

Research paper

Supplementation of antipsychotic treatment with sarcosine – GlyT1 inhibitor – causes changes of glutamatergic ^1H NMR spectroscopy parameters in the left hippocampus in patients with stable schizophrenia



Dominik Strzelecki ^{a,*}, Michał Podgórski ^b, Olga Kałużyńska ^a, Oliwia Gawlik-Kotelnicka ^a, Ludomir Stefańczyk ^b, Magdalena Kotlicka-Antczak ^a, Agnieszka Gmitrowicz ^c, Piotr Grzelak ^b

^a Department of Affective and Psychotic Disorders, Medical University of Łódź, Central Clinical Hospital, ul. Pomorska 251, 92-213 Łódź, Poland

^b Department of Radiology and Diagnostic Imaging, Medical University of Łódź, Poland

^c Department of Adolescent Psychiatry, Medical University of Łódź, Poland

HIGHLIGHS

- Sarcosine decrease hippocampal glutamatergic parameters in schizophrenia.
- Sarcosine simultaneously improve mental state of patients with schizophrenia.
- Sarcosine is an effective augmentation of antipsychotic treatment.

ARTICLE INFO

Article history:

Received 11 May 2015

Received in revised form 30 July 2015

Accepted 20 August 2015

Available online 22 August 2015

Keywords:

Schizophrenia

Hippocampus

Sarcosine

Glutamate

NMDA receptor

^1H NMR spectroscopy

ABSTRACT

Glutamatergic system, the main stimulating system of the brain, plays an important role in the pathogenesis of schizophrenia. Hippocampus, a structure crucial for memory and cognitive functions and rich in glutamatergic neurons, is a natural object of interest in studies on psychoses. Sarcosine, a glycine transporter (GlyT-1) inhibitor influences the function of NMDA receptor and glutamate-dependent transmission.

The aim of the study was to assess the effects of sarcosine on metabolism parameters in the left hippocampus in patients with schizophrenia. Assessments were performed using proton nuclear magnetic resonance (^1H NMR) spectroscopy (1.5T).

Fifty patients diagnosed with schizophrenia (DSM-IV-TR), with dominant negative symptoms, in stable clinical condition and stable antipsychotics doses were treated either with sarcosine ($n=25$) or placebo ($n=25$). Spectroscopic parameters were evaluated within groups and between two groups before and after 6-month intervention. All patients were also assessed with the Positive and Negative Syndrome Scale (PANSS).

In the sarcosine group, after 6-month treatment, we found significant decrease in hippocampal Glx/Cr (Glx-complex of glutamate, glutamine and GABA, Cr-creatine) and Glx/Cho (Cho-choline), while *N*-acetylaspartate (NAA), myo-inositol (mI), Cr and Cho parameters remained stable along the study and also did not differ significantly between both groups.

This is the first study showing that a pharmacological intervention in schizophrenia, particularly augmentation of the antipsychotic treatment with sarcosine, may reverse the pathological increase in glutamatergic transmission in the hippocampus. The results confirm involvement of glutamatergic system in the pathogenesis of schizophrenia and demonstrate beneficial effects of GlyT-1 inhibitor on the metabolism in the hippocampus and symptoms of schizophrenia.

© 2015 Elsevier Ireland Ltd. All rights reserved.

* Corresponding author. Fax: +48 426757403.

E-mail address: dominik.strzelecki@umed.lodz.pl (D. Strzelecki).

1. Introduction

Schizophrenia is a serious global health problem, with lifetime prevalence of 0.30–0.66%, and incidence of 10.2–22.0 per 100,000 person-years [1]. Antipsychotic drugs, whose mechanism is mostly based on the blockade of dopamine and serotonin receptors, are effective in the treatment of psychotic symptoms (delusions, hallucinations, disorganized thinking and behavior). However, their effectiveness is limited regarding negative symptoms (autism, emotional flatness, social withdrawal), affective symptoms (sadness, anxiety) and cognitive dysfunction (impairment of executive functions, concentration, attention), which to a large degree, determine the outcome, everyday functioning and quality of life. In majority of patients the disease leads to disability, while only 13.5% of patients meet the recovery criteria [2].

Lately, it was proposed that glutamatergic and GABAergic systems may be involved in the pathogenesis of schizophrenia. NMDA (*N*-methyl-D-aspartate) receptors are located all over the brain, but location on inhibitory GABAergic neurons is especially an object of interest. Lack of adequate control on glutamatergic transmission (also influencing dopaminergic system) causes information bias and both negative and cognitive symptoms in schizophrenia [3–5].

Sarcosine – an exogenous amino acid – is serving in the brain as a glycine transporter type 1 (GlyT-1) inhibitor and as a source of glycine (natural coagonist of the NMDA receptor, metabolized from sarcosine by sarcosine dehydrogenase) [6]. It was reported to be effective in treating negative and cognitive symptoms [7].

