D-serine serum levels in patients with schizophrenia: Relation to psychopathology and comparison to healthy subjects

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Abstract

OBJECTIVES: The main objective was to test the hypothesis of the association between D-serine serum levels and negative symptoms in patients with schizophrenia. Secondary objective was to examine the assumption of D-serine serum levels difference between a population of mostly chronic patients with schizophrenia and healthy controls.

METHODS: We recruited outpatients with schizophrenia and age and gender matched healthy controls for the study. D-serine and total serine serum levels were measured by high-performance liquid chromatography (HPLC). The Positive and Negative Syndrome Scale (PANSS) and The Scale for the Assessment of Negative Symptoms (SANS) were used to assess schizophrenic symptoms. Non-parametric statistics was used to test the differences in D-serine and total serine serum levels and rank correlation was used to detect the associations with psychopathology.

RESULTS: We did not find any differences between patients (n=50) and controls (n=50) in D-serine serum levels. Patients had significantly lower total serine serum levels and higher D-serine/total serine ratio. D-serine serum levels were not associated with the PANSS or the SANS total and subscales scores. Total serine serum levels inversely correlated with the SANS total and the PANSS negative symptom subscale scores.

CONCLUSION: Decreased, not increased, serum levels of total serine negatively associated with intensity of negative symptoms were detected in patients with schizophrenia. We did not find any relationship between D-serine serum levels and negative symptoms among the patients. These findings do not agree with the previous reports of decreased D-serine and increased total serine serum levels in schizophrenia.

ORIGINAL ARTICLE

INTRODUCTION

Recently, there has been an accumulating evidence on the links between N-methyl-D-aspartate (NMDA) receptor dysfunction and pathogenesis of schizophrenia (Krystal *et al*. 2002; Moghaddam & Jackson, 2003). The interest in the role of NMDA receptor dysfunction in schizophrenia is primarily supported by the psychotomimetic action of the NMDA receptor antagonists (Luby *et al*. 1959; Javitt & Zukin, 1991; Krystal *et al*. 1994; Lahti *et al*. 1995) and the results of adjuvant treatment trials with the NMDA receptor modulators D-cycloserine (Goff *et al*. 1995; Goff *et al*. 1996; Goff *et al*. 1999; Heresco-Levy *et al*. 1998; Heresco-Levy *et al*. 2002; Evins *et al*. 2002), glycine (Javitt *et al*. 1994; Heresco-Levy *et al*. 1996; Heresco-Levy *et al*. 1999; Javitt *et al*. 2001; Heresco-Levy *et al*. 2004) and D-serine (Tsai *et al*. 1998; Heresco-Levy *et al*. 2005). The competitive NMDA receptor antagonists, phencyclidine and ketamine, were consistently reported to induce psychopathology similar to schizophrenia in animal models (Noda *et al*. 2000; Becker *et al*. 2003; Wass *et al*. 2006; Nabeshima *et al*. 2006; Murai *et al*. 2007) and in human subjects including cognitive and negative symptoms (Javitt & Zukin, 1991; Krystal *et al*. 1994; Malhotra *et al*. 1996; Micallef *et al*. 2003; Krystal *et al*. 2005).

Excitatory amino acids like glycine and D-serine were used as adjunctive treatment to enhance glutamatergic neurotransmission in the treatment of schizophrenic symptoms. However, the systematic review and meta-analysis of clinical effects of the NMDA receptor agonists evaluated in 18 short-time trials provided inconsistent results: D-cycloserine (the NMDA receptor partial agonist) did not appear effective, glycine and D-serine (the NMDA receptor co-agonists) improved negative symptoms, but had no effects on positive symptoms and cognitive deficit (Tuominen *et al*. 2005).

Augmentation by D-serine improved negative, positive, cognitive (Tsai *et al*. 1998; Heresco-Levy *et al*. 2005) and depressive symptoms (Heresco-Levy *et al*. 2005) in patients treated with second generation antipsychotics. In contrast, studies with clozapine (Tsai *et al*. 1999) and risperidone (Lane *et al*. 2005) did not detect any treatment effect of augmentation by D-serine. This has been tentatively explained by the partially agonistic effect of these antipsychotics at the NMDA receptor.

