Cover Page

Protocol Number: 7725 Version Date: 6-26-20

Protocol Title: D-serine augmentation of neuroplasticity-based auditory learning in schizophrenia

Principal Investigator: Joshua Kantrowitz MD

[redacted]

[redacted]

[redacted] [redacted]

Lay Summary

This section is intended to provide a basic overview of the study including a description of its purpose, methods, and subject population. The summary should provide a concise overview of the study for non-scientific and scientific members of the IRB. Please avoid medical or technical terminology. In general, the abstract of a grant does not provide a suitable lay summary.

Please also paste of a copy of the Lay Summary into the PRISM PSF Form.

Schizophrenia is a major public health problem associated with cognitive deficits, such as short and long term memory, executive functioning and attention that are amongst the strongest predictors of which patient will have ongoing disability and which will recover. *In addition, schizophrenia patients show reduced "plasticity", defined as reduced learning of new information.* This project trains subjects to improve an auditory measure of plasticity—"tone-matching threshold," defined as the ability to distinguish between two tones that differ in pitch (sound frequency). As improved tone-matching can help improve overall cognitive deficits that are closer to daily functioning, improving plasticity represents an unmet clinical need and a necessary first step prior to remediating cognition and overall function.

D-Serine is a naturally occurring substance in the brain that activates the *N*-methyl-d-aspartate-type glutamate receptor (NMDAR). This receptor is thought to be important in both schizophrenia and plasticity (learning). My model proposes that problems with NMDAR within the brain leads to impaired plasticity, which in turn leads to impaired cognition. d-serine is an ideal NMDAR activator to study because it balances efficacy, availability and safety. Most d-serine studies have used a low dose, but the evidence for efficacy is even stronger for high dose d-serine, as will be tested in the current study. There has only been limited summary of higher dose d-serine, which is another important reason for this study.

In addition to testing a potentially viable treatment in schizophrenia, a positive result would provide opportunities for use of D-serine in other populations (e.g. anxiety disorders or dementia) and stimulate the pharmaceutical industry to utilize this methodology to assess the efficacy of novel NMDAR modulators, using d-serine as a "gold-standard."

The ultimate goal of this two part grant (R61-R33) study is to improve cognitive remediation by augmenting with D-serine.

First, during the R61-phase, we will assess 3 doses of D-serine treatment over three sessions of a program designed to measure auditory plasticity across 45 schizophrenia patients.

During the three-year R33-phase, we will conduct a larger study of D-serine of 60 schizophrenia patients, assessing the effects of D-serine over 16 sessions of this program. Most successful, cognitive remediation programs are limited by lengthy (30-50 hours) treatments. Hypothesizing that adding D-serine will increase efficiency of cognitive remediation, successful completion of the R33-phase is defined as significant improvement in global cognition after 16 hours of treatment, and will serve as a pilot study to determine whether future, definitive clinical trials are warranted.

The structure of this grant requires successful completion of the R61 phase over 2 years prior to initiation of the R33 phase. We will amend the PSF and consent to include a full description of the R33 phase prior to its initiation.

Background, Significance, and Rationale

In this section, provide a brief summary of the status quo of the relevant work field, and how the proposed study will advance knowledge. Specifically, identify the gaps in knowledge that your project is intended to fill. If no gaps exist that are obviously and directly related to your project, explain how your proposed research will contribute to the overall understanding of your field. Describe potential impacts of your project within your field of study and in a broader context. Provide a critical evaluation of existing knowledge. The literature review does not have to be exhaustive.

Study overview: Schizophrenia (Sz) is a major public health problem that affects ~1% of the population worldwide. Sz is associated with deficits in neurocognition[1-4] that represent a core feature of the disorder and that may precede illness onset[6], and predict impaired psychosocial function[7,8]. When comprehensive neurocognitive batteries are used, patients show deficits across a large variety of cognitive domains, suggesting widespread cortical involvement. In addition, Sz patients show reduced neuroplasticity in tasks designed to produce learning, leading to reduced ability to benefit from various forms of cognitive remediation[9-11]. The effects of intervention strategies aimed at remediating neuroplasticity deficits, however, have been evaluated to only a limited degree. *Thus, remediating neuroplasticity is a rate-limiting first step prior to remediating cognition and overall function. Plasticity represents an unmet clinical need and a clinically relevant target. The goal of this NIMH sponsored two-site, two-phase R61-R33 study is to enhance efficacy and efficiency of auditory cognitive remediation by augmenting with D-serine.*

For both the R61 and R33 phase, we utilize the same neuroplasticity-based auditory remediation program which has demonstrated a dynamic, direct link between behavioral/neurophysiological plasticity and cognitive improvements.

The R61 phase is designed to assess whether D-serine has dose dependent target engagement over 3 sessions (1x week) of auditory remediation.

During the R61, we will confirm target engagement, pharmacodynamics, functional relationships and the optimal dose (80 vs.100 vs. 120 mg/kg, IND: 122821) of D-serine treatment combined with 3 sessions of our auditory remediation program. *As previously, D-serine will be given 30 minutes before sessions, allowing for auditory remediation during peak serum levels and a pharmacodynamic assessment*. Successful completion of the R61 (go/no go) is defined by ≥moderate effect size change in auditory plasticity, MMN and θ-ITC, plus a moderate effect size correlation with functionally relevant cognitive measures ("auditory cognition": without safety issues.

In the R33, we directly evaluate functional improvement as an outcome, assessing the sustained effects of D-serine + 16 sessions (1x week) of the same auditory remediation program used in the R61.

Successful completion of this two-phase study will pave the way for a larger, definitive study pairing D-serine with auditory remediation or testing alternative dose intervals (1x vs. 2x week). In addition to testing a potentially viable treatment, this project will stimulate industry to utilize this methodology to assess the efficacy of novel NMDAR modulators, using d-serine as a "gold-standard."