Supplementation of sarcosine at a 2 grams daily dose is supposed to increase glycine concentration and normalize hypo-function of the NMDA receptors, which are present in high density in the prefrontal cortex and hippocampus—areas associated with development of cognitive and negative symptomatology [5,8,9].

We assume that neurochemical (and clinical) changes may also affect other parameters including chemical compounds typically assessed in proton magnetic resonance spectroscopy (^1H NMR) as Glx (complex of glutamate, glutamine and GABA), *N*-acetylaspartate (NAA), myo-inositol (ml), creatine (Cr) and choline (Cho).

^1H NMR studies on schizophrenia in general did not allow to draw definite conclusions about changes in metabolic parameters depending on the studied brain regions, symptoms severity, stages of the disease or the treatment strategy [10–13].

Several projects assessed glutamatergic parameters in the hippocampus. Majority of them reported no significant differences in glutamate levels between medicated chronic patients [14,15], drug naïve [16] and medicated first episode patients [14]. Other observations indicated increase in Glu (glutamate) [17] and Glx levels in the hippocampal area in patients with medicated chronic schizophrenia [18] as well as without antipsychotic treatment [19]. Glu level in the left hippocampus was also increased in 22q11 deletion syndrome with schizophrenia symptoms [20].

Effect of antipsychotic treatment on Glx level in the hippocampus was also analyzed. Polish researchers reported no significant differences in Glx level after four weeks of treatment with risperidone [21].

In schizophrenia, reduced concentration of *N*-acetylaspartate (NAA) in various brain areas was most frequently observed. NAA is considered to be a marker of density and integrity of neurons [22], and its decrease is a derivative of neuronal loss or mitochondrial dysfunction [23].

In schizophrenia, there is less spectroscopic data concerning the hippocampus than the prefrontal cortex and the anterior cingulate gyrus. In the studies on the hippocampus, patients in chronic phase of the disease, had reduced NAA concentrations [14,24–30] and NAA/Cr ratio [31–37]. On the other hand, results of other studies

did not show significant changes in these parameters in comparison to healthy controls [38–41]. Results of meta-analyses are also ambiguous. According to earlier meta-analysis, levels of NAA in the hippocampus were significantly reduced [10], while in more recent paper no significant difference was reported [41].

Myoinositol, inositol stereoisomer and a precursor in the phosphatidylinositol second messenger system is accepted as a glial marker in spectroscopic studies. In neurodegenerative diseases increase of ml levels co-occur with reduced NAA concentrations [42]. Other compounds as Cr and Cho are described as relatively stable during therapeutic interventions, therefore when determining the concentrations of substances is not possible, ratios to Cr and Cho are commonly used.

We aim to evaluate effects of sarcosine therapy on the concentrations of NAA, Glx, ml, Cho and Cr in the left hippocampus in patients with schizophrenia. Our study can bring new facts on the role of the glutamatergic system in the pathogenesis of schizophrenia and also support basic information on psychopharmacological suitability of sarcosine and GlyT-1 inhibitors in schizophrenia.

2. Materials and methods

2.1. Subjects

Subjects diagnosed with schizophrenia (according to DSM-IV-TR criteria), with dominant negative symptoms, in stable clinical condition, were eligible to enter the study. Fifty right-handed, aged 18–60 years, physically, neurologically and endocrinologically healthy patients having normal laboratory tests results (routine blood tests, biochemical tests including TSH, lipid profile, ECG) were randomly assigned to either a sarcosine or a placebo group in a double blinded manner. In both groups ^1H -NMR spectroscopy was performed at the beginning of the study and 6 months later according to the protocol described below. Sarcosine or placebo were added to the ongoing antipsychotic treatment. Patients in the study group were given plastic capsules containing 2 grams of the amino acid, while subjects in control group received capsules with microcrystalline cellulose. Subjects in both groups were administered to drink dissolved contents of one capsule once a day in the morning. All patients were treated with stable doses of antipsychotic drugs for a minimum 3 months before a baseline visit. Doses of antipsychotic drugs were calculated for defined daily doses (DDD) developed by the World Health Organization. Severity of schizophrenia symptoms was assessed with the Positive and Negative Syndrome Scale (PANSS) [43] in the day of spectroscopy. Antidepressants were used as a supportive therapy for affective and negative symptoms [44]. They were administered to 14 patients in the sarcosine group and 11 in the control group, however the difference was not significant ($p = 0.43211$). Characteristic of study groups is presented in Table 1.

Subjects were recruited from an outpatient clinic and informed about aims and methods of the study. All individuals gave their written informed consent for participation in this study. The study protocol was in accordance with the Declaration of Helsinki and was approved by the local Bioethics Committee. For further details of the project – Polish Sarcosine Study (PULSAR) – please see acknowledgments.

2.2. Spectroscopy

Imaging was performed using 1.5T MR scanner (Siemens Avanto 1.5) equipped with a standard head coil.

