 cm ³ ; spatial extrapolation to 16 × 16 × 16 1.5 × 1.5 × 1.5 cm ³ voxels; TA = 45 min
Data post-processing	MRI	Segmentation (FreeSurfer, http://surfer.nmr.mgh.harvard.edu/) and co-registration of MRI data with CSI data to determine GM, WM and CSF fractions in spectroscopic voxels
	^1H -CSI	Quantitation with LCModel (Provencher, 1993) by using a vendor provided basis set including 15 model spectra of, e.g., NAA, creatine, total choline, myo-inositol, glutamate and glutamine
	^{31}P -CSI	Determination of absolute concentrations (in mmol/l) by using water as internal concentration reference Quantitation of PME, PDE, PCr, Pi and ATP intensities with jMRUI (www.mrui.uab.es/mrui/) (Stefan et al., 2009); AMARES tool (Vanhamme et al., 1997) and subsequent normalization with the total phosphorous amount in the spectrum. For an example of a ^1H - and ^{31}P -spectrum, see Fig. 1d and e, respectively

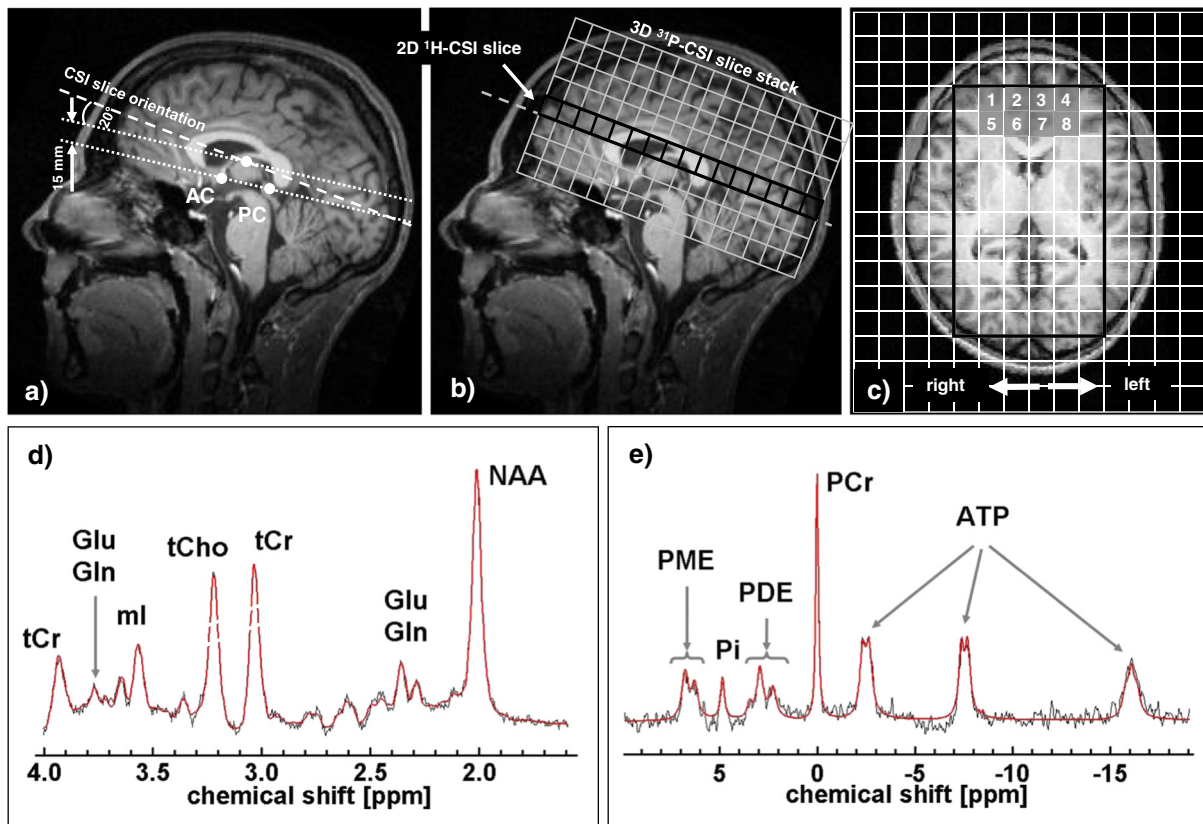


Fig. 1. The brain landmarks *anterior and posterior commissure* ((a) AC-PC line) were used to identically position the ^1H -CSI and ^{31}P -CSI slice stacks using re-sliced MP-RAGE imaging data ((b) and (c)). Due to the strong magnetic susceptibility gradients between brain tissue and the air filled nasal cavity sufficient field homogeneity could not be achieved across the complete brain volume. Therefore, the shim volume was reduced to the frontal part of the brain. Analysis of ^1H - and ^{31}P -compounds was therefore performed in the 8 CSI voxels within the marked region of interest (c). These 8 voxels were assigned to the following brain regions: voxels 1 and 4: right/left frontal/prefrontal cortex (77%) and adjacent white matter; voxels 2 and 3: right/left anterior cingulate cortex (ACC) (65%); voxels 5 and 8: right/left dorsolateral prefrontal cortex (DLPFC) (26%) and adjacent frontal white matter; voxels 6 and 7: right/left anterior cingulate cortex (ACC) (52%) and adjacent cerebral white matter. Subplots (d) and (e) show representative ^1H - and ^{31}P -MR spectra together with calculated fit curves (black solid lines) and major proton and phosphorous compounds