Association between increased serum D-serine and cognitive gains induced by intensive cognitive training in schizophrenia

Rogerio Panizzutti [a,b,c,d,*], Melissa Fisher [e], Coleman Garrett [d], Wai Hong Man [d], Walter Sena [b,c], Caroline Madeira [b], Sophia Vinogradov [e]

[a] W.M. Keck Foundation Center for Integrative Neurosciences, 675 Nelson Rising Lane, San Francisco, CA, USA
[b] Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, CCS, Cidade Universitaria, Rio de Janeiro, RJ, Brazil
[c] Instituto de Psiquiatria, Universidade Federal do Rio de Janeiro, Av. Venceslau Braz, 71, Rio de Janeiro, RJ, Brazil
[d] Department of Psychiatry, UCSF School of Medicine, 982 Mission St, San Francisco, CA, USA
[e] Department of Psychiatry, University of Minnesota, 2312 S 6th St., Minneapolis, MN, USA

ARTICLE INFO

ABSTRACT

Neuroscience-guided cognitive training induces significant improvement in cognition in schizophrenia subjects, but the biological mechanisms associated with these changes are unknown. In animals, intensive cognitive activity induces increased brain levels of the NMDA-receptor co-agonist D-serine, a molecular system that plays a role in learning-induced neuroplasticity and that may be hypoactive in schizophrenia. Here, we investigated whether training-induced gains in cognition were associated with increases in serum D-serine in outpatients with schizophrenia. Ninety patients with schizophrenia and 53 healthy controls were assessed on baseline serum D-serine, L-serine, and glycine. Schizophrenia subjects performed neurocognitive tests and were assigned to 50 h of either cognitive training of auditory processing systems (N = 47) or a computer games control condition (N = 43), followed by reassessment of cognition and serum amino acids. At study entry, the mean serum D-serine level was significantly lower in schizophrenia subjects vs. healthy subjects, while the glycine levels were significantly higher. There were no significant changes in these measures at a group level after the intervention. However, in the active training group, increased D-serine was significantly and positively correlated with improvements in global cognition and in Verbal Learning. No such associations were observed in the computer games control subjects, and no such associations were found for glycine. D-Serine may be involved in the neurophysiologic changes induced by cognitive training in schizophrenia. Pharmacologic strategies that target D-serine co-agonism of NMDA-receptor functioning may provide a mechanism for enhancing the behavioral effects of intensive cognitive training.

© 2018 Elsevier B.V. All rights reserved.

1. Introduction

Schizophrenia is characterized by a range of neurocognitive deficits, including impairments in Verbal Learning. We have performed a series of studies investigating the effects of targeted cognitive training of auditory processing and Verbal Learning to enhance verbal memory function in schizophrenia, based on principles of learning-induced neuroplasticity (Fisher et al., 2009). This program of intensive cognitive training (e.g., 50 h over 10 weeks) focuses on the accuracy, the temporal resolution and the power of auditory inputs feeding working and explicit memory processes. In studies of both persistently ill participants (average age of 40 years) and in recent-onset subjects (average age of

21 years), we have found significant improvements in Verbal Learning and in general cognition, when compared to a computer games control condition (Fisher et al., 2009, 2015, 2016). Cognitive gains persisted 6 months after cessation of training (Fisher et al., 2010); showed an inverse association with anticholinergic burden (Vinogradov et al., 2009b); and were associated with variations in the catechol-*O*-methyltransferase (COMT) gene (Panizzutti et al., 2013). Subjects who were in the active training group also demonstrated a significant increase in serum brain-derived neurotrophic factor (BDNF) levels (Vinogradov et al., 2009a). Despite these promising results, many open questions remain about the neurophysiologic mechanisms harnessed during successful training.

It is now well-established that activation of NMDA receptors is necessary for experience-dependent neuroplasticity (Clem et al., 2008), likely through their role in long-term potentiation, a key cellular mechanism involved in learning and memory (Whitlock et al., 2006). Long-term potentiation is modulated by D-serine, an endogenous co-agonist

of NMDA receptors (Panatier et al., 2006). Impaired NMDA receptor activity is believed to be involved in the pathophysiology of schizophrenia (Cho et al., 2016; Coyle, 2006), and several lines of evidence suggest that decreased availability of D-serine contributes to this hypofunction of NMDA receptors and impaired cortical plasticity (Kantrowitz et al., 2016). For example, D-serine levels have been found to be decreased in the blood (Calcia et al., 2012; Hashimoto et al., 2003; Yamada et al., 2005; Yamamori et al., 2014; Cho et al., 2016) and in the cerebrospinal fluid (Bendikov et al., 2007; Hashimoto et al., 2005) of schizophrenia patients, although conflicting results for blood levels have also been reported (Hons et al., 2008; Ohnuma et al., 2008; Ozeki et al., 2016). Also in corroboration to the hypofunction evidences, genetic studies show an association between schizophrenia and the G72 gene, whose product appears to interact with the D-serine degrading enzyme D-amino acid oxidase (Chumakov et al., 2002; Detera-Wadleigh and McMahon, 2006). Postmortem studies demonstrate increased activity of D-amino acid oxidase in schizophrenia patients (Burnet et al., n.d.; Madeira et al., 2008), while clinical trials with D-serine yield improved positive, negative and cognitive symptoms (Heresco-Levy et al., 2005; Kantrowitz et al., 2010; Tsai et al., 1998).