MRI acquisition:

Table 1

Characteristic of groups.

Features		Group		p-Value
		Sarcosine (n = 25)	Control (n = 25)	
Gender	Female	14	12	>0.05
	Male	11	13	>0.05
Age (mean)		36.5	40	>0.05
Mean number of hospitalizations		5	4	>0.05
Mean duration of the illness [years]		12.3	13.2	>0.05
Mean timespan of education per patient		14.2	14.4	>0.05
Antipsychotic treatment (DDD)		1.94	1.97	>0.05
Antidepressive treatment (DDD)		0.58	0.6	>0.05
PANSS total (\pm SD)		71.4 \pm 14	73.3 \pm 13	>0.05

Abbreviations: DDD—defined daily dose, PANSS—the Positive and Negative Syndrome Scale.

1. FLAIR sequences in axial plane with following parameters: Repetition Time (TR) = 9000 ms; Echo Time (TE) = 105 ms; inversion time (TI) = 2500 ms; flip angle = 150°; voxel size 1.4 × 1.3 × 3 mm.
2. T2-weighted sequences were obtained in coronal plane with following parameters: TR = 5000 ms; TE = 100 ms; flip angle = 50°; voxel size 0.6 × 0.6 × 5.0 mm.
3. T1 weighted sequences in transverse plane with following parameters: TR = 400 ms; TE = 7.8 ms; flip angle, 90° g; voxel size 0.9 × 0.9 × 0.5 mm.

¹H-MRS data was acquired by single voxel spectroscopy (SVS), using a point resolved spin echo (PRESS) sequence 128 averages; TR = 3000 ms; TE = 30 ms; voxel size 15 × 15 × 15 mm. The region of interest was placed in a left hippocampus by the neuroradiologist (Fig. 1). During the second spectroscopy, voxel localization parameters were copied and adjusted to the position of patient. Automated procedures were used to optimize radiofrequency pulse power, field homogeneity, and water suppression, as well as to convert the lines into a Gaussian shape. No absolute concentrations of metabolites were determined, but ratios to Cr and Cho.

2.3. Statistical analysis

Continuous variables are expressed as the mean ± standard deviation (SD). The Shapiro-Wilk test was used to determine the normality of the data. In comparison of ratios of substance concentrations between both groups, the Mann-Whitney test was employed, while the Wilcoxon sing rank test was performed within the same group. Statistical analysis was performed using Statistica for Windows (version 12.0, StatSoft). A p-value of ≤0.05 was considered significant.

3. Results

At baseline examination there were no differences regarding spectroscopic parameters between both studied groups (Table 2). There were also no significant differences in substance concentration ratios between sarcosine and placebo group on second assessment. However, after the therapy in the experimental group, Glx/Cr and Glx/Cho ratios significantly decreased comparing to baseline values (21.4% and 21.2%, respectively).

At the beginning of the experiment, there was no significant difference in the PANSS scores between both groups. However at the end of the experiment patients treated with sarcosine had significantly lower results in negative and general psychopathology subscales and total score in the PANSS (Table 3).

4. Discussion

To our knowledge, this is the first study assessing the effects of sarcosine (or glutamatergic agents) on spectroscopic parameters in the hippocampus of patients with schizophrenia. Differences in Glx/Cr and Glx/Cho ratios in the experimental group indicate that 2 g of sarcosine daily is a sufficient dose to cross the blood-brain barrier and affect the glutamatergic function within hippocampus as well as improve mental status of patients with schizophrenia.

4.1. Glx

A significant decrease in Glx/Cho and Glx/Cr ratios in the experimental group may indicate a positive effect of sarcosine in managing schizophrenia.

An increase in hippocampal parameters associated with the glutaminergic transmission was observed in schizophrenia [17–19,21]. It was speculated that psychotic process results from

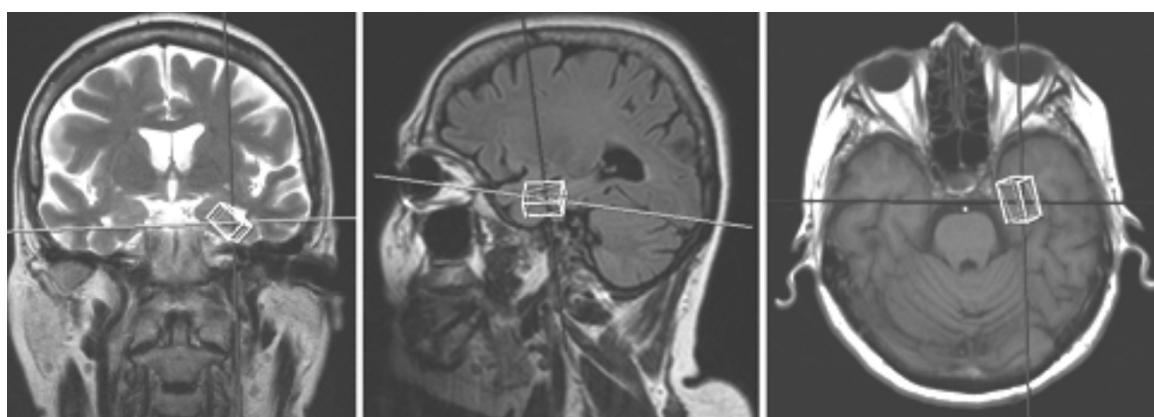


Fig. 1. Images showing voxel location in the left hippocampus.