from the left anterior cingulate cortex (voxel 3). Assessed metabolites: (d) ^1H -CSI: glutamate (Glu), glutamine (Gln), N-acetylaspartate (NAA), creatine (Cr), myo-Inositol (ml), total choline (tCho) [mmol/l]; and (e) ^{31}P -CSI: phosphomonoesters (PME), phosphodiesteres (PDE), phosphocreatine (PCr), anorganic phosphate (Pi), and adenosine triphosphate (ATP, sum value of all three subpeaks).

3.3. Correlations between metabolite values and psychopathology

No significant correlations were found between Glu and psychopathological measures (Glu/PANSS total score $r = 0.28$, $p = 0.572$; Glu/PANSS pos. $r = 0.32$; $p = 0.763$; Glu/PANSS neg. $r = 0.27$, $p = 0.543$).

3.4. Group effects of Glu values and dependency on GM fraction

Glu was significantly increased in voxel 1 to 4, voxel 5 and voxel 8 (Table 3), though only the results of voxels 1–4 survived Bonferroni correction. Glu increases in the latter voxels were not associated statistically with possible group differences of GM fraction in these voxels (group by GM interaction of univariate ANCOVA: vo 1 $F_{(1,58)} = 0.053$, $p = 0.819$; vo 2 $F = 0.437$, $p = 0.511$; vo 3 $F = 0.021$, $p = 0.887$; vo 4 $F = 0.520$, $p = 0.474$). Group comparisons of PME, PDE, PCr, and ATP revealed no significant differences in none of the investigated voxels.

3.5. Associations between glutamate, energy and phospholipid metabolism

We performed two multivariate analyses of covariance (one using PCr and ATP, the other using PME and PDE as dependent variables) in each voxel that showed significantly increased Glu levels (vo 1–4, 5, and 8). We focussed on group by Glu interactions that indicate an influence of illness on the associations between Glu and PCr (or ATP),

and PDE (or PME), respectively. Results of multivariate tests are presented in Table 4; corresponding results of univariate tests and mean values with standard deviations are summarized in Table 5.

The most pronounced effects (i.e., reaching significance levels in multivariate tests, see Table 4) were observed in voxels assigned to the right frontal and anterior cingulate cortex (vo 1 and 2).