The brain is the sole significant source of endogenous D-serine, which is synthesized from the racemization of L-serine catalyzed by serine racemase (De Miranda et al., 2000; Wolosker et al., 1999). D-Serine crosses the blood-brain barrier bi-directionally and is detectable in serum; serum levels correlate with brain levels in animal studies (Bauer et al., 2005; Hashimoto, 2002; Hashimoto and Chiba, 2004). Although D-serine has been proposed as a serum biomarker for schizophrenia, its levels are not static; a study showed that serum D-serine was significantly increased in schizophrenia patients from the time of admission to the time of discharge as their clinical symptoms improved (Ohnuma et al., 2008). Such changes in serum D-serine may possibly be due to increased NMDA receptor activity; indeed, activation of both NMDA and non-NMDA glutamate receptors has been shown to increase extracellular D-serine levels (Kartvelishvily et al., 2006; Kim et al., 2005; Mustafa et al., 2009; Ribeiro et al., 2002; Schell et al., 1995).

Given the putative role of D-serine in schizophrenia, as well as the established role of NMDA receptors and of D-serine in neuroplasticity and experience-dependent learning, we asked whether serum D-serine levels in schizophrenia participants could be affected by a course of intensive cognitive training. To investigate this hypothesis, we posed two questions retrospectively: 1) Could we replicate earlier findings of reduced serum D-serine levels in schizophrenia outpatients who participated in our cognitive training studies? 2) Were the cognitive gains in the schizophrenia subjects who underwent intensive "neuroplasticity-informed" auditory system training associated with changes in serum D-serine levels?

To answer these questions, we conducted a post hoc analysis using levels of the free amino acids D-serine, L-serine and glycine from serum samples collected at baseline (study entry), and after 40–50 h (8–10 weeks) of either auditory processing and auditory working memory training exercises or commercial computer games, in 90 schizophrenia subjects who participated in our studies and on whom we had serum samples.

2. Methods

2.1. Participants

Subjects were obtained from our completed randomized controlled trials (RCT) of neuroplasticity-informed computerized cognitive training in adults with persistent schizophrenia (Fisher et al., 2009, 2016; Vinogradov et al., 2009a, 2009b), N = 74, and in young adults with recent-onset schizophrenia (who were within 5 years of illness onset), (Fisher et al., 2015) N = 57 (Fig. 1). The RCTs were pre-registered in ClinicalTrials.gov (NCT00312962 and NCT00694889 respectively).
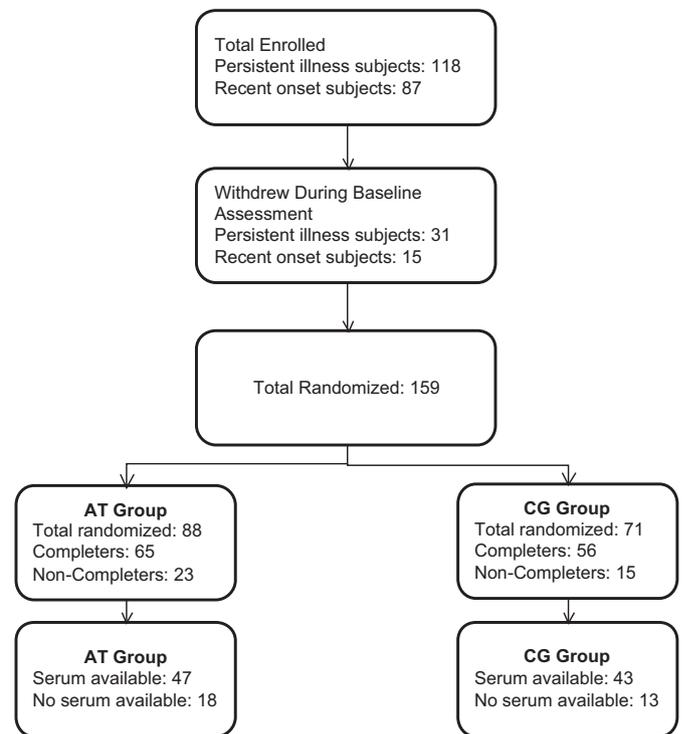

Fig. 1. Diagram of recruitment and enrollment of auditory training subjects (AT) and computer games control subjects (CG) from two cognitive training trials, for pooled serum and neurocognitive data.

Since the trial design and the assessment and cognitive training protocols were virtually identical between the two studies, serum data and neurocognitive data were pooled for the purposes of the present analysis. This resulted in a total sample of 90 clinically stable schizophrenia outpatients with a mean age of 36.72 (SD = 13.79). Fifty-three healthy comparison subjects were recruited from the community. Inclusion criteria were: Axis I diagnosis of schizophrenia or schizoaffective disorder (determined by the DSM-IV SCID) or, for healthy subjects, no Axis I or Axis II psychiatric disorder (SCID-NP); no substance dependence or current substance abuse; good general physical health; English as first language. Table 1 presents subject demographics, baseline clinical status, and smoking status. In order to be included in the study, schizophrenia subjects had to have outpatient status for at least one month prior to study entry. All subjects provided written informed consent for study participation, and received nominal payment for each successful day and week of participation that was contingent on attendance only.

2.2. Neuroplasticity-informed computerized cognitive training exercises

Software for cognitive training of auditory and verbal processing (auditory training) was provided by Posit Science Corporation. In these computerized exercises subjects were driven to make progressively more accurate distinctions about the spectro-temporal fine-structure of auditory stimuli and speech under conditions of increasing working memory load (Fisher et al., 2009). Improvements in auditory signal salience were incorporated and generalized into real-world language comprehension and working memory rehearsal. In the control condition, subjects rotated through a series of 16 commercial computer games for the same number of hours as the auditory training subjects. Both groups rated their experiences as equally enjoyable on the 7-item subscale of interest/enjoyment from the Intrinsic Motivation Inventory (Ryan et al., 1991).

Table 1
Subject characteristics.