Table 2

Comparison of ratios of substance concentrations in the study group.

Compared ratios	Baseline			After 6 months			Baseline vs. after 6 months	Baseline vs. after 6 months
	Sarcosine (mean ± SD)	Placebo (mean ± SD)	p-Level	Sarcosine (mean ± SD)	Placebo (mean ± SD)	p-Level		
NAA/Cr	3.38 (2.55)	2.43 (1.79)	>0.05	2.03 (0.53)	2.75 (1.37)	>0.05	>0.05	>0.05
Cho/Cr	1.31 (0.87)	1.21 (0.84)	>0.05	1.02 (0.34)	1.05 (0.86)	>0.05	>0.05	>0.05
ml/Cr	0.63 (0.69)	0.70 (0.90)	>0.05	0.28 (0.18)	0.45 (0.35)	>0.05	>0.05	>0.05
Glx/Cr	1.40 (0.70)	0.97 (0.43)	>0.05	1.10 (0.74)	0.92 (0.50)	>0.05	0.028	>0.05
NAA/Cho	2.72 (1.96)	3.21 (1.69)	>0.05	1.89 (0.87)	4.26 (4.14)	>0.05	>0.05	>0.05
ml/Cho	1.63 (4.93)	0.83 (1.04)	>0.05	0.35 (0.31)	0.80 (1.51)	>0.05	>0.05	>0.05
Glx/Cho	1.18 (0.50)	1.49 (0.81)	>0.05	0.93 (0.50)	1.38 (0.82)	>0.05	0.0425	>0.05

Abbreviations: NAA—N-acetylaspartate, Cr—creatinine, Cho—choline, ml—myo-inositol, Glx—glutamine, glutamate and GABA.

Table 3

Differences in PANSS subscales and total score.

PANSS	Baseline			After 6 months			p-Level
	Sarcosine (mean ± SD)	Placebo (mean ± SD)	p-Level	Sarcosine (mean ± SD)	Placebo (mean ± SD)	p-Level	
Positive	10.8 (3.1)	10.9 (3.7)	0.8937	9.9 (2.6)	10.8 (3.5)	0.4021	
Negative	26.0 (4.7)	25.8 (5.0)	0.8931	19.2 (6.3)	25.5 (5.0)	0.0014	
General	34.6 (8.1)	36.5 (7.6)	0.4489	28.6 (8.9)	35.2 (7.6)	0.0170	
Total	71.4 (14.0)	73.3 (12.8)	0.6736	57.7 (15.0)	71.5 (13.0)	0.0049	

impaired glutamate transmission within the dentate gyrus in the hippocampus complex, which in consequence leads to hyperactivity of glutamatergic transmission within the CA3, CA1 (a part of the Cornu Ammonis) and the subiculum of the hippocampus [45,46]. Dysfunctional CA3 neurons may cause increased neuronal excitability, along with increased cerebral perfusion and inadequate plasticity changes, such as disturbances of a long-term potentiation process (LTP) in the mentioned subfield [45,47]. Impaired transmission in the dentate gyrus can be explained by the changes in the structure of NMDA receptor (decreased number of NR1 subunit in the left hippocampus) [48], defective neurogenesis [49], DISC1 [50] or NRG1 [51] gene effects, or disturbances of inhibitory GABAergic transmission [52].

Disturbances of inhibitory processes, which in schizophrenia result from hypofunction of NMDA receptors on GABAergic interneurons, may be of particular importance [53]. An increased glutamate stimulation in the hippocampus, is generally believed to be responsible for the attention deficits and cognitive dysfunction [45]. Rüsch observed, that increased glutamate concentrations in hippocampus were correlated with poorer cognitive function (assessed with the Wisconsin Card Sorting Test (WCST)) in patients with schizophrenia, but not in healthy controls [14].

Insufficient control of the stimulatory system may also lead to the development of hallucinations, delusions and formal thought disorders typical for the acute psychosis [54]. These inhibitory control dysfunctions may be expressed in disturbances of coherent neuronal oscillation at a rate below 0.1 Hz [55] and changes in gamma rhythms (25–100 Hz) [53,56], which both may resemble increased information noise.

Our results indicate that sarcosine may reduce pathological hyperactivity within the hippocampus. It is the first evidence that the application of pharmacological agents can reverse tendency to increase the glutamate concentration in the hippocampus across the course of schizophrenia with a simultaneous improvement in mental status.