The structure of this grant requires successful completion of the R61 phase over 2 years prior to initiation of the R33 phase. We will amend the PSF and consent to include a full description of the R33 phase prior to its initiation.

Rationale for auditory plasticity and remediation in Sz*:* Specific deficits in early auditory processing and plasticity have been replicated over recent years by multiple independent groups[8,28-38], and have investigated the functional consequences of these impairments on more complex forms of information processing dysfunction, including higher cortical functions such as global cognition[7,39], occupational functioning (e.g. reading[40] and working memory[11,41-44]) and social cognition [e.g., auditory emotion recognition (AER)[28,33,37,45-50], perceptual music disorders[30] and sarcasm detection[51,52]]. Although the present study targets the auditory system specifically, impaired plasticity is felt to be a core deficit in Sz across cortical systems, and thus the present project is relevant across sensory/cognitive domains.

Limitations of current treatments for cognition: There are no FDA approved pharmacological treatments for cognitive enhancement in Sz. An alternative strategy to enhance cognitive function in Sz includes cognitive remediation, commonly defined[53] as "a behavioral training-based intervention that aims to improve cognitive processes." Meta-analyses of cognitive remediation trials[53,54] in Sz indicate a moderate effect size for improvement in cognitive performance (d=0.45). However, up to 45% of people with Sz demonstrate minimal improvement after undergoing a therapeutic dose (≥32 hours) of cognitive training[55], suggesting that further research is needed. Cognitive remediation programs vary in the skills they target[56], with some focused on complex skills like executive functioning and others targeting more basic skills[56-62]. Posit Science, a program that is unique in its focus on auditory remediation and plasticity, has shown significant, moderate-large (d=0.56-0.86) effect-size improvements in global cognition compared to a videogame control in Sz[63,64]. Subsequent reports using this program have been mixed[11,65], and furthermore, the clinical burden of a ~50-hour treatment administered 3 to 5x a week limits feasibility. *Thus, the ultimate goal of the present project is to enhance efficacy and efficiency of cognitive, particularly auditory remediation, fulfilling an unmet clinical need.*

Mismatch negativity (MMN) as a measure of target engagement: The present project utilizes MMN as both a neurophysiological proxy of plasticity and a measure of NMDAR target engagement. MMN[66,67] is a neurophysiological response (ERP) elicited most commonly in the context of an auditory oddball paradigm. In this paradigm, a sequence of repetitive standards is interrupted infrequently by a physically different oddball stimulus. Deviants may differ from standards in one or more physical and/or abstract dimensions, including frequency, intensity or duration[68,69]. Deficits in auditory MMN generation in Sz were first demonstrated in the early 1990's and have been replicated extensively since then[7,70-76]. In addition, test-retest reliability of MMN is high, showing an ICC of 0.9[72], encouraging its use as a neurophysiological biomarker for new treatment development[32,77]. Deficits in MMN correlate extensively with poor premorbid function and impaired psychosocial outcome even following covariation for more general demographic and neurocognitive factors[35,37,70,76,78-80].

In addition to assessing plasticity, MMN has been linked to NMDAR dysfunction across rodent[147,148], monkey[77,149] and human[150-155] investigations, and confirmed in a recent meta-analysis[155]. MMN is tied to NMDAR function at the level of auditory sensory cortex[156-160]. MMN has also been linked to glutamatergic functioning by recent studies[161,162] showing that MMN deficits predict parallel deficits in proton magnetic resonance spectroscopy (^{1}H MRS) measured glutamate. By contrast, MMN is unaffected by treatment with antipsychotics[76,163,164], suggesting relative specificity for glutamatergic vs. dopaminergic mechanisms.

Rationale for use of D-serine to assess the NMDAR target for enhancing auditory neuroplasticity: Recent studies of NMDAR modulators have shown the ability to enhance neuroplasticity both in specific patient populations[86-90] and healthy volunteers[12,13], suggesting a potentially useful role in addressing cognitive deficits in schizophrenia[18], yet one which has not yet been translated into clinical practice. The use of NMDAR modulators stems from the well-characterized role of brain NMDAR function in both schizophrenia pathophysiology and neuroplasticity, based upon the ability of phencyclidine (PCP), ketamine and similar compounds to induce deficits closely resembling those of Sz by blocking neurotransmission at NMDAR[14,17,91-95]. In ketamine challenge studies of normal volunteers, significant increases in positive, negative and cognitive symptoms were observed, in similar proportions to Sz. As previously reviewed[14], NMDAR antagonists also reproduce core neuropsychological abnormalities of Sz, including executive functioning[17,96-98], attention/vigilance[96,97,99-102], verbal fluency[17,103,104], working memory[96,97,101-103,105-111] and MMN. By contrast, similar effects are not observed during exposure to dopaminergic (e.g. methylphenidate[112]) or serotonergic (e.g. psilocybin[113,114]) agents, suggesting that such deficits in Sz are attributed most parsimoniously to NMDAR dysfunction[115].

In addition to a proposed role in Sz in general, NMDAR have a well characterized role in learning and neuroplasticity, including acquisition, consolidation, and retrieval[116,117]. Much cortical and hippocampal plasticity does appear to have strong NMDAR contribution, so that broad impairments of learning and plasticity would be expected with NMDAR dysfunction, as are seen in Sz.

Specific rationale for use of D-serine: In the present project, we propose that D-serine is the ideal NMDAR modulator to study because it balances efficacy, availability and safety[18]. D-Serine is a direct agonist at the glycine modulatory site of the NMDAR[16,19] that was originally reported to be beneficial in Sz based upon studies conducted in Taiwan[120] and Israel[86,121]. We recently conducted a dose-escalation study in Sz and observed beneficial, dose dependent clinical effects at doses up to 120 mg/kg[122]. The target engagement of D-serine using MMN is clear relative to other potentially available NMDAR agonists[20], such as glycine type I (GlyT1) transport inhibitors.