	Healthy subjects		Schizophrenia subjects		Statistics[a]	Schizophrenia groups				Statistics[a]
	N = 53		Total sample N = 90			Auditory training N = 47		Computer games N = 43		
	Mean	SD	Mean	SD	p	Mean	SD	Mean	SD	p
Female/male	26/27		23/67		0.004	15/32		8/35		0.23
Age (years)	26.2	13.6	36.7	13.8	<0.0001	36.2	13.2	37.3	14.5	0.71
Education (years)	13.1	3.0	13.3	2.2	0.65	13.3	2.3	13.2	2.2	0.83
IQ	113.9	12.5	100.7	14.4	<0.0001	100.4	13.0	101.1	16.0	0.72
PANSS score	NA		67.8	17.0	NA	67.7	17.8	67.8	13.4	0.93
gaf total	NA		47.0	13.7	NA	47.4	15.1	46.5	12.0	0.89
Age of onset	NA		20.8	7.1	NA	20.2	4.6	21.4	9.0	0.40
Hospitalizations (n)	NA		7.5	8.9	NA	7.3	7.1	7.7	10.5	0.85
Years of illness	NA		21.9	11.8	NA	21.5	10.9	22.3	13.0	0.81
CPZ equivalent (mg)	NA		420.1	447.9	NA	392.7	407.5	450.2	491.4	0.55
Smokers/non-smoker	4/49		41/49		<0.0001	24/23		17/26		0.27
Cigarettes/day	7.3	5.3	18.5	10.0	<0.0001	18.8	11.0	18.1	8.7	0.64

[a] Chi-squared test for sex and smoking status, and Independent Samples t-test (two-tailed) for the others. IQ, intelligence quotient. PANSS, Positive and Negative Syndrome Scale. GAF, Global Assessment of Functioning scale. CPZ, chlorpromazine.

2.3. Measurement of serum amino acids

Serum samples for amino acid analysis were obtained from healthy volunteers at baseline and from schizophrenia participants at two time points: after completion of baseline neurocognitive testing; and ~12 weeks later, after completion of the intervention. Samples were drawn at the same time of the day (~1 PM ± 1 h); the serum was separated by centrifugation and stored at −70 °C. D-Serine, L-serine and glycine levels were measured using HPLC (Hashimoto et al., 1992) by investigators (RP, CM and WHM) blind to the group identity of the samples.

2.4. Procedures

All schizophrenia subjects underwent cognitive and clinical testing over a 1–2 week period, followed by the first blood draw. Cognitive measures recommended by MATRICS (Measurement and Treatment Research to Improve Cognition in Schizophrenia) were used (Kern et al., 2008; Nuechterlein et al., 2008). Raw scores were converted to z-scores using published normative data. Clinical status was assessed via the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and the modified Global Assessment of Functioning scale (GAF) (Hall, 1995).

After baseline assessments, the schizophrenia subjects were stratified by age, education, gender, and symptom severity and randomly assigned to either computerized auditory system training (50 h over ~10 weeks for the persistently ill adults; 40 h over ~8 weeks for the recent-onset subjects) or an active computer games control group. After the intervention, all schizophrenia subjects were re-assessed on clinical and cognitive measures by personnel "blind" to group assignment. Cognitive change scores were computed as the z-score difference (post-training minus baseline scores). A Global Cognition composite score was computed as the mean of all measures of the MATRICS-defined cognitive domains.

2.5. Data analysis

The distributions of amino acid levels and of neurocognitive measures were evaluated for normalcy and missing values. Winsorized means were calculated for eight outlying amino acid values and constituted 1.5% of the total amino acid data. Cognitive measures were normally distributed after winsorising of outlying values.

Independent Samples t-tests (two-tailed) or Chi-squared tested for group differences in demographic variables between healthy comparison and schizophrenia subjects, and between auditory training and computer games schizophrenia subgroups. Pearson correlations (2-tailed) between serum amino acids and the following variables were conducted to identify potential covariates: age, gender, education, IQ, smoking quantity (number of cigarettes per day), and symptom severity.

ANCOVA was used to examine group differences in amino acids between the healthy subjects and the schizophrenia subjects at baseline, with age, gender, IQ and smoking quantity entered as covariates. ANCOVA was used to test for auditory system training and computer games schizophrenia group differences from baseline to post-training, with age, gender, and baseline D-serine entered as covariates. Pearson and partial correlations (2-tailed) tested the association at baseline between the cognitive measures and D-serine or glycine levels (with age and gender entered as covariates), and the association of changes after intervention in cognitive measures to the changes in D-serine and glycine (with age, gender, and baseline D-serine, glycine or L-serine entered as covariates).

Partial correlations, with age and gender as covariates, were conducted between D-serine and the composite cognitive measure of Global Cognition. Additionally, we conducted analysis on the composite measures of cognitive subdomains where significant effects were found in the two RCTs: Speed of Processing and Verbal Learning.

3. Results

3.1. Baseline results

Baseline amino acid levels are presented for all subject groups in Table 2. Since the schizophrenia and healthy control subjects showed an imbalance in gender, age, IQ, and smoking quantity, these measures were entered as covariates in the analyses. Serum D-serine levels were significantly lower in the schizophrenia subjects as compared to healthy subjects, with a large effect size (Cohen's $d = 8.11$). L-Serine and glycine levels were significantly lower in the schizophrenia subjects as compared to healthy subjects, also with large effect sizes (Cohen's $d = 5.83$ and 2.56, respectively). The ratios of D-serine to L-serine, and L-serine to glycine were also significantly different in the schizophrenia subjects as compared to healthy subjects. At baseline, D-serine in the schizophrenia group showed a significant positive correlation with Global Cognition ($r = 0.37$, $P < 0.0001$). Baseline D-serine showed no significant correlations with smoking history or symptom severity (data not shown).

Subjects in the two schizophrenia groups, the auditory training and the computer games groups, were similar at baseline in IQ, symptoms

Table 2
Baseline serum amino acid levels in healthy comparison and schizophrenia subjects.