4.2. NAA

Changes in NAA/Cho and NAA/Cr ratios between spectroscopies in both experimental and control group were not significant. Differences between the groups were also not significant. However, what

we take as surprise in light of previous reports, comparison of the average values in both parameters shows a decrease in sarcosine group (NAA/Cr of 40%, NAA/Cho 30%) and an increase in controls (13% and 32%, respectively). These differences can be interpreted as lowering of overall neuronal metabolism in sarcosine group as a result of a reduction of excessive glutamatergic activity in the hippocampus, which is composed mainly of glutamatergic neurons.

4.3. ml

Although our results did not reach statistical significance, in the study group there was a massive reduction of mean ml/Cho ratio and a marked of ml/Cr ratio comparing to the baseline. In the control group we observed also a non-significant decrease in ml/Cho ratio, but ml/Cr ratio was relatively stable in both assessments.

4.4. Limitations of the study

Due to the small number of patients and the technical limitations of spectroscopy performed with 1.5T magnetic field, conclusions should be drawn carefully.

The main technical limitation is 1.5T field. In spectroscopy Glx pick was a combined signal of glutamate, glutamine and partially GABA, which cannot be separated with 1.5T magnetic field. However, Glx along with Glx/Cr or Glx/Cho ratios can be regarded as a derivative of glutamate transmission because glutamate concentration is 5-fold higher than glutamine and 10-fold higher than GABA concentration [57]. Moreover, Glu and Gln are closely associated. After Glu is released from neurons, it is reabsorbed by glia and transformed to Gln, which is subsequently transferred to neurons and then again transformed to Glu [58]. In the future, assessment of GABA with at least 3T field, would be particularly interesting due to the influence of sarcosine on GABA interneurons function.

Other important limitation of this work is application of ratios of metabolites concentrations instead of exact concentrations. Despite possible changes of Cr and Cho along the course of schizophrenia, it was proved that treatment with either atypical or typical medication does not alter Cr or Cho levels [58]. Thus, ratios might have a good intra-subject validity [58].

5. Conclusion

We conclude that augmentation of the antipsychotic treatment with sarcosine may reverse the increase in glutamatergic transmission in the left hippocampus in schizophrenia along with improvement of mental state, assessed with the PANSS. The results confirm involvement of glutamatergic system in the pathogenesis of schizophrenia and demonstrate beneficial effects of GlyT-1 inhibitor on the metabolism in the hippocampus.

Conflict of interest

No potential conflict of interest.

Acknowledgments

NMR study described here is a part of a larger project—PULSAR study (Polish Sarcosine Study in Schizophrenia) supported by the Polish Ministry of Science and Higher Education (grant N402 268836). More details about the project is available on Clinicaltrials.gov site, number NCT01503359.

