Official Title: Targeting a Genetic Mutation in Glycine Metabolism With D-cycloserine (DCS)

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DETAILED PROTOCOL

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I. Background and Significance

a. Historical background

Multiple rare structural variants of relatively recent evolutionary origin are recognized as important risk factors for schizophrenia (SZ) and other neurodevelopmental disorders [e.g., autism spectrum disorders, mental retardation, epilepsy] with odds ratios as high as 7-30 (Sebat et al. 2009; Malhotra et al. 2011; Heinzen et al. 2010; Weiss et al. 2008; McCarthy et al. 2009). We have found a *de novo* structural rearrangement on chromosome 9p24.1. In addition to other genes, the rearranged region contains the gene encoding glycine decarboxylase (*GLDC*), which affects brain glycine (Gly) metabolism. A specific focus of this project is on potential contributions of this gene to abnormal glycine homeostasis and N-methyl-D-aspartate receptor (NMDAR) dysfunction in SZ. This mutation is an obvious 'smoking gun.' *GLDC* encodes the glycine decarboxylase or glycine cleavage system P-protein, which is involved in degradation of glycine (Gly) in glia cells. Carriers of the *GLDC* triplication would be expected to have low levels of brain Gly, resulting in NMDA receptor-mediated hypofunction, which has been strongly implicated in the pathophysiology of SZ (Olney & Farber, 1995; Coyle, 2006; Javitt, 2007). Individuals with mutations that lower brain Gly or alter other aspects of glutamatergic transmission are obvious candidates for Gly augmentation or complementary NMDAR modulatory strategies, which have been used with varying degrees of success (Goff et al. 1999; Javitt et al. 2001; Lane et al. 2005; Heresco-Levy et al. 2004; Buchanan et al. 2007). Genetic risk factors, including variants that impact the synthesis and breakdown of Gly, are likely to contribute to this variability.

b. Previous pre-clinical or clinical studies leading to and supporting the proposed research

There is an extensive literature on the effects of NMDA enhancing agents on positive, negative, and depressive symptoms and on neurocognitive function (see Tsai & Lin, 2010; Lin et al. 2011 for reviews). Although many studies have reported positive results in at least one symptom domain (Heresco-Levy et al. 1996, 1999, 2004; Tsai et al. 1998, 1999, 2004a, 2006; Javitt et al. 2001; Goff et al. 1996; Lane et al. 2008), the results of other studies have been negative or ambiguous (Goff et al. 1999; Evins et al. 2000; Duncan et al. 2004; van Berckel et al. 1999). Individual agents also differ in their impact on various domains of psychopathology and cognition. Factors likely to contribute to this variability are: mechanism of action of the agent, compliance, concurrent treatment with first- vs second generation antipsychotic drugs, baseline Gly blood levels, presence/absence of kynurenine pathway metabolic abnormalities [e.g., increased kynurenic acid) (KYNA)] (Wonodi et al. 2010; Erhardt et al. 2007) and individual differences in brain Gly uptake and metabolism (Kaufman et al. 2009; Buchanan et al. 2007). Genetic variants that impact the synthesis and breakdown of Gly, Glu, or other modulators of NMDAR function are also likely to have significant effects. Although NMDAR modulators has shown variable efficacy in patients ***unselected*** for having a mutation that would be expected to lower brain Gly levels, the *GLDC* triplication in the two carriers would be expected to result in *unusually low* brain Gly levels, supporting the therapeutic potential of augmenting agents in these individuals.

In previous work Kaufman and colleagues have shown that 14 days of oral Gly increases brain Gly compared with baseline levels (Kaufman et al. 2009). Oral high dose Gly also increases total brain and plasma Gly levels in mice, and the increments in brain and plasma Gly covary for several hours (Toth & Lajtha, 1986). This protocol includes magnetic resonance spectroscopy (MRS). It is possible to obtain J-resolved proton ^{1}H MRS that provides high quality information on glutamine, glycine, and glutamate (Ongur et al. 2008; Jensen et al. 2009; Prescott et al. 2006). GABA can be quantified separately from MEGAPRESS difference spectra (Jensen et al. 2009), permitting the measurement of levels of other metabolites that may be impacted by the *GLDC* triplication.

In a separate IRB-approved protocol (Neurobiology of a Mutation in Glycine Metabolism in Psychotic Disorders), we treated two carriers of the *GLDC* triplication with oral glycine using a double blind placebo-controlled cross over design, followed by open-label glycine. During both active treatment arms, the subjects experienced substantial improvement in clinical symptoms. The clinical gains that were achieved during Gly treatment were lost during the placebo arm and when the open-label treatment period ended. We obtained an administrative supplement from NIMH to continue to treat the subjects with open-label glycine for six months. The six-month extension is approved by the IRB. Due to the high doses of Gly required for therapeutic effects (~0.8 g/kg), it cannot be administered in capsule(s) without producing an osmotic load resulting in vomiting. As a result, the glycine powder (~60 g/d) has to be mixed with soft food or a drink, ingested slowly after each meal, and taken on a TID schedule, making it a cumbersome long-term treatment.