In vo 1 (assigned to the right frontal/prefrontal cortex), illness affected the correlation between Glu and PDE (i.e., metabolites of phospholipid degradation) (Fig. 2a). While in healthy individuals higher Glu values were associated with lower PDE levels, the opposite held true in patients. In vo 2 (assigned to the right ACC), illness affected the correlation between Glu and PCr, the main interim energy storage (Fig. 3b). While in HC higher Glu values were linked to lower PCr levels, patients were lacking this association.

Weaker results (trend level in multivariate tests, see Table 4) were obtained in voxels assigned to the left frontal/prefrontal and dorsolateral prefrontal cortex (vo 4 and 8). Here, illness seemed to influence also the association between Glu and PME, i.e., metabolites of phospholipid synthesis and membrane restoration (Fig. 2c and d). While in healthy subjects Glu and PME were not observably associated, higher Glu values were connected with higher PME values in patients. Although not significant in the multivariate test, an effect similar to the finding in the right ACC (Fig. 3b) was observed for the energy metabolites in the left frontal/prefrontal cortex (Fig. 3c). The negative correlation between Glu and PCr in healthy controls was absent in patients.

Finally, we considered general effects of Glu without taking into account group differences (i.e., group by Glu interactions in multivariate

Table 3

Results of group comparisons for Glu levels and GM fractions after applying T-tests. The voxel number together with the corresponding assigned region and the calculated GM fraction in each voxel are also included. Results of univariate ANCOVA in terms of group by GM interactions are given in the main text. (Bold numbers: results surviving Bonferroni correction ($p = 0.00625$)).

Brain region	FEP		HC		Group statistics	
	Mean	SD	Mean	SD	T	p
vo 1	Right frontal/prefrontal cortex (77%) and adjacent white matter					
GM	0.762	0.139	0.715	0.130	1.324	0.191
Glu	10.539	0.859	9.510	1.005	4.190	0.000
vo 2	Right anterior cingulate cortex (ACC) (65%)					
GM	0.650	0.099	0.620	0.092	1.171	0.247
Glu	10.500	0.754	9.805	0.534	3.906	0.000
vo 3	Left anterior cingulate cortex (ACC) (65%)					
GM	0.679	0.064	0.651	0.083	1.371	0.176
Glu	11.396	0.733	10.767	0.909	2.850	0.006
vo 4	Left frontal/prefrontal cortex (78%) and adjacent white matter					
GM	0.777	0.122	0.758	0.107	0.642	0.523
Glu	11.613	1.088	10.491	1.684	3.027	0.004
vo 5	Right dorsolateral prefrontal cortex (26%) and frontal white matter					
GM	0.321	0.128	0.242	0.110	2.468	0.017
Glu	8.369	0.661	8.031	0.478	2.196	0.032
vo 6	Right anterior cingulate cortex (ACC) (52%) and adjacent white matter					
GM	0.592	0.099	0.597	0.087	-0.197	0.844
Glu	8.603	0.689	8.883	0.387	-1.841	0.071
vo 7	Left anterior cingulate cortex (ACC) (52%) and cerebral white matter					
GM	0.616	0.093	0.623	0.088	-0.263	0.794
Glu	9.186	0.617	9.080	0.630	0.600	0.551
vo 8	Left dorsolateral prefrontal cortex (26%) and adjacent frontal white matter					
GM	0.352	0.125	0.275	0.138	2.187	0.033
Glu	9.082	0.682	8.517	1.072	2.333	0.023

tests). In vo 3, assigned to the left ACC, both groups showed a positive correlation between Glu and PDE values (see Fig. 2b).