Direct evidence for D-serine enhancement of plasticity: In our preliminary study Kantrowitz 2016), 21 Sz patients (44.0±11.5, 90% men) received three neuroplasticity-based remediation sessions separated by 1-week, paired with either D-serine 60 mg/kg or placebo, which were administered in an interleaved fashion across subjects. Analyses focused on effects of both initial and repeated D-serine administration. 13 Sz were studied in a double-blind randomized control design, with 8 additional Sz studied in an open label design to increase power for EEG analysis. Behavioral data are presented only from Sz subjects who participated in the blinded study, while EEG were analyzed from all subjects. D-Serine was given 30 minutes before sessions to allow for training at peak levels[9].

On their initial treatment day, there was no significant between group difference in plasticity (p=0.96). Change over repeated D-serine administration was then evaluated by comparing effects of treatment order in subjects who received either D-serine after their initial placebo day (placebo/D-serine) or placebo after their initial D-serine day (D-serine/placebo) vs. those who received a second consecutive D-serine treatment (D-serine/D-serine). A significant difference was observed across orders, reflecting a significant D-serine effect (p=0.044).

Furthermore, following just two consecutive D-serine treatments, Sz outcomes for behavioral plasticity were normalized vs. controls (p=0.36). In prior studies, alterations in cognition were observed following ~50 hours of training[61,66]. *Thus, a noteworthy result of this study is the observed large effect size improvement following only 2 weekly sessions paired with D-serine.*

Sz patients receiving two consecutive sessions of D-serine had a significantly larger pre-post change in MMN than those receiving placebo (p=0.02, d=0.7), demonstrating target engagement of D-serine. A relationship between plasticity and functional target engagement was demonstrated by a significant correlation between changes in MMN and plasticity improvements (r=-0.34, p=0.034).

In addition to MMN, we will also assessed time frequency outcomes as functionally relevant, target engagement biomarkers. Electrophysiological activity is divided conventionally into discrete θ (4-7 Hz), α (7-12 Hz), β (12-24 Hz) and γ (>24 Hz) bands, which reflect differential underlying local-circuit processes[71,168-170]. In our published work, across all subjects and sessions, θ-ITC correlated significantly with plasticity (r=-0.39, p=0.002), which remained significant after control for group status (r=-0.32, p=0.013). Thus, plasticity was associated with both functionally relevant outcomes and NMDAR dependent changes in early auditory processing (MMN/θ-ITC).

We hypothesize that adding D-serine will further increase efficiency of cognitive remediation, leading to improvement with an abbreviated regimen.

For these reasons, D-serine is the best available agent for assessing NMDAR-based plasticity enhancement and target engagement. In addition to testing a potentially viable treatment in Sz, a positive result would provide opportunities for use of D-serine in other populations (e.g. phobia extinction) and stimulate industry to utilize this methodology to assess the efficacy of novel NMDAR modulators, using D-serine as a "gold-standard."

Dose Finding: In our published preliminary study (Kantrowitz 2016) we observed significant effects using D-serine 60 mg/kg. Whether or not maximal effectiveness is achieved at this dose, however, has not been determined. Our dose-escalation study (Kantrowitz 2010, 2018) supports the potential superiority of D-serine up to 120 mg/kg[9], which is the highest dose with human safety testing. In this study, we evaluated the potential for dose dependent target engagement across D-serine doses (30 (n=5), 60 (n=19) and 120 (n=6) mg/kg/d). Subjects received 4 to 6 weeks of daily D-serine treatment.

Dose-dependent improvements in target engagement, clinical symptoms and cognition were seen. A dose response for target engagement was supported by a large effect size improvement in MMN during high dose D-serine treatment vs. placebo (p=0.001, d=1.7), consistent with our double-blind study (d=2.3). By contrast, the 30 mg/kg dose was not individually significant vs. placebo (Fp=0.23, d=0.7). Definitive analysis of the individual doses was limited by a small sample. While all doses produced significant improvement on the PANSS total (all p<0.05), only the 120 mg/kg produced specific, significant improvement on PANSS negative symptoms. Similarly, large effect size treatment effects were observed for the MCCB at both the 60 (p<0.001; d=1.1) and 120 (p=0.01; d=0.99) mg/kg doses, but not at 30 mg/kg (p=0.39; d=0.25). A significant dose effect was supported by significantly greater improvement at ≥60 mg/kg vs. 30 mg/kg dose for the MCCB composite (p=0.017), with specific improvement in auditory cognition (Verbal Memory Domain, p=0.035). Finally, pharmacodynamic analysis also supports a dose effect, as increasing peak serum levels predicted MCCB improvements (r_s=0.38, p<0.01).

The present project will be the first to assess plasticity and target engagement of weekly treatment of D-serine in doses >60 mg/kg.

See main grant for references

Specific Aims and Hypotheses

Concisely state the objectives of the study and the hypothesis or primary research question(s) being examined. There should be one hypothesis for every major study procedure or intervention. For pilot studies, it is important not to overstate the study's objectives. If there are no study hypotheses, describe broad study goals/aims.