References

- [1] J. McGrath, S. Saha, D. Chant, J. Welham, Schizophrenia: a concise overview of incidence, prevalence, and mortality, *Epidemiol. Rev.* 30 (2008) 67–76.
- [2] E. Jääskeläinen, P. Juola, N. Hirvonen, J.J. McGrath, S. Saha, M. Isohanni, J. Veijola, J. Miettunen, A systematic review and meta-analysis of recovery in schizophrenia, *Schizophr. Bull.* 39 (2013) 1296–1306.
- [3] A. Carlsson, N. Waters, S. Waters, M.L. Carlsson, Network interactions in schizophrenia –therapeutic implications, *Brain Res. Brain Res. Rev.* 31 (2000) 342–349.
- [4] J.T. Coyle, The glutamatergic dysfunction hypothesis for schizophrenia, *Harv. Rev. Psychiatry* 3 (1996) 241–253.
- [5] J.W. Olney, J.W. Newcomer, N.B. Farber, NMDA receptor hypofunction model of schizophrenia, *J. Psychiatr. Res.* 33 (1999) 523–533.
- [6] J. Kantrowitz, D.C. Javitt, Glutamatergic transmission in schizophrenia: from basic research to clinical practice, *Curr. Opin. Psychiatry* 25 (2012) 96–102.
- [7] S.P. Singh, V. Singh, Meta-analysis of the efficacy of adjunctive NMDA receptor modulators in chronic schizophrenia, *CNS Drugs* 25 (2011) 859–885.
- [8] D.T. Mattson, M. Berk, M.D. Lucas, A neuropsychological study of prefrontal lobe function in the positive and negative subtypes of schizophrenia, *J. Genet. Psychol.* 158 (1997) 487–494.
- [9] A. Qiu, T.A. Tuan, P.S. Woon, M.F. Abdul-Rahman, S. Graham, K. Sim, Hippocampal-cortical structural connectivity disruptions in schizophrenia: an integrated perspective from hippocampal shape, cortical thickness, and integrity of white matter bundles, *Neuroimage* 52 (2010) 1181–1189.
- [10] R.G. Steen, R.M. Hamer, J.A. Lieberman, Measurement of brain metabolites by ¹H magnetic resonance spectroscopy in patients with schizophrenia: a systematic review and meta-analysis, *Neuropsychopharmacology* 30 (2005) 1949–1962.
- [11] S. Brugge, J.M. Davis, S. Leucht, J.M. Stone, Proton magnetic resonance spectroscopy and illness stage in schizophrenia—a systematic review and meta-analysis, *Biol. Psychiatry* 69 (5) (2011) 495–503.
- [12] N.V. Kraguljac, M. Reid, D. White, R. Jones, J. den Hollander, D. Lowman, A.C. Lahti, Neurometabolites in schizophrenia and bipolar disorder—a systematic review and meta-analysis, *Psychiatry Res.* 203 (2012) 111–125.
- [13] A. Marsman, M.P. van den Heuvel, D.W. Klomp, R.S. Kahn, P.R. Luijten, H.E. Hulshoff Pol, Glutamate in schizophrenia: a focused review and meta-analysis of ¹H-MRS studies, *Schizophr. Bull.* 39 (2013) 120–129.
- [14] N. Rüsch, L. Tebartz van Elst, G. Valerius, M. Buchert, T. Thiel, D. Ebert, J. Hennig, H.M. Olbrich, Neurochemical and structural correlates of executive dysfunction in schizophrenia, *Schizophr. Res.* 99 (2008) 155–163.
- [15] N.V. Kraguljac, M.A. Reid, D.M. White, J. den Hollander, A.C. Lahti, Regional decoupling of N-acetyl-aspartate and glutamate in schizophrenia, *Neuropsychopharmacology* 37 (2012) 2635–2642.
- [16] R. Bartha, Y.M. al-Semaan, P.C. Williamson, D.J. Drost, A.K. Malla, T.J. Carr, M. Densmore, G. Canaran, R.W. Neufeld, A short echo proton magnetic resonance spectroscopy study of the left mesial-temporal lobe in first-onset schizophrenic patients, *Biol. Psychiatry* 45 (1999) 1403–1411.
- [17] L.T. van Elst, G. Valerius, M. Buchert, T. Thiel, N. Rusch, E. Bubl, J. Hennig, D. Ebert, H.M. Olbrich, Increased prefrontal and hippocampal glutamate concentration in schizophrenia: evidence from a magnetic resonance spectroscopy study, *Biol. Psychiatry* 58 (2005) 724–730.
- [18] L. Chang, J. Friedman, T. Ernst, K. Zhong, N.D. Tsopelas, K. Davis, Brain metabolite abnormalities in the white matter of elderly schizophrenic subjects: implication for glial dysfunction, *Biol. Psychiatry* 62 (2007) 1396–1404.
- [19] N.V. Kraguljac, D.M. White, M.A. Reid, A.C. Lahti, Increased hippocampal glutamate and volumetric deficits in unmedicated patients with schizophrenia, *JAMA Psychiatry* 70 (2013) 1294–1302.
- [20] F. da Silva Alves, E. Boot, N. Schmitz, A. Nederveen, J. Vorstman, C. Lavini, P.J. Pouwels, L. de Haan, D. Linszen, T. van Amelsvoort, Proton magnetic resonance spectroscopy in 22q11 deletion syndrome, *Plos One* 6 (2011) e21685.
- [21] A. Szulc, B. Galińska, E. Tarasów, W. Dzienis, B. Kubas, B. Konarzewska, J. Walecki, A.S. Alathiaki, A. Czernikiewicz, 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 (2005) 214–219.
- [22] J.R. Moffett, B. Ross, P. Arun, C.N. Madhavarao, A.M. Namboodiri, *N*-acetylaspartate in the CNS: from neurodiagnostics to neurobiology, *Prog. Neurobiol.* 81 (2007) 89–131.