D-cycloserine (DCS) is a reasonable, and much less cumbersome, alternative. There are three reasons why DCS may be particularly advantageous in treating the mutation carriers. First, at the low dose (50 mg) we will use, DCS acts as a partial agonist at the glycine modulatory site (GMS); the *GLDC* mutation impacts glycine homeostasis. DCS has 40-70% of the potency of Gly to potentiate excitation of the NMDAR (Emmett et al. 1991; Henderson et al. 1990; Hood et al. 1989 McBain et al. 1989; Watson et al. 1990). Although the lower potency of DCS might reduce its efficacy, DCS crosses the blood brain barrier more readily than Gly (Morton et al. 1955-1956). In addition, the magnitude of the augmentation needed to normalize Gly homeostasis in our subjects was substantially lower than anticipated - the maximum tolerated Gly dose was lower (~0.7 g/kg) than what is thought to be optimally therapeutic (0.8 g/kg) and the clinical benefits began at doses well below the maximum dose used. Second, DCS inhibits the formation of kynurenic acid (KYNA), a glutamate receptor antagonist with a particularly high affinity for the GMS, as least in *in vitro* studies (Baran & Kepplinger, 2014). Schwarcz was unable to replicate this effect in live rats, however (personal communication). Whether this is a difference between *in vitro* and *in vivo* effects or whether effects in rats generalize to humans is unclear. Unbeknownst to us when we designed the original Gly study, our subjects have unusually high levels of KYNA. Elevated KYNA may potentiate the deleterious effect of the *GLDC* triplication at the GMS and may have attenuated the magnitude of the clinical effects we observed with Gly. If the Baran and Kepplinger findings hold up in people, it is conceivable that DCS may normalize KYNA, which Gly did not. Third, low dose DCS enhances learning and memory in animals, at least acutely (Watson et al., 1990; Collingridge, 1987; Monahan et al. 1989; Quartermain et al. 1994), and in SZ patients, but is time-limited (Goff et al. 2008, 2012; Gottlieb et al. 2011). Gly produced no change in neurocognition in our subjects; conceivably, the combination of GMS agonism and KYNA inhibition may result in greater neurocognitive benefit. Whether these effects are sustainable is an empirical question. This tripartite rationale for using DCS *in these specific subjects* outweighs the relatively sparse and variable data on the clinical efficacy of DCS (Goff et al. 1999a, b, 2005, 2008; Duncan et al. 2004; van Berckel et al. 1999;

Rosse et al. 1996; Heresco-Levy et al. 1998, 2002). Of note, when DCS works, the effect size is large (0.8) (Goff et al. 1999b).

DCS has been widely used as an anti-tubercular drug at doses substantially higher (500-2000 mg) than the 50 mg dose we plan to use. At high doses, DCS acts as an antagonist at the GMS (Emmett et al. 1991) and can cause seizures, anxiety and psychosis (Kendig et al. 1956; Crane, 1959). Some studies suggest that 50 mg of DCS selectively worsens negative symptoms in patients being treated with clozapine (Goff et al. 1996, 1999a, b), but improves negative symptoms when used in conjunction with typical antipsychotics (Goff et al. 1995, 1999b). At the low dose we plan to use, the only reported side effect was a modest and time-limited worsening of negative symptoms in a small minority of patients who were taking clozapine; this effect may be related to having a high DCS plasma level (Goff et al. 1996, 1999a, b). Interestingly, this mild worsening was not "particularly distressing to patients or clinicians" (Goff et al. 1999a).

NMDAR modulatory agents have generally not shown therapeutic efficacy in patients taking CLZ (Tsai et al. 1999, 2010; Evins et al. 2000;), but there are exceptions (Heresco-Levy et al. 1996; Tsai et al. 2010). Both of our patients will continue to take clozapine during the DCS study, as they did during the Gly study. One possible reason why they benefited from Gly despite being treated with clozapine is that the *GLDC* triplication is likely to cause unusually low levels of brain glycine. In such a situation, clozapine alone may not normalize synaptic levels of glycine in the presence of the accelerated degradation caused by the mutation. We will be monitoring DCS plasma levels every month as part of the monthly blood tests (see schema). Only one of the two carriers has negative symptoms and we will be following both of them closely (see below), as we did in the previous study.

c. Rationale behind the proposed research and potential benefits to patients and/or society

The successful discovery of genetic variants underlying adverse and optimized treatment responses underscores the high potential for genetic discoveries to impact personalized medicine (Chung et al. 2004; McCormack et al. 2011; Chen et al. 2011; Ge et al. 2009; Tanaka et al. 2009; Suppiah et al. 2009; O'Brien, 2009; Klein et al. 2009; Takeuchi et al. 2009; Cooper et al. 2008; Bollag et al 2010; Daly et al. 2009; Mallal et al. 2008; Sorlie et al. 2001; Loi et al. 2010; Bennett et al. 2012; Nathwani et al. 2011). Of particular importance, the identification of mutations in specific genes can lead to "medically actionable" treatment interventions tailored to the underlying disease biology, with positive therapeutic effects (Bainbridge et al. 2011; Worthey et al. 2011; Cirak et al. 2011). Such individually tailored treatments may not only improve clinical response and outcome in *appropriately selected* patients, but also may reduce the heterogeneity in symptom reduction in clinical trials.

The rationale for including the ophthalmological and pattern evoked electroretinogram (PERG) exam is as follows: Dr. Robert Miller at the University of Minnesota has shown that NMDA receptors in retinal ganglion cells require d-serine to function normally. He has been studying schizophrenic patients and shown abnormalities in the pattern evoked electroretinogram (PERG) consistent with NMDA hypofunction. A similar pattern is observed in serine racemase mice. We know from work that we have done to date that the two carriers have multiple indices of NMDAR hypofunction (reduced serine; increased kynurenine and kynurenic acid and quinolinic acid all of which are consistent with glycine dysregulation). In light of this evidence, there is a compelling scientific justification for studying their PERGs during the open-label DCS augmentation arm. Although it would be interesting to obtain the PERG data after chronic DCS treatment, there are two reasons why we will not do so: 1) because of the blind, we would have to repeat the PERG procedures at least twice more, and 2) the procedure is rather arduous

since it takes 2 hours, involves having the pupils dilated, and would mean two trips to Minneapolis.

d. Overview of Design

The design is shown in the schema. If subjects are using glycine, this will be discontinued. All of their other psychotropic medications will remain unchanged. They will be evaluated clinically every two weeks during the washout to establish the point at which they show a 25% worsening on the BPRS and/or when their CGI severity scores change from borderline mentally ill (their current ratings) to moderately mentally ill (a worsening of 2 severity points). These assessments are carried out by Dr. Levy and Mr. Coleman, both of whom have over 30 years of experience with psychotic patients and who know the symptom profiles of these patients quite well. Dr. Bodkin is kept fully informed about changes in clinical symptoms. When the subjects have returned to their baselines, Dr. Bodkin will assess the subjects as well to confirm that he is in agreement. This will establish a new clinical baseline (time point 0). Based on our previous experience with glycine discontinuation, we anticipate that this worsening will occur within about one month. Once notable clinical changes have started to occur, the formal clinical ratings will be carried out every week until the criteria for a new baseline have been met. Note that subjects are in at least weekly contact with the PI to monitor symptoms and side effects independent of completing the clinical scales, so the need for a change in the frequency of the formal clinical assessments can be readily ascertained.