	Healthy subjects N = 53		Schizophrenia subjects N = 90		Statistics ANCOVA
	Mean	SD	Mean	SD	
D-Serine (nmol/ml)	1.96	0.09	1.34	0.06	$F(1,133) = 28.0$ $P < 0.001$[a]
L-Serine (nmol/ml)	161.58	5.54	133.72	3.87	$F(1,133) = 14.6$ $P < 0.001$[a]
Glycine (nmol/ml)	385.7	146.4	116.5	25.72	$F(1,118) = 63.3$ $P < 0.001$[a]
D-Serine/L-Serine	0.010	0.003	0.01	0.01	$F(1,133) = 3.64$ $P = 0.06$
L-Serine/Glycine	0.367	0.164	1.15	0.25	$F(1,118) = 278$ $P < 0.001$[a]

[a] ANCOVA controlling for gender, age, IQ, and smoking quantity (number of cigarettes per day).

and cognition (Tables 1 and 3). Also, there were no significant differences between the two schizophrenia groups in the number of subjects taking 1st generation antipsychotics versus those taking 2nd generation antipsychotics (X^2 (1, N = 74) = 0.83, P = 0.36), or in the number of subjects in each group taking antidepressants, benzodiazepines, mood stabilizers, or anticholinergic medications (Supplemental Table 1). Of note, the auditory training group included more females than the computer games group (Table 1). Although the difference was not significant (P = 0.20), the mean age and D-serine at baseline was lower in the auditory training group (Tables 1 and 3). Thus, we included sex, age and baseline D-serine as covariates in the further analysis comparing the auditory training and computer games groups of schizophrenia subjects.

3.2. Post-intervention results

Table 3 shows mean values at baseline and after intervention for serum D-serine and cognitive performance for the auditory training and the computer games schizophrenia groups; the subject groups did not differ in the change in D-serine. After the intervention, the two groups differed significantly in the change in Global Cognition and Verbal Learning, and at trend level in Speed of Processing. The change in Verbal Learning survived the Bonferroni correction for multiple comparisons, considering the 7 cognitive domains included in MATRICS. No significant group differences were found in the change in PANSS scores.

In the auditory training group, increased D-serine was significantly associated with improvements in Global Cognition ($r = 0.41$, P = 0.005) (Fig. 2A, *left*). In contrast, no significant correlations between D-serine and change in Global Cognition were observed in the computer games control condition ($r = 0.15$, P = 0.33) (Fig. 2A, *right*). There were no significant correlations between cognitive gain and changes in glycine or L-serine levels in either subject group (Supplemental Table 2).

Additionally, we studied the association between the changes in D-serine and the changes in the cognitive domains significantly affected by training in the full RCTs: Verbal Learning and Speed of Processing

(Fisher et al., 2015, 2016). Increased D-serine was significantly associated to improvements in Verbal Learning in the auditory training group ($r = 0.54$, P < 0.0001) (Fig. 2B, *left*) but not in the computer games control group ($r = 0.28$, P = 0.07) (Fig. 2B, *right*). The association between increased D-serine and improvements in Verbal Learning in the auditory training group survived the Bonferroni correction for multiple comparisons, considering the 7 cognitive domains included in MATRICS. There was no significant association between changes in D-serine and changes in Speed of Processing. There were no significant associations between changes in glycine or L-serine and changes in Global Cognition, Verbal Learning or Speed of Processing. All results of the partial correlations (2-tailed) with age, gender, and baseline amino acid entered as covariates are shown in the Supplemental Table 2.

4. Discussion

To the best of our knowledge, this is the first study to investigate changes in serum D-serine levels during a cognitive enhancing treatment in schizophrenia. We found that, at baseline, serum D-serine was significantly lower in 90 schizophrenia outpatients as compared to healthy subjects, consistent with prior reports (Calcia et al., 2012; Hashimoto et al., 2003; Yamada et al., 2005). D-Serine was also associated with baseline performance in Global Cognition. Although the relationship between serum D-serine and neurocognition is unknown, these results are consistent with a clinical trial of D-serine that reported a significant positive correlation between peak levels of plasma D-serine at the beginning of the trial and the final MATRICS measure of Global Cognition (Kantrowitz et al., 2010).

More importantly, we found a significant positive correlation between *increases* in serum D-serine and *cognitive improvement* in the schizophrenia subjects who performed the neuroplasticity-informed auditory training, but not in the subjects assigned to the computer games control condition. Specifically, we observed a significant association with improvement in Verbal Learning, functions that are targeted by the training. Our premise is that training-induced improvement in

Table 3
D-Serine and cognitive performance at baseline and after training in schizophrenia subjects participating in either auditory training or computer games control condition.

	Auditory training N = 47						Computer games N = 43						ANCOVA		
	Baseline		Post-training		Paired t-test		Baseline		Post-training		Paired t-test				
	Mean	SD	Mean	SD	t	p	Mean	SD	Mean	SD	t	p	F	P	η2p
D-Serine[a]	1.26	0.34	1.31	0.36	−0.75	0.46	1.36	0.34	1.35	0.30	0.05	0.96	<0.01	0.99	<0.001
Global cognition[b]	−1.05	0.90	−0.81	0.90	−4.42	<0.001	−1.01	0.97	−1.05	0.90	−1.09	0.28	4.54	0.04	0.05
Speed of processing[b]	−0.63	0.95	−0.39	0.93	−3.05	0.004	−0.69	0.77	−0.65	0.87	−0.45	0.66	2.70	0.10	0.03
Verbal learning[b]	−2.06	1.50	−1.72	1.61	−2.20	0.03	−1.88	1.27	−2.20	1.19	2.14	0.04	9.51	0.003	0.10