Amino acid D-serine is an endogenous selective full co-agonist at the glycine modulatory site of the NMDA receptor and acts as a modulator of glutamatergic neurotransmission, neuronal migration and long term potentiation (LTP) (Scolari *et al*. 2007). D-serine levels in postmortem schizophrenic brains did not differ from healthy controls in prefrontal and parietal cortex and the regional distribution of free D-serine was associated with the distribution of the NMDA receptors (Kumashiro *et al*. 1995). Contrary to post-mortem studies, increased total serine and L-serine levels and decreased D-serine levels were found in the blood serum of patients with schizophrenia in comparison to healthy controls (Hashimoto *et al*. 2003; Yamada *et al*. 2005). Bendikov *et al*. (2007) detected decreased D-serine levels and D/L-serine ratio in cerebrospinal fluid (CSF) of patients with schizophrenia. The low ratio of D-serine to total serine was also found in CSF of drug-naive first episode patients with schizophrenia (Hashimoto *et al*. 2005).

The objective of this study was to test the hypothesis that low D-serine serum concentrations in patients with schizophrenia are associated with negative symptoms. We also compared D-serine serum levels in mostly chronic patients with schizophrenia to the serum levels in healthy controls to confirm the findings of low D-serine serum concentration in schizophrenia.

SUBJECTS AND METHODS

Study population

We recruited fifty patients with schizophrenia (33 males and 17 females) and fifty individually age and sex matched healthy subjects as controls into the study. Patients with schizophrenia were recruited at the Outpatient clinic of the Department of Psychiatry, University Hospital Hradec Kralove, Czech Republic. They were 18 years or older and had no history of substance abuse or positive urine toxicology prior to screening, no electroconvulsive therapy within the last 5 months and no pregnancy. They were diagnosed by two experienced psychiatrists in agreement with the ICD-10 Diagnostic Criteria for Research. Paranoid type of schizophrenia was the most frequent diagnosis (n=35), the other types were residual (n=7), undifferentiated (n=5), hebephrenic (n=2) and simple (n=1). The medical history of neurological disorders, cardiovascular disorders or renal dysfunction reported by patients or documented in their records led to exclusion from the study population. The patients were physically healthy upon examination and their laboratory assessments were within physiological limits. The baseline characteristics of patients are in Table 1. All patients, with a single exception, were treated with antipsychotic medication. The patient who was drug-naive had 2 years duration of psychosis and she was clinically stable. The antipsychotic medication that the patients used at the time of the assessments is in Table 1. Healthy controls had no history of psychiatric or neurological disorders or renal dysfunction and were free of any psychotropic medication.

Determination of D-serine and total serine

After 12 hours of overnight fasting, the blood samples for the determination of D-serine and total serine (D- and L-serine) serum levels were obtained by venipuncture from both, patients and healthy controls. The blood samples were collected between 8.00 and 9.30 a.m. (before breakfast) from all participants (in order to avoid the bias by different D-serine content in food).

Table 1. The baseline characteristics of the patients (n = 50).

Sex	
Males	n = 33
Females	n = 17
Types of schizophrenia (ICD-10)	
Paranoid (F 20.0)	n = 35
Hebephrenic (F 20.1)	n = 2
Undifferentiated (F 20.3)	n = 5
Residual (F 20.5)	n =7
Simple (F 20.6)	n = 1
Medication	
Monotherapy (dose)	
fluphenazine decanoate (25 mg/14–35d)	n = 3
chlorpromazine (50 mg/d)	n = 1
amisulpride (100–800 mg/d)	n = 3
ziprasidone (80–160 mg/d)	n = 3
long acting risperidone (25–37.5 mg/14d)	n = 6
sertindole (12–24 mg/d)	n = 2
olanzapine (5–20 mg/d)	n = 9
quetiapine (600–1400 mg/d)	n = 3
clozapine (100–500 mg/d)	n = 8
aripiprazole (15 mg/d)	n = 2
Combination (dose)	
haloperidol decanoate (50 mg/14–21d) + risperidone (1.5–6 mg/d)	n = 2
haloperidol decanoate (50 mg/21d) + olanzapine (35 mg/d)	n = 1
haloperidol (3 mg/d) + risperidone (6 mg/d)	n = 1
haloperidol (1.5 mg/d) + quetiapine (800 mg/d)	n = 1
clozapine (150–450 mg/d) + aripiprazole (15 mg/d)	n = 2
long acting risperidone (25–37.5 mg/14d) + aripiprazole (15 mg/d)	n = 2