We temporarily suspended the glycine augmentation, because the TID dosing had become so burdensome that the subjects asked to stop, at least temporarily, after 16 weeks of augmentation, even though they both recognized that they were substantially better. Both subjects have now resumed taking glycine due to a recurrence of symptoms. Assuming that they continue to take it until this protocol is approved, they will both undergo the washout described above. If one or both discontinue taking glycine before this protocol is approved, they may already have returned to their baseline.

After the discontinuation period is completed, the subjects will receive open-label DCS (50 mg/d) for 8 weeks. Subjects will be formally evaluated on all of the clinical measures (see below and schema) every two weeks but will be in weekly phone contact with the PI throughout. Although we do not anticipate that DCS will cause a worsening of their symptoms, we cannot rule out this possibility in advance. If the BPRS shows an additional 25% worsening or severity of specific symptoms changes by two additional points (compared to their new baseline), that subject will be discontinued. Dr. Bodkin will review the clinical data before a subject is discontinued. . Subjects who complete the open label DCS trial will be randomized to DCS or placebo for 6-week periods in a double-blind design. The McLean Hospital research pharmacist, Laura Godfrey, will determine the randomization and will not be blind to drug or placebo condition, but the subjects and all other study staff will be. There will be a total of four 6-week double-blind exposures to DCS or placebo, each followed by one week of washout. A one-week washout is more than sufficient for both a behavioral and biochemical washout (half-life of DCS is 10 hours, so technically, two days should be sufficient). The two possible randomization schemas are: DCS-placebo-DCS-placebo or placebo-DCS-placebo-DCS, to be determined by the research pharmacist. We are limiting the length of each double-blind arm to six weeks to minimize the length of symptom exacerbation experienced by the subjects when they are receiving placebo. These four double-blind exposures (two each for DCS and placebo) will be followed by a 6-week single-blind exposure to DCS (subjects and the person doing the clinical assessments will not know if they are getting DCS or placebo, but Drs. Levy, Bodkin, and Ongur will know that they are getting DCS). After a one-week washout, this single-blind exposure will

be followed by 6 weeks of open-label DCS. Subjects will be aware of the design and thus will know that if they experience a worsening of symptoms in any clinical arm, it will be time-limited.

II. Specific Aims

a. Objectives/hypotheses

The broad specific aims of this study are 1) to treat the carriers of this mutation with an NMDAR modulatory compound that can be administered as a pill, D-cycloserine (DCS), and 2) to compare the clinical and neurobiological changes achieved with DCS to those achieved with Gly. If the subjects experience similar clinical benefits, DCS augmentation could potentially be a long-term treatment for these individuals.

Specific Aim 1: To assess the acute (8 weeks) effects of open-label DCS augmentation on positive, negative, and mood symptoms and on neurocognition. Hypothesis: Improvement during acute and chronic treatment, but not during placebo. Greater improvement on DCS than on Gly. To assess changes in the same clinical and cognitive dimensions just mentioned during discontinuation of DCS and DCS treatment (6 weeks/arm).

Specific Aim 2: To assess the acute (8 weeks) effects of DCS augmentation on brain structure (3.0 Tesla) and chemistry using magnetic resonance spectroscopy (MRS, 4.0 Tesla). Hypothesis: Normalization of brain Gly level, decreased baseline GABA and increased baseline glutamate (Glu). Greater normalization on DCS than on Gly.

Specific Aim 3: To assess the acute (8 weeks) effects of DCS augmentation on auditory evoked response potentials (ERPs). Hypothesis: Greater normalization of P50, mismatch negativity (MMN), P300, gamma oscillations on DCS than on Gly.

Specific Aim 4: To assess the acute (8 weeks) effects of DCS augmentation on NMDAR in retinal ganglion cells. Hypothesis: Pattern electroretinogram amplitudes will increase more (i.e., greater saturation) on DCS than on Gly.

Specific Aim 5: To assess the acute (8 weeks) and subsequent (6 weeks) effects of DCS augmentation or placebo on amino acid levels and other metabolites (e.g., kynurenic acid, KYNA). Hypothesis: Serine and Gly levels will increase, and KYNA levels will decrease, more on DCS than on Gly, and more on DCS and Gly than on placebo.

III. Subject Selection

Inclusion Criteria: Subjects will be selected on the basis of having a triplication of *GLDC* and a psychotic disorder and having participated in the protocol entitled Neurobiology of a Mutation in Glycine Metabolism in Psychotic Disorders (2012P001597). Exclusion criteria: meeting inclusion criteria but having a creatinine clearance less than 50 mL/min. Prior to beginning the study, subjects will be called by the PI and all of the procedures and time frames will be reviewed. Written informed consent will be obtained by the PI and Dr. J. Alexander Bodkin, a licensed physician who is medical back-up for the study.

IV. Subject Enrollment

The total projected sample size is two individuals who are carriers of the 9p24.1 duplication/triplication. One has a diagnosis of bipolar disorder with psychotic features and the other has a diagnosis of schizo-affective disorder. They will be recruited as outpatients. Their

current ages are 33 (male) and 61 (female). The total projected sample size is two individuals who are carriers of the 9p24.1 duplication/triplication.