D-serine is presented as nmol/ml. Global Cognition, Speed of Processing and Verbal Learning are presented as Z-scores.
[a] Repeated Measures ANCOVA controlling for sex, age, and baseline D-serine (df = 1.85).
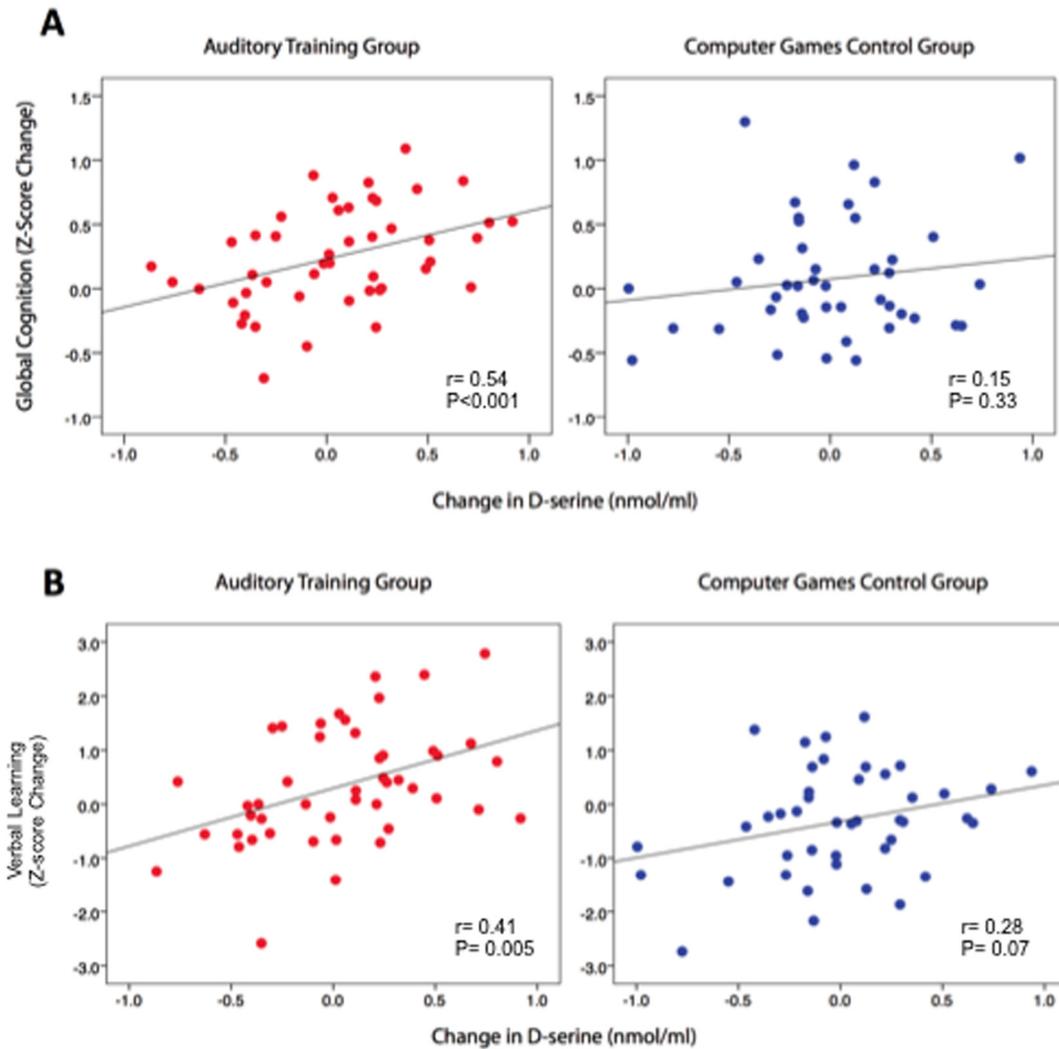[b] Repeated Measures ANCOVA controlling for sex and age (df = 1.86).

Fig. 2. Correlation between Changes in D-serine and Changes in Global Cognition and Verbal Learning. Change in D-serine was positively correlated to z-score changes in Global Cognition (A, *left*) and Verbal Learning (B, *left*) after neuroplasticity-informed auditory training. No significant correlations were observed in the computer games control group (A and B, *right*). Inserts show R and *P* values of the Pearson correlation (2-tailed).

auditory and verbal signal salience feeds forward and facilitates adaptive neuroplastic changes in verbal cognitive systems, resulting in more efficient higher-order operations. It is possible that enhanced endogenous D-serine activity plays a role in this generalization of auditory processing efficiency to improved Verbal Learning through mechanisms of synaptic plasticity.

What biological mechanisms might account for our findings? The only known site of action of endogenous D-serine in the brain is the NMDA receptor; co-activation of NMDA receptors by D-serine is a key component of synaptic plasticity (Panatier et al., 2006). Serine racemase is activated by both NMDA and non-NMDA glutamate receptors, resulting in an increase in extracellular D-serine levels (Kartvelishvily et al., 2006; Kim et al., 2005; Mustafa et al., 2009; Ribeiro et al., 2002; Schell et al., 1995). We have shown that performance of a cognitive task increases D-serine levels in the brains of rodents (Vargas-Lopes et al., 2011). It is conceivable that intensive cognitive training and successful learning could enhance synaptic glutamatergic activity in the brain, and that this would result in increased D-serine formation. However, the precise nature of the relationship between serum D-serine levels and D-serine signaling in the brain is unknown, and nothing in our study addresses the association between central D-serine and behavioral response. Thus, our interpretations – while plausible – remain highly speculative at the present time.