Aim #1 (R61): To determine target engagement and safety of D-serine enhancement of auditory plasticity. 45 Sz patients will be randomized to receive three auditory remediation sessions + a double-blind dose of D-serine (80, 100 or 120 mg/kg) vs. placebo. *Hypothesis: Based on our pilot data, we hypothesize that D-serine will be safe and lead to greater plasticity, MMN and θ-ITC changes than placebo, with the largest effect at 120 mg/kg.*

Aim #2 (R61): To confirm the functional relationship of auditory plasticity improvements. In prior studies, auditory plasticity deficits have been related to impairments in higher, functionally relevant auditory functions. *Hypothesis: We hypothesize that plasticity outcomes will be related to functionally relevant outcomes (auditory cognition[3], primary), emotion recognition (AER)[10], MMN, θ-ITC, other functional outcomes and pharmacodynamics.*

Aim #3 (R33): To evaluate effects of D-serine-enhanced auditory plasticity on auditory cognition. 60 Sz patients will be randomized to auditory remediation + D-serine or placebo. 16 sessions (1x week) of treatment will be utilized, with dose dependent on R61 results. *Hypothesis: We hypothesize that D-serine treated subjects will have greater improvements in auditory cognition[3] than placebo. Plasticity, AER, MMN, θ-ITC, reading, other cognitive/functional measures and pharmacodynamics will be secondary outcomes.*

Inclusion/Exclusion Criteria

This section details your study sample(s) and addresses the requirement for risk minimization.
You may choose to divide your sample by population (healthy controls vs. subjects) or by procedure (subjects who will have an MRI) and then define different sets of criteria for each.

For each sample, create or insert a table to describe detailed criteria for study inclusion and exclusion and the method you will use to ascertain each criterion. The method of ascertainment may describe tests, scales and instruments. When relevant, indicate the level of training of the person who will make the assessment (e.g. clinical interview by a psychiatrist).

Inclusion/Exclusion Criteria needs to be numbered and listed in outline form (see Table template below).

CRITERION	METHOD OF ASCERTAINMENT
Inclusion:	
1. Age between 18-50	Self-report
2. DSM-V diagnosis of schizophrenia or schizoaffective disorder	SCID
3. **Auditory Cognitive impairment demonstrated by:** **1. MATRICS composite score (MCCB) composite domain score less than or equal to 0.5 standard deviation below normal (T score less than or equal to 45)** **2. And at least one of the following:** **a. MCCB verbal memory domain score less than or equal to 0.5 standard deviation below**	Screening MCCB

normal (T score less than or equal to 45) **b. Tone matching score of less than or equal to 77.7%**	
4. Willing to provide informed consent	Physician evaluation
5. Medically stable for study participation	Medical history, physical examination and screening laboratory parameters, information from clinical team, review of medical record.
6. Taking an antipsychotic medication other than clozapine at a stable dose for at least 4 weeks	Self report
7. Judged clinically not to be at significant suicide or violence risk.	The Columbia Suicide Severity Rating Scale (C-SSRS); clinical interview
8. Clinically stable for 2 months (CGI≤4)	Screening CGI
9. Moderate or lower cognitive disorganization (PANSS P2 ≤4)	Screening PANSS
10. Visual acuity corrected to at least 20/30	Screening exam
11. An estimated Glomerular Filtration Rate (GFR) ≥60	Screening labs
12. Fluent English speaker	Screening exam
13. Normal conversational hearing	Screening exam
14. Willing to use qualified methods of contraception for the study duration and up to 2 months after its end	Screening exam

CRITERION	METHOD OF ASCERTAINMENT
Exclusion:	
1. Substance abuse (excluding nicotine) within last 60 days	Physician evaluation/SCID
2. ECG abnormality that is clinically significant in the context of study participation in the opinion of the study cardiologist	ECG
3. Current clozapine use. Clozapine is excluded for two reasons: to avoid the potential confound of treatment resistant patients and because of clozapine's intrinsic NMDA agonist	Self report.

4. Participation in study of investigational medication/device within 4 weeks	Self report
5. Pregnant women or women of child-bearing potential, who are either not surgically-sterile or for outpatients, using appropriate methods of birth control. Women of child-bearing potential must have a negative serum β-hCG pregnancy test at screening.	Instructions to study subject, pregnancy tests as per protocol and review of the medical record.
6. Presence of positive history of unstable significant medical or neurological illness	Medical history and screening labs
7. Positive toxicology screen for any substances of abuse	Urine toxicology screen. Positive tests may be repeated at the discretion of the study investigator for suspected false positive
8. Subjects with suicidal ideation with intent or plan (indicated by affirmative answers to items 4 or 5 of the Suicidal Ideation section of the baseline C-SSRS) in the 6 months prior to screening or subjects who represent a significant risk of suicide in the opinion of the investigator	Screening C-SSRS

Study Procedures

Provide a clear, concise narrative of study procedures with special attention to the subjects' involvement. Detail the overall study timeline and location of study procedures, list all interventions, assessments and interviews, estimate the duration of each procedure, provide dosing schedules, identify study personnel involved in each procedure, and provide credentials for relevant personnel. For complicated study designs, we strongly encourage attaching tables, flow-charts, and study algorithms.

In the R61, we will confirm target engagement of D-serine + auditory remediation, assess relationships of plasticity changes and cognition, safety and assess the optimal dose of D-serine treatment. In the R33, we conduct a randomized, placebo-controlled, parallel group study with the superior dose of D-serine combined with 16 weekly sessions of the same neuroplasticity-based auditory remediation. The structure of this grant requires successful completion of the R61 phase over 2 years prior to initiation of the R33 phase. We will amend the PSF and consent to include a full description of the R33 phase prior to its initiation.

The study will be conducted in groups of 15 subjects in which 12 subjects will receive D-serine for each of the three treatment visits and 3 will receive placebo for each of the three treatment visits. The first group of 15, will receive 80 mg/kg or placebo, and the second group of 15 subjects will receive 100 mg/kg or placebo, followed by a third group receiving 120 mg/kg or placebo. As previous studies noted minimal carry over effect of D-serine effects (Kantrowitz 2010), subjects may be permitted to participate in more than one phase with at least a six

month space in between.

If there are tolerability concerns at the 80 or 100 mg/kg doses, we will add a 60 mg/kg arm, ensuring the assessment of multiple doses. After each cohort, we will obtain permission from both the FDA and the IRB via an amendment, prior to the beginning of the next cohort. We require this safety milestone to be reached in order to begin the next cohort.