In summary, Glu levels were increased in the frontal/prefrontal cortex bilaterally and in the anterior cingulate cortex of patients. These results are stable also if controlled for multiple comparisons (Bonferroni). In the patient group, higher Glu was associated with increased left frontal/prefrontal PME and right frontal/prefrontal PDE, while healthy controls were lacking these associations. In HC, higher Glu levels were associated with lower PCr or ATP values bilaterally in the frontal/prefrontal cortex and in the right ACC. These associations

Table 4

Multivariate test results of MANCOVA with interaction term. The main focus is on the general level of group by Glu interactions. (Bold numbers: results $p \leq 0.05$; *italics*: results at trend level $p \leq 0.1$). Correcting for multiple comparisons, none of these results survives the corrected significance levels (6 tests on two hypotheses: $p_{corr} = 0.00416$).

Brain region	Group		Glutamate		Group by glutamate	
	F	p	F	p	F	p
vo 1	Right frontal/prefrontal cortex (77%) and adjacent white matter					
PME/PDE	3.411	0.041	0.128	0.880	3.482	0.039
PCr/ATP	0.466	0.630	2.464	0.096	0.205	0.816
vo 2	Right anterior cingulate cortex (ACC) (65%)					
PME/PDE	0.638	0.533	1.891	0.163	0.582	0.563
PCr/ATP	4.172	0.022	2.307	0.111	4.622	0.015
vo 3	Left anterior cingulate cortex (ACC) (65%)					
PME/PDE	0.301	0.741	<i>3.081</i>	<i>0.055</i>	0.349	0.707
PCr/ATP	0.139	0.871	0.202	0.817	0.122	0.885
vo 4	Left frontal/prefrontal cortex (78%) and adjacent white matter					
PME/PDE	2.368	0.105	3.301	0.045	2.575	<i>0.087</i>
PCr/ATP	1.634	0.205	0.094	0.910	1.935	0.155
vo 5	Right DLPFC (26%) and adjacent frontal white matter					
PME/PDE	0.485	0.619	0.566	0.571	0.494	0.613
PCr/ATP	1.815	0.174	4.433	0.017	1.826	0.172
vo 8	Left DLPFC (26%) and frontal white matter					
PME/PDE	2.374	0.104	1.235	0.300	2.438	<i>0.099</i>
PCr/ATP	2.859	<i>0.068</i>	1.065	0.354	2.662	<i>0.081</i>

Table 5

Univariate test results of MANCOVA with interaction term. The main focus here is on the effects of group by Glu interactions on single metabolites. Also included are mean values and standard deviations (SD) of metabolite values and assigned brain regions to each voxel. (Bold numbers: results $p \leq 0.05$; *italics*: results at trend level $p \leq 0.1$).