We have described another biological response to this method of cognitive training in persistently ill schizophrenia subjects who overlap in part with the present sample: a significant increase in serum BDNF in the auditory training group but not the computer games control group that shows no relationship to cognitive improvement (Vinogradov et al., 2009a). It appears that increased serum BDNF may reflect biological effects of the "neuroplasticity-informed" training, but that these effects may be non-specific or independent of successful learning. In contrast, in the current study we did not find a significant group-by-time interaction for serum D-serine in the two subject groups; however, we did find significant positive correlations in the auditory training subjects (but not the control subjects) between cognitive improvement and increases in D-serine. This result raises the possibility that an increase in D-serine may reflect some integral component of the brain's ability to generate neurocognitive change and training-induced plasticity (engage in successful learning) as a result of intensive training.

We do not know if the D-serine findings we report here are seen only in schizophrenia subjects, or if they would also be seen in healthy subjects after cognitive training. We also cannot say whether the observed associations are found only in the kind of neuroplasticity-informed auditory training studied here, or whether they would also be found in other successful cognitive, behavioral, or pharmacological interventions. A recent study showed that serum D-serine levels increased significantly in chronic

schizophrenia inpatients between the time of hospital admission and discharge, and correlated with improvement in positive symptoms; however, the D-serine levels of these schizophrenia patients at the time of the admission were elevated compared to healthy controls (Ohnuma et al., 2008). Last, in contrast to prior reports (Hashimoto et al., 2003; Kantrowitz et al., 2010) we did not find an association between D-serine levels and clinical symptoms or medication status, neither at baseline nor after training, although subjects in the present study were stable outpatients with average symptom severity in the mild range.

Limitations of this study include the unrepresentative nature of our participants, with a higher IQ and more education than the general schizophrenia population. Further, we did not control for the medication status of the subjects we studied, who were treated with a range of agents. Finally, we must consider whether the observed changes in serum D-serine could reflect processes occurring peripherally. While serine racemase is located in both brain and peripheral organs, such as kidney, liver and heart (De Miranda et al., 2000; Wolosker et al., 1999), D-serine is very low in peripheral tissues because of the presence of a large amount of the D-serine degrading enzyme D-amino acid oxidase (Nagata et al., 1989). Although this argues against a peripheral source for the D-serine changes we observed, we have no direct evidence to rule out this possibility.

In conclusion, we found a significant association between increases in serum D-serine levels and cognitive improvements induced by intensive neuroplasticity-informed auditory training in clinically stable schizophrenia outpatients, but not in subjects undergoing a computer games control condition. This association was not present for serum L-serine. Our data raise intriguing questions about the role of D-serine and glutamate-NMDA receptors in the response to this form of cognitive enhancement in schizophrenia. Future studies should investigate the biology of D-serine and NMDA receptor function as a possible means of augmenting the gains generated by cognitive training in individuals with schizophrenia and in other clinical populations.

Funding

Contributors

RP and SV designed the study and wrote the protocol. RP and WS managed the literature searches. RP, WHM and CM conducted the biochemical analysis. CG and MF conducted the cognitive analysis. RP and MF undertook the statistical analysis, and RP wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

Dr. Vinogradov is a paid consultant on a BRDG-SPAN grant to Brain Plasticity Inc., a company with a commercial interest in the cognitive training software used in this study. Dr. Panizzutti is the founder of NeuroForma LTDA, a company with a financial interest in cognitive training. None of the other authors have any financial interest in Posit Science.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2018.04.011.

References

Bauer, D., Hamacher, K., Broer, S., Pauleit, D., Palm, C., Zilles, K., Coenen, H.H., Langen, K.J., 2005. Preferred stereoselective brain uptake of D-serine—a modulator of glutamatergic neurotransmission. Nucl. Med. Biol. 32 (8), 793–797.

Bendikov, I., Nadri, C., Amar, S., Panizzutti, R., De Miranda, J., Wolosker, H., Agam, G., 2007. A CSF and postmortem brain study of D-serine metabolic parameters in schizophrenia. Schizophr. Res. 90 (1–3), 41–51.

Burnet, P.W.J., Eastwood, S.L., Bristow, G.C., Godlewska, B.R., Sikka, P., Walker, M., Harrison, P.J., 2008. d-Amino acid oxidase activity and expression are increased in schizophrenia. Mol. Psychiatry 13 (7), 658–660.

Calcia, M.A., Madeira, C., Alheira, F.V., Silva, T.C., Tannos, F.M., Vargas-Lopes, C., Goldenstein, N., Brasil, M.A., Ferreira, S.T., Panizzutti, R., 2012. Plasma levels of D-serine in Brazilian individuals with schizophrenia. Schizophr. Res. 142 (1–3), 83–87.

Cho, S.E., Na, K.S., Cho, S.J., Kang, S.G., 2016. Low D-serine levels in schizophrenia: a systematic review and meta-analysis. Neurosci. Lett. 634, 42–51.

Chumakov, I., Blumenfeld, M., Guerassimenko, O., Cavarec, L., Palicio, M., Abderrahim, H., Bougueleret, L., Barry, C., Tanaka, H., La Rosa, P., Puech, A., Tahri, N., Cohen-Akenine, A., Delabrosse, S., Lissarrague, S., Picard, F.P., Maurice, K., Essioux, L., Millasseau, P., Grel, P., Debailleul, V., Simon, A.M., Caterina, D., Dufaure, I., Malekzadeh, K., Belova, M., Luan, J.J., Bouillot, M., Sambucy, J.L., Primas, G., Saumier, M., Boubkiri, N., Martin-Saumier, S., Nasroune, M., Peixoto, H., Delaye, A., Pinchot, V., Bastucci, M., Guillou, S., Chevillon, M., Sainz-Fuertes, R., Meguenni, S., Aurich-Costa, J., Cherif, D., Gimalac, A., Van Duijn, C., Gauvreau, D., Ouellette, G., Fortier, I., Raelson, J., Sherbatich, T., Riazanskaia, N., Rogaev, E., Raeymaekers, P., Aerssens, J., Konings, F., Luyten, W., Macciardi, F., Sham, P.C., Straub, R.E., Weinberger, D.R., Cohen, N., Cohen, D., 2002. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc. Natl. Acad. Sci. U. S. A. 99 (21), 13675–13680.