R61 Design (Table 1):

R61 Design

Table 1 (R61)	**Baseline**	Treatment Day			**Follow-up[1]**
		1	**2**	**3**	
	Day -30 to -1	Day 1	Day 8±2	Day 15±2	
Consent	x				
Medical screening	x				
D-serine/Placebo		x	x	x	
MMN		x	x	x	
Auditory Remediation/EEG		x	x	x	
Cognitive and behavioral ratings	x				
Clinical laboratory/pregnancy/vitals	x	x	x	x	x
SAFTEE/C-SSRS	x	x	x	x	x
1. If necessary to follow-up any abnormalities.					

Screening/Informed consent: Informed consent will be obtained before any study procedures, including screening procedures, are initiated. After providing informed consent, subjects will undergo full medical screening (medical history, physical examination, vital signs, laboratories for basic chemistries, blood counts, liver function tests, urinalysis, urine toxicology, serum pregnancy test for women, and thyroid tests, electrocardiogram [EKG]) and psychiatric screening (i.e., Structured Clinical Interview for DSM V Axis I Disorders (SCID) to confirm eligibility. Screening will occur over an up to 31-day screening period.

Baseline ratings: Eligible subjects will then undergo baseline cognitive and behavioral tests:

Randomization: Assignment to treatment groups will be on a random basis. Randomization will be stratified by baseline tone matching.

Auditory remediation visits: Subjects will then undergo three auditory remediation/EEG sessions. Each visit will follow the same format. Sessions are administered once a week±2 days. We utilize an auditory remediation program, in which participants are presented with paired tones (e.g. Stimulus 1 ("reference") and Stimulus 2 ("test"): S1 and S2) and indicate which tone is higher in pitch (frequency). In the first pair, the ratio is 50% (e.g. 1000±500 Hz), and the difficulty level is adjusted to maintain a steady (~70% correct) level of performance across the 80 repeats. Ratios in each pair are log-transformed and averaged across 10-trial pairs (e.g., pairs 1-10, 11-20, etc.). When the reference (S1) remains constant ("fixed"), highly significant improvement is seen.

After a negative urine pregnancy test for fecund women and the Columbia Suicide Severity Rating Scale (C-SSRS), each visit will begin with a pretreatment EEG/capping. Subjects will then receive D-serine or placebo. Auditory remediation will begin 30 minutes after study drug administration, to allow for training during peak D-serine levels (Kantrowitz, Malhotra et al. 2010). EEG will be recorded during sessions, to assess theta-ITC and secondary EEG measures. Immediately after auditory remediation, subjects will complete post treatment MMN. A D-serine level will be drawn immediately after the session using established methods to allow for functional pharmacodynamics readout, along with urinalysis and clinical laboratory assessments. The SAFTEE will be used to assess general side effects. Columbia/NKI EEG labs are both under the supervision of Drs. Sehatpour and Javitt and have previously conducted parallel studies, ensuring cross-site validation.

Study drug and maintenance of the blind: D-Serine and placebo will be administered as a solution, prepared (premixed) in water and prescribed only as "study medication." D-serine will be dosed by weight (e.g. 80 mg/kg

for the 1st cohort). Study medication will be dispensed to subjects in individual, identical appearing bottles, and dispensed on the day of the session by an un-blinded pharmacist otherwise uninvolved in the study. As previously, D-serine will be obtained from Evonik, and an artificial sweetener will be used as placebo.

The un-blinded pharmacist, based upon predetermined assignment schedule, will make study assignment. Envelopes containing treatment assignment will be kept in pharmacy and in patients' clinical charts. Envelopes will be unsealed only in cases of medical necessity. Blind-breaks will be on individual subject basis only. The pharmacist does not participate in assessing any dependent variable and conveys no information about drug status to patients or staff except in a medical emergency.

Permitted medications: Subjects will be receiving antipsychotics at doses within the PORT criteria (Lehman, Kreyenbuhl et al. 2004) whenever possible. Patients will be allowed to receive the following adjunctive medications during the course of the study: anticholinergic agents; beta-blockers; mood stabilizers, antidepressants; and anti-anxiety agents. PRN doses of clinically determined benzodiazepines or antipsychotics will be permitted. Given a possible detrimental effect on cognition, patients will be asked to not take these medications the night before or on the day of testing/training session if clinically feasible. The clinical decision will largely be based on a discussion with the subject. The study doctor will ask the subject about their use of PRN benzodiazepines or antipsychotics. For example, if the subject reports that they tend to sleep poorly or have excessive anxiety without them, then they will be permitted. We will err on the side of allowing PRN's. These procedures have been successfully used in our prior published D-serine studies with good effect.

Safety: The primary concern during D-serine treatment is potential for nephrotoxicity. Safety procedures, will be similar to prior D-serine studies (see criteria for early discontinuation). While primary safety focus is on renal function (serum creatinine, BUN, microscopic urinalysis), other measures obtained are liver function tests, CBC, fasting glucose, and general chemistry.

Plasma D-serine levels: Venous blood samples (10 mL per blood draw) will be drawn after the auditory remediation session for assay at the Analytical Psychopharmacology laboratory.

A full description of outcomes is found in section 4.3 of the main grant.

Milestones and Rationale (Go/No-Go Criteria):

Per the R61-R33 mechanism, we are required to meet predesignated milestones prior proceed to the R33. These include target engagement criteria of specific improvement in plasticity, MMN amplitude and θ-ITC, a relationship between plasticity and MMN and safety. Full detail is found in the main application, section 3C4 (page 111).

End of Participation

Care at termination of the study will transfer back to their previous providers and if subjects are hurt by the study their medical care will be provided, but their insurance will be billed for the care. Any relevant health information revealed during the study will be shared with the subject or their health care provider at the subject's request.