Sample preparation: Venous blood was collected in Serum-SST™ II Advance tubes (BD Diagnostics, GB) and transported to the laboratory within one hour after collection. The serum was centrifuged and deproteinated by ultrafiltration in Microcon columns (Millipore, USA). We used D-norvaline as internal standard. Clear filtrate was stored at –20°C until analysis. Derivatization: The derivatization reagent contained 50 mg OPA (o-phthaldialdehyde) in 5 ml methanol, 10 ml 0.2 mol/l boric acid in 0.2 mol/l KCl (potassium chloride), 10 ml 0.2 mol/l NaOH (sodium hydroxide) and thiol – 2 ml MPA (3-mercaptopropionic acid) for total serine or 1.52 g NAC (N-acetyl-L-cysteine) for D-serine determination (pH = 9.3). The volume ratio of derivatization reagent and sample was 2:1 and reaction time was 10 minutes (Vasanits *et al*. 2000; Kutlan *et al*. 2002; Kutlan *et al*. 2003; Molnar-Perl, 2003). Analysis: Chromatographic determination of D-serine on HPLC system type LC-10A vp (Shimadzu, Japan) has been performed in the gradient mode made of two components: (A) eluent that contained 0.21 mol/l sodium acetate adjusted to pH 5.6 with concentrated acetic acid, while (B) eluent was methanol (Zhao *et al*. 1995). There were three mobile phases for the analysis of total serine: (A) 0.05 mol/l acetate buffer (pH 7.3), (B) 0.1 mol/l sodium acetate – acetonitrile – methanol (46:44:10) and (C) methanol (Vasanits *et al*. 2000; Kutlan *et al*. 2002; Kutlan *et al*. 2003). Separations were performed on 250 × 4 mm Lichrospher RP-18e column containing 5 μm particles (Merck, Germany). Signal was detected by fluorescence detector (λem/λex 454/337 nm for D-serine, λem/λex 455/230 nm for total serine) (Zhao *et al*. 1995; Vasanits *et al*. 2000; Kutlan *et al*. 2002). Average of reproducibility was 4.54% for D-serine and 8.91% for total serine. Bias values were 1.58% for D-serine and 1.91% for total serine. We also determined limits of detection 0.27 μmol/l for D-serine and 2.58 μmol/l for total serine.

Assessments

The assessments of psychopathology were performed on the same day in patients within the 4 hours period after the blood collection. The Positive and Negative Syndrome Scale (PANSS, 30 items, score 1–7) (Kay *et al*. 1987) and The Scale for the Assessment of Negative Symptoms (SANS, 30 items, score 0–5) (Andreasen, 1989) were employed for psychopathology assessments. The fully qualified and trained psychiatrist with experience in rating (JH) did all rating scales in patients.

Statistical Analysis

Because of lacking normality of data distribution, non-parametric tests were used for mean comparisons. Mann-Whitney *U* test and Kolmogorov-Smirnov test were used for group comparisons of D-serine, total serine and D-serine/total serine ratio mean values. Correlations of variables were computed by Spearman Rank Correlation (Pair-Wise Deletion, $\alpha = 0.05$). Box and scatter plots were used for graphical presentation of data (Figure 1, 2).

Ethical Issues

All participants were thoroughly informed on its nature and requirements, and they signed the informed consent before admission to the study. The study protocol was approved by the Ethics Committee of the University Hospital Hradec Králové, Czech Republic.

RESULTS

The values of mean serum levels of D-serine, total serine and D-serine/total serine ratio are in Table 2. The mean serum level of D-serine in patients with schizophrenia did not differ from that of healthy controls (Mann-Whitney *U* test, *p* value = .410). The mean total serine serum level was significantly lower (Kolmogorov-Smirnov test, *p*= .006) in comparison to the mean of healthy controls (Figure 1). D-serine/total serine ratio was in patients significantly higher (Kol-