	FEP		HC		Group		Glutamate		Group by glutamate	
	Mean	SD	Mean	SD	F	p	F	p	F	p
vo 1	Right frontal/prefrontal cortex (77%) and adjacent white matter									
PME	0.115	0.016	0.112	0.008	0.302	0.585	0.001	0.979	0.376	0.543
PDE	0.148	0.017	0.147	0.013	6.769	0.012	0.259	0.613	6.855	0.012
PCr	0.166	0.009	0.160	0.014	0.710	0.404	4.535	0.038	0.321	0.574
ATP	0.128	0.011	0.134	0.013	0.339	0.563	0.881	0.353	0.139	0.711
vo 2	Right anterior cingulate cortex (ACC) (65%)									
PME	0.122	0.011	0.117	0.008	0.101	0.753	1.251	0.270	0.142	0.708
PDE	0.151	0.010	0.153	0.010	1.118	0.296	2.222	0.143	0.957	0.333
PCr	0.155	0.008	0.150	0.011	8.031	0.007	3.403	0.072	8.891	0.005
ATP	0.127	0.014	0.132	0.010	0.647	0.425	1.169	0.285	0.724	0.399
vo 3	Left anterior cingulate cortex (ACC) (65%)									
PME	0.119	0.007	0.117	0.009	0.570	0.454	0.302	0.585	0.628	0.432
PDE	0.153	0.011	0.153	0.011	0.022	0.884	6.172	0.016	0.050	0.824
PCr	0.152	0.011	0.149	0.011	0.050	0.824	0.042	0.838	0.020	0.888
ATP	0.132	0.011	0.133	0.010	0.170	0.682	0.290	0.592	0.184	0.670
vo 4	Left frontal/prefrontal cortex (78%) and adjacent white matter									
PME	0.120	0.012	0.117	0.008	4.809	0.033	6.656	0.013	5.235	0.026
PDE	0.147	0.009	0.149	0.013	0.057	0.812	0.561	0.458	0.068	0.796
PCr	0.158	0.013	0.156	0.012	<i>3.330</i>	<i>0.074</i>	0.076	0.784	<i>3.895</i>	<i>0.054</i>
ATP	0.135	0.011	0.129	0.012	0.219	0.641	0.070	0.792	0.064	0.801
vo 5	Right dorsolateral prefrontal cortex (26%) and frontal white matter									
PME	0.109	0.014	0.111	0.008	0.158	0.693	0.399	0.530	0.189	0.666
PDE	0.152	0.015	0.151	0.009	0.917	0.343	0.900	0.347	0.914	0.344
PCr	0.169	0.008	0.158	0.013	0.108	0.744	4.771	0.034	0.365	0.548
ATP	0.129	0.012	0.135	0.010	3.258	<i>0.077</i>	2.670	0.109	2.864	<i>0.097</i>
vo 8	Left dorsolateral prefrontal cortex (26%) and adjacent frontal white matter									
PME	0.115	0.014	0.114	0.006	4.845	0.033	2.173	0.147	4.982	0.030
PDE	0.152	0.010	0.148	0.011	0.001	0.971	0.447	0.507	0.015	0.902
PCr	0.154	0.012	0.151	0.009	1.749	0.193	1.710	0.198	1.496	0.228
ATP	0.135	0.007	0.134	0.010	2.797	0.101	0.137	0.713	2.749	0.104

were not observed in patients in the right ACC and in the left frontal/prefrontal cortex. All results of the interaction analyses by MANCOVA do not survive adjustment for multiple comparisons.

4. Discussion

We hypothesized that glutamatergic functioning is impaired in never treated FEP. Indeed, we observed increased Glu levels in the frontal and prefrontal cortex bilaterally and in the anterior cingulate cortex. These results remained stable after adjustment for multiple comparisons. With about 80% of stimulus-released Glu being derived from Gln (Erecinska and Silver, 1990; Ross, 1991), increased Glu likely reflects increased glutamatergic activity. This corresponds to the increased glutamatergic levels reported in the medial frontal cortex (meta-analysis by Marsman 2013 (Marsman et al., 2013)) and in the medial prefrontal and anterior cingulate cortex (ACC) (Theberge et al., 2002, 2007) of never-medicated drug-naïve patients (review by Poels et al. (2014)).

The interpretation of focally increased Glu levels is supported by the observed associations of Glu with energy metabolites and membrane lipid compounds. These results are, although significant statistically less powerful as the Glu group differences, as they would not survive adjustment for multiple comparisons. However, concerning energy metabolites, higher Glu levels were bilaterally associated with lower PCr or ATP values in frontal/prefrontal regions and in the right ACC of healthy individuals. Higher glutamatergic activity is obviously associated with higher energy demands met by ATP and higher strain of intercellular phosphate buffer (reflected by decreased PCr). Of note, Glu transport, being part of the metabolic Glu/Gln cycling, is energy consuming and closely connected to the mitochondrial PCr/ATP metabolism (Farooqui et al., 2000; Kanai et al., 2013). The observation that patients did not show negative correlations between Glu and PCr or ATP in the right

