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Divergent Effects of Augmenting NMDA Receptor Signaling on Plasticity and Working Memory in Healthy Adults versus Schizophrenia Patients: Importance of Mechanisms Underlying Distinct Cognitive Functions

Author

Forsyth, Jennifer K.

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Divergent Effects of Augmenting NMDA Receptor Signaling on Plasticity and Working Memory in Healthy Adults versus Schizophrenia Patients: Importance of Mechanisms Underlying Distinct Cognitive Functions

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Psychology

by

Jennifer K. Forsyth

2016

ABSTRACT OF THE DISSERTATION

Divergent Effects of Augmenting NMDA Receptor Signaling on Plasticity and Working Memory in Healthy Adults versus Schizophrenia Patients: Importance of Mechanisms Underlying Distinct Cognitive Functions

by

Jennifer K. Forsyth

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2016

Professor Robert F. Asarnow, Co-Chair

Professor Constance L. Hammen, Co-Chair

The *N*-methyl-D-aspartate glutamate receptor (NMDAR) is a critical substrate underlying experience-dependent plasticity and working memory. NMDAR signaling is impaired in schizophrenia and NMDAR hypofunction may contribute to deficits in plasticity and working memory in schizophrenia. Augmenting NMDAR signaling using the partial agonist d-cycloserine (DCS) may ameliorate such deficits. However, given that divergent properties of the NMDAR underlie its roles in plasticity versus working memory and that various aspects of NMDAR function are abnormal in schizophrenia, examining the effects of DCS in both healthy and patient populations is crucial.

In two study samples, we therefore investigated 100 mg DCS versus placebo on working memory, using a spatial *n*-back task, and plasticity, using an EEG paradigm that utilizes high frequency visual stimulation (HFvS) to induce long-term potentiation (LTP) in visual cortex neurons and two LTP-dependent learning tasks, the weather prediction and information integration tasks.

In study one among healthy participants, participants who received DCS (n = 32) showed enhanced plasticity compared to placebo (n = 33), as demonstrated by enhanced LTP following HFvS and accelerated acquisition of both learning tasks. Conversely, there were no group differences in working memory. In study two among patients with schizophrenia, patients who received DCS (n = 24) showed enhanced neural responsivity and working memory compared to placebo (n = 21), with no differences on the EEG or learning measures of plasticity.

In healthy participants, DCS therefore enhanced plasticity without affecting working memory, consistent with evidence that beyond a threshold of NMDAR activation needed to generate recurrent firing in working memory circuits, further NMDAR activation should have limited benefits. Conversely, the mirror effects of DCS on baseline neural responsivity and working memory in schizophrenia with no effects on plasticity suggest that DCS ameliorated reductions in NMDAR signaling in schizophrenia, but that increased NMDAR activation was not translated into the structural synaptic changes that support experience-dependent plasticity. Results are consistent with emerging evidence that NMDAR abnormalities in schizophrenia involve not only the receptor but also NMDAR-associated proteins critical for plasticity and highlight the importance of considering how distinct NMDAR properties contribute to individual cognitive deficits in schizophrenia.

The dissertation of Jennifer K. Forsyth is approved.

Carrie E. Bearden

Michael F. Green

Barbara Knowlton

Robert F. Asarnow, Committee Co-Chair

Constance L. Hammen, Committee Co-Chair

University of California, Los Angeles

2016

DEDICATION

For my parents and for Karen, for your immense support, for inspiring me to love learning and to work hard, and for giving me every opportunity to pursue my dreams. Without you, none of this would be.

For Devin, for your unwavering love and support, and for bringing me joy and laughter every day that has nourished my soul and my work.

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LIST OF TABLES AND FIGURES

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Jennifer K. Forsyth

EDUCATION

University of California, Los Angeles, Los Angeles, CA
C. Phil. in Psychology, June 2014
M.A. in Psychology, December 2011

Queen's University, Kingston, Canada
B.A. with Distinction in Psychology, April 2008
Dean's Honor List with Distinction; W.R. Thompson Prize for best undergraduate thesis

PEER-REVIEWED PUBLICATIONS

Forsyth, J. K., Bachman, P., Mathalon, D. M., Roach, B. J., Asarnow, R. F. (2015). Augmenting NMDA Receptor Signaling Boosts Experience-Dependent Plasticity in the Adult Human Brain. *Proceedings of the National Academy of Sciences, 112*(50), 15331-15336.

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CHAPTER 1

Augmenting NMDA Receptor Signaling Boosts Experience-Dependent Plasticity in the Healthy Adult Brain without Affecting Working Memory

Augmenting NMDA receptor signaling boosts experience-dependent neuroplasticity in the adult human brain

Jennifer K. Forsyth[a], Peter Bachman[b], Daniel H. Mathalon[c,d], Brian J. Roach[d], and Robert F. Asarnow[a,e,1]

[a]Department of Psychology, University of California, Los Angeles, CA 90095; [b]Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15213; [c]Department of Psychiatry, University of California, San Francisco, CA 94143; [d]San Francisco Veterans Affairs Medical Center, San Francisco, CA 94121; and [e]Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, CA 90095

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Experience-dependent plasticity is a fundamental property of the brain. It is critical for everyday function, is impaired in a range of neurological and psychiatric disorders, and frequently depends on long-term potentiation (LTP). Preclinical studies suggest that augmenting *N*-methyl-D-aspartate receptor (NMDAR) signaling may promote experience-dependent plasticity; however, a lack of noninvasive methods has limited our ability to test this idea in humans until recently. We examined the effects of enhancing NMDAR signaling using D-cycloserine (DCS) on a recently developed LTP EEG paradigm that uses high-frequency visual stimulation (HFvS) to induce neural potentiation in visual cortex neurons, as well as on three cognitive tasks: a weather prediction task (WPT), an information integration task (IIT), and a *n*-back task. The WPT and IIT are learning tasks that require practice with feedback to reach optimal performance. The *n*-back assesses working memory. Healthy adults were randomized to receive DCS (100 mg; $n = 32$) or placebo ($n = 33$); groups were similar in IQ and demographic characteristics. Participants who received DCS showed enhanced potentiation of neural responses following repetitive HFvS, as well as enhanced performance on the WPT and IIT. Groups did not differ on the *n*-back. Augmenting NMDAR signaling using DCS therefore enhanced activity-dependent plasticity in human adults, as demonstrated by lasting enhancement of neural potentiation following repetitive HFvS and accelerated acquisition of two learning tasks. Results highlight the utility of considering cellular mechanisms underlying distinct cognitive functions when investigating potential cognitive enhancers.

D-cycloserine | NMDA receptor | neuroplasticity | long-term potentiation | learning

Experience-dependent neuroplasticity is the capacity of the brain to change in response to environmental input, learning, and use. It is a fundamental property of the brain and is critical for everyday functioning. It allows us to learn and remember patterns, predict and obtain reward, and refine and accelerate response selection for adaptive behavior (1). During development, experience-dependent plasticity interacts with genetic programming to organize neurons into the structurally and functionally connected circuits that characterize a mature brain. Although this basic circuitry is established by early adulthood, experience-dependent plasticity continues to shape connectivity within these circuits such that important inputs and action outputs are represented by larger and more coordinated populations of neurons. Given that these changes are the primary means through which the adult brain enables new behavior and that such plasticity is impaired in a range of neurological and psychiatric disorders (2), identifying manipulations that can harness experience-dependent plasticity offers exciting possibilities. Here, we tested whether augmenting *N*-methyl-D-aspartate receptor (NMDAR) activity could enhance experience-dependent plasticity in the adult human brain.

The classical mechanism underlying experience-dependent plasticity is long-term potentiation (LTP) or depression (LTD) of synaptic strength. The brain encodes external and internal events through spatiotemporal patterns of activity generated by populations of neurons. Lasting changes in synaptic strength via LTP and LTD shapes these patterns of activity and are thought to be the primary cellular mechanism for representing new information in the brain (1, 3). In animals, LTP is identified electrophysiologically as an enduring increase in postsynaptic cellular currents using single-cell or local field recordings and is observed following high-frequency electrical stimulation or new learning. In mature animals, LTP has been observed at subcortical and sensory cortex synapses, including in the amygdala, hippocampus, and striatum, as well as in visual, auditory, and somatosensory cortex (1–6). Although a lack of noninvasive methods has traditionally limited our ability to investigate LTP in humans, recent research indicates that protocols using high-frequency, repetitive presentation of visual or auditory stimuli provide a naturalistic method for inducing LTP in humans and animals. Studies in rodents demonstrated that changes in neural responses following repetitive sensory stimulation show the cardinal features of synaptic LTP, including persistence (>1 h), input specificity, and NMDAR dependency (7, 8). Furthermore, these LTP-like changes can be measured noninvasively as changes in sensory evoked potentials, which are stimulus-synchronized electroencephalograph (EEG) signals that result from postsynaptic potentials in populations of sensory neurons. High-frequency sensory stimulation has thus been shown to induce lasting potentiation of visual and auditory evoked potentials in human adults (9, 10) and has been used to demonstrate that

Significance

Experience-dependent plasticity is the capacity of the brain to undergo changes following environmental input and use, and is a primary means through which the adult brain enables new behavior. In the current study, we provide evidence that enhancing signaling at the glutamate *N*-methyl-D-aspartate receptor (NMDAR) can enhance the mechanism underlying many forms of experience-dependent plasticity (i.e., long-term potentiation of synaptic currents) and also enhance experience-dependent learning in healthy adult humans. This suggests exciting possibilities for manipulating plasticity in adults and has implications for treating neurological and neuropsychiatric disorders in which experience-dependent plasticity is impaired.

Author contributions: J.K.F. and R.F.A. designed research; J.K.F. performed research; P.B., D.H.M., and B.J.R. contributed new reagents/analytic tools; P.B. provided EEG equipment and guidance in EEG data acquisition and analysis; D.H.M. provided LTP task and guidance on analysis of LTP task; B.J.R. provided guidance on analysis of LTP task and wrote custom Matlab script for LTP analysis; J.K.F., D.H.M., and B.J.R. analyzed data; and J.K.F. and R.F.A. wrote the paper.

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[1]To whom correspondence should be addressed. Email: rasarnow@mednet.ucla.edu.

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LTP-like processes are impaired in patients with depression (11), bipolar disorder (12), and schizophrenia (13, 14). Sensory LTP protocols therefore provide a valuable window into the cellular mechanism thought to underlie many forms of experience-dependent plasticity.

One potential method for promoting experience-dependent plasticity is to augment NMDAR signaling. The NMDAR is a primary glutamate receptor and is critical for triggering LTP at many synapses in the brain. This role stems from the receptor's unique biophysical properties, including that (*i*) NMDARs are blocked by a magnesium ion at rest such that they are dually voltage and ligand gated and therefore detect coincident presynaptic and postsynaptic activity; (*ii*) NMDARS are calcium permeable and therefore initiate signaling cascades when activated, leading to structural synaptic changes such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptor upregulation and enlarged dendritic spines on which synapses are localized; and (*iii*) NMDARs have slow excitatory postsynaptic potential (EPSP) decay kinetics that facilitate temporal summation of EPSPs and sustained neural excitation (15, 16). Studies of transgenic and knockout mice showed that blocking NMDARs impairs LTP as well as learning and memory performance. Conversely, enhancing NMDAR activity enhanced LTP and the acquisition and retention of information (17). Given the role of NMDARs in triggering the cellular machinery that supports experience-dependent plasticity, augmenting NMDAR signaling may offer a powerful means to promote LTP and learning in humans.

In the current study, we used the NMDAR agonist D-cycloserine (DCS) to examine how augmenting NMDAR signaling affects LTP-like processes and learning in the adult human brain. NMDARs are tetramers composed of two NR1 and two NR2 subunits. Activation requires binding of glutamate to the NR2 subunit and concurrent binding of glycine or D-serine to the NR1 subunit (18). Although direct enhancement of NMDAR signaling via the glutamate site can produce excitotoxicity, indirect stimulation via the glycine site offers a safer method for facilitating activity. DCS is a partial agonist at the glycine site that readily crosses the blood–brain barrier, is approved by the Food and Drug Administration for daily use as an antituberculosis drug, and has few side effects at low doses. Thus, DCS offers a safe means to augment NMDAR signaling at low doses.