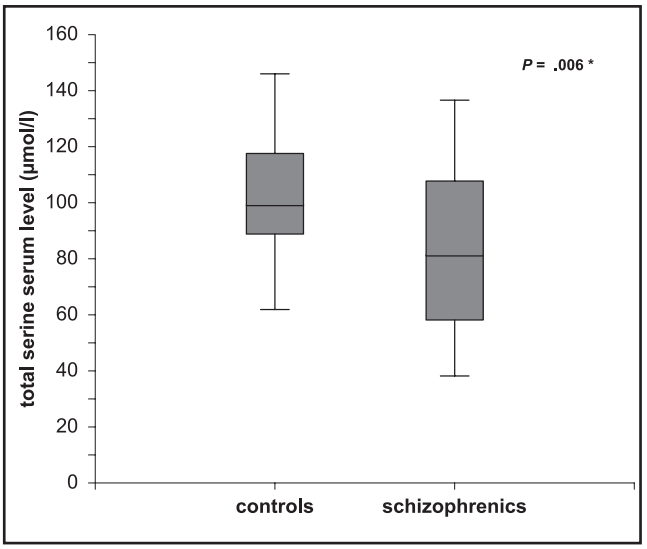

Figure 1. Total serine serum level: comparison of study groups (box plot; * $p < .05$ was considered as significant).

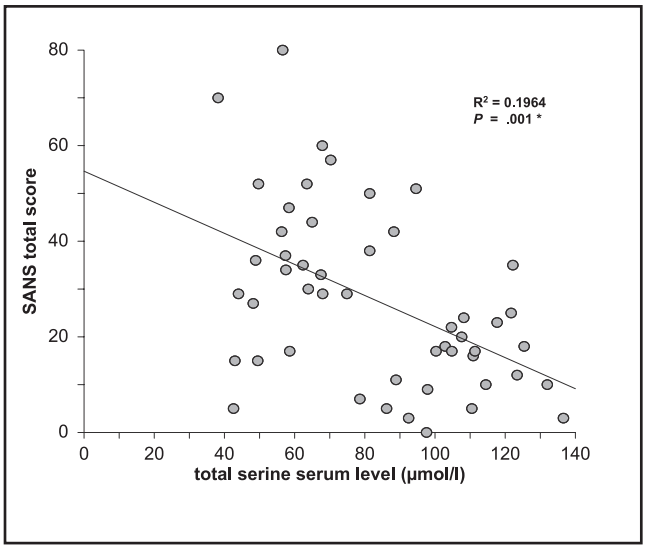

Figure 2. Total serine serum level relation to SANS total score in patients (n=50) (scatter plot with the linear regression curve; * $p < .05$ was considered as significant).

Table 2. Amino acids serum levels: comparison of study groups.

		Patients (n=50)	**Controls** (n=50)	***p Value***
D-serine (µmol/l)	mean ± SD	3.3 ± 1.53	3.1 ± 1.59	.410 [1]
	median	2.75	2.73	
Total serine (µmol/l)	mean ± SD	83.1 ± 27.7	100.9 ± 21.7	.006 * [2]
	median	81.4	99.4	
D-serine ratio to total serine	mean ± SD	0.043 ± 0.023	0.031 ± 0.014	.022 * [2]
	median	0.036	0.029	

Commentary: * $p < .05$ was considered as significant; [1] Mann-Whitney *U* test; [2] Kolmogorov-Smirnov test.

Table 3. The results of the assessments of patients (n= 50).

Variable	**Mean ± SD**	**Median**	**Minimum - Maximum**
Age (years)	35.1 ± 11.0	31.5	21 – 62
Illness duration (years)	10.4 ± 10.4	6	1 – 40
Dose of medication in chlorpromazine equivalents (mg)	344 ± 323	200	0 – 1867
PANSS total score	49.1 ± 10.9	46.5	33 – 79
PANSS: P1-P7 score	11.1 ± 4.0	9	7 – 24
PANSS: N1-N7 score	14.0 ± 5.7	13	7 – 33
PANSS: G1-G16 score	24.1 ± 4.3	23	16 – 36
SANS total score	27.7 ± 18.5	24.5	0 – 80
SANS: affective flattening or blunting score	8.4 ± 6.0	8.5	0 – 26
SANS: alogia score	3.7 ± 3.9	3	0 – 16
SANS: avolition-apathy score	3.8 ± 3.5	3	0 – 13
SANS: anhedonia-asociality score	7.6 ± 6.1	6.5	0 – 22
SANS: attention score	4.2 ± 3.3	4	0 – 12

Commentary: PANSS, The Positive and Negative Syndrome Scale; SANS, The Scale for the Assessment of Negative Symptoms; P1-P7, positive symptom subscale; N1-N7, negative symptom subscale; G1-G16, general psychopathology subscale.