- [23] T.N. Sager, S. Topp, L. Torup, L.G. Hanson, B. Egestad, A. Moller, Evaluation of CA1 damage using single-voxel ¹H-MRS and un-biased stereology: can non-invasive measures of *N*-acetyl-aspartate following global ischemia be used as a reliable measure of neuronal damage? *Brain Res.* 892 (2001) 166–175.
- [24] R.F. Deicken, L. Zhou, N. Schuff, G. Fein, M.W. Weiner, Hippocampal neuronal dysfunction in schizophrenia as measured by proton magnetic resonance spectroscopy, *Biol. Psychiatry* 43 (1998) 483–488.
- [25] R.F. Deicken, M. Pegues, D. Amend, Reduced hippocampal *N*-acetylaspartate without volume loss in schizophrenia, *Schizophr. Res.* 37 (1999) 217–223.
- [26] G. Ende, D.F. Braus, S. Walter, W. Weber-Fahr, F.A. Henn, Multiregional ¹H-MRSI of the hippocampus, thalamus, and basal ganglia in schizophrenia, *Eur. Arch. Psychiatry Clin. Neurosci.* 253 (2003) 9–15.
- [27] M. Maier, M.A. Ron, G.J. Barker, P.S. Tofts, Proton magnetic resonance spectroscopy: an *in vivo* method of estimating hippocampal neuronal depletion in schizophrenia, *Psychol. Med.* 25 (1995) 1201–1209.
- [28] M. Maier, M.A. Ron, Hippocampal age-related changes in schizophrenia: a proton magnetic resonance spectroscopy study, *Schizophr. Res.* 22 (1996) 5–17.
- [29] W. Weber-Fahr, G. Ende, D.F. Braus, P. Bachert, B.J. Soher, F.A. Henn, C. Büchel, A fully automated method for tissue segmentation and CSF-correction of proton MRSI metabolites corroborates abnormal hippocampal NAA in schizophrenia, *Neuroimage* 16 (2002) 49–60.
- [30] A.A. Klär, M. Ballmaier, K. Leopold, I. Häke, M. Schaefer, R. Brühl, F. Schubert, J. Gallinat, Interaction of hippocampal volume and *N*-acetylaspartate concentration deficits in schizophrenia: a combined MRI and 1H-MRS study, *Neuroimage* 53 (2010) 51–57.
- [31] A. Bertolino, S. Nawroz, V.S. Mattay, A.S. Barnett, J.H. Duyn, C.T.W. Moonen, J.A. Frank, G. Tedeschi, D.R. Weinberger, Regionally specific pattern of neurochemical pathology in schizophrenia as assessed by multislice proton magnetic resonance spectroscopic imaging, *Am. J. Psychiatry* 153 (1996) 1554–1563.
- [32] A. Bertolino, S. Kumra, J.H. Callicott, V.S. Mattay, R.M. Lestz, L. Jacobsen, I.S. Barnett, J.H. Duyn, J.A. Frank, J.L. Rapoport, D.R. Weinberger, Common pattern of cortical pathology of childhood-onset and adult-onset schizophrenia as identified by proton magnetic resonance spectroscopic imaging, *Am. J. Psychiatry* 155 (1998) 1376–1383.
- [33] A. Bertolino, J.H. Callicott, I. Elman, V.S. Mattay, G. Tedeschi, J.A. Frank, A. Breier, D.R. Weinberger, Regionally specific neuronal pathology in untreated patients with schizophrenia: a proton magnetic resonance spectroscopic imaging study, *Biol. Psychiatry* 43 (1998) 641–648.
- [34] A. Bertolino, J.H. Callicott, S. Nawroz, V.S. Mattay, J.H. Duyn, G. Tedeschi, J.A. Frank, D.R. Weinberger, Reproducibility of proton magnetic resonance spectroscopic imaging in patients with schizophrenia, *Neuropsychopharmacology* 18 (1998) 1–9.
- [35] A. Bertolino, D. Scioti, F. Brudaglio, M. Altamura, G. Blasi, A. Bellomo, N. Antonucci, J.H. Callicott, T.E. Goldberg, T. Scarabino, D.R. Weinberger, M. Nardini, Working memory deficits and levels of *N*-acetylaspartate in patients with schizopreniform disorder, *Am. J. Psychiatry* 160 (2003) 483–489.
- [36] J.H. Callicott, M.F. Egan, A. Bertolino, V.S. Mattay, F.J.P. Langheim, J.A. Frank, D.R. Weinberger, Hippocampal *N*-acetyl aspartate in unaffected siblings of patients with schizophrenia: a possible intermediate neurobiological phenotype, *Biol. Psychiatry* 44 (1998) 941–950.
- [37] D. Fannon, A. Simmons, L. Tennakoon, S. O'Ceallaigh, A. Sumich, V. Doku, C. Shew, T. Sharma, Selective deficit of hippocampal *N*-acetylaspartate in antipsychotic-naïve patients with schizophrenia, *Biol. Psychiatry* 54 (2003) 587–598.
- [38] P. Delamillieure, J.M. Constans, J. Fernandez, P. Braze, K. Benali, P. Courtheoux, F. Thibaut, M. Petit, S. Dollfus, Proton magnetic resonance spectroscopy (1H MRS) in schizophrenia: investigation of the right and left hippocampus, thalamus, and prefrontal cortex, *Schizophr. Bull.* 28 (2002) 329–339.
- [39] L.S. Kegeles, D.C. Shungu, S. Anjilvel, S. Chan, S.P. Ellis, E. Xanthopoulos, D. Malaspina, J.M. Gorman, J.J. Mann, M. Laruelle, C.A. Kaufmann, Hippocampal pathology in schizophrenia: magnetic resonance imaging and spectroscopy studies, *Psychiatry Res* 98 (2000) 163–175.
- [40] E.S. Lutkenhoff, T.G. van Erp, M.A. Thomas, S. Therman, M. Manninen, M.O. Huttunen, J. Kaprio, J. Lonnqvist, J. O'Neill, T.D. Cannon, Proton MRS in twin pairs discordant for schizophrenia, *Mol. Psychiatry* 15 (2010) 308–318.
- [41] H.M. Olbrich, G. Valerius, N. Rusch, M. Buchert, T. Thiel, J. Hennig, D. Ebert, L.T. Van Elst, Frontolimbic glutamate alterations in first episode schizophrenia:

- evidence from a magnetic resonance spectroscopy study, *World J. Biol. Psychiatry* 9 (2008) 59–63.
- [42] J.R. Bustillo, Use of proton magnetic resonance spectroscopy in the treatment of psychiatric disorders: a critical update, *Dialogues Clin. Neurosci.* 15 (2013) 329–337.
- [43] S.R. Kay, A. Fiszbein, L.A. Opfer, The Positive and Negative Syndrome Scale (PANSS) for Schizophrenia, *Schizophr. Bull.* 13 (1987) 261–276.
- [44] S.P. Singh, V. Singh, N. Kar, K. Chan, Efficacy of antidepressants in treating the negative symptoms of chronic schizophrenia: meta-analysis, *Br. J. Psychiatry* 197 (2010) 174–179.
- [45] C.A. Tamminga, A.D. Stan, A.D. Wagner, The hippocampal formation in schizophrenia, *Am. J. Psychiatry* 167 (2010) 1178–1193.
- [46] X.-M. Guo, K. Sakai, R.C. Roberts, R.R. Conley, B. Dean, C.A. Tamminga, Ionotropic glutamate receptors and expression of *N*-methyl-D-aspartate receptor subunits in subregions of human hippocampus: effects of schizophrenia, *Am. J. Psychiatry* 157 (2000) 1141–1149.
- [47] R.C. Malenka, M.F. Bear, LTP and LTD: an embarrassment of riches, *Neuron* 44 (2004) 5–21.
- [48] M. Vrajdová, F. Stastný, J. Horáček, J. Lochman, O. Serý, S. Peková, J. Klaschka, C. Höschl, Expression of the hippocampal NMDA receptor GluN1 subunit and its splicing isoforms in schizophrenia: postmortem study, *Neurochem. Res.* 35 (2010) 994–1002.
- [49] A. Reif, A. Schmitt, S. Fritzen, K.P. Lesch, Neurogenesis and schizophrenia: dividing neurons in a divided mind? *Eur. Arch. Psychiatry Clin. Neurosci.* 257 (2007) 290–299.
- [50] X. Duan, J.H. Chang, S. Ge, R.L. Faulkner, J.Y. Kim, Y. Kitabatake, X.B. Liu, C.H. Yang, J.D. Jordan, D.K. Ma, C.Y. Liu, S. Ganesan, H.J. Cheng, G.L. Ming, B. Lu, H. Song, Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain, *Cell* 130 (2007) 1146–1158.
- [51] B. Li, R.S. Woo, L. Mei, R. Malinow, The neuregulin-1 receptor erbB4 controls glutamatergic synapse maturation and plasticity, *Neuron* 54 (2007) 583–597.
- [52] V. Varga, A. Losonczy, B.V. Zemelman, Z. Borhegyi, G. Nyiri, A. Domonkos, B. Hangya, N. Holderith, J.C. Magee, T.F. Freund, Fast synaptic subcortical control of hippocampal circuits, *Science* 326 (2009) 449–453.
- [53] J.E. Lisman, J.T. Coyle, R.W. Green, D.C. Javitt, F.M. Benes, S. Heckers, A.A. Grace, Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia, *Trends Neurosci.* 31 (2008) 234–242.
- [54] S.M. Cohen, R.W. Tsien, D.C. Goff, M.M. Halassa, The impact of NMDA receptor hypofunction on GABAergic neurons in the pathophysiology of schizophrenia, *Schizophr. Res.* (2015) <http://dx.doi.org/10.1016/j.schres.2014.12.026>
- [55] M.D. Fox, M.E. Raichle, Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging, *Nat. Neurosci. Rev.* 8 (2007) 700–711.
- [56] G. Gonzalez-Burgos, D.A. Lewis, NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia, *Schizophr. Bull.* 38 (2012) 950–957.
- [57] A. Szulc, B. Konarzewska, B. Galińska-Skok, J. Łazarczyk, N. Waszkiewicz, E. Tarasów, R. Milewski, J. Walecki, Proton magnetic resonance spectroscopy measures related to short-term symptomatic outcome in chronic schizophrenia, *Neurosci. Lett.* 547 (2013) 37–41.
- [58] A. Schwerk, F.D. Alves, P.J. Pouwels, T. van Amelsvoort, Metabolic alterations associated with schizophrenia: a critical evaluation of proton magnetic resonance spectroscopy studies, *J. Neurochem.* 128 (2014) 1–87.