Using a double-blind design, we randomized healthy adults to receive DCS or placebo. We examined the effects of augmenting NMDAR signaling on two indices of experience-dependent plasticity: (*i*) LTP and (*ii*) incremental learning. Participants completed the visual LTP task using high-frequency visual stimulation (HFvS) to induce potentiation of visual cortex neurons, followed by a weather prediction task (WPT) (19), an information integration task (IIT) (20), and an *n*-back task. The WPT and IIT are incremental learning tasks in which stimulus–feedback associations are thought to be encoded by LTP at corticostriatal synapses (21, 22). The *n*-back is a spatial working memory task. Working memory relies on reverberating activity in cortical microcircuits over short delays to maintain information in the absence of stimuli and, thus, does not rely on LTP (23). To facilitate dissociation of the effects of DCS on experience-dependent plasticity versus working memory, the *n*-back task was designed to be identical to the IIT in stimuli and trial structure. Thus, the only difference participants experienced between the tasks was whether they were asked to learn about the stimuli (i.e., for the IIT) or recall whether stimuli were in the same location on the screen as recently shown stimuli (i.e., for the *n*-back). To assess potential delayed effects of DCS, participants returned to the laboratory the following day to repeat cognitive testing. No drug or placebo was administered on the second day. Although the idea of using NMDAR agonists to enhance cognition is not new, past studies examining diverse cognitive domains have yielded mixed results (24–36). Difficulty reconciling divergent effects has limited our ability to harness NMDAR agonists as cognitive enhancers. To our knowledge, this is the first human study to systematically test the hypothesis that increasing NMDAR signaling enhances experience-dependent plasticity, and the first study to combine behavioral measures with assessment of a mechanism thought to underlie experience-dependent plasticity. We hypothesized that participants who received DCS would show (*i*) enhanced neural potentiation following HFvS on the LTP task; (*ii*) enhanced performance on the WPT and IIT; and (*iii*) similar performance on the *n*-back task, compared with Placebo participants.

Results

Participants. Sixty-five healthy adults enrolled in the study and received DCS ($n = 32$) or placebo ($n = 33$). Randomization yielded groups that were well-matched in age [$t_{(63)} = 0.16$, $P = 0.87$], gender [$\chi^2 = 0.01$, $P = 0.91$], and IQ [$t_{(63)} = -0.08$, $P = 0.94$] (Table 1).

Visual Evoked Potential Responses. To examine the effects of enhancing NMDAR signaling on LTP-like processes in the human brain, we compared changes in visual evoked potentials (VEPs) to a black-and-white checkerboard stimulus following HFvS in participants who received DCS versus those who received placebo. VEPs were assessed for 4 min immediately before HFvS to establish baseline neural responses, and during four post-HFvS blocks that occurred at 2–4, 4–6, 20–22, and 120–122 min following HFvS (*SI Methods*). The VEP complex was prominent at midline parietal-occipital channels and included a negative component, C1, that peaked at Oz at 100.79 ms (SD = 7.20) in Placebo participants and 102.06 ms (SD = 9.07) in DCS participants, and a positive component, P2, that peaked at Oz at 195.40 ms (SD = 29.03) in Placebo participants and 197.39 ms (SD = 24.10) in DCS participants (Fig. 1 *A* and *B*). C1 and P2 latencies did not differ between groups. For description of the time course of C1 and P2 plasticity following HFvS, see *SI Results*.

DCS Enhanced Potentiation of VEP Components. There were no differences in baseline amplitude of C1 [$t_{(63)} = 0.39$, $P = 0.70$] or P2 [$t_{(63)} = 0.24$, $P = 0.81$] between DCS and Placebo participants, indicating that DCS did not affect general neural excitability (*SI Results*). HFvS modulated C1 and P2 in both groups (*SI Results*), however, DCS significantly enhanced potentiation of both C1 and P2 following HFvS. Repeated-measures ANOVA on C1 amplitude change from baseline across the four post-HFvS blocks revealed that DCS participants showed greater potentiation overall compared with Placebo [$F_{(1,63)} = 4.92$, $P = 0.03$], due to less depression of C1 during early post-HFvS blocks and greater potentiation of C1 during the last post-HFvS block (Fig. 2*A*). The Group by Block interaction was not significant.

Similarly for P2, repeated-measures ANOVA revealed a significant effect of Group overall, due to the DCS group showing greater potentiation of P2 across all post-HFvS blocks compared with Placebo [$F_{(1,63)} = 6.08$, $P = 0.02$] (Fig. 2*B*). The Group by Block interaction was not significant. Parallel analyses using C1–P2 peak-to-peak amplitude also showed enhanced potentiation across post-HFvS blocks in the DCS group (*SI Results*). These results indicate that enhancing NMDAR signaling augmented potentiation of neural responses for 2 h following HFvS compared with Placebo, consistent with our first hypothesis.

Table 1. Demographic characteristics of Placebo and DCS participants

Group	*n*	Age (SD)	Sex	WASI IQ (SD)
Placebo	33	20.55 (2.41)	19 F/14 M	120.42 (9.33)
DCS	32	20.59 (2.69)	18 F/14 M	120.78 (8.23)

F, female; M, male; WASI, Weschler Abbreviated Scale of Intelligence.

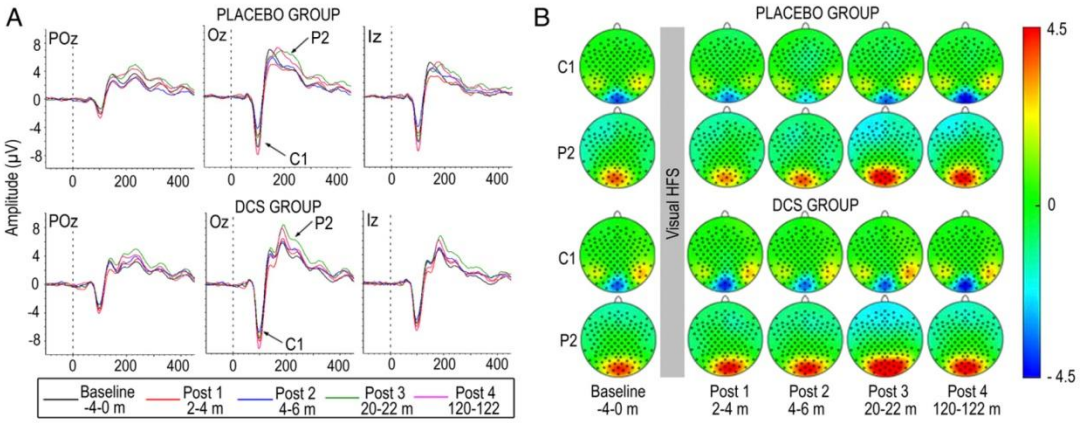

Fig. 1. (*A*) Grand average VEPs elicited by the standard checkerboard stimulus in example midline parietal-occipital channels for Placebo (*Top*) and DCS (*Bottom*) participants across VEP assessment blocks. (*B*) Scalp topography of C1 and P2 for Placebo (*Top*) and DCS (*Bottom*) participants across VEP assessment blocks. C1 analyses were based on average peak amplitudes from four channels surrounding Oz and Iz (i.e., Oz, Iz, O1, OI1h); P2 analyses were based on average peak amplitudes from six channels surrounding POz, Oz, and Iz (i.e., POz, Oz, Iz, POO1, O1, OI1h).

DCS Enhanced Experience-Dependent Learning. The WPT is a probabilistic classification learning task in which participants viewed combinations of cues that probabilistically predicted sun or rain. Following each response, participants were shown feedback regarding the actual outcome for each trial (*SI Methods*). Successful learning of associations between cues and probabilistic outcomes is thought to depend on LTP at corticostriatal synapses (21, 22). Both groups showed successful learning on the WPT, as indicated by improved performance over trial blocks [$F_{(3,180)}$ = 10.35, $P < 0.001$] and days [$F_{(1,180)} = 20.05$, $P < 0.001$]. However, the DCS group showed enhanced performance overall [$F_{(1,60)}$ = 5.60, $P = 0.02$]. Improved performance in the DCS group was evident within the first block of trials, indicating that DCS participants learned more rapidly and maintained gains over Placebo, despite no drug being given on the second day (Fig. 3*A*). No interactions of Group with Block or Day were significant.

The IIT is a classification learning task in which participants viewed sine-wave grating stimuli that varied in bar width and orientation. Participants were instructed to integrate the two dimensions and use auditory feedback to learn whether stimuli belonged to category A or B (*SI Methods*). Modeling of participant responses confirmed that the majority of participants (95%) used the optimal information integration decision strategy to learn the IIT (*SI Results*). Similar to the WPT, although both groups showed learning across trial blocks [$F_{(3,171)} = 7.48$, $P < 0.001$] and days [$F_{(1,171)} = 27.03$, $P < 0.001$], participants who received DCS showed enhanced learning compared with participants who received placebo. Enhanced performance in the DCS group was particularly evident during early learning, as indicated by a significant Group by Block by Day interaction [$F_{(3,171)} = 4.76$, $P = 0.003$], due to the DCS group showing significantly enhanced performance during the first ($P = 0.03$) and second trial blocks on day 1 ($P = 0.02$). The DCS group also showed a trend toward enhanced performance during the fourth trial block on day 1 ($P = 0.06$) (Fig. 3*B*). Although correct responses remained higher for DCS than Placebo participants on the second day of testing when no drug was administered, this effect was not significant. Thus, consistent with our second hypothesis, DCS significantly enhanced acquisition of two incremental learning tasks.

In contrast to the effects of DCS on the IIT and WPT, DCS did not affect performance on the *n*-back. The *n*-back was a spatial working memory task with four memory loads (0- to 3-back). Both groups performed better at lower working memory loads [$F_{(3,177)} = 146.26$, $P < 0.001$] and showed practice effects over testing days [$F_{(1,177)} = 35.33$, $P < 0.001$]. However, consistent with our third hypothesis, DCS and Placebo participants did not differ in working memory performance at any load. Thus, the main effect of Group and interactions of Group with Day and

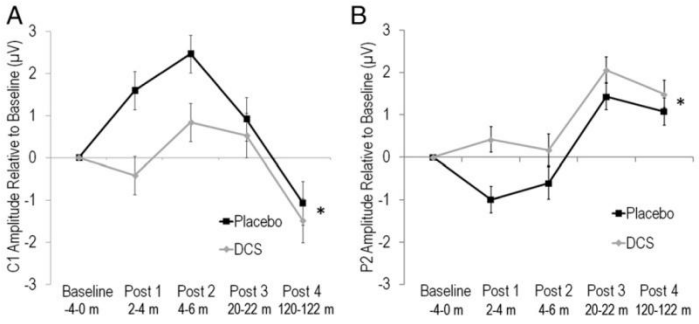

Fig. 2. (*A*) Mean ± SE. C1 amplitude change from baseline for Placebo and DCS participants. *DCS participants showed enhanced potentiation of C1 across post-HFvS blocks compared with Placebo ($P = 0.03$). (*B*) Mean ± SE. P2 amplitude change from baseline for Placebo and DCS participants. *DCS participants showed enhanced potentiation of P2 across post-HFvS blocks compared with Placebo ($P = 0.02$).

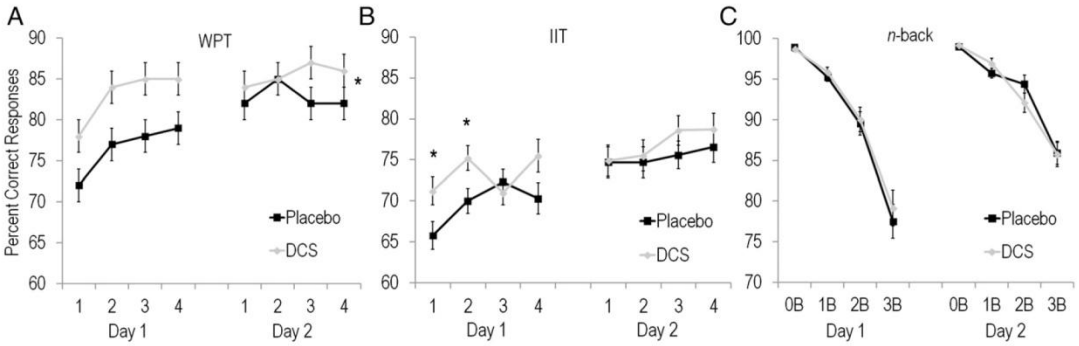

Fig. 3. (*A*) Mean ± SE percent correct responses per 80-trial blocks of the weather prediction task (WPT) for Placebo ($n = 31$) and DCS participants ($n = 31$). *DCS participants performed significantly better than Placebo participants overall ($P = 0.02$). (*B*) Mean ± SE percent correct responses per 80-trial blocks of the information integration task (IIT) for Placebo ($n = 29$) and DCS participants ($n = 30$). *DCS participants performed significantly better than Placebo participants during blocks 1 ($P = 0.03$,) and 2 ($P = 0.02$). (C) Mean ± SE percent correct responses per 80-trial blocks for the 0-back (0B), 1-back (1B), 2-back (2B), and 3-back (3B) conditions for Placebo ($n = 29$) and DCS participants ($n = 32$). There were no group differences between Placebo and DCS participants on the *n*-back.

memory load were not significant (values of $P > 0.05$) (Fig. 3*C*). This lack of effect of DCS on the *n*-back task was evident despite identical stimuli, trial structure, and auditory feedback to the IIT (*SI Methods*). Furthermore, there were no group differences in reaction times on any task (*SI Results*).