Clem, R.L., Celikel, T., Barth, A.L., 2008. Ongoing in vivo experience triggers synaptic metaplasticity in the neocortex. Science 319 (5859), 101–104.

Coyle, J.T., 2006. Glutamate and schizophrenia: beyond the dopamine hypothesis. Cell. Mol. Neurobiol. 26 (4–6), 365–384.

De Miranda, J., Santoro, A., Engelender, S., Wolosker, H., 2000. Human serine racemase: molecular cloning, genomic organization and functional analysis. Gene 256 (1–2), 183–188.

Detera-Wadleigh, S.D., McMahon, F.J., 2006. G72/G30 in schizophrenia and bipolar disorder: review and meta-analysis. Biol. Psychiatry 60 (2), 106–114.

Fisher, M., Holland, C., Merzenich, M.M., Vinogradov, S., 2009. Using neuroplasticity-based auditory training to improve verbal memory in schizophrenia. Am. J. Psychiatry 166 (7), 805–811.

Fisher, M., Holland, C., Subramaniam, K., Vinogradov, S., 2010. Neuroplasticity-based cognitive training in schizophrenia: an interim report on the effects 6 months later. Schizophr. Bull. 36 (4), 869–879.

Fisher, M., Loewy, R., Carter, C., Lee, A., Ragland, J.D., Niendam, T., Schlosser, D., Pham, L., Miskovich, T., Vinogradov, S., 2015. Neuroplasticity-based auditory training via laptop computer improves cognition in young individuals with recent onset schizophrenia. Schizophr. Bull. 41 (1), 250–258.

Fisher, M., Mellon, S.H., Wolkowitz, O., Vinogradov, S., 2016. Neuroscience-informed auditory training in schizophrenia: a final report of the effects on cognition and serum brain-derived neurotrophic factor. Schizophr. Res. Cogn. 3, 1–7.

Hall, R.C., 1995. Global assessment of functioning. A modified scale. Psychosomatics 36 (3), 267–275.

Hashimoto, A., 2002. Effect of the intracerebroventricular and systemic administration of L-serine on the concentrations of D- and L-serine in several brain areas and periphery of rat. Brain Res. 955 (1–2), 214–220.

Hashimoto, A., Chiba, S., 2004. Effect of systemic administration of D-serine on the levels of D- and L-serine in several brain areas and periphery of rat. Eur. J. Pharmacol. 495 (2–3), 153–158.

Hashimoto, A., Nishikawa, T., Oka, T., Takahashi, K., Hayashi, T., 1992. Determination of free amino acid enantiomers in rat brain and serum by high-performance liquid chromatography after derivatization with N-tert.-butyloxycarbonyl-L-cysteine and o-phthaldialdehyde. J. Chromatogr. 582 (1–2), 41–48.

Hashimoto, K., Fukushima, T., Shimizu, E., Komatsu, N., Watanabe, H., Shinoda, N., Nakazato, M., Kumakiri, C., Okada, S., Hasegawa, H., Imai, K., Iyo, M., 2003. Decreased serum levels of D-serine in patients with schizophrenia: evidence in support of the N-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. Arch. Gen. Psychiatry 60 (6), 572–576.

Hashimoto, K., Engberg, G., Shimizu, E., Nordin, C., Lindström, L.H., Iyo, M., 2005. Reduced D-serine to total serine ratio in the cerebrospinal fluid of drug naive schizophrenic patients. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 29 (5), 767–769.

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

Hons, J., Zirko, R., Ulrychova, M., Cermakova, E., Libiger, J., 2008. D-Serine serum levels in patients with schizophrenia: relation to psychopathology and comparison to healthy subjects. Neuro Endocrinol. Lett. 29 (4), 485–492.

Kantrowitz, J.T., Malhotra, A.K., Cornblatt, B., Silipo, G., Balla, A., Suckow, R.F., D'Souza, C., Saksa, J., Woods, S.W., Javitt, D.C., 2010. High dose D-serine in the treatment of schizophrenia. Schizophr. Res. 121 (1–3), 125–130.

Kantrowitz, J.T., Epstein, M.L., Beggel, O., Rohrig, S., Lehrfeld, J.M., Revheim, N., Lehrfeld, N.P., Reep, J., Parker, E., Silipo, G., Ahissar, M., Javitt, D.C., 2016. Neurophysiological mechanisms of cortical plasticity impairments in schizophrenia and modulation by the NMDA receptor agonist D-serine. Brain 139 (Pt 12), 3281–3295.

Kartvelishvily, E., Shleper, M., Balan, L., Dumin, E., Wolosker, H., 2006. Neuron-derived D-serine release provides a novel means to activate N-methyl-D-aspartate receptors. J. Biol. Chem. 281 (20), 14151–14162.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13 (2), 261–276.

Kern, R.S., Nuechterlein, K.H., Green, M.F., Baade, L.E., Fenton, W.S., Gold, J.M., Keefe, R.S.E., Mesholam-Gately, R., Mintz, J., Seidman, L.J., Stover, E., Marder, S.R., 2008. The MATRICS consensus cognitive battery, part 2: co-norming and standardization. Am. J. Psychiatry 165 (2), 214–220.

Kim, P.M., Aizawa, H., Kim, P.S., Huang, A.S., Wickramasinghe, S.R., Kashani, A.H., Barrow, R.K., Huganir, R.L., Ghosh, A., Snyder, S.H., 2005. Serine racemase: activation by glutamate neurotransmission via glutamate receptor interacting protein and mediation of neuronal migration. Proc. Natl. Acad. Sci. U. S. A. 102 (6), 2105–2110.