At the present time, the subjects are taking the following medications:

5459: clozapine (62.5 mg/d); lithium (600 mg/d); gabapentin (300/d); cymbalta (120/d), mirapex (0.3 mg/d)

3363: Clozapine (100 mg/d); abilify (15 mg/d); celexa (40 mg/d)

V. Study Procedures

1) Baseline procedures previously completed at McLean prior to the glycine augmentation study that will **not** be repeated at baseline for this study: The previously collected data will be used for comparison with data collected during the open-label DCS treatment arm.

Physical exam/EKG/weight (by Dr. Ongur).
Structural MRI (3T - 15 minutes), proton ^{1}H MRS (4T) for GABA levels (MEGAPRESS - 1 hour); Proton ^{1}H MRS (4T-J-Resolved) for glycine and glutamate and GABA levels (1 hour). All scan procedures were approved by the McLean IRB. Dr. Ongur will continue to be responsible for medical oversight of the imaging components of the study.
Auditory evoked response data (by Dr. Hall).

2) Baseline procedures that **will** be collected at time point 0 (defined above and below), the new baseline, even if they were previously obtained as part of the glycine study.

Movement disorders exam (extrapyramidal side effects, tardive dyskinesia - AIMS/SAS) (Guy, 1976; Simpson & Angus, 1970) (by Dr. Levy, in person).
Clinical ratings: PANSS (Kay et al. 1987); Clinical Global Impression Scale (Guy, 1976); Brief Psychiatric Rating Scale (Overall & Gorham, 1962); Young Mania Rating Scale (Young et al. 1978); Hamilton Depression Scale (Hamilton, 1960) (by Michael Coleman, M.A., using a secure skype-like connection – vidyo – see below). Dr. Bodkin will confirm that the subject meets criteria for a new baseline (a 25% worsening on the BPRS and/or when their CGI severity scores worsen by 2 severity points).
Neurocognitive testing (MATRICS Consensus Cognitive Battery-MCBB (Kern et al. 2011) (by Dr. Levy, in person).
Physical Exam/EKG/Weight: These procedures will be carried out by the subjects' internist, Dr. Colette Kirchhoff, in Bozeman MT.

Fasting blood tests for amino acid levels, psychotropic drug levels, DCS levels, and routine clinical labs [comprehensive metabolic profile (CMP), lipid panel, GGT, uric acid, CBC with differential, CRP-hs]: Blood will be drawn at the Clinical Lab of Bozeman-Deaconess Hospital. Centrifuged plasma will be frozen for later analysis of amino acid, psychotropic drug, and DCS levels at Nathan Kline Institute. Blood for routine clinical labs and one subject's lithium level will be processed by the Clinical Lab of Bozeman-Deaconess Hospital on the day the sample is obtained.

Prior to the beginning of the open-label arm (within three days) and at the end of each of the six double-blind placebo controlled treatment arms fasting blood tests will be done for the following:

Both subjects:
6 cc - tryptophan plasma level;
6 cc - small neutral/excitatory amino acid plasma levels;
4 cc – GABA plasma level;
6 cc- homocysteine plasma level;
7 cc – KYNA plasma level, KYN plasma level, and quinolinic acid plasma levels
5 cc – DCS level
1 cc – comprehensive metabolic profile (CMP);
1 cc - lipid panel
1 cc - GGT
1 cc – uric acid
4 cc - CBC with differential
1 cc – CRP-hs
Subtotal: 43 cc

Subject 5459:
6 cc - cymbalta steady state plasma level;
6 cc- clozaril steady state plasma level;
5 cc - lithium steady state plasma level;
6 cc - gabapentin steady state plasma level, all prior to AM dose.
Subtotal: 23 cc
Thus, for this subject a total of 66 cc will be drawn at each time point.

Subject 3363:
6 cc- clozaril steady state plasma level;
6 cc – abilify steady state plasma level;
6 cc – celexa steady state plasma level, all prior to AM dose.
Subtotal: 18 cc
Thus, for this subject a total of 61 cc will be drawn at each time point.

If a subject's medications change prior to the initiation of the DCS study, plasma levels for specific dugs may change. If this results in a change in the amount of blood required, the IRB will be informed.

This amount of blood is well within safe guidelines for any healthy individual weighing at least 35 pounds according to the McLean IRB and OHRP guidelines (< 550 ml in an 8 week period). The total amount of blood that will be drawn over the course of the entire 50 weeks of the study will be 854 cc (about 58 tablespoons) for one subject and 896 cc (about 61 tablespoons) for the other.

The personal physician of the two subjects has been informed about their planned participation in a study that involves exposure to DCS and has agreed to its safety (physician ok has been uploaded). Before the study begins, the internist will be notified in writing (sample letter to practitioner has been uploaded).

3) Glycine Washout. Glycine augmentation will be discontinued and subjects will be monitored clinically every week (at least once a week). During the washout all other psychotropic medications will remain unchanged. The subjects will receive clinical ratings (see above) every two weeks to establish the point at which they show a 25% worsening on the BPRS and/or when their CGI severity scores change from borderline mentally ill (their current ratings) to

mildly or moderately mentally ill (a worsening of 1-2 severity points) (new clinical baseline). Based on our previous experience with glycine discontinuation, we anticipate that this worsening will occur within about one month. Once notable clinical changes have started to occur, the clinical ratings will be carried out every week until the criteria for a new baseline have been met. Note that subjects are in at least weekly contact with the PI to monitor symptoms and side effects independent of completing the formal clinical scales, so the need for a change in the frequency of the formal clinical assessments can be readily ascertained. The end of the glycine washout will be equivalent to week 0.

4) Open-label DCS (daily dose of 50 mg) for 8 weeks – Treatment Arm A: Product Description of DCS (seromycin), has been uploaded with the application.

Dr. Bodkin will be responsible for medical oversight of the DCS augmentation component of the study. Dr. Ongur will serve as back-up medical coverage.