Discussion

Augmenting NMDAR signaling using the partial agonist DCS enhanced experience-dependent plasticity as shown by persisting enhancement of neural potentiation following repetitive HFvS on the LTP task and enhanced acquisition of two incremental learning tasks. Thus, DCS augmented potentiation of the C1 and P2 components following HFvS, without affecting baseline neural excitability. DCS also improved acquisition of the WPT and IIT without affecting performance on the *n*-back working memory task. Together, these results suggest that DCS enhanced both a mechanism (i.e., LTP) and behavioral correlates of experience-dependent plasticity (i.e., incremental learning), and provide compelling evidence that enhancing NMDAR signaling can boost experience-dependent plasticity in the adult human brain.

LTP of synaptic currents is the most well-studied form of activity-dependent plasticity. It persists into adulthood, is frequently NMDAR dependent, and has been observed at subcortical and sensory cortex synapses (1–6). Although classical LTP studies used high-frequency electrical stimulation to induce LTP, HFvS also induces lasting potentiation of neural responses (8–14), and potentiated neural responses following HFvS show cardinal features of synaptic LTP (7, 8). In the current study, participants who received DCS showed greater potentiation of the VEP following HFvS compared with participants who received placebo. This is consistent with prior findings that DCS augmented increases in motor cortex excitability following anodal transcranial direct-current stimulation in humans (37) and augmented LTP in rat hippocampus following high-frequency electrical stimulation (38, 39). Our finding is also consistent with preclinical studies demonstrating that potentiation of the VEP following HFvS is NMDAR dependent (8).

It is interesting to note that both Placebo and DCS participants showed depression of C1 in the early minutes following HFvS, before showing potentiation at 2 h post-HFvS. C1 is the first major visual event-related potential component and is generated by neurons in primary visual cortex (40). Although one study using a similar LTP paradigm in humans found that C1 was potentiated up to 22 min following HFvS (13), additional studies found no change or a trend toward reduced C1 amplitude up to 28 min following HFvS (11, 12). Given that VEPs measured by EEG capture the electrical discharge of populations of neurons, this variable direction of C1 plasticity may reflect interactive effects of homosynaptic LTP and heterosynaptic LTD across visual cortex synapses, where homosynaptic synapses are those synapses that are directly activated by electrical stimulation and heterosynaptic synapses are nearby synapses that are not directly activated by stimulation. Traditional investigations of LTP have focused on homosynaptic plasticity. However, a growing body of research indicates that the same procedures that induce plasticity in one direction in a given synaptic pathway frequently induce inverse plasticity at adjacent synapses (41–43). Thus, stimulation that results in LTP at tetanized, homosynaptic synapses has been shown to produce LTD at non-tetanized, heterosynaptic synapses, such that minimal change in total synaptic weight occurs over a population of neurons (42). Such heterosynaptic plasticity is thought to represent a homeostatic mechanism to provide stability at the neural system level (for review, see ref. 41). Importantly, whereas homosynaptic plasticity is associative and usually NMDAR dependent, heterosynaptic plasticity is nonassociative and usually depends on non-NMDAR mechanisms such as retrograde signaling by nitric oxide and endocannabinoids. Few studies have investigated the time course of heterosynaptic plasticity in neocortical regions of mature animals; however, at least one study found that heterosynaptic depression induced in somatosensory neurons following high-frequency stimulation was more transient (<10 min) than the induced homosynaptic LTP (6). Although speculative, we suggest that the variable direction of C1 plasticity during early post-HFvS blocks in the current study may reflect the net effect of homosynaptic potentiation and heterosynaptic depression at the neuron population level. Nevertheless, our finding that DCS participants showed enhanced potentiation of C1 and P2 across post-HFvS assessments compared with Placebo is consistent with evidence that homosynaptic LTP is usually NMDAR dependent, and suggests that enhancing NMDAR signaling resulted in enhanced homosynaptic LTP in visual cortex neurons.

Enhancing NMDAR signaling also enhanced performance on the WPT and the IIT. The WPT and IIT both depend on incremental, feedback-based learning that is generally mediated by corticostriatal circuits, although prefrontal and medial temporal lobe structures may also be involved (21, 22, 44, 45). In particular, rapid acquisition of stimulus–outcome contingencies that coincides with early gains in learning is thought to be mediated by NMDAR-dependent LTP at dorsomedial striatal (DMS) synapses (21, 22, 46).

As stimulus–outcome contingencies are consolidated and motor responses are increasingly automatized during late learning, performance asymptotes. In contrast to early learning, later expression of automatized responses is thought to be supported by LTD at dorsolateral striatal synapses following metabotropic glutamate and D2 dopamine receptor activation (22, 46). In the current study, enhanced performance in the DCS group was evident within the first block of 80 trials for both the IIT and the WPT. This suggests that augmenting NMDAR signaling facilitated the encoding of stimulus–outcome contingencies, possibly by accelerating or augmenting LTP at DMS synapses. Our findings of enhanced acquisition of the WPT and IIT are consistent with other findings of enhanced incremental learning following DCS administration, including on category learning (28), motor learning (25), and mental rotation learning tasks (47).

In contrast to the WPT and IIT, DCS participants did not differ from Placebo participants on the *n*-back, despite identical stimuli and trial structure between the IIT and *n*-back. This dissociation of cognitive effects is consistent with an emerging neurobiological framework that suggests that transient plasticity that underlies working memory is modulated in a fundamentally different way than lasting plasticity changes that support learning and memory consolidation (23, 48). Thus, whereas experience-dependent learning is thought to be mediated by lasting structural changes at dendritic spines following NMDAR-induced signaling cascades, short-term representation of stimuli for spatial working memory is thought to be mediated by transient, persistent firing of dorsolateral prefrontal cortex (dlPFC) microcircuits over brief delays. These working memory microcircuits involve glutamatergic pyramidal neurons in layer III dlPFC with similar spatial tuning that excite each other via AMPA and NMDA receptors, and GABAergic interneurons that inhibit neurons with dissimilar spatial tuning. Sufficient NMDAR activity is necessary to generate reverberating activity; however, it is lateral inhibition from GABAergic interneurons and dynamic modulation from acetylcholine and dopamine that is thought to enhance neuron firing for preferred directions, reduce firing for nonpreferred directions, and sculpt network activity to define the specifics of mental representation (23). This framework suggests that, given the minimum NMDAR activation necessary to produce transient, persistent firing, further NMDAR activation should have relatively limited effect on working memory performance. Our finding of similar working memory performance between DCS and Placebo participants is consistent with this theory and is in line with prior studies that failed to find effects of DCS on working memory in healthy volunteers or patient groups (27, 34–36). This dissociation of effects of DCS on experience-dependent learning versus working memory highlights the importance of considering the cellular mechanisms underlying distinct cognitive functions and demonstrates how investigating targeted hypotheses that capitalize on basic cognitive neuroscience can help reconcile discrepant effects of potential cognitive-enhancing drugs.

Some limitations to the current study should be noted. First, the current investigation was limited to healthy young adults. Studies in healthy participants provide critical information about potential mechanisms through which procognitive drugs exert their effects, as well as early feedback on how drugs affect specific cognitive domains and ideas for how effects may best be investigated in patient groups (49). Nevertheless, given that a key motivation for investigating effects of enhancing NMDAR signaling is to identify manipulations that can ameliorate plasticity deficits in patient populations, parallel studies in older adults and patient groups are an obligatory next step. Additionally, investigation of prolearning effects of DCS in the current study was limited to classification learning tasks that depend primarily on corticostriatal circuits. NMDAR-dependent LTP is thought to underlie distinct forms of learning in distributed circuits. For example, fear learning is thought to be encoded by LTP at lateral amygdala synapses (4), whereas motor sequence learning is thought to be encoded by LTP in motor cortex synapses (2). Other studies have shown that DCS can enhance learning mediated by other circuits, including fear (24) and motor learning (25), and indeed, some of the most reliable effects of DCS have been found when administered before cognitive behavioral therapy (CBT) exposure sessions to enhance fear extinction (ref. 29; but see also refs. 30 and 31). Future studies that systematically assess the effects of DCS on learning across multiple neural circuits will strengthen our understanding of the distributed effects of augmenting NMDAR signaling on experience-dependent plasticity. Finally, the current study did not assess how varying the parameters of DCS administration affects the efficacy of DCS. For example, some evidence suggests that the NMDAR complex may show desensitization when DCS is administered repeatedly (50). Although beyond the scope of the current study, future studies that characterize the optimal time window for DCS administration and examine whether effects of DCS persist with chronic dosing will be critical before practical applications of DCS can be realized.

Taken together, the current results provide compelling evidence that enhancing NMDAR signaling can augment experience-dependent plasticity in the adult human brain. Effects of DCS were seen across tasks probing both the mechanism thought to underlie many forms of experience-dependent plasticity (i.e., LTP) and behavioral correlates of experience-dependent plasticity (i.e., acquisition of incremental learning). These findings suggest exciting possibilities for using NMDAR agonists to help ameliorate plasticity deficits in neurodegenerative and psychiatric disorders. Our results complement a growing literature that suggests that DCS can enhance new learning during CBT interventions (29, 32) and cognitive training programs (33, 34). The dissociation of effects of DCS on incremental learning versus working memory highlights the importance of capitalizing on progress in basic cognitive neuroscience to develop more specific hypotheses for targets of cognitive-enhancing drugs.

Methods

Participants and Procedures. Healthy volunteers between 18 and 30 y completed the study (*SI Methods*). Study procedures were approved by the University of California, Los Angeles, Human Participants Institution Review Board, and written informed consent was obtained from all participants.

Testing consisted of a 2-d, randomized, double-blind 100-mg DCS versus placebo design. On the first day, DCS or placebo was administered as encapsulated pills. EEG testing began 3 h later, followed by cognitive testing (*SI Methods*). To explore potential delayed effects of DCS on memory consolidation (51), participants returned to the laboratory the next day to repeat the cognitive tasks. No drug or placebo was administered on the second day.

LTP Task. The LTP task was adapted from Cavuş et al. (13) and assessed VEPs in 2-min blocks before and after exposure to HFvS. VEPs were assessed to a standard circle stimulus filled with a black-and-white checkerboard pattern, presented at 0.83 Hz. The HFvS block consisted of repeated presentation of the standard circle at ~8.87 Hz for 2 min. For additional details on the task and on EEG recording and processing, see *SI Methods*.

Cognitive Tasks. The WPT was a probabilistic classification task adapted from Wagshal et al. (19). The WPT consisted of 320 trials during which participants predicted the weather based on cue combinations that probabilistically related to "sun" and "rain." Cues were presented for a maximum of 5 s. Following response, feedback showing the cue combination and actual outcome for the trial was presented for 1 s. The IIT was a category learning task adapted from Waldschmidt and Ashby (20). The IIT consisted of four blocks of 80 trials separated by 10-s rest periods, for 320 total trials. Stimuli belonged to category A or B, and consisted of circular sine-wave gratings that varied in bar width and bar orientation. On each trial, a single stimulus was presented in one of four quadrants of the screen for 2 s. Participants were instructed to integrate information about the two dimensions and choose whether stimuli belonged to category A or B; following response, participants were provided auditory feedback for 500 ms indicating whether they made a correct or incorrect response. The *n*-back was a spatial working memory task with four memory loads and was designed to be identical in stimuli and trial structure to the IIT. Thus, participants viewed a sequence of 80 stimuli at each memory load, and on each trial, were asked to respond "yes" or "no," whether the stimulus was in the same quadrant

on the screen as the stimulus they saw *n* trials earlier. See *SI Methods* for additional details on the cognitive tasks.

Statistical Analyses. Statistical analyses were conducted with SPSS, version 22.0 (SPSS). Independent-samples *t* tests assessed group differences in age and IQ, and a χ^2 test assessed differences in gender.