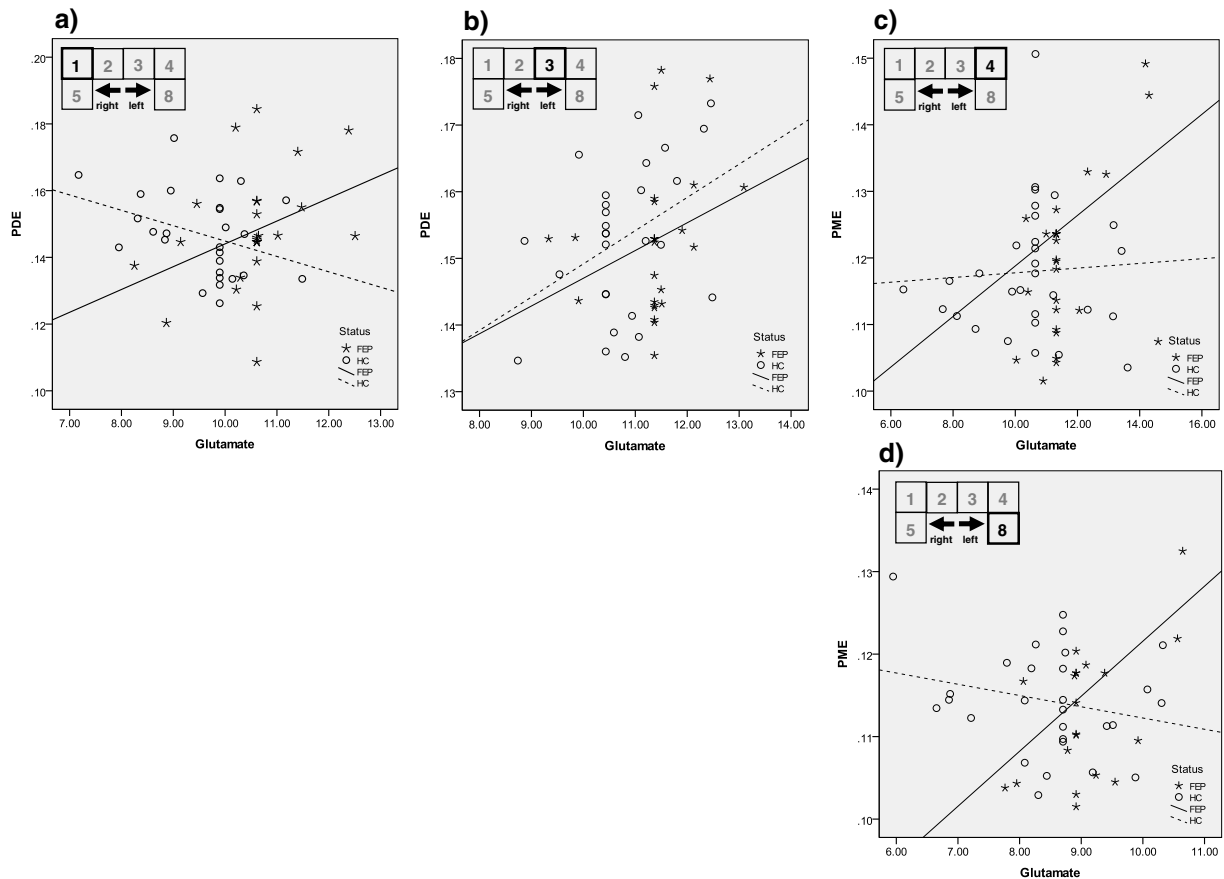


Fig. 2. Illustration of interactions as indicated by MANCOVA with interaction term (see also Tables 4 and 5). Each scatter plot reflects differences (or lack of differences) between FEP and HC in terms of correlation (strictly speaking “associations”) between Glu values and metabolite values of phospholipid metabolism (PME, PDE).

ACC and left frontal and prefrontal cortex points to a focal decoupling of glutamate and energy metabolism under the condition of illness, which might be clinically reflected as negative symptomatology or impaired cognitive function (Kato et al., 1995; Volz et al., 1998). Thus, increased glutamatergic activity seems to be ineffective in warranting adequate social/cognitive functioning. This is in line with the finding of Bartha and colleagues (Bartha et al., 1997) who also reported co-occurrence of increased medial frontal Gln and increased (instead of decreased) PCr.