The patients' usual psychotropic drug regimen will not be altered as part of the study, but an attempt will be made to keep those medications unchanged throughout the study if possible. Any changes will be left up to the patients' psychiatrists. Both psychiatrists agreed to the DCS study before the DCS grant was submitted to NIMH. Copies of those letters have been uploaded.

The McLean Hospital Pharmacy will order pharmaceutical grade DCS from The Chao Center. The DCS will be stored at room temperature in closed containers in a dry area in the McLean Hospital Pharmacy, avoiding humidity, sunlight and high temperature.

DCS is supplied in 250 mg capsules. The McLean Research Pharmacy will compound DCS 50 mg double-blind capsules and Placebo double blind capsules for dispensing to subjects. Although unlikely at the low dose of DCS (50 mg) that will be used, to ensure that subjects do not develop B12 or folate deficiency, they will be prescribed one vitamin B-complex tablet per day. Prescriptions for DCS 50 mg/Placebo double blind capsules and vitamin B-complex tablets will be dispensed by the McLean Hospital Research Pharmacy and shipped by Fed Ex to subjects in 4-6 week supplies.

B-Complex vitamins will be procured and dispensed to subjects by the McLean Hospital Research Pharmacy in the following USP approved formulation: Nature Made Super B Complex® Tablets (UPC # 031604027278).

It is anticipated that all of the DCS that is ordered will be used. However, if there is any unused DCS, we will collect and dispose of all unused study medications. After documenting the return and de-identifying the product, non-controlled study medication is destroyed with biohazard waste and documented as such by the McLean Hospital Pharmacy.

Subjects will keep a daily log to mark the time each dose was taken (sample log has been uploaded).

Weekly
Monitoring of clinical changes and side effects weekly (or more often as needed).

At the end of weeks 2, 4, 6, and 8:

Clinical ratings using “skype-like” video conferencing (see below for details on measures that will be taken to ensure confidential and secure connections under the auspices of Partners Collaborative Media).

During week 4:
a) Weight measurement
b) Blood work (66 cc and 61 cc) (drawn in the Clinical Laboratory at Bozeman-Deaconess Medical Center; frozen plasma for amino acid and psychotropic blood levels will shipped to the Analytical Psychopharmacology Laboratory at the Nathan Kline Institute (NKI) for processing. Routine laboratory tests, such as CBC with differential, etc as well as DCS levels will be performed by Clinical Laboratory at Bozeman-Deaconess Medical Center.

At the end of week 7 (on the way to Boston for week 8 procedures detailed below):
Ophthalmology and Electroretinogram Exams at the University of Minnesota:

The subjects will be flying through Minneapolis to get to Boston for their week 8 procedures during the open-label DCS treatment arm. For reasons explained above, this is a unique opportunity to collect data related to NMDA receptor function. The procedures are routine and do not pose significant risk to the subjects. The details of the eye exam are described below and in the consent form. The eye exam will be carried out in collaboration with Dr. Robert F. Miller, 3M Bert Cross Professor of Visual Neuroscience, Professor in Neuroscience and Ophthalmology Department of Neuroscience, University of Minnesota Medical School. He currently has IRB approval to carry out these procedures in schizophrenia patients at the University of Minnesota and is revising the protocol to include bipolar patients. Copies of his currently approved consent form and IRB approval have been uploaded. Both subjects have completed these procedures uneventfully twice.

There are two components to the eye exam, both of which were previously approved by the McLean IRB:

Component I is a routine clinical eye exam to evaluate the health of the eyes. A board certified ophthalmologist shines a light into the eyes and views them through a magnifying lens to examine internal structures of the eyes. This procedure requires dilated using a standard dilation eye-drops (phenlyephrine and tropicamide). The exam will also include photography of the inside of the eyes with a specialized camera. This, too, is a routine procedure and will be conducted by a trained photographer. The subject will sit in front of a camera that is equipped with a head rest and chin rest for comfort. The subject will be asked to look at a visual target while the photographer takes the picture. Both procedures (exam and photography) take only a few minutes to complete and should involve no physical discomfort. The camera is non-contact, FDA-approved, and there are no known risks associated with them other than the dilation.

Component II involves four tests that measure physical and functional features of the retina. Three of the tests are electroretinogram (ERGs). The fourth test is called OCT. The three types of ERGs are the 1) Pattern ERG, 2) Full Field ERG, and 3) Multifocal ERG. Collectively, these tests will measure how well different parts of the retina work. For these tests, anesthetic eye-drops are administered to make the subject comfortable during the procedures (the subject should feel minimal or no discomfort. Short breaks can be taken if necessary). For the full field ERG, multifocal ERG and the OCT test, the pupils will be fully dilated using standard dilation eye-drops (phenlyephrine and tropicamide).

Below is a more detailed description of the ERG and OCT procedures:

Pattern ERG (pERG) and Fullfield ERG (ffERG): The pERG provides information about the ganglion cell in the retina. The Full Field ERG (ffERG) is a standard clinical test that looks at the overall function of the retina. For both tests, a flexible conducting fiber will be placed just inside the margin of the lower eyelid so it comes in contact with the tear film of the eye. The conducting fiber will be held in place by 2 sticky pads on the skin. Four additional conducting fibers (two for each eye recording) will be placed on the skin near the outer corner of the eye, on the earlobe, on the forehead or on the forearm/hand. For the pERG, subjects will have a vision test and wear corrective lenses if necessary. Both eyes will be tested at the same time. Subjects will be asked to look at black and white checkerboard images as part of the task. This procedure should last between 45 and 75 minutes, which includes the time required for the vision test. For the ffERG, each eye is tested separately. Subjects will sit in a dark room for about 15 minutes prior to the beginning of the procedure in order to dark-acclimate. During the testing, subjects will place their chins on a chin rest and forehead against a headrest and look straight ahead, while lights of that differ in brightness are flashed in front of their eyes. Next, subjects will sit in a more brightly lit room and view the flashes as before. This procedure should last between 45 and 60 minutes.