LTP analyses. C1 and P2 components were identified at midline occipital channels and were analyzed using the channels available for all participants that showed the largest amplitude for each component. Thus, C1 analyses used Oz, Iz, O1, OI1h, and P2 analyses used POz, Oz, Iz, POO1, O1, OI1h. As initial analyses showed no interactions of channel with Group or Block, average amplitude across the four C1 channels and six P2 channels were used for all analyses. Paired-samples *t* tests showed no differences between the two baseline VEP blocks for either group for C1 or P2; thus, amplitudes for the two baseline blocks were averaged to yield one baseline. To first characterize the time course of HFvS effects, component amplitudes were investigated using Group by Block repeated-measures ANOVA, followed by tests of simple contrast to baseline (*SI Results*). Next, to obtain a measure of potentiation induced by HFvS, baseline VEP amplitude was subtracted from each post-HFvS amplitude. Group differences in potentiation were assessed using Group by Block repeated-measures ANOVA.

Cognitive analyses. Percent correct responses per 80-trial blocks were calculated for each cognitive test. For the WPT, trials for cue combinations that equally predicted sun and rain (i.e., probability of 0.5) were excluded. Group differences in accuracy and reaction time were assessed using Group by Block by Day repeated-measures ANOVA. An α value of 0.05 was used to determine significance for all analyses.

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Supporting Information

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SI Methods

Inclusion Criteria. Participants were eligible for the study if they were between 18 and 30 y of age; were comfortable reading in English; had no history of seizures, neurologic disease, or allergies to antibiotics; had normal vision and hearing; were not prescribed psychotropic medication; were not pregnant; had not used recreational drugs in the past month; and had an IQ > 70, as assessed by the vocabulary and matrix reasoning tests of the Weschler Abbreviated Scale of Intelligence (WASI) (52). Participants completed a brief medical screen and were asked to abstain from alcohol use for 24 h before testing.

For data analyses, participants were excluded from analysis of a given cognitive test if they missed more than 5% of trials on a testing day. This resulted in exclusion of two Placebo and one DCS participants for the IIT; two Placebo and one DCS participants for the WPT; and four Placebo participants for the *n*-back. One Placebo participant who performed poorly on the IIT (51.6% correct on day 1) reported that he had not understood the instructions; one Placebo participant performed below chance on the IIT and was considered an outlier (28.4% correct overall); and one DCS participant was erroneously administered a rule-based version of the IIT; data for these participants on the IIT were excluded. No participants were excluded from LTP data analyses.

DCS Properties and Administration Schedule. The administration schedule of DCS was chosen based on pharmaceutical information that peak plasma levels are reached in 3–4 h (King's Pharmaceuticals), and is consistent with other studies showing beneficial effects of DCS when administered 3–4 h before learning or exposure therapy (e.g., refs. 26 and 28). However, it is possible that an alternate administration schedule would have yielded stronger effects of DCS. For example, a recent meta-analysis of DCS and fear extinction indicated that beneficial effects of DCS were most reliable when DCS was administered 1–2 h before exposure sessions (30). Nevertheless, given that the half-life of DCS is 8–12 h (King's Pharmaceuticals), it is likely that maximal or near-maximal effects of DCS were present during testing in the current study.

It is also important to note that the effects of DCS can differ at low versus high doses. This dose–response pattern may reflect the differential efficacy of DCS at NMDARs with different subunit compositions. Specifically, DCS has been shown to increase the channel open time and open probability of NMDARs containing the NR2C subunit with ∼200% efficacy compared with glycine. However, at NMDARs containing NR2A or 2B subunits, DCS has ∼50% efficacy compared glycine (36). Thus, although DCS is a potent agonist at NMDARs with NR2C subunits regardless of dose, at NMDARs with NR2B and NR2A subunits, DCS may act as an agonist at low doses (e.g., 50–250 mg) by stimulating unoccupied glycine sites, but as an antagonist at high doses (e.g., 1,000 mg) (53) by displacing endogenous glycine from the glycine site. Given this activity profile, the current study used a low dose of 100 mg of DCS that has been shown to be an agonist in other studies (e.g., refs. 25 and 37).

LTP Task. VEP assessment blocks during the LTP task consisted of a pseudorandom oddball sequence of 90% standard and 10% target stimuli presented for 33 ms (jittered 1- to 1.33-s stimulus onset asynchrony). The standard stimulus was a circle filled with a black-and-white checkerboard pattern, presented at 0.83 Hz. To maintain attention, participants were asked to press a button whenever they saw a target square containing a blue-and-white checkerboard pattern. Unrelated auditory and resting tasks were performed between the HFvS and post-HFvS blocks 1 through 3. The cognitive tasks were performed between post-HFvS blocks 3 and 4. The standard stimulus and time course of the LTP task are shown in Fig. S1.

Continuous EEG was amplified and recorded using a Biosemi Active-Two system (BioSemi, Amsterdam) employing 128 electrodes in an elastic cap and arranged according to the extended 10–20 EEG system. Horizontal eye movements were measured via two electrodes located 1 cm lateral to the lateral canthi of the right and left eyes, and vertical eye movements and blinks were measured by two electrodes located 1 cm above and below the orbit of the right eye. Due to recording error, data from the 64 right hemisphere channels were not retained for the first 33 participants; however, posterior midline electrodes were available for all participants.

EEG data were processed using Brain Vision Analyzer (Brain Products) and custom MATLAB (Mathworks) scripts. Continuous data were rereferenced to nose, bandpass filtered at 0.5–50 Hz, and 600-ms (−100 to 500 ms) epochs time-locked to the standard stimulus onsets were extracted for each VEP block. Epochs were baseline corrected using the prestimulus period and subjected to ocular correction using the Gratton et al. (54) algorithm. Epochs with voltage exceeding ±100 μV between 0 and 250 ms after stimulus onset at parietal or occipital sites were excluded. VEP blocks following artifact rejection contained a minimum of 57 segments (Placebo, M = 89.15, SD = 2.37; DCS, M = 89.43, SD = 1.70).

Epochs were averaged generating VEPs for the two baseline and four post-HFvS blocks. A custom MATLAB script identified the negative peak with the greatest amplitude between 80 and 120 ms for C1 and the positive peak with the greatest amplitude between 150 and 250 ms for P2 for each participant, averaged across VEP blocks. C1 and P2 latencies and peak amplitudes for each VEP block were extracted.

Cognitive Tasks.

WPT. On each trial of the WPT, between one and three out of four possible cues appeared, yielding 14 different combinations. Cues were presented for a maximum of 5 s, and participants were instructed to respond using buttons for "rain" or "sun." Following response, feedback showing the cue combination and actual outcome for the trial was presented for 1 s (Fig. S2). The probabilities for different cue combinations predicting rain or sun ranged from 0.875 to 0.125. Thus, if a given cue combination was 0.875 associated with sun, the cue combination was associated with sun for 87.5% of trials and with rain for 12.5% of trials. The probability structure of the WPT is summarized in Table S1. Responses were counted as correct if the most likely outcome was selected.

IIT. On each trial of the IIT, a single stimulus was presented in one of four quadrants of the screen for 2 s. IIT stimuli consisted of circular sine-wave gratings that varied across trials on bar width and bar orientation. Stimuli belonged to category A or B and were defined by a set of points (x,y) randomly sampled from a 100 × 100 stimulus space and converted to a disk stimulus by defining frequency as $f = 3(x/100) - 1$, and orientation as $o = (3\pi/8)(y/100) + (\pi/11)$. Category A stimuli were generated from a multivariate normal distribution with the following parameters (55): $\mu_A = (43,57)$; $\Sigma_A = \{155\ 145; 145\ 155\}$. The same sampling method was used to generate category B stimuli: $\mu_B = (57, 43)$;

$\Sigma_A = \Sigma_B$. Participants were instructed to integrate information about the two dimensions and decide whether each stimulus belonged to category A or B by pressing the corresponding keys. Following response, participants were provided auditory feedback for 500 ms using a pure tone at 262 Hz for a correct response and a sawtooth (harsher) tone centered at 440 Hz for an incorrect response. Auditory feedback was followed by a 1,500-ms delay before the next trial (Fig. S3*A*). If participants did not respond during stimulus presentation, "please respond faster" was displayed for 1,500 ms. Participants were told that they would learn the categories by attending to the auditory feedback, that perfect accuracy was possible, and that stimulus location on the screen did not matter. Participants completed four practice trials before testing to ensure that they understood the task.

n-back. In the *n*-back task, participants viewed a sequence of 80 stimuli at each of four working memory loads (0- to 3-back), and on each trial, were asked to respond "yes" or "no," whether the stimulus was in the same quadrant on the screen as the stimulus they saw *n* trials earlier. For the 0-back control condition, participants were instructed to press "yes" when the stimulus appeared on the left side of the screen, and "no" when the stimulus appeared on the right side of the screen. Load conditions were separated by a 10-s rest during which instructions were displayed to inform participants of the upcoming condition. Participants were provided auditory feedback for 500 ms following each response, parallel to the IIT (Fig. S3*B*). Participants completed 13 practice trials per block in ascending memory load difficulty before testing to ensure that they understood the task.

IIT Model-Based Analyses. The WPT and IIT are most commonly thought of as striatal-dependent implicit learning tasks. However, debate remains regarding the extent to which individuals use explicit strategies to learn these tasks, and accordingly, the extent to which executive prefrontal and medial temporal lobe structures are also involved. Given that the optimal multidimensional rules that govern the WPT and IIT are more difficult to learn and verbalize than one-dimensional rules, it is thought that multidimensional rules are more likely learned implicitly, whereas one-dimensional rules are more likely learned explicitly (44). Interestingly, on the WPT, the rate of positive feedback that participants receive if they use a multicue strategy is not much higher than the rate of positive feedback received if they ignore all cues other than the single most predictive cue. For example, in the current study, a participant who integrated information from all four cues would receive positive feedback at a rate of 74%, whereas a participant who ignored all cues except cue 1 would receive positive feedback at a rate of 72.7% (Table S1). Thus, it has been suggested that participants may be likely to use a single-cue strategy on the WPT rather than the more difficult multicue strategy, given the small difference in positive feedback between these strategies (44). In contrast, on the IIT, the rate at which participants receive positive feedback if they use a one-dimensional strategy is much lower than the rate of positive feedback if they learn the optimal information integration strategy (e.g., 71% versus 100% in the current study). One method for assessing whether participants use implicit or explicit strategies is therefore to model participant responses to discern whether responses are dominated by multidimensional or one-dimensional rules. Such modeling is difficult to complete on the WPT given the complexity of task design, probabilistic nature of feedback, and the fact that a variety of decision-making strategies yield similar performance levels. However, decision-bound modeling has been reliably used to provide insight into the strategies being used by participants to solve the IIT (20, 56). Thus, we used decision-bound modeling to test whether participant responses on the IIT were dominated by one-dimensional versus multidimensional rules.

Briefly, decision-bound models assume that participants partition the perceptual space into response regions, determine which region the percept is in for each trial, and respond accordingly. In the current study, four models were fit to the each participant's data across testing days and for each testing day separately: (*i*) a unidimensional X model; (*ii*) a unidimensional Y model; (*iii*) a guessing model; and (*iv*) a general linear classifier model (GLC), the optimal multidimensional model. The unidimensional X and unidimensional Y models assume that participants set a criterion on one perceptual dimension (i.e., spatial frequency or line orientation, respectively) and make category A versus B decisions based on explicit evaluations of the stimulus on that dimension. The guessing model assumes that participants guess on each trial. The GLC is a multidimensional model that assumes that participants divide the stimulus space using a linear decision bound that integrates the frequency and orientation dimensions (i.e., the line $x = y$). The GLC was the optimal IIT strategy in the current study. For detailed information on decision-bound modeling, please see ref. 56.

The model parameters were estimated using maximum likelihood and the goodness-of-fit statistic was the Bayesian information criterion (BIC), which is defined as follows:

$$\mathrm{BIC} = r \times \ln(N) - 2 \times \ln(L),$$

where N is the sample size, r is the number of free parameters, and L is the likelihood of the model given the data. The best model was identified as the model with the smallest BIC score.

SI Results

No Significant Effect of DCS on Baseline Excitability. To further assess potential effects of DCS on baseline excitability, we extracted average C1 and P2 amplitude for each of the 4 min of baseline. Repeated-measures ANOVA for C1 indicated no significant effect of Group [$F_{(1,63)} = 0.07$, $P = 0.79$] or Group by Block interaction [$F_{(3,189)} = 0.50$, $P = 0.65$] (Fig. S4*A*). Similarly, for P2, there was no significant effect of Group [$F_{(1,63)} = 0.01$, $P = 0.92$] nor Group by Block interaction [$F_{(3,189)} = 0.49$, $P = 0.66$] (Fig. S4*B*). Thus, DCS did not affect basal synaptic transmission even though it is likely that peak plasma levels of DCS were present during the baseline assessment.