Table 4. The correlations between variables and amino acids serum levels in patients (n= 50).

Variable	D-serine		total serine	
	r	*p* value	*r*	*p* value
Age	– 0.07	.64	– 0.20	.16
Illness duration	0.13	.36	– 0.14	.33
Dose of medication in chlorpromazine equivalents	0.23	.10	0.19	.19
PANSS total score	0.01	.93	– 0.22	.12
PANSS: P1-P7 score	0.08	.60	0.14	.34
PANSS: N1-N7 score	0.02	.89	– 0.39	.005 *
PANSS: G1-G16 score	0.01	.94	– 0.17	.25
SANS total score	–0.08	.58	– 0.44	.001 *
SANS: affective flattening or blunting score	–0.003	.98	– 0.38	.006 *
SANS: alogia score	–0.16	.27	– 0.37	.008 *
SANS: avolition-apathy score	–0.04	.76	– 0.25	.08
SANS: anhedonia-asociality score	–0.15	.29	– 0.52	.00009 *
SANS: attention score	0.07	.65	– 0.26	.07

Commentary: * $p < .05$ was considered as significant; *r*, Spearman Correlation Coefficient; PANSS, The Positive and Negative Syndrome Scale; SANS, The Scale for the Assessment of Negative Symptoms; P1-P7, positive symptom subscale; N1-N7, negative symptom subscale; G1-G16, general psychopathology subscale.

mogorov-Smirnov test, $p = .022$) than that of healthy controls (Table 2).

The mean scores of psychopathology assessments are listed in Table 3. We did not detect any association between the mean D-serine serum level and the mean scores of the total PANSS (Spearman Correlation Coefficient, $r = 0.01$, $p = .93$) or the total SANS ($r = – 0.08$, $p = .58$). Either subscales mean scores of the PANSS or the SANS did not correlate with D-serine serum levels. The correlation coefficients are listed in Table 4.

We found statistically significant inverse correlation between the mean total serine serum level and the mean scores of the PANSS negative symptom subscale ($r = –0.39$, $p = .005$), the total SANS ($r = – 0.44$, $p = .001$) (Figure 2), the SANS affective flattening or blunting subscale ($r = – 0.38$, $p = .006$), the SANS alogia subscale ($r = – 0.37$, $p = .008$) and the SANS anhedonia-asociality subscale ($r = – 0.52$, $p = .00009$).

Serum levels of D-serine or total serine did not relate to the age or the illness duration and chlorpromazine dose equivalent in patients (Table 4). D-serine ($r = 0.19$, $p = .19$) or total serine ($r = 0.10$, $p = .47$) serum levels were not associated with the age in healthy controls.

DISCUSSION

We did not find a significant association between serum level of D-serine and negative symptoms. In a large sample of patients, with stabilized, mostly chronic schizophrenia, whose serum levels of D-serine were analysed, we did not confirm the association of schizophrenia with decreased D-serine serum levels (Hashimoto *et al*. 2003; Yamada *et al*. 2005). On the contrary, the total serine serum levels in our patients were lower and D-serine/total serine ratio higher than in healthy controls. We found lower total serine serum levels associated with the higher intensity of negative symptoms assessed by the PANSS and the SANS. These findings has not been previously reported and may be related to the effects of the NMDA receptor agonists on negative symptoms in clinical studies (Tsai *et al*. 1998; Evins *et al*. 2002; Heresco-Levy *et al*. 2005; Tuominen *et al*. 2005).

Higher plasma concentrations of total serine and lower plasma activity of serine hydroxymethyltransferase were reported in patients with psychosis in comparison to non-psychotic patients or healthy controls (Waziri *et al*. 1983; Waziri *et al*. 1985; Wilcox *et al*. 1985; Waziri & Mott, 1986), and a decade later several studies confirmed the results in schizophrenia (Macciardi *et al*. 1990; Baruah *et al*. 1991; Sumiyoshi *et al*. 2004). However there were also reports of no difference in total serine plasma concentrations (Perry & Hansen, 1985; Carl *et al*. 1992; Neeman *et al*. 2005) and total serine CSF concentrations (Perry & Hansen, 1985) between schizophrenics and healthy controls. The only report of significantly lower total serine serum levels has been published in patients with treatment resistant schizophrenia (Tortorella *et al*. 2001).