Coordination with outside providers:

All subjects not receiving care at the Lieber Schizophrenia clinic will be asked to sign a release to contact their current community psychiatrist or therapist prior to randomization. The community psychiatrist or therapist will be updated on the status of the subject prior to randomization, at the beginning and end of each treatment period and upon study completion. The outside provider will be provided with the 24 hour contact for the study physician. These updates will be documented in the study chart.

COVID-19 safety procedures:

I attest to follow the COVID-19 Safety Guidelines for Columbia Psychiatry and NYSPI Re-Entry outlined in the NYSPI Director's June 1st memo, which include but are not limited to:

- ***Infection Control/PPE – Guidelines***
- ***Research participants will only come on-site if absolutely necessary for study procedures.***
- ***No volunteers/externs on-site during Stage 1.***
- ***Clinical research teams will screen their participants for COVID symptoms (night before and day of onsite visit, documenting this in the chart), and escort them in and out of the building.***
- ***COVID/COVID-like symptoms in participants will be reported to the IRB via PRISM as an SAE.***

Section References:

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

Lehman, A. F., J. Kreyenbuhl, R. W. Buchanan, F. B. Dickerson, L. B. Dixon, R. Goldberg, L. D. Green-Paden, W. N. Tenhula, D. Boeresu, C. Tek, N. Sandson and D. M. Steinwachs (2004). "The Schizophrenia Patient Outcomes Research Team (PORT): Updated Treatment Recommendations 2003." Schizophr Bull **30**(2): 193-217.

Criteria for Early Discontinuation

Define criteria that will be used to exit or drop subjects from the study. Indicate the time points when such criteria will be applied, and describe the rating instruments, parameters, and thresholds that will lead to a decision to terminate a subject's participation. In addition, explain procedures for managing subjects who are dropped from the protocol.

For treatment studies: To minimize risks to subjects, operationalized drop-out criteria should be defined so that subjects who worsen, or in some cases, fail to improve, are removed from the study and offered standard care. The threshold for drop-out should consider the level of risk associated with non-improvement for the specific disorder, the availability of alternatives, and the typical required duration of treatment. For example, emergence of suicidal intent, or psychosis, should prompt immediate clinical evaluation and withdrawal from the study.

The following safety procedures will be followed, as approved under our IND.

a) Urinalysis with microscopics will be done at every visit.

b) Immediately discontinue D-serine for unexplained serum creatinine increase >0.3 mg/dL over the pre-study value or for >1 granular or muddy casts. Treat as serious adverse event (SAE) possibly related to study medication. Repeat until clear x 2 to demonstrate reversibility

c) Hold D-serine for >1 hyaline casts, and repeat lab. Ask subject to eat more salt and drink more water. If absent on repeat, reinstate D-serine and treat as adverse event (AE). If present on repeat, continue to hold d-serine and repeat lab once again. If still present on second repeat, discontinue D-serine and treat as SAE possibly related to study medication. Repeat until clear x 2 to demonstrate reversibility.

d) Hold D-serine for proteinuria > 100 mg/dl or unexplained glucose >250 g/dl (both equivalent to 2+). If absent on repeat, resume D-serine and treat as AE. If still present on repeat, discontinue D-serine. Repeat until clear x 2 to demonstrate reversibility. This would be treated as SAE possibly related to study medication. Unexplained glycosuria is defined as increased urine glucose in absence of corresponding increase in serum glucose levels, in patients without glycosuria at baseline.

e) Continue D-serine for proteinuria >30 but <100 mg/dl (1+), or unexplained glycosuria (>100 but < 250 g/dl) but repeat. If absent on repeat, continue D-serine and treat as AE. If still present on repeat, hold D-serine and repeat

once more. If absent on repeat, resume D-serine and treat as AE. If still present on second repeat, discontinue D-serine and treat as SAE possibly related to study medication. Repeat until clear x 2 to demonstrate reversibility.

f) For other kidney related measures (e.g., ketones, bilirubin, WBC, RBC, bacteria, crystals), repeat, but no need to discontinue even if present on repeat, since unlikely to be D-serine related. Manage in consultation with medical specialist.

g) Contaminated samples (hemolyzed/non-clean catch) will be repeated.

In addition

A subject will not receive any further doses of the study drug should any of the following events occur:

- Any serious adverse event. If drug relationship can be ruled out (e.g., hospitalization due to traumatic injury), no decisions will be taken for the study as a whole.

- Any other physical examination finding, change in vital signs, adverse event, or laboratory abnormality that in the opinion of the Investigator would cause an excessive risk if the subject continued the study.

-A CGI-improvement (CGI-I) score of 6 or 7

-Positive urine pregnancy test at any visit

Blood and other Biological Samples

Describe how the sample will be used and indicate, when relevant, the amount of the sample. The IRB wants to know that the sample is sufficient for the purposes of the study, but that sampling is limited to what is minimally necessary.

If you've indicated that you intend to store a sample for future use, indicate where the sample will be stored, how long the sample will be stored, and to what purposes the sample will eventually be put. Check the IRB website at http://irb.nyspi.org/irbdnn/Policies/GeneticResearch/tabid/96/Default.aspx for specific guidance and additional information about future use of DNA samples.

A total of up to 200 mL of blood (equal to 13.5 tablespoons) will be drawn during the entire study on different days, no more than one tablespoon at a time. Blood will be drawn for safety assessments and for pharmacokinetic sampling.

Assessment Instruments

List all assessment instruments, indicate who will administer them, and provide an estimate the duration of each. The IRB wants to know that assessments instruments are appropriate measures for the purposes of the study and are no more burdensome than is necessary. The IRB will consider the burden of assessment instruments (in terms of time, sensitivity of material, etc.) in the risk/benefit analysis. Please attach copies or otherwise provide all non-standard instruments.