Time Course of C1, P2, and C1–P2 Peak-to-Peak Plasticity. HFvS modulated the C1 and P2 components in both groups. Repeated-measures ANOVA to characterize the time course of HFvS effects on C1 revealed a significant effect of Block [$F_{(4,252)} = 16.96$, $P < 0.001$] and a significant Block by Group interaction [$F_{(4,252)} = 2.79$, $P = 0.04$]; the main effect of Group was not significant. Follow-up tests within groups showed a significant Block effect for both groups (values of $P < 0.001$). Tests of simple contrast to baseline indicated that Placebo participants showed depression of C1 during the early post-HFvS blocks from 2 to 4 and 4 to 6 min (values of $P < 0.01$) and tended toward potentiation from 120 to 122 min post-HFvS ($P = 0.07$). In contrast, DCS participants showed significant potentiation of C1 at 120–122 min ($P = 0.003$) and only showed depression at 4–6 min post-HFvS ($P = 0.03$).

For P2, there was also a significant effect of Block [$F_{(4,252)} = 26.83$, $P < 0.001$]; the main effect of Group and Group by Block interaction were not significant. Follow-up contrasts comparing post-HFvS blocks to baseline indicated that Placebo and DCS participants showed significant potentiation of P2 at 20–22 min and 120–122 min post-HFvS (values of $P < 0.001$).

Exploratory analyses also assessed modulation of C1–P2 peak-to-peak amplitude following HFvS. C1–P2 peak-to-peak amplitude was calculated by subtracting the amplitude of C1 from P2 for baseline and each post-HFvS block. Channel Oz was used, given that both components showed maximal amplitude at Oz. Repeated-measures ANOVA showed a significant effect of

Block [$F_{(4,252)}$ = 19.53, P < 0.001] and a significant Block by Group interaction [$F_{(4,252)}$ = 3.18, P = 0.03]. Follow-up tests within each group showed a significant Block effect for both groups (values of P < 0.001). Simple contrasts to baseline indicated that Placebo participants showed depression of C1–P2 during the early post-HFvS blocks from 2 to 4 and 4 to 6 min (values of P = 0.001) and significant potentiation at 120–122 min post-HFvS (P = 0.04). In contrast, DCS participants showed significant potentiation of C1–P2 at 20–22 (P = 0.02) and 120–122 min (P < 0.001), and only showed depression at 2–4 min post-HFvS (P = 0.04).

DCS Enhanced Potentiation of C1–P2 Peak-to-Peak Amplitude. There were no group differences in baseline C1–P2 peak-to-peak amplitude (i.e., before exposure to HFvS) [$t_{(63)}$ = 0.49, P = 0.63]. However, repeated-measures ANOVA for C1–P2 amplitude change from baseline across the four post-HFvS blocks revealed that DCS participants showed greater potentiation overall compared with Placebo [$F_{(1,63)}$ = 8.75, P = 0.004] (Fig. S5). The Group by Block interaction was not significant (P > 0.05). Thus, potentiation of C1–P2 peak-to-peak amplitude was enhanced in the DCS group for 2 h following HFvS, similar to results for the C1 and P2 components separately.

IIT Model-Based Results. When IIT responses were modeled across testing days, the GLC model provided the best fit for ∼95% of participants, which corresponded to 26 of 29 Placebo participants and 30 of 30 DCS participants. The remaining three Placebo participants were best fit by a unidimensional X model. When responses were modeled for each testing day separately, the best-fitting model was more variable. On day 1, 26 Placebo and 27 DCS participants were best fit by the GLC model, 3 Placebo and 2 DCS participants were best fit by a unidimensional X model, and 1 DCS participant was best fit by a unidimensional Y model. On day 2, 24 Placebo and 27 DCS participants were best fit by the GLC model, 2 Placebo and 1 DCS participants were best fit by a unidimensional X model, 1 Placebo and 2 DCS participants were best fit by a unidimensional Y model, and 1 Placebo participant was best fit by a guessing model. Thus, overall, participant responses were dominated by the optimal information integration strategy. However, there was greater variability in the best-fitting model when participant responses were modeled across individual testing sessions, suggesting some switching between decision-making strategies during task acquisition. Analysis of the Group effect on day 1 of the IIT confined to participants who were best fit by the GLC model yielded results that were similar to the analysis with all included participants. Thus, among participants who relied primarily on the optimal information integration decision strategy on day 1, DCS participants performed significantly better than Placebo participants during block 2 [$F_{(1,51)}$ = 5.88, P = 0.02] and tended toward better performance compared with Placebo participants during blocks 1 [$F_{(1,51)}$ = 3.33, P = 0.07] and 4 [$F_{(1,51)}$ = 3.95, P = 0.05].

Reaction Time on Cognitive Tasks. ANOVA for reaction time for each task revealed no significant main effect of Group or interactions involving Group for the IIT or WPT (values of P > 0.47). On the n-back, there was a significant interaction of Day by Group [$F_{(1,177)}$ = 4.26, P = 0.04]; however, follow-up tests for each testing day showed no significant effects of Group for day 1 or 2 (values of P > 0.18). Thus, reaction times for each task were similar between groups (Fig. S6).

Relationships Between Plasticity Measures. Exploratory correlational analyses were conducted to assess potential relationships between the EEG and cognitive measures of neuroplasticity. To minimize the number of comparisons, summary plasticity scores were used for each task. Thus, mean amplitude change across post-HFvS blocks relative to baseline was computed and used for each LTP component (i.e., C1, P2, and C1–P2 peak-to-peak amplitude), and mean percent correct responses across testing days was used for the IIT and WPT. Although plasticity measures within the LTP task were significantly related (values of P < 0.01), there was only one statistically significant correlation between measures of plasticity across tasks. Increased C1 amplitude relative to baseline positively predicted performance on the WPT [$r_{(62)}$ = 0.25, P = 0.049], suggesting that depression of C1 predicted better WPT performance. However, this relationship did not survive correction for multiple comparisons. No other relationships between the learning tasks or between the LTP and learning tasks were significant (Table S2). Given that the EEG measure of plasticity assessed LTP-like processes in visual cortex neurons and that acquisition of the WPT and IIT are thought to depend on plasticity at corticostriatal synapses, it may not be surprising that a clear relationship between the EEG and cognitive measures of plasticity was not detected. However, it is surprising that performance on the two learning tasks were not related.

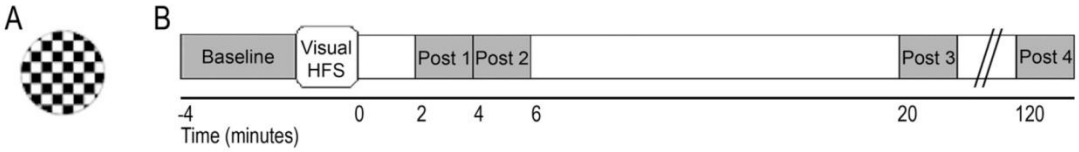


Fig. S1. (*A*) Standard circle black-and-white checkerboard stimulus presented at 0.83 Hz during visual evoked potential (VEP) assessment blocks and at ∼8.87 Hz during high-frequency visual stimulation (HFvS). (*B*) Time course of the long-term potentiation (LTP) paradigm. Adapted from ref. 13.

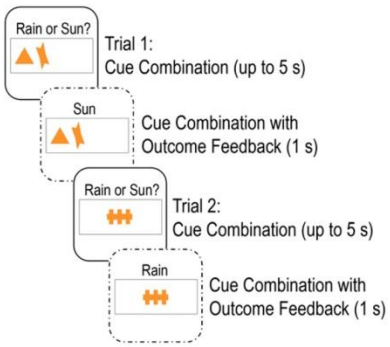


Fig. S2. Two example weather prediction task (WPT) trials.

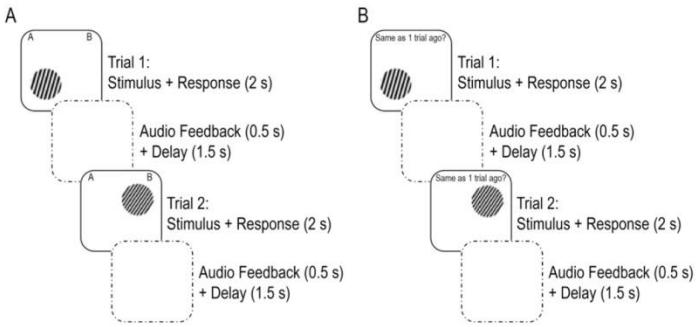


Fig. S3. (*A*) Two example information integration task (IIT) trials and (*B*) two example trials for the 1-back condition of the *n*-back task. The IIT and *n*-back task were identical in stimuli, trial structure, and feedback such that the only difference participants experienced was whether they were asked to learn about the stimuli (i.e., for the IIT) or recall whether stimuli were in the same location on the screen as recently shown stimuli (i.e., for the *n*-back).

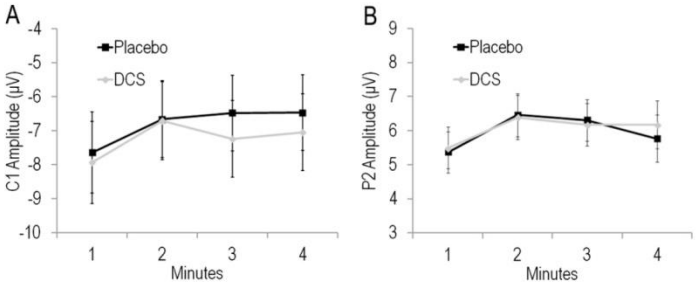


Fig. S4. Mean ± SE (*A*) C1 and (*B*) P2 amplitude for Placebo and DCS participants across 4 min of baseline assessment (i.e., pre-HFvS).

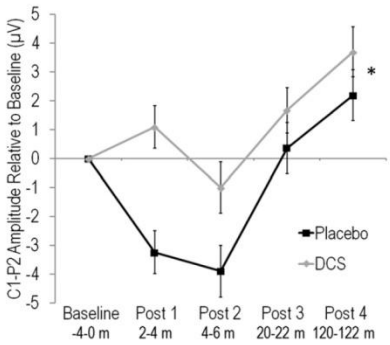


Fig. S5. Mean ± SE. C1–P2 peak-to-peak amplitude change relative to baseline for Placebo and DCS participants. *DCS participants showed enhanced potentiation of C1–P2 peak-to-peak amplitude across post-HFvS blocks ($P = 0.004$).

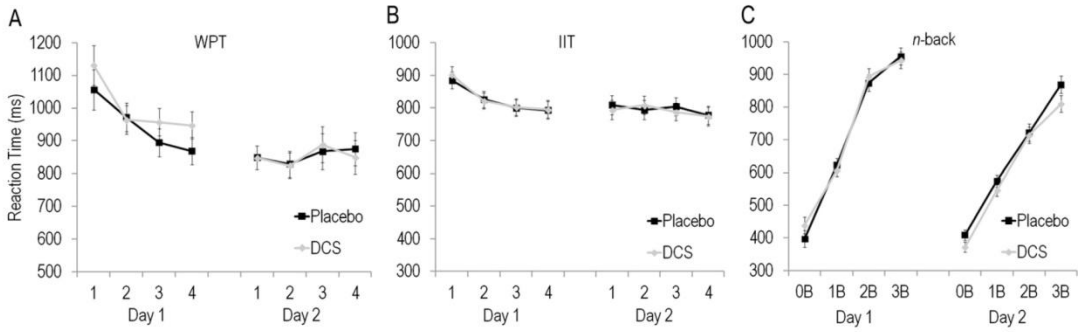


Fig. S6. Mean ± SE reaction time per 80-trial blocks across testing days for Placebo and DCS participants for the (*A*) weather prediction task (WPT), (*B*) information integration task (IIT), and (*C*) *n*-back task.

Table S1. Probability structure of WPT

Combination	Cue 1	Cue 2	Cue 3	Cue 4	*P* (Combination)	*P* (Sun|| Combination)
1	1	0	0	0	0.14	0.857
2	0	0	1	0	0.06	0.333
3	1	0	1	0	0.08	0.75
4	0	1	0	0	0.06	0.667
5	1	1	0	0	0.08	0.75
6	0	1	1	0	0.04	0.5
7	1	1	1	0	0.04	0.5
8	0	0	0	1	0.16	0.125
9	1	0	0	1	0.04	0.5
10	0	0	1	1	0.14	0.143
11	1	0	1	1	0.02	1
12	0	1	0	1	0.04	0.5
13	1	1	0	1	0.04	0.5
14	0	1	1	1	0.06	0.333
P (Sun|| Overall)	0.727	0.556	0.409	0.280		

For each cue combination, each card could be present (1) or absent (0). The bottom row lists the overall probability of Sun for the four cues across all cue combinations.