Madeira, C., Freitas, M.E., Vargas-Lopes, C., Wolosker, H., Panizzutti, R., 2008. Increased brain D-amino acid oxidase (DAAO) activity in schizophrenia. Schizophr. Res. 101 (1–3), 76–83.

Mustafa, A.K., van Rossum, D.B., Patterson, R.L., Maag, D., Ehmsen, J.T., Gazi, S.K., Chakraborty, A., Barrow, R.K., Amzel, L.M., Snyder, S.H., 2009. Glutamatergic regulation of serine racemase via reversal of PIP2 inhibition. Proc. Natl. Acad. Sci. U. S. A. 106 (8), 2921–2926.

Nagata, Y., Akino, T., Ohno, K., 1989. The presence of free D-amino acids in mouse tissues. Experientia 45 (4), 330–332.

Nuechterlein, K.H., Green, M.F., Kern, R.S., Baade, L.E., Barch, D.M., Cohen, J.D., Essock, S., Fenton, W.S., Frese III, F.J., Gold, J.M., Goldberg, T., Heaton, R.K., Keefe, R.S.E., Kraemer, H., Mesholam-Gately, R., Seidman, L.J., Stover, E., Weinberger, D.R., Young, A.S., Zalcman, S., Marder, S.R., 2008. The MATRICS consensus cognitive battery, part 1: test selection, reliability, and validity. Am. J. Psychiatry 165 (2), 203–213.

Ohnuma, T., Sakai, Y., Maeshima, H., Hatano, T., Hanzawa, R., Abe, S., Kida, S., Shibata, N., Suzuki, T., Arai, H., 2008. Changes in plasma glycine, L-serine, and D-serine levels in patients with schizophrenia as their clinical symptoms improve: results from the Juntendo University Schizophrenia Projects (JUSP). Prog. Neuro-Psychopharmacol. Biol. Psychiatry 32 (8), 1905–1912.

Ozeki, Y., Sekine, M., Fujii, K., Watanabe, T., Okayasu, H., Takano, Y., Shinozaki, T., Aoki, A., Akiyama, K., Homma, H., Shimoda, K., 2016. Phosphoserine phosphatase activity is elevated and correlates negatively with plasma D-serine concentration in patients with schizophrenia. Psychiatry Res. 237, 344–350.

Panatier, A., Theodosis, D.T., Mothet, J.P., Touquet, B., Pollegioni, L., Poulain, D.A., Oliet, S.H., 2006. Glia-derived D-serine controls NMDA receptor activity and synaptic memory. Cell 125 (4), 775–784.

Panizzutti, R., Hamilton, S.P., Vinogradov, S., 2013. Genetic correlate of cognitive training response in schizophrenia. Neuropharmacology 64, 264–267.

Ribeiro, C.S., Reis, M., Panizzutti, R., de Miranda, J., Wolosker, H., 2002. Glial transport of the neuromodulator D-serine. Brain Res. 929 (2), 202–209.

Ryan, R., Koestner, R., Deci, E.L., 1991. Ego-involved persistence: when free-choice behavior is not intrinsically motivated. Motiv. Emot. 15, 185–205.

Schell, M.J., Molliver, M.E., Snyder, S.H., 1995. D-Serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proc. Natl. Acad. Sci. U. S. A. 92 (9), 3948–3952.

Tsai, G., Yang, P., Chung, L.C., Lange, N., Coyle, J.T., 1998. D-Serine added to antipsychotics for the treatment of schizophrenia. Biol. Psychiatry 44 (11), 1081–1089.

Vargas-Lopes, C., Madeira, C., Kahn, S.A., Albino do Couto, I., Bado, P., Houzel, J.C., De Miranda, J., de Freitas, M.S., Ferreira, S.T., Panizzutti, R., 2011. Protein kinase C activity regulates D-serine availability in the brain. J. Neurochem. 116 (2), 281–290.

Vinogradov, S., Fisher, M., Holland, C., Shelly, W., Wolkowitz, O., Mellon, S.H., 2009a. Is serum brain-derived neurotrophic factor a biomarker for cognitive enhancement in schizophrenia? Biol. Psychiatry 66 (6), 549–553.

Vinogradov, S., Fisher, M., Warm, H., Holland, C., Kirshner, M.A., Pollock, B.G., 2009b. The cognitive cost of anticholinergic burden: decreased response to cognitive training in schizophrenia. Am. J. Psychiatry 166 (9), 1055–1062.

Whitlock, J.R., Heynen, A.J., Shuler, M.G., Bear, M.F., 2006. Learning induces long-term potentiation in the hippocampus. Science 313 (5790), 1093–1097.

Wolosker, H., Blackshaw, S., Snyder, S.H., 1999. Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. Proc. Natl. Acad. Sci. U. S. A. 96 (23), 13409–13414.

Yamada, K., Ohnishi, T., Hashimoto, K., Ohba, H., Iwayama-Shigeno, Y., Toyoshima, M., Okuno, A., Takao, H., Toyota, T., Minabe, Y., Nakamura, K., Shimizu, E., Itokawa, M., Mori, N., Iyo, M., Yoshikawa, T., 2005. Identification of multiple serine racemase (SRR) mRNA isoforms and genetic analyses of SRR and DAO in schizophrenia and D-serine levels. Biol. Psychiatry 57 (12), 1493–1503.

Yamamori, H., Hashimoto, R., Fujita, Y., Numata, S., Yasuda, Y., Fujimoto, M., Ohi, K., Umeda-Yano, S., Ito, A., Ohmori, T., Hashimoto, K., Takeda, M., 2014. Changes in plasma D-serine, L-serine, and glycine levels in treatment-resistant schizophrenia before and after clozapine treatment. Neurosci. Lett. 582, 93–98.