Electrophysiology: **2-3** hours

MCCB: 1 hour

CGI-S/CGI-S: 5 minutes

SCID: 1 hour

PANSS: 30 minutes

C-SSRS ratings:10 minutes

Auditory and social cognition: 2-3 hours. In particular, auditory cognition is defined as the HVLT and letter number sequence test of the MCCB. In all, the ratings visit is expected to take 3-4 hours

Research Related Delay to Treatment

Research involving participants who are in need of treatment invariably involves delay to care, and this delay is associated with risk. Scheduling of procedures must be carefully organized to minimize delay. Other delay must involve only that minimally necessary to accomplish the aims of the research while respecting subject well- being and safety. Describe the delay, by virtue of research participation in this study, before a participant can receive treatment of known efficacy or standard care routinely offered in the community.

No. All subjects will be offered four months of free treatment after the study.

Clinical Treatment Alternatives

Describe what other treatment or assessment options are available to subjects who do not participate in research.

There are currently no FDA-approved treatments for cognitive deficits in patients with schizophrenia. Therefore, no proven treatment will be withheld from subjects. There are behavioral therapies such as Cognitive Remediation Therapy that may address cognitive deficits.

Risks/Discomforts/Inconveniences

"Risk" is a broad term used to convey the potential for harm, burden, and inconvenience related to research participation. Use this section to provide a comprehensive description of foreseeable physical, psychological, social, interpersonal, and economic risks introduced by the research. Include the source of the information. Consider both the probability and magnitude of harm and its impact. Describe the foreseeable harms associated with the research (untoward effects of a medication) and those related to delay to individualized treatment. Include data from the literature, and local data, if available, on risk rates and subject experiences with research procedures. Describe procedures in place to minimize risk. In general, please create a numbered list of risks/categories of risk, and in general put the list in the order of significance or level of risk, the most significant risks first followed by others.

Risks associated with D-serine

Please see the main grant application for a full description of D-serine safety, including animal studies and pharmacokinetics.

The PI has an active IND for D-serine (122821), specifically permitting use up to 120 mg/kg in this protocol.

D-serine is a naturally occurring amino acid that is present in high concentrations in the human brain. D-serine is cleared almost exclusively by the kidney.

The primary safety concern of D-serine is the potential for nephrotoxicity. Nephrotoxicity is a theoretical concern during D-serine treatment, as rats receiving D-serine experience a reversible acute tubular necrosis. When present, this toxicity leads to high levels of glucose and protein being present in the urine. This effect, however appears to be isolated to rats, in that other rodent species (e.g., mice) to not show similar sensitivity to D-serine, nor is toxicity observed in non-rodent species (e.g., rabbit, dog, monkey)[219]. Further, toxic effects of D-serine, even in rats, are fully reversible even with continued D-serine treatment. The specific sensitivity of rats appears to be due to the presence of a D-serine transporter in rat kidney that actively reabsorbs D-serine from the urine, leading to buildup of high levels within the rat kidney. The presence of this transport mechanism is apparent from the low levels of D-serine in rat urine relative to that of other species, despite relatively similar serum levels.

In humans, there is no evidence of reabsorption of D-serine by the kidney. The safety of high dose D-serine in non-rat animal species has been further established as part of the safety reports provided to the FDA for my IND.

14 human trials have been published with D-serine (Table), including 431 subjects and treatment duration up to 16 weeks of daily dosing. 102 subjects received high dose (>30 mg/kg), including 16 patients at 120 mg/kg. Across all of the studies, only one subject was reported to have abnormal renal values related to D-serine treatment. The abnormality occurred in a subject receiving 4 weeks of the 120 mg/kg dose. Even at that dose, the abnormality was extremely mild in that it involved only an increase in protein (2+ by dipstick) without accompanying increase in glycosuria or change in creatinine level. The abnormal urinalysis values were observed 4 weeks following treatment onset, and fully resolved within a few days of stopping treatment.

Overall, this 1 case represents 0.2% of all D-serine treated subjects, <1% of subjects receiving continuous high dose D-serine and one of sixteen (6.3%) of subjects treated continuously with 120 mg/kg, emphasizing safety. Moreover, in the present study D-serine will be used intermittently (1x week) instead of daily, so that the overall exposure will be 1/7 of that in prior studies.

Non-renal side effects:

No significant changes in laboratory values or EKG have been noted in D-serine trials. No other clinical or statistical changes have been noted in extrapyramidal symptoms as measured by the BAS or the SAS.

All full listing of published D-serine studies, which emphasize renal safety, is found in the Table.

Table: Renal safety of D-serine

Reference	Active D-serine "n" & diagnosis	Dose	Renal Abnormalities
High dose			
(Kantrowitz et al. 2015)	20 CHR (prodrome)	60 mg/kg/day for 16 weeks	None
(Kantrowitz et al. 2016)	21 Sz	60 mg/kg single dose x1week for 3 weeks	None
(Kantrowitz et al. 2018)	16 Sz	60 mg/kg/day for 6 weeks	None
(Ermilov et al. 2013)	10 Sz	3 gram/day for 6 weeks (~45 mg/kg)	None
(Kantrowitz et al. 2010)	47 Sz	12 Sz at 30 mg/kg 19 at 60 mg/kg 16 at 120 mg/kg for 4 weeks	1 subject showed 2+ proteinuria without glycosuria after 4 weeks of 120 mg/kg, without change in creatinine. Proteinuria resolved following D-serine discontinuation.
Low Dose			
(Tsai et al. 1998)	14 Sz	30 mg/kg/day for 6 weeks	None
(Tsai et al. 1999)	10 Sz	30 mg/kg/day for 6 weeks	None
(Heresco-Levy et al. 2005)	19 Sz	30 mg/kg/day for 6 weeks	None
(Lane et al. 2005)	21 Sz	2 gram/day for 6 weeks (~30 mg/kg)	None
(Lane et al. 2010)	20 Sz	2 gram/day for 6 weeks (~30 mg/kg)	None
(D'Souza et al. 2013)	51 Sz	30 mg/kg/day for 12 weeks	None
(Weiser et al. 2012)	97 Sz	2 gram/day for 16 weeks (~30 mg/kg)	None
(Levin et al. 2015)	35 healthy controls	2.1 gram single dose (~30 mg/kg)	None
(Avellar et al. 2016)	50 healthy older adults	30 mg/kg single dose	None