Table S2. Pearson correlation coefficients between plasticity on the LTP and learning tasks

Measure	1	2	3	4
1. Mean C1 plasticity	—			
2. Mean P2 plasticity	−0.35**	—		
3. Mean C1–P2 plasticity	−0.87***	0.72***	—	
4. WPT percent correct responses	0.25*	0.14	−0.12	—
5. IIT percent correct responses	−0.08	0.20	0.17	0.17

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

CHAPTER 2

Augmenting NMDA Receptor Signaling in Schizophrenia Enhances Neural Responsivity and Working Memory without Affecting Experience-Dependent Plasticity

INTRODUCTION

Schizophrenia is a debilitating neuropsychiatric disease that affects 1% of the population worldwide. Despite considerable efforts, much remains to be understood about the core biological processes that underlie the disorder and existing pharmacologic treatments yield disappointing outcomes for many patients. This lack of success may reflect several issues, including the clinical and neurobiological heterogeneity of the disorder and the traditional focus on psychotic symptoms as a lens for etiology and treatment research (Insel, 2010). Although psychotic symptoms are often the most prominent feature of schizophrenia, they fluctuate considerably over the course of illness and rarely emerge before late adolescence. In contrast, cognitive dysfunction is present prior to psychosis onset, may be the most enduring and debilitating feature of the illness, and is the best predictor of long-term outcome (Asarnow & MacCrimmon, 1978; Green, Kern, & Heaton, 2004; Heaton et al., 2001; Green & Nuechterlein, 1999). Thus, cognitive dysfunction is a core feature of the illness and may provide a window into both its pathophysiology and novel therapeutics (Green & Nuechterlein, 1999). One path towards improving our understanding of the neurobiology of schizophrenia and identifying potential therapeutics is therefore to: 1) identify biological pathways that may underlie cognitive deficits in schizophrenia; and 2) to interrogate the effects of manipulating these pathways systematically.

The *N*-methyl-D-aspartate receptor (NMDAR)

Hypofunction at the *N*-methyl-D-aspartate receptor (NMDAR) may be one such critical pathway in schizophrenia. The NMDAR is a glutamate receptor that is present throughout the brain and is involved in essential brain functions ranging from basic excitatory neurotransmission to complex learning functions (Zito & Scheuss, 2009). NMDARs are

composed of obligatory NR1 subunits and additional NR2 or NR3 subunits that surround a central ion pore. NMDARs are unusual in that they require concurrent binding of two ligands for normal activation, glutamate and glycine or D-serine, as well as membrane depolarization from activation of non-NMDARs such as amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptors (AMPARs) to relieve blockade from a magnesium ion (Mg+) at resting potential. NMDAR opening leads to an influx of cations, including sodium ions (Na^{+}), which contribute to postsynaptic depolarization, and calcium ions (Ca^{2+}), which activate intracellular signaling cascades that ultimately modulate synaptic strength (Hardingham & Bading, 2003). Relative to non-NMDA receptors which typically have rapid rise (0.2-0.4 ms) and deactivation times (~2 ms), the speed at which NMDARs activate (10-50 ms) and deactivate (~50-500 ms) is markedly slow (Zito & Scheuss, 2009). The slow delay kinetics of NMDARs allows NMDAR activation to easily accumulate to high or saturating levels, even at low firing rates, which is critical for enhancing the computational power of neurons and generating sustained neural excitation in local recurrent circuits (Vargas-Caballero & Robinson, 2003).

The Role of NMDARs in Schizophrenia, Working Memory, and Experience-Dependent Plasticity

Convergent pharmacological, imaging, post-mortem, and genetic evidence implicate the NMDAR in schizophrenia. Blocking NMDAR signaling using antagonists such as phencyclidine and ketamine induces positive, negative, and cognitive symptoms in healthy individuals strikingly similar to those seen in schizophrenia (Luby, Cohen, Rosenbaum, Gottlieb, & Kelley, 1959; Krystal et al., 1994), and in patients with schizophrenia, NMDAR antagonism profoundly exacerbates existing symptomatology (Javitt & Zukin, 1991). Post-mortem studies revealed

aberrant expression of multiple NMDAR subunit transcripts (Beneyto & Meador-Woodruff, 2008; Akbarian et al., 1996; Sokolov, 1998; Dracheva et al., 2001) and NMDAR-associated proteins in patients with schizophrenia (Beneyto & Meador-Woodruff, 2008; Ohnuma et al., 2000; Toyooka et al., 2002), as well as decreased phosphorylation of the NMDAR (Emamian, Karayiorgou & Gogos, 2004). Neuroimaging studies found reduced NMDAR binding in the hippocampus of medication-free schizophrenia patients (Pilowsky et al., 2006). Further, studies of cerebrospinal fluid revealed reduced availability and altered metabolism of the endogenous NMDAR co-agonist D-serine in schizophrenia (Hashimoto et al., 2005; Bendikov et al., 2007; Verrall, Burnet, Betts, & Harrison, 2010). Finally, large-scale genomic studies of tens of thousands of patients (PGC-SCZ, 2014; Purcell et al., 2009) and convergent functional genomics analyses (Ayalew et al., 2012) identified genes intrinsic to the NMDAR and those modulating NMDAR signaling as among those most robustly associated with schizophrenia. Thus, convergent evidence implicates impaired NMDAR signaling in schizophrenia.

The unique biophysical characteristics of the NMDAR confer it a specialized role in a subset of distinct cognitive processes (Kantrowitz & Javitt, 2010; Javitt, 2004). These include working memory, which is the ability to transiently hold and manipulate information to guide immediate goal-directed behavior, and experience-dependent plasticity, which is the capacity of the brain to undergo lasting changes with environmental input and use to encode relationships that guide future behavior. Experience-dependent plasticity allows us to learn and remember patterns, predict and obtain reward, and refine and accelerate response selection for adaptive behavior (Feldman, 2009). The classical mechanism underlying experience-dependent plasticity is long-term potentiation (LTP) or depression (LTD) of synaptic strength. Preclinical studies using transgenic and knockout mice demonstrated that blocking NMDARs impairs working

memory, as well as synaptic plasticity and learning and memory. Conversely, enhancing NMDAR activity enhanced LTP and the acquisition and retention of information (Lee & Silva, 2009).

Consistent with NMDAR hypofunction, patients with schizophrenia show deficits in working memory and experience-dependent plasticity. Meta-analyses indicate that impaired working memory is found in schizophrenia across diverse methods of assessment, is present in both visual-spatial and verbal working memory domains, and is present even over short delays of 1 second (Lee & Park, 2005; Forbes, Carrick, McIntosh, & Lawrie, 2009). Patients with schizophrenia also show deficits in electrophysiological and behavioral (i.e. learning) measures of experience-dependent plasticity. Patients show impaired acquisition of various learning tasks including simple reinforcement learning (Murray et al., 2008), probabilistic classification learning (Weickert et al., 2002; Morris, Heerey, Gold, & Holryd, 2008; Horan et al., 2008; Wagshal et al., 2012), and explicit and implicit sequence learning (Siegert, Watherall, & Bell, 2008; Pederson et al., 2008). Patients with schizophrenia also show deficits on a recently developed electrophysiological measure of LTP. In animals, LTP is induced using high-frequency electrical stimulation and is observed as an enduring increase in the post-synaptic response of glutamate synapses in neurons. Lack of noninvasive methods historically limited our ability to investigate LTP in humans; however, recent research indicates that protocols utilizing high-frequency, repetitive presentation of visual or auditory stimuli provide a naturalistic method for inducing LTP in humans. Studies in rodents demonstrated that changes in neural responses following repetitive sensory stimulation show the cardinal features of synaptic LTP, including persistence (> 1 hour), input specificity, and NMDAR dependency (Clapp, Eckert, Teyler, & Abraham, 2006; Cooke & Bear, 2010). Further, these LTP-like changes can be measured

noninvasively as changes in sensory evoked potentials, which are stimulus-synchronized electroencephalograph (EEG) signals that result from postsynaptic potentials in populations of sensory neurons. Thus, in healthy adults, high-frequency sensory stimulation induces lasting potentiation of visual and auditory evoked potentials (Kirk et al., 2010; Teyler et al., 2005). Such potentiated neural responses were found to be impaired in patients with schizophrenia (Cavus et al., 2012; Mears & Spencer, 2012).

Importantly, the characteristics of the NMDAR that are responsible for its specialized role in working memory differ from those that allow it to play a critical role in experience-dependent plasticity. During spatial working memory, transient representation of stimuli in the absence of sensory input is thought to depend upon recurrent excitation in dorsolateral prefrontal cortex (dlPFC) microcircuits (Arnsten, Wang, & Paspalas, 2012). These microcircuits involve glutamatergic pyramidal neurons with similar spatial tuning that excite each other via AMPA and NMDA receptors, and GABAergic interneurons that inhibit neurons with dissimilar spatial tuning. Whereas AMPAR activation is thought to contribute to the membrane depolarization that permits NMDAR activation, it is the slower kinetics of NMDARs that generates sustained neural excitation over delay periods (Wang et al., 2013). A threshold of NMDAR activity is therefore required to generate reverberating activity for working memory function; however, beyond this threshold, it is lateral inhibition from GABAergic interneurons and dynamic modulation from acetylcholine and dopamine that is thought to enhance neuron firing for preferred directions, reduce firing for non-preferred directions, and sculpt network activity to define the specifics of mental representation (Arnsten et al., 2012).

In contrast to the transient changes in neural firing that subserve working memory, in experience-dependent plasticity, the brain undergoes lasting structural changes to represent

important inputs and outputs by larger and more coordinated populated of neurons (Vinogradov, Fisher, & de Villers-Sidani, 2012). Changes in synaptic strength via LTP and LTD generate these lasting alterations in neural activity and are thought to be the primary mechanism for encoding new information in the brain (Feldman, 2009; Citri & Malenka, 2008). NMDARs are critical for triggering the cellular machinery that supports experience-dependent plasticity in these circuits. NMDARs are localized to cell membranes in large signaling complexes that physically link the receptor to a network of kinases, phosphatases and downstream signaling proteins. NMDAR-mediated rises in Ca^{2+} activate this network of molecules to generate intracellular signal transduction cascades that promote gene transcription and structural changes at the synapse, such as AMPAR insertion into the membrane, and ultimately result in LTP and LTD (Zito & Scheuss, 2009). Thus, through divergent biophysical features of the receptor, NMDARs are critically involved in both experience-dependent plasticity and working memory function.

Using NMDAR Agonists to Enhance Cognition

Given aberrant NMDAR functioning in schizophrenia, interest has grown in targeting the NMDAR to treat cognitive deficits in schizophrenia. While enhancing NMDAR signaling via direct stimulation of the glutamate site can lead to excitotoxicity, indirect stimulation via the glycine co-agonist site offers a safer method to augment activity. D-cycloserine (DCS) is a partial NMDAR agonist that binds to the NMDAR glycine site and increases NMDAR channel open time and open probability (Henderson, Johnson, & Ascher, 1990; Dravid et al., 2010). In our recent study in healthy individuals, acute DCS enhanced experience-dependent plasticity, as indicated by enhanced potentiation of the visual evoked potential (VEP) following high

frequency visual stimulation (HFvS) on the EEG LTP task and accelerated acquisition of two cortical-striatal LTP-dependent learning tasks: the weather prediction task (WPT; Knowlton, Squire, & Gluck, 1994; Wagshal et al., 2013) and information integration task (IIT; Waldschmidt & Ashby, 2011). Conversely, DCS showed no effects on the *n*-back spatial working memory task, despite the fact that the *n*-back was designed to be identical to the IIT in stimuli and trial structure such that the only difference participants experienced between the tasks was whether they were asked to learn about the stimuli (i.e. for the IIT) or recall whether stimuli were in the same location on the screen as recently shown stimuli (i.e. for the *n*-back). The lack of effect of DCS on working memory in healthy individuals is consistent with the above described framework suggesting that beyond a threshold of NMDAR activation necessary to produce transient, persistent firing in dlPFC microcircuits, further NMDAR activation should have relatively limited effects on working memory performance in individuals with normal NMDAR function. The dissociation of effects of DCS on measures of experience-dependent plasticity versus working memory in healthy individuals highlights the importance of using mechanistic-driven hypotheses to guide investigations of NMDAR-agonists as potential cognitive enhancers.