Multifocal ERG (mfERG): The mfERG looks the functioning of different areas of the retina. The procedure will require fully dilated eyes using drops commonly used in an ophthalmology clinic (phenylephrine and tropicamide). Each eye will be tested separately. A contact lens, which is attached to a flexible conducting fiber, will be used to record biological signals from the retina. The upper and lower eyelids will be held open by a special device. A thick artificial tear drop (Goniosol) will be placed between the contact lens and the surface of your eye for comfort during data collection. Subjects will be asked to view black and white hexagon patterns and to minimize eye movement in order to avoid irritating the eye. The total duration of this procedure is approximately 45-60 minutes (and includes testing both eyes).

Optical coherence tomography (OCT): The OCT test uses light to measure structures in the eye. This machine also takes a picture of the inside of the eyes with a specialized camera. A trained photographer will conduct this test. The subject will sit in front of the OCT machine and be asked to look at a target. The head and chin will rest against a head rest and chin rest. The OCT machine will shine a light into the eye and a photograph will be taken. The test takes about 1-2 minutes per eye. This procedure is non-contact, FDA approved, and no risks are known to be associated with it other than the dilation.

Dilation drops may need to be applied to the eyes twice in each visit if the dilation effects wear off before all procedures are completed. There should be no additional risk associated with a second application of dilation drops.

During week 8:

a) Weight measurement
b) Repeat physical exam and EKG (Dr. Ongur, at McLean)
c) Blood work (66 cc and 61 cc) (drawn in the Clinical Laboratory at McLean Hospital; frozen plasma for amino acid and psychotropic blood levels will shipped to the Analytical Psychopharmacology Laboratory at the Nathan Kline Institute (NKI) for processing (routine laboratory tests, such as CBC with differential, etc as well as DCS levels will be performed by North Shore Medical Center).
d) Movement disorders assessment (extrapyramidal side effects, tardive dyskinesia - AIMS/SAS – by Dr. Levy or Dr. Bodkin, in person at McLean)

e) MCCB (MATRICS) - by Dr. Levy, in person.
f) EEG – Dr. Hall, at McLean

f) Structural MRI (3T - 15 minutes), proton ^{1}H MRS (4T) for GABA and glutamate levels (MEGAPRESS - 1 hour); Proton ^{1}H MRS (4T-J-Resolved) for glycine levels (1 hour). All scan procedures are approved by the McLean IRB. All of these are brain imaging scans that were previously performed at McLean Hospital as part of the glycine study baseline procedures.

Subjects will first receive an occipital cortex echo time averaged (TEAV) MRS scan; an MRS technologist will provide oversight. Head and MRS voxel position coordinates at will be determined using brain structural landmarks and will be recorded.

In addition, since both subjects will be taking DCS during the open-label phase, they will receive one MRS scan for glycine levels prior to their dose of DCS (above), followed by a scan shortly after taking the daily dose (50 mg) (90 minutes). We will be able to compare brain uptake after an acute dose (baseline) and after chronic treatment, including uptake immediately after dosing.

Subjects will first receive a baseline occipital cortex echo time averaged (TEAV) MRS scan; an MRS technologist will provide oversight. Head and MRS voxel position coordinates at will be determined using brain structural landmarks and will be recorded. After their DCS dose, subjects will be re-positioned in the scanner for the brain and blood measurements over the next two hours, as described above.

Clinical Oversight:
The subjects are well known to the PI and study staff, who will be in touch with them by phone on a weekly basis or more often as needed. Both subjects' psychiatrists will also be monitoring their clinical states and side effects. Dr. Levy will inform Dr. Bodkin and Dr. Ongur immediately if any side effects or clinical changes are reported that warrant medical follow-up.

At the end of the first week of DCS, and at the end of weeks 3 and 5 (more often if necessary), the subjects will be called by a study physician (Dr. Bodkin) to assess how they are reacting to the DCS. The subjects will also be given emergency contact numbers for Drs. Levy, Bodkin, and Ongur. The subjects will also be told to call Dr. Levy immediately if they experience any acute side effects (e.g., exacerbation of psychotic or negative symptoms).

Week 9 will be a washout week during which neither DCS nor placebo will be administered.

5) <u>6 Weeks on DCS (50 mg/d) or Placebo</u> (weeks 10-15) – Treatment Arm B.

The McLean Pharmacy will prepare and label prescriptions for DCS capsules, Placebo capsules, and vitamin B-12 Complex tablets, which will be FedEx'ed in 4-6-week supplies. The placebo is supplied in opaque gelatin capsules filled with microcrystalline cellulose. The McLean Hospital Pharmacy will provide the placebo.

Subjects will keep a daily log to mark the time each dose was taken.

<u>At the end of weeks 11, 13, and 15</u>:

Clinical ratings as described above using "skype-like" video conferencing.

At the end of week 12:
Blood work as described above.
Weight measurement.

An assessment of movement disorders (extrapyramidal side effects, tardive dyskinesia - AIMS/SAS - Dr. Levy, by "skype."

Clinical Oversight:
The subjects are well known to the PI, who will be in touch with them by phone on a weekly basis or more often as needed. Both subjects' psychiatrists will also be monitoring their clinical states and side effects. See also above.

At the end of the first week of DCS or placebo (week 10), and at the end of weeks 12 and 14, the subjects will be called by a study physician (Dr. Bodkin) to assess how they are reacting to the DCS or placebo. The subjects will also be told to call Dr. Levy immediately if they experience any acute side effects (e.g., exacerbation of psychotic or negative symptoms).

Week 16 will be a washout week during which neither DCS nor placebo will be administered.

6) 6 Weeks on DCS (50 mg/d) or Placebo (weeks 17-36) – Treatment Arms C-E.

Procedures identical to those described above in section 5 (and at corresponding time points) will be administered except for the MATRICS battery, which will not be re-administered until week 50 (see 8 below).

Weeks 23, 30, and 37 will be washout weeks during which neither DCS nor placebo will be administered.