According to our hypothesis, effects of illness on Glu are also affecting those phospholipid compounds that maintain membrane structure. Higher Glu was associated with increased left frontal/prefrontal PME and right frontal/prefrontal PDE in patients, whereas healthy controls did not show such associations. We therefore argue that the previously reported increased PDE levels in frontal/prefrontal regions of neuroleptic-naïve patients (Keshavan et al., 1989; Pettegrew et al., 1991; Shioiri et al., 1997; Stanley et al., 1994) and associations with cognitive impairment and increased negative symptoms (Deicken et al., 1995; Shioiri et al., 1994, 1997; Volz et al., 1998) are related to the glutamatergic regulation state. Taking into account the findings of animal studies mentioned before (Grace, 1993), enhanced “phasic” glutamatergic activity may predispose increased stress-induced dopamine release and concomitantly induce excitotoxicity and oxidative membrane damage (current literature on Glu induced excitotoxicity reviewed by (Plitman et al., 2014)). This pathway could explain focally increased membrane breakdown as indicated by higher PDE levels, or increased efforts of compensatory membrane regeneration and repair as reflected by increased PME levels.

Our direct group comparisons were unable to replicate the previously reported findings of increased prefrontal/frontal PDE or decreased PME (Keshavan et al., 1989; Pettegrew et al., 1991; Stanley et al., 1995). We speculate that the extent of membrane damage and respective processes

of restoration are dynamic and occur during the early course of acute illness. While in our patient population membrane turnover has been supposedly still in balance, it may progressively deteriorate with continuing active illness, as reported by other groups.

Regarding the left ACC we emphasize our observation that in all participants higher Glu levels were associated with higher PDE levels. This likely reflects a general close connection between brain activity and membrane lipid turnover in this brain region, which also implies high susceptibility to hyperexcitation and excitotoxicity. Again, in our population of early acute patients metabolism of the ACC did not differ (yet) from that of healthy individuals.

5. Limitations

Differing amounts of metabolites in gray or white matter (Hetherington et al., 2001; Jensen et al., 2006) or inter-individual variations of gray and white matter composition in voxels do influence spectroscopic measurements. To control for partial volume effects, we compared the gray matter fraction of each voxel between the groups and used it as a covariate in our group comparisons of Glu. Although we observed neither group differences in gray matter fractions nor significant group by GM interactions, we nevertheless stress that small partial volume effects cannot be completely ruled out.

As mentioned before, even significant results of our complex statistical model need to be interpreted with caution. While the highly significant increase of Glu in patients survived the conservative Bonferroni correction, this was not the case for the results of our interaction analyses. Despite this statistical weakness, we consider our results quite plausible and mainly in line with previous findings. Furthermore, we have combined for the first time ^1H - and ^{31}P -MRS in a clinical population, which explains its partly explorative character. Certainly, our results