The Present Research: Effects of DCS on Deficits in Working Memory and Experience-Dependent Plasticity in Schizophrenia

For patients with schizophrenia who show abnormalities in structure and function of the NMDAR, deficits in experience-dependent plasticity including on the EEG LTP task and the WPT, and deficits in working memory, we hypothesized that augmenting NMDAR signaling using DCS would ameliorate deficits in experience-dependent plasticity and working memory. To test this hypothesis, we carried out a randomized, single dose 100 mg DCS versus Placebo

double-blind study in patients with schizophrenia. Some evidence suggests that chronic DCS dosing may lead to desensitization of the NMDAR complex over time (Quartermain, Mower, Rafferty, Herting, & Lanthorn, 1994; Parnas, Weber, & Richardson, 2005; Mickley et al., 2012). Thus, a single dose of DCS was used to probe the effects of increasing NMDAR signaling on cognitive deficits in schizophrenia without introducing potential confounding effects of tolerance. Forty-five schizophrenia patients completed the EEG LTP task, WPT, and IIT to assess experience-dependent plasticity, as well as the *n*-back task to assess spatial working memory. To facilitate comparison of effects of DCS in healthy individuals versus patients with schizophrenia, the study design closely paralleled that used in our healthy control participant study. Interestingly, and in contrast to our results in healthy participants in which DCS enhanced electrophysiological and learning measures of experience-dependent plasticity, in patients with schizophrenia, acute DCS enhanced baseline neural responsivity and working memory performance without significantly affecting neural potentiation on the EEG LTP task or learning on the WPT or IIT.

METHODS

Participants

Forty-five patients with a psychotic disorder completed the study. Participants were recruited in collaboration with the Aftercare Research Program and the Center for the Assessment and Prevention of Prodromal States at the University of California, Los Angeles (UCLA) Semel Institute for Neuroscience. Eligible participants were between 18 and 50 years; comfortable reading in English; had no history of seizures, neurologic disease, or allergy to DCS; were not currently taking Clozapine; had normal vision and hearing; were not pregnant; had not

used recreational drugs for 48 hours or alcohol for 24 hours prior to testing; had an IQ > 70 as assessed by the Weschler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999); and met DSM-IV criteria for Schizophrenia, Schizoaffective, or Schizophreniform Disorder. Participants completed a brief medical screen to confirm eligibility. Study procedures were approved by the UCLA Human Participant Institution Review Board and written informed consent was obtained from all participants.

Study Procedures

Testing consisted of a 1-day, randomized, double-blind 100 mg DCS versus Placebo design. Prior to study entry, participants completed a brief telephone screening to assess eligibility for participation based on inclusion/exclusion criteria. Participants who met inclusion criteria were invited to visit the lab to complete the consent and medical screening process. Medical screening was completed by a study physician or nurse-practitioner. Presence of a psychotic disorder was confirmed by review of diagnostic interviews conducted by collaborating labs within 2 years of study entry (i.e. Structured Clinical Interview for DSM-IV Axis I Disorders; First, Spitzer, Gibbon & Williams, 1997). Upon completion of consent and medical screening, participants were randomized to receive 100 mg of DCS (n = 24) or placebo (n = 21). DCS and placebo were administered orally as an encapsulated pill.

Immediately after taking the study drug, patients completed an assessment of general intellectual functioning using the 2 subtest version of the WASI that includes the vocabulary and matrix reasoning subtests (FSIQ-2). The WASI is one of the most commonly used brief assessments of intellectual functioning. It has high to excellent internal reliability (FSIQ-2 reliability coefficients ≥ .93 for adult age groups), test-retest reliability (FSIQ-2 reliability

coefficient = .88 for adults), and inter-rater reliability (reliability coefficients ≥ .95 per subtest in adults), and shows high correlations with other measures of intellectual ability and achievement (Wechsler, 1999). Following WASI administration, patients completed an assessment of symptom severity using the expanded 24-item version of the Brief Psychiatric Rating Scale (BPRS; Overall, Hollister, & Pichot, 1967, Ventura et al., 1993). The BPRS is one of the most frequently used symptom rating scales, is an effective and sensitive measure of psychiatric symptoms and change in symptoms, and has high inter-rater reliability with training (i.e. ICCs ≥ 0.85; Ventura et al., 1993; Roncone et al., 1999). Scores for each item range from 1 (not present) to 7 (extremely severe). Total Symptom Severity was calculated by summing scores on all items (range: 24 - 168). In addition, scores for Thought Disturbance (sum of Unusual Thought Content, Hallucinations, and Conceptual Disorganization items; range 3 - 21), and Withdrawal-Retardation (sum of Blunted Affect, Emotional Withdrawal, and Motor Retardation items; range 3 - 21) were calculated to index positive and negative symptoms, respectively (Ventura, Nuechterlein, Subotnik, Gutkind, & Gilbert, 2000). EEG testing began approximately 3 hours after administration of DCS or Placebo, followed by cognitive testing. This schedule was selected to ensure maximal effects of DCS given that peak plasma levels are reached in 3-4 hours and plasma half-life is 8-12 hours (King's Pharmaceuticals Ltd, product information).

Long Term Potentiation (LTP) Task

The LTP task assessed VEPs in 2-minute blocks before and after exposure to HFvS (Cavus et al., 2012). VEP assessment blocks consisted of a pseudorandom oddball sequence of 90% standard and 10% target stimuli presented for 33 ms. VEPs were assessed to the standard stimulus, a circle filled with a black and white checkerboard pattern presented at .83 Hz (jittered

1-1.33s stimulus onset asynchrony; Fig. 1A). To maintain attention, participants were asked to press a button whenever they saw a target square containing a blue and white checkerboard pattern. VEP blocks occurred from 4-2 minutes and 2-0 minutes before HFvS to provide a baseline, and from 2-4 minutes, 4-6 minutes, 20-22 minutes, and in a 2-minute block approximately 2 hours after the HFvS. The HFvS block designed to induce potentiation consisted of repeated presentation of the standard circle at ~8.87 Hz for 2 minutes. Unrelated auditory and resting tasks were performed between the HFvS and post-HFvS blocks 1 through 3. The cognitive tasks were performed between post-HFvS blocks 3 and 4. The timeline of the LTP task is depicted in Fig. 1B.

EEG Recording and Data Processing

In an electromagnetically shielded, sound-attenuating room, continuous EEG was amplified and recorded using a Biosemi Active-Two system (BioSemi, Amsterdam, Netherlands) employing 128 electrodes in an elastic cap and arranged according to the extended 10–20 EEG system. Biosemi replaces the grounding configuration used in conventional systems with two separate electrodes: Common Mode Sense active electrode and Driven Right Leg passive electrode (see http://www.biosemi/faq/cms&drl.htm for further detail). Electro-oculogram (EOG) was recorded using two bipolar pairings of electrodes: horizontal eye movements were measured via two electrodes located 1 cm lateral to the lateral canthi of the right and left eyes, and vertical eye movements and blinks were measured by two electrodes placed on top of the orbicularis oculi muscle, 1 cm above and below the orbit of the right eye.

EEG data were processed using Brain Vision Analyzer (Brain Products, Munich, Germany) and custom MATLAB (Mathworks, Natick, Massachusetts) scripts. Continuous data were re-referenced to nose, band-pass filtered at 0.5-50 Hz, and 600 ms (-100 to 500 ms) epochs

time-locked to the standard stimulus onsets were extracted for each VEP block. Epochs were baseline corrected using the pre-stimulus period and subjected to ocular correction using the Gratton, Coles, & Donchin (1983) algorithm. Epochs with voltage exceeding ±100 μV between 0-250 ms post stimulus onset at parietal or occipital sites were excluded. VEP blocks following artifact rejection contained a minimum of 68 segments (Placebo M = 88.42, SD = 2.99; DCS M = 88.37, SD = 3.11).

Epochs were averaged generating VEPs for the 2 baseline and 4 post-HFvS blocks. A custom MATLAB script identified the negative peak with the greatest amplitude between 65-125 ms for C1 and the positive peak with the greatest amplitude between 155-255 ms for P2 for each participant, averaged across VEP blocks. C1 and P2 latencies were extracted for each VEP block. Given slight variations in the latency of VEP component peaks between assessment blocks, mean C1 amplitude for 10 ms around peak C1 latency and mean P2 amplitude for 40 ms around peak P2 latency were extracted and used for all analyses.

Cognitive Tasks

Weather Prediction Task

The WPT was a probabilistic classification task consisting of 240 trials during which participants predicted the weather based on cue combinations that probabilistically related to "sun" and "rain" (Wagshal et al., 2013). On each trial, between one and three out of four possible cues appeared, yielding 14 different combinations. Cues were presented for a maximum of 5 s, and participants were instructed to respond using buttons for “rain” or “sun.” Following response, feedback showing the cue combination and actual outcome for the trial was presented for 1 s (Fig. 2). Due to programming error, there were slight variations in the probability

structure per cue combination and number of trials per cue combination that each patient received. However, the number of trials each patient completed per cue combination and probability associated with each cue combination was similar across groups. Across groups, the average probabilities for different cue combinations predicting rain or sun ranged from 0.120 to 1.00 (Table 1). Thus, if a given cue combination was 0.120 associated with sun, the cue combination was associated with sun on 12% of trials and with rain on 88% of trials. Responses were counted as correct if the most likely outcome was selected. Trials for cue combinations that equally predicted sun and rain (i.e. probability ≈0.5; cue combinations 6, 7, 9, 12, 13) were excluded from analysis.

Information Integration Task

The IIT was a category learning task in which participants learned to categorize circular sine-wave grating stimuli that varied in bar width and bar orientation (Waldschmidt & Ashby, 2011). Stimuli belonged to category A or B and were defined by a set of points (*x*,*y*) randomly sampled from a 100 x 100 stimulus space and converted to a disc stimulus. Example categories for an IIT are depicted in Fig. 3. In the current study, frequency was defined by $f = 3(x/100) - 1$, and orientation was defined by $o = (3\pi/8)(y/100) + (\pi/11)$. Category A stimuli were generated from a multivariate normal distribution with the following parameters (55): $\mu_A = (43,57)$; $\Sigma_{\square} = \{155\ 145; 145\ 155\}$. The same sampling method was used to generate Category B stimuli: $\mu_B = (57,43)$; $\Sigma_A = \Sigma_B$.

The task consisted of 3 blocks of 80 trials separated by 10 s rest periods, for 240 total trials. On each trial, a single stimulus was presented in one of four quadrants of the screen for a maximum of 3 s with the category labels “A” and “B” displayed at the top of the screen (Fig. 4A). Participants were instructed to integrate information about the two dimensions (i.e. bar

width and bar orientation) and decide whether each stimulus belonged to category A or B by pressing the corresponding keys. After 2 s and following response, participants were provided auditory feedback for 500 ms using a pure tone at 262 Hz for a correct response and a sawtooth (harsher) tone centered at 440 Hz for an incorrect response. If participants did not respond within 3 s during stimulus presentation, "please respond faster" was displayed for 1500 ms. Participants were told that they would learn the categories by attending to the auditory feedback and that stimulus location on the screen did not matter. Auditory feedback was followed by a 1500 ms delay before the next trial. Participants completed 4 practice trials before testing to ensure that they understood the task.

N-Back Task

The *n*-back was a spatial working memory task with 3 memory load conditions in 80 trial blocks: 0-back, 1-back, and 2-back conditions. Stimuli presentation and trial structure for the *n*-back was identical to the IIT except that the task-level (e.g. 2-back) was shown at the top of the screen during stimulus presentation rather than category labels (Fig. 4B). In the 0-back control condition, participants indicated whether stimuli were on the left or right side of the screen. In the 1- and 2-back working memory conditions, participants indicated whether each new stimulus was in the same location on the screen as the stimulus shown 1 or 2 trials ago, respectively. Each condition was completed once in random order for a total of 240 trials. Load conditions were separated by a 10 s rest during which instructions were displayed to inform participants of the upcoming condition. Participants were provided auditory feedback for 500 ms following each response, parallel to the IIT. Participants completed 13 practice trials per block in ascending memory load difficulty prior to testing to ensure that they understood the task.

Statistical Analyses

Statistical analyses were conducted with SPSS, version 22.0 (SPSS, Chicago, Illinois). Antipsychotic information was available for all but one patient who was taking Navane daily but was not able to provide dose information. Chlorpromazine dose equivalency was calculated for all remaining patients (Danivas & Venkatasubramanian, 2013). Independent samples t-tests assessed potential group differences in age, IQ, BPRS scores, and antipsychotic dose, and a χ^2 test assessed group differences in gender.