Incostintent results can be due to different populations of patients, to the influence of individual diet, the effects of antipsychotic medication or by different

assay techniques. Our outpatients with schizophrenia were stabilized and capable of functioning outside of the hospital. They were treated mostly with second generation antipsychotics. In contrast, higher total serine plasma levels were found in the medication-free population of mixed inpatients and outpatients (Sumiyoshi *et al*. 2004) or an inpatient group with a mixed medication status (Baruah *et al*. 1991). Hashimoto did not find differences in D-, L- or total serine serum levels between medicated or drug-naive schizophrenics (Hashimoto *et al*. 2003). He also didn´t find an association of D-, L- or total serine serum levels with the medication dose in chlorpromazine equivalents. On the other hand, the decrease of total serine levels after treatment with first generation antipsychotics was reported (Sumiyoshi *et al*. 2004).

There were concerns regarding the confounding effects of the amino acids contained in food on the amino acid serum levels in patients (Friedman, 1999). Fasting blood samples for the assays of excitatory amino acids were in some studies obtained in patients on special diet (Baruah *et al*. 1991; Tortorella *et al*. 2001). Other studies did not control for the effects of the amino acids in the food (Hashimoto *et al*. 2003; Neeman *et al*. 2005). In this study, we drew blood samples in all participants after 12 hours of overnight fasting, which should have eliminated the effect of external amino acid intake.

Additional source of different results may originate in the techniques of total serine and D-serine assays. Especially D-serine, the physiological levels of which in healthy humans are low may be sensitive to assay methods. Gas chromatography (Waziri & Mott, 1986), ion-exchange amino acid chromatography (Perry & Hansen, 1985; Neeman *et al*. 2005), gas chromatography-mass spectrometry (Baruah *et al*. 1991) or high pressure liquid chromatography (Waziri & Mott, 1986; Carl *et al*. 1992) were used for total serine plasma level determinations in the published clinical studies. The high-performance liquid chromatography (HPLC), that we employed to assay serum amino acids levels was used in studies with conflicting results (Tortorella *et al*. 2001; Hashimoto *et al*. 2003; Sumiyoshi *et al*. 2004; Yamada *et al*. 2005), which makes a systematic effect of the assay technique on the results unlikely. Our results correspond only with Tortorella´s report, but contradict the other findings.

It is difficult to account for the differences in findings of total serine and D-serine serum levels in patients with schizophrenia, although its practical significance appear to be considerable for the targeted treatment with the NMDA receptor agonists. Endogenous D-serine is synthesized from L-serine mainly in the cytosolic matrix of astrocytes in the brain (Schell *et al*. 1997). However, D-serine occurs in very low levels in blood serum, saliva and urine. It is synthesized de novo outside of the brain (Hashimoto *et al*. 1997) in hepatocytes, that express serine racemase (Wolosker *et al*. 1999), and then released into circulation. At the periphery, D-serine probably interacts with the NR1 subunit of the NMDA receptors expressed in the peripheral tissues (e.g. megakaryocytes, osteoclasts). Its metabolism by D-amino acid oxidase (DAAO) and consequent elimination takes place in the kidney cells (Schell *et al*. 2004). The transport through the blood-brain barrier for neutral amino acids is considered as limited (Hashimoto *et al*. 1997). D- and L-serine share common transport systems. The major uptake mechanism of D- and L-serine in the blood-brain barrier supplies peripherally synthesised D-serine into the brain (Yamamoto *et al*. 2001). The preference for a stereoselective transport of D-serine through the blood-brain barrier was reported (Bauer *et al*. 2005). In comparison to L-serine, D-serine from hepatocytes may have better access to the brain. We can assume, that the effects of long-term treatment on liver functions, can lead to lower peripheral synthesis of D-serine. Serum assays of endogenous D-serine may become a biomarker for its role at the NMDA receptor in the brain and eventually treatment sensitivity to exogenous excitatory amino acids in the future. However, the transport mechanisms of excitatory amino acids across the blood-brain barrier, the length and the preceding medication will have to be taken into consideration. Additional studies of excitatory amino acids in the periphery as well as in CSF may help to clarify the role of treatment related NMDA receptor dysfunction.

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