7) 6 weeks of DCS: Single-blind (weeks 38-43) – Treatment Arm F.
Subjects will receive 50 mg/d of DCS. Subjects and the person doing the clinical assessments will not know if they are getting DCS or placebo, but Drs. Levy, Bodkin, and Ongur will know that they are getting DCS.

Procedures identical to those described above (and at corresponding time points) will be administered.
Week 44 will be a washout week during which neither DCS nor placebo will be administered.

8) 6 weeks of DCS: Open-label (weeks 45-50) – Treatment Arm G.
Procedures identical to those described above (and at corresponding time points) will be administered.
The MATRICS battery will be re-administered in week 50.

9) Processing of Amino acid levels. The consent forms contain a provision for subjects to give permission for Dr. Raymond Suckow and the Nathan Kline Institute to provide Dr. Levy and her colleagues at McLean with the results of any blood samples that are analyzed at NKI, and for the Clinical Laboratories at McLean Hospital and at the medical center in the subjects' home city to provide Dr. Levy and her colleagues with the results of any blood tests.

Blood samples drawn at McLean or in the subject's city of residence will be sent to the Analytical Psychopharmacology Laboratory at NKI for analysis (SMA-20, large neutral & excitatory amino acid, KYNA, and psychotropic drug plasma levels). The urinalyses and blood

samples for lithium levels will be done either by the clinical lab of the local medical center or by the clinical lab at McLean. All samples will be coded with the subject's 4-digit ID number, the date and the time of day. Dr. Suckow will not know the identities of the subjects whose blood samples he receives.

10) Total Amount of Blood Drawn: The total amount of blood drawn at each scheduled time point is 66 cc and 61 cc for each of the two non-mutation carriers, respectively. The total amount of blood will not exceed 550 cc in any 8-week period. The subjects will be advised not to donate blood for at least one month after completing the study.

11) Neuropsychological Assessment: Cognitive functioning will be assessed using the battery developed by the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative (Kern et al. 2011). The MATRICS battery (MCCB) includes 10 tasks that measure processing speed (Brief Assessment of Cognition in Schizophrenia, Symbol Coding, Animal Fluency, Trails A), attention (Continuous Performance Test), working memory (WMS-III Spatial Span, Letter-Number Span), verbal learning (Hopkins Verbal Learning Test – Revised), visual learning (Brief Visuospatial Memory Test - Revised), problem solving (Neuropsychological Assessment Battery) and social cognition (Mayer-Salovey-Caruso Emotional Intelligence Test). Total administration time is 60-90 minutes. The MATRICS is well tolerated by patients and can be administered repeatedly. Use of this battery will permit comparisons between baseline, post-glycine, and post-placebo effects on neurocognition. Material on the MATRICS battery has been uploaded.

12) Partners Collaborative Media (PCM) will provide oversight (for a fee) to ensure that the clinical and movement disorders assessments taking place every two weeks using skype-like video conferencing are secure. Specific web cameras recommended by PCM will be used by McLean staff and by the subjects. All calls will be initiated from McLean using a Partners computer. This computer will have the Cisco program, "blue jeans," installed. PCM will create generic credentials for the subjects such that they will not be using their own actual skype credentials. Thus, if the skype material is stolen, it cannot be linked to the subjects themselves. A McLean clinician will call the subjects by clicking on customized phone numbers and allow the McLean caller to lock the virtual meeting room.

A technical statement from PCM about how the secure connection is implemented has been uploaded.

VI. Biostatistical Analysis

All data acquisition and processing will be carried out blind to mutation status. The McLean investigators have extensive imaging databases involving controls and patients with psychotic disorders. Thus, we are well positioned to compare MRS spectra between carriers and non-carriers even if formal statistical testing is not always possible. At a minimum, using data from groups scanned on the same scanners under the same conditions, we can establish where our subjects fall in the range of values compared with other subject groups using z-scores or % difference scores (e.g., Glu, GABA, Gln metabolite levels; Gly and other metabolite concentrations; Gln:Glu ratios). It will be possible to assess the magnitude of change in clinical and neurocognitive function and to correlate change in amino acid levels with clinical and neurocognitive change scores during DCS vs placebo. This is not a population study; it is designed to provide mechanistic insights about the effects of this mutation in the brain. Dr. Miller has PERG data on other patients and on controls.

VII. Risk and Discomforts

The imaging procedures have been used extensively at McLean without complications and subjects will be carefully screened.

The procedures described above pose no serious physical risk to subjects and no psychological, social, or legal risks are anticipated.

Although there are no known general risks associated with MRI scans, there are risks to individual subjects who have contraindications to MRI scanning, including those with metal implants in their body (pacemaker, aneurysm clips, metal screws and plates for orthopedic purposes, hearing implants, certain kinds of tattoos, sheetmetal workers with lodged metal fragments in the eyes). Subjects are screened carefully and excluded if there are even suspected contraindications to scanning. All subjects are asked to remove jewelry, belts and other metal-containing objects. Surgical records will be retrieved prior to scan for subjects who have had metal placed in their body intra-operatively to ensure the hardware is MRI safe, even if the subject has been told the hardware is MRI-safe or if they have had MRI scans since the operation. As an additional precaution, subjects are screened with a handheld metal-detection wand prior to the scan to ensure that no unidentified metal objects remain on the subject. The noise generated by the pulsing of the gradients can lead to temporary decrease in hearing. The use of disposable earplugs is an easy and reliable means of preventing hearing loss. The risks associated with Specific Absorption Rate (SAR) are related to the fact that given a large enough SAR, heating of the tissue may occur. These experiments will comply with all FDA guidelines with regard to RF power deposition. There is also the potential risk of injury from a projectile (i.e., ferromagnetic objects being attracted into the magnet); and of asphyxiation due to large amounts of cryogenic gases generated during a quench (i.e., the event which occurs when a magnet makes the sudden transition from superconducting to resistively conducting). Routine safety procedures are in place at both scanning centers to screen subjects prior to scanning, maintain security of the restricted access areas, and ensure that system security features are in good working order. Both imaging centers associated with this study (McLean, NKI) are very experienced with MRI scanning and have impeccable safety records. The effects of MRI on the fetus are not well characterized. Therefore, females of childbearing age must be sexually inactive or be using a contraceptive measure for three months prior to being scanned.