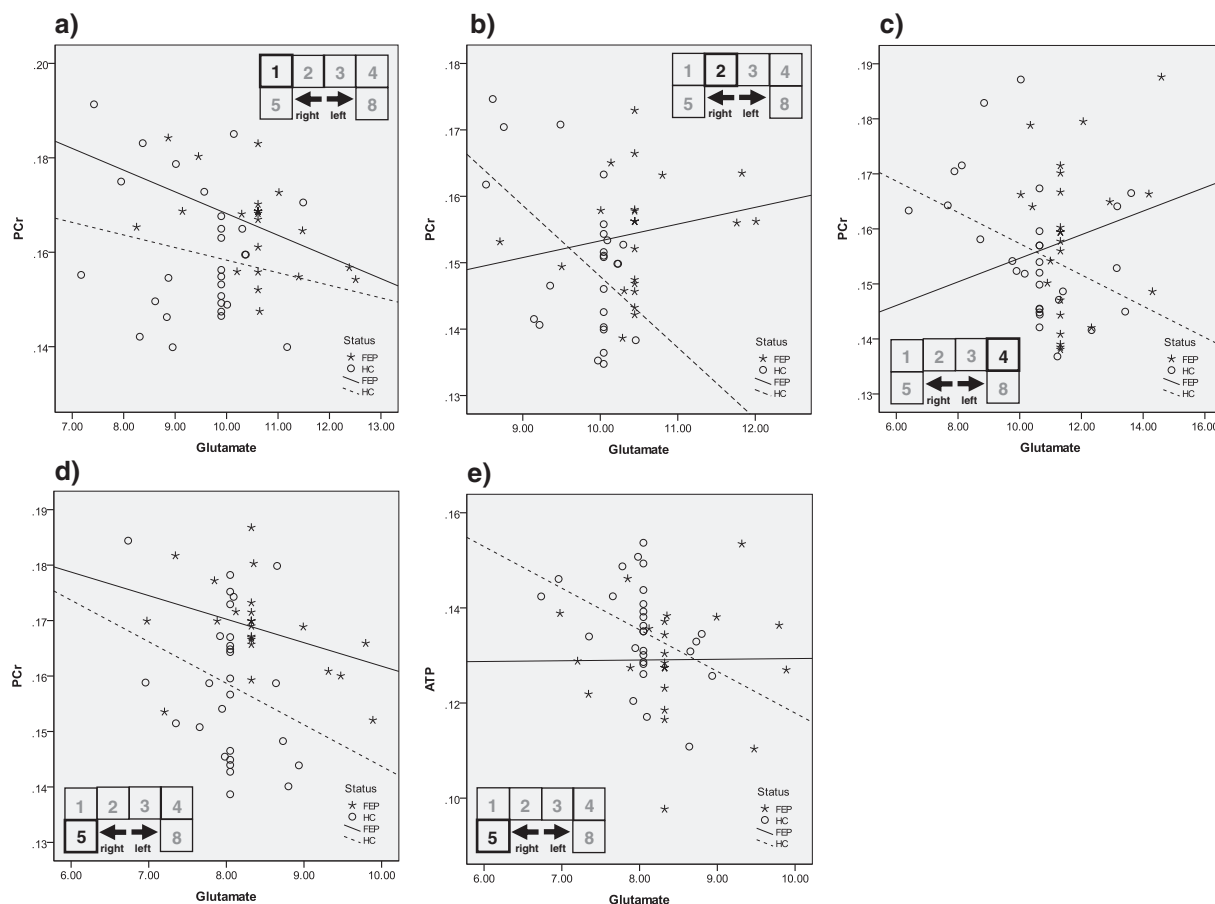


Fig. 3. Energy metabolism (PCr, ATP). The schematic presentation of the 6 voxels with increased Glu values (see Table 3) outlines the respective focal assignment of the interaction effect.

need confirmation by independent replication presumably in a multi-center setting.

6. Conclusion

Glutamatergic activity is increased in never treated early course acute schizophrenic patients in the bilateral prefrontal/frontal and anterior cingulate cortex. This hyperactivity seems disconnected from physiologically regulated energy metabolism but associated with increased membrane breakdown in right frontal and increased membrane restoration in left frontal regions. Considering findings of other research groups, this pathology is likely dynamic during the course of first acute illness and might be associated with negative symptoms and impairment of cognitive performance. Our findings underline the importance of further research on neuroprotective treatment options, e.g., restoration of polyunsaturated fatty acid deficits by supplementation (Berger et al., 2008; Hoen et al., 2013), during the early acute or even better for the ultra-high risk state of psychotic illness.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Author contributions

Drs Smesny and Gussew had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis; all authors contributed to the writing of the paper and have approved the final version.

Conflict of interest statement

We declare that we have no conflict of interest.

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