Dose escalation plan: This study has a built-in space between doses, maximizing safety. Furthermore, no side effects were noted in our prior, once weekly intermittent treatment study. For the present study, potential nephrotoxicity will be monitored through serum chemistry and urine microscopic examination looking for evidence of active sediment (e.g. casts), proteinuria or glycosuria after each dose, as per FDA suggestions. No subjects with baseline renal impairment, as evidenced by a GFR<60 or clinically abnormal laboratories will be enrolled in the study. As attached, FDA has approved our IND, which incorporates the monitoring procedures described above as well as a sequential dose escalation to identify the maximum tolerated dose.

Placebo

The primary risk with placebo is that patients' symptoms may get worse or may not improve. Since patients will remain on their antipsychotic, and the risk of placebo is small.

Potential subjects will be informed about the results of previous studies and will be encouraged to seek treatment outside of the study as an alternative to study participation if they wish.

Antipsychotics:

All subjects will be asked to remain on their pre-study antipsychotics during the study, and any dose changes for this 6 week study, if any, will be included as co-variates for analysis

EEG Recordings

These studies entail the recording of EEG from the scalp employing standard sensors and amplification methods. These procedures are well standardized and there are few known risks. These are principally due to equipment malfunction. The recording equipment used in these studies meet the current design criteria for subject safety, including isolation from potential electrical hazards. Regular preventative maintenance and careful attention to recording procedures further minimize the already insignificant risks of electrophysiological recording. Electrodes and materials used for affixing them in place are kept scrupulously clean and sterilized and materials are utilized which minimize the chance of skin and scalp irritation. Urine pregnancy tests will be conducted at the time of EEG on all female patients to confirm that the patient is not pregnant prior to study drug administration.

Interviews and Neuropsychological Assessments

Interviews and neuropsychological assessments are associated with minimal risk. Some participants may find the interviews and assessments tiring or distressing. Risks are to be minimized by allowing as much flexibility in the interview process (e.g., doing the interview in several meetings, giving breaks) as possible. If subjects have emotional responses, appropriate psychological support is given. Most patients find the interviews and assessments helpful.

Pregnant or Nursing Females

We will not administer experimental medication to pregnant females. For these reasons, pregnant females are excluded from the study. All female patients will demonstrate a negative urine human chorionic gonadotropin (HCG) test prior to sessions to confirm that the patient is not pregnant.

Emergencies

If, during the course of interviewing and assessment procedures, study staff identifies a condition that mandates immediate clinical intervention or official reporting (e.g., homicidality/suicidality), all necessary steps will be taken, and

the emergency procedures of the Division of Experimental Therapeutics, inpatient unit NKI, and the Lieber Clinic will be followed. In the case that staff determine that the participant is at significant risk for self or other destructive behavior, any mental health professional currently treating the participant will be contacted and necessary treatment steps will be taken (e.g., hospitalization, continuous observation, referral to a care provider, etc.). All patients will be given numbers to call in the event of an emergency during treatment: the primary phone number of the treating clinician and the number for the Lieber clinic (both the front desk number and the number for the Doctor- on-call beeper). If the treating clinician, a covering MD in the Lieber clinic, NKI, or the Doctor- on- Call is immediately unavailable, the subject will be instructed to not delay and to go to their nearest Emergency Room.

COVID-19 risks

Going out in public and traveling involves some risk of infection with COVID-19. There is risk of COVID-19 infection during in-office visits and during travel for research purposes.

These risks can be reduced by taking recommended precautions. These include always wearing a mask in public and while traveling, practicing hand hygiene, and staying at least 6 feet away from others. We may be able to arrange alternative transportation for subjects to avoid the subway and the bus.. We have also minimized in-office visits to lessen this risk.

We will keep subjects informed about current public health recommendations, such as federal and local government guidelines and directives.

Methods to Protect Confidentiality

Describe the data management plan and the methods you will employ to protect subject privacy and the confidentiality of research data. The section should detail how information will be collected, recorded, coded, stored, transmitted, and as applicable, shared with other investigators so as to minimize risks related to breach of confidentiality. Confirm that identifiers are removed, to the extent possible, from research data, and explain if there are links between subject identity and research data, or if the data is anonymous. Also, indicate where the data is stored, who is responsible for its safekeeping, and who has access to subject identity and codes, if any, which cross-link research data and subject identity. Confirm that identifiable data is not collected, stored, or transmitted by mail, fax, on removable drives, laptops, or via the internet without proper protections, e.g. encryption.

Describe methods to protect confidentiality
Blood and urine samples, behavioral assessments, EEG recordings, and all other clinical/neuropsychological data will be obtained from the subjects for specific research purposes. Data will include self-report information, observer records, and physiological and behavioral information collected during test sessions. Each subject is assigned a unique ID and all data related to that subject is entered at the site by the site data entry personnel.
Study information will be collected at NYSPI, and entered into a secure electronic database.

For remote visits, we will use HIPAA-compliant videoconferencing, phone and web-based platforms.

Direct Benefits to Subjects

Describe only benefits to individual subjects that are likely to accrue during the study itself. Do not include subject compensation or treatment to be provided at the end of the study, as these do not figure into the IRB's risk benefit

considerations. Do not describe diagnostic and evaluation components unless subjects receive clinical feedback. Do not describe the anticipated scientific benefits of the research. Some studies offer no direct benefit to subjects.

This study was not designed to benefit individual subjects; however, it is possible that some patients might receive some temporary improvement in their symptoms during this study.

References

See main protocol