The scans involve use of a standard clinical MRI scanner (3T) as well as a high field (4T) MRI scanner. The 4T scanner is not used for routine clinical studies in children or adults, but the FDA has determined (July 14, 2003) that scanners with magnetic field strengths of less than 8 Tesla do not represent a significant risk to adults, children, or infants older than 1 month. [164] Most people experience no ill effects from 3T or 4 T scans, but some do report claustrophobia, dizziness, mild nausea, headaches, a metallic taste in their mouth, back tingling, double vision, or sensation of flashing lights. These symptoms, if present, disappear shortly after leaving the scanner. During the scan, the examiner can see and hear the subjects and will ask them to report any problems so the scan can be stopped if necessary. A magnetic resonance scan may be uncomfortable due to claustrophobia, lying still for an hour, or loud sounds. Subjects who express serious concern about these will not be included. The scan will be stopped if the subject expresses discomfort. Total time in the scanner for the structural scan and 2 MRS scans at McLean is 150 minutes, with breaks occurring between scans (structural: 15 minutes; MEGAPRESS: 60 minutes; J-PRESS: 75 minutes). Subjects will be allowed to leave the scanner between scans and can be re-positioned for the next scan, as described in the application. Both subjects have successfully completed imaging procedures.

DCS has been widely used to augment standard psychotropic drug treatment and is well tolerated. The daily dose of 50 mg is low enough to be a partial agonist of the GMS and has no side effects. The subjects will be monitored closely by the research team and by their own psychiatrists. Blood chemistries, including liver/kidney function tests, will be monitored at baseline and at the end of each treatment arm.

Periodic blood samples involve the slight discomfort of a needle stick and the small risk of a bruise. Every attempt is made to have the subject feel comfortable and at ease with the environment and the staff.

The eye exam procedures are routine and should cause minimal or no discomfort.

Risks to privacy and confidentiality are minimal. All subjects are assigned a random 4-digit ID number, which is used to code all material.

VIII. Potential Benefits

The general goal of this study is to clarify the neurobiology of a mutation in glycine mutation and to determine whether carriers of this mutation may preferentially benefit from DCS augmentation of their medication regimen. Although subjects receive no immediate benefit from the brain imaging procedures beyond contributing to important research and reasonable monetary compensation for the time commitment, the potential scientific yield could have a major impact on identifying causal mechanisms in psychotic disorders. If the DCS augmentation is beneficial, these two subjects may experience a significant reduction in psychotic symptoms and improvement in neurocognition, which may also help other subjects with mutations impacting the glycine metabolic pathway and NMDA receptor function.

IX. Monitoring and Quality Assurance

The scientific integrity of the study and protection of participant safety will be monitored by the investigators using an Adverse Event Tracking Log, including the detection and reporting of adverse events. The safety data include all imaging procedures, the neurocognitive function battery, as well as the double-blind placebo-controlled and open-label DCS augmentation trials. Efficacy data includes all DCS-related procedures.

All adverse events will be promptly reported to the Partners Human Research Committee (PHRC) and NIMH as appropriate. At a minimum, the investigators will review all adverse events at the end of each treatment arm, but more often if needed. Unanticipated problems involving risks to subjects or others including adverse events will be reported to the PHRC in accordance with PHRC unanticipated problems reporting guidelines.
At a minimum, the investigators will review all adverse events at the end of each treatment arm, but more often if needed. The research will be altered or stopped if subjects have adverse reactions to any of the procedures (e.g., claustrophobia in the scanners) or significant side effects to DCS.

The subjects will also be given emergency contact numbers for Drs. Levy, Bodkin, Ongur, and Kaufman. The subjects will also be told to go the nearest emergency room if they experience any acute side effects (e.g., vomiting, diarrhea). Dr. Goff, a consultant on this project, has a great deal of experience in using DCS to augment the therapeutic effects of antipsychotic medication and will be available to advise the study team about any needed changes (i.e., dose reduction, discontinuation) based on side effects or changes in clinical state. Decisions about how best to proceed will be made in consultation among Drs. Levy, Bodkin, (Ongur if Dr. Bodkin is not available), Goff, and the McLean pharmacist. Based on Dr. Goff's extensive experience

using this dose of DCS, it is very unlikely that it will be necessary to discontinue the trial altogether. Should there be changes in any arm of the study, such as temporary discontinuation or extending the time period, the IRB will be notified. If subjects develop side effects that make them too uncomfortable or that make it medically necessary to discontinue the study, the study will be stopped. This level of oversight for subjects who are not local seems reasonable for monitoring subjects taking a compound that has been safely used as an augmentation strategy. The same procedures were used in the initial glycine augmentation study and the current extended glycine trial and have worked effectively.

The PI has talked all of the people involved in the study (Drs. Kaufman, Ongur, Bodkin, Goff, Visscher, and Vuckovic; Ms. Godfrey) about the adequacy of the plan to provide medical and/or psychiatric monitoring of these patients while they are taking a novel compound; they have agreed that the proposed plan is acceptable. Dr. Visscher is the psychiatrist for one of the subjects and Dr. Vuckovic is the psychiatrist for the other. Blood work results are faxed to them and they are kept apprised of any notable developments (e., beginning and end of any phase of the study, worsening of clinical state, improvement in clinical state). They, in turn, contact the PI if they become concerned about changes in clinical state or side effects.

The PI will monitor the validity and integrity of the data and ensure that all appropriate forms (e.g., consent forms) have been thoroughly completed and that all blood samples are collected and shipped in accordance with the approved protocol. Monitoring will be done on an ongoing basis in close collaboration with the co-investigators.

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