LTP Analyses

C1 and P2 components were identified at midline occipital channels and were analyzed using the channel with the largest amplitude, Oz, for each component. One Placebo participant who had a small blind spot in one eye had an unusual VEP; data for this participant were excluded from the LTP analyses. Due to equipment difficulties, one DCS participant had invalid data at channel Oz, and one DCS participant had excessive 60 Hz noise at channel Oz across VEP assessment blocks. One additional DCS participant was unable to keep his eyes open throughout the HFvS and VEP assessment blocks and had an unusual VEP. Data for these participants were also excluded from LTP analyses. Among DCS participants, paired samples t-tests showed no significant differences in baseline 1 (M = -8.90, SD = 6.46) and baseline 2 (M = -7.44, SD = 4.85) amplitude for C1, $t(20) = -1.51$, $p = .15$, or between baseline 1 (M = 4.41, SD = 3.03) and baseline 2 (M =4.77, SD = 3.16) amplitude for P2, $t(20) = -.97$, $p = .34$. For Placebo participants, there was a trend level difference between C1 amplitude for baseline 1 (M = -5.35, SD = 5.22) and baseline 2 (M = -4.48, SD = 4.95), $t(19) = -2.03$, $p = .06$. P2 amplitude was similar across baseline 1 (M = 4.73, SD = 3.50) and baseline 2 (M = 4.94, SD =3.39), $t(19) = -.42$, $p = .68$. Amplitudes for the 2 baseline blocks were therefore averaged to yield one baseline

per VEP component and used for subsequent analyses. To first characterize the timecourse of HFvS effects, component amplitudes were investigated using Drug Condition x Block repeated measures ANOVA, followed by tests of simple contrasts compared to baseline. Next, to obtain a measure of potentiation induced by HFvS, plasticity scores were computed by subtracting baseline VEP amplitude from each post-HFvS amplitude. Group differences in potentiation were assessed using Drug Condition x Block repeated measures ANOVA.

Cognitive Analyses

Percent correct responses per 80-trial blocks were calculated for each cognitive test. Group differences in accuracy and reaction time were assessed using Dug Condition x Block repeated measures ANOVA. Participants who missed more than 5% of trials on a given task were excluded for that cognitive test. This resulted in exclusion of 2 DCS participants for the IIT; 3 DCS and 1 Placebo participants for the WPT; and 1 DCS and 1 Placebo participants for the n-back. Additionally, to ensure that null effects of drug on cognitive tasks were not due to general difficulties understanding or engaging in cognitive tasks, analyses were re-run for each task restricted to patients performing above chance. For the IIT and WPT, this was defined as patients with ≥ 55% accuracy during the last block of 80 trials. For the *n*-back, this was defined as patients with ≥75% accuracy during the control 0-back condition and ≥55% accuracy during the 1-back and 2-back conditions.

Parallel analyses including sex, age, IQ, and chlorpromazine equivalent antipsychotic doses as covariates, or excluding patients who were on antidepressants were conducted for all outcome measures. Effect sizes used partial eta square (η^2_p) which describes the amount of variance in scores attributable to the effect. Suggested norms for partial eta square are small =

.01, medium = .06, large = .14. An alpha of 0.05 was used to determine significance for all analyses.

Secondary Analyses Comparing Effects of DCS in Healthy Controls

To facilitate comparison of effects of DCS in patients with schizophrenia to control participants, data from the original 2-day study in healthy control participants were extracted, re-processed, and analyzed to parallel the current study as closely as possible.

Briefly, sixty-five healthy adults completed the original study and were randomized to receive DCS (n = 32) or Placebo (n = 33). Control participants were between 18 and 30 years of age; were comfortable reading in English; had no history of seizures, neurologic disease, or allergies to antibiotics; had normal vision and hearing; were not prescribed psychotropic medication; were not pregnant; had not used recreational drugs in the past month; and had an IQ > 70 as assessed by the WASI. Randomization yielded groups that were well-matched in age, $t(63)=0.16$, $p=0.87$, gender, $\chi^2=0.01$, $p=0.91$, and IQ, $t(63)=-0.08$, $p=0.94$. Demographic characteristics of the healthy participants are shown in Supp. Table 1. Participants completed a brief medical screen and were asked to abstain from alcohol use for 24 hours prior to testing. See Forsyth et al., 2015 for full description of procedures.

Control participants originally completed 320 trials for the WPT, IIT, and *n*-back task, including completing an additional 3-back condition for the *n*-back. Data for the first 240 trials of testing on the WPT and IIT, and data for the 0-, 1-, and 2-back conditions of the *n*-back were therefore extracted for comparison to data in the current study. The stimuli and trial structure of the WPT, IIT, and *n*-back in the control study were identical to that in the current study, except

that control participants were only allowed up to 2 seconds to respond on each trial of the IIT and *n*-back.

Data for the LTP task was re-extracted to parallel the current study. While continuous EEG processing steps were identical between the two studies, VEP peak extraction was modified slightly to account for the greater variability in C1 and P2 latencies and more centralized topography of the VEP response among schizophrenia patients. Thus, control participant data was re-extracted using the custom Matlab script to identify the negative peak with the greatest amplitude between 65-125 ms for C1 and the positive peak with the greatest amplitude between 155-255 ms for P2. Mean C1 amplitude for 10 ms around the peak C1 latency and mean P2 amplitude for 40 ms around peak the P2 latency were used for all analyses and analyses were restricted to Oz where the C1 and P2 components showed the largest amplitude in schizophrenia patients.

Effect sizes of DCS in healthy participants for each of the LTP and cognitive measures are presented and compared to those in the current study.

RESULTS

Demographic Characteristics of Schizophrenia Patients

Demographic and clinical characteristics of schizophrenia patients who received Placebo and DCS are shown in Table 2. Randomization yielded Placebo and DCS groups that were similar in age, $t(43) = .64$, $p = .54$; sex, $\chi^2 = .98$, $p = .32$; IQ, $t(43) = .47$, $p = .64$; positive, $t(43) = .63$, $p = .53$, negative, $t(43) = -.55$, $p = .58$, and total symptom levels, $t(43) = -.06$, $p = .95$; and chlorpromazine equivalent anti-psychotic dose, $t(36) = .117$, $p = .91$.

LTP Task in Schizophrenia Patients

Baseline VEP Responses Within Groups

To examine the effects of enhancing NMDAR signaling on neural responsivity and LTP-like processes in the human brain, we compared visual evoked potentials (VEPs) to a black and white checkerboard stimulus before and after HFvS in patients who received DCS versus Placebo. VEPs were assessed for 4 minutes immediately prior to HFvS to establish baseline neural responses, and during four post-HFvS blocks that occurred at 2-4, 4-6, 20-22, and approximately 120-122 minutes following HFvS.

The VEP complex was prominent at midline parietal-occipital channels and included a negative component, C1, that peaked at Oz at 96.10 ms (*SD*=19.13) in Placebo patients and 104.62 ms (*SD*=6.48) in DCS patients, and a positive component, P2, that peaked at Oz at 200.70 ms (*SD*=28.81) in Placebo patients and 203.19 ms (*SD*=24.07) in DCS patients (Fig. 5A,B). C1 latency tended towards being earlier in Placebo patients, $F(1,39) = 3.72$, $p = .06$, $\eta^2_p = .087$. P2 latency was similar between groups, $F(1,39) = .09$, $p = .77$. Interestingly, C1 amplitude at baseline was significantly larger in patients who received DCS ($M = -8.17$, $SD = 5.27$) compared to patients who received Placebo ($M = -4.91$, $SD = 5.00$), $F(1,39) = 4.12$, $p = .049$, $\eta^2_p = .096$, indicating that DCS enhanced baseline neural responsivity in patients with schizophrenia. Increased baseline C1 amplitude in patients who received DCS remained after controlling for age, sex, antipsychotic dose, and IQ, $F(1,34) = 6.50$, $p = .015$, $\eta^2_p = .161$. Baseline P2 amplitude was similar between patients who received DCS ($M = 4.59$, $SD = 2.98$) and Placebo ($M = 4.84$, $SD = 3.25$), $F(1,39) = .06$, $p = .80$, $\eta^2_p = .002$, including after controlling for age, sex, antipsychotic dose, and IQ, $p = .98$.

Timecourse of VEP Plasticity Following HFvS

Repeated measures ANOVA to characterize the timecourse of HFvS effects on C1 amplitude revealed a significant effect of block, $F(4,156) = 5.45$, $p < 0.005$, $\eta^2_p = .12$, and a significant effect of drug overall, $F(1,39) = 4.86$, $p = .03$, $\eta^2_p = .11$, due to the DCS group showing larger C1 amplitude across assessment blocks, starting at baseline, compared to the Placebo group (Fig. 6). The drug x block interaction was not significant, $F(4,156) = .72$, $p = .50$, $\eta^2_p = .018$. Simple contrasts to baseline indicated that DCS and Placebo patients showed depression of C1 at 4-6 minutes post-HFvS, $p = .001$, $\eta^2_p = .24$, and tended towards depression of C1 at 2-4 minutes post-HFvS, $p = .07$, $\eta^2_p = .083$. Neither patient group showed significant modulation of C1 at 20-22, $p = .68$, $\eta^2_p = .004$, or 120-122 minutes post-HFvS, $p = .10$, $\eta^2_p = .068$.

Repeated measures ANOVA to characterize the timecourse of HFvS effects on P2 showed a significant effect of block, $F(4,156) = 7.10$, $p < .001$, $\eta^2_p = .154$. There was no significant effect of drug, $F(1,39) = .01$, $p = .92$, $\eta^2_p < .001$, nor drug x block effect, $F(4,156) = 2.14$, $p = .10$, $\eta^2_p = .052$ (Fig. 7). Simple contrasts to baseline indicated that both DCS and Placebo patients showed potentiation of P2 at 120-122 minutes post-HFvS, $p < .001$, $\eta^2_p = .272$. However, P2 amplitude did not differ from baseline at 2-4, $p = .22$, $\eta^2_p = .039$; 4-6, $p = .24$, $\eta^2_p = .035$; or 20-22 minutes post-HFvS, $p = .13$, $\eta^2_p = .058$, in either group.

Exploratory analyses also assessed modulation of C1-P2 peak-to-peak amplitude following HFvS. C1-P2 peak-to-peak amplitude was calculated by subtracting the amplitude of C1 from P2 for baseline and each post-HFvS block. Repeated measures ANOVA using C1-P2 peak-to-peak amplitude showed a significant effect of block, $F(4,156) = 9.40$, $p < .001$, $\eta^2_p = .194$. The drug effect, $F(1,39) = 2.81$, $p = .10$, $\eta^2_p = .067$, and drug x block interaction, $F(4,156) = .054$, $p = .96$, $\eta^2_p = .001$, were not significant (Fig. 8). Simple contrasts to baseline indicated

that both DCS and Placebo patients showed significant depression of C1-P2 peak-to-peak amplitude at 2-4, $p = .021$, $\eta^2_p = .129$, and 4-6 minutes post-HFvS, $p = .001$, $\eta^2_p = .27$, and significant potentiation of C1-P2 peak-to-peak amplitude at 120-122 minutes post-HFvS, $p = .006$, $\eta^2_p = .181$.

DCS Did Not Enhance Potentiation of the VEP

Although DCS significantly enhanced baseline amplitude of C1 compared to placebo, DCS did not enhance potentiation of C1 following HFvS. Thus, there was no significant effect of drug, $F(1,39) = .14$, $p = .71$, $\eta^2_p = .004$, nor significant drug x block interaction, $F(3,117) = .81$, $p = .44$, $\eta^2_p = .02$, for C1 potentation among schizophrenia patients (Fig. 9).

There was also no significant effect of drug on P2 potentation following HFvS in schizophrenia patients, $F(1,39) = .18$, $p = .67$, $\eta^2_p = .005$. There was a trend towards a drug x block interaction, $F(3,117) = 2.48$, $p = .08$, $\eta^2_p = .06$; however, this was not significant (Fig. 10).

Analyses using C1-P2 peak-to-peak amplitude similarly showed no significant effect of drug condition, $F(1,39) = .304$, $p = .58$, $\eta^2_p = .008$, and no significant drug x block interaction, $F(3,117) = .02$, $p = .98$, $\eta^2_p = .001$ (Fig. 11). Thus, despite effects of DCS on baseline neural responsivity in patients with schizophrenia, DCS did not enhance potentiation of the VEP compared to Placebo, indicating no significant effect of DCS on the electrophysiological measures of experience-dependent plasticity. Parallel analyses including sex, age, IQ and antipsychotic dose as covariates, or excluding patients who were on anti-depressants yielded a similar lack of effect of DCS on potentiation of the VEP components following HFvS.

Cognitive Tasks in Schizophrenia Patients

DCS Did Not Enhance Learning

The WPT is a probabilistic classification learning task in which patients viewed combinations of cues that probabilistically predicted sun or rain. Following response selection, patients were shown feedback regarding the actual outcome for each trial. Both the DCS and Placebo group showed impaired learning on the WPT, as indicated by no significant effect of block, $F(2,78) = .98$, $p = .38$, $\eta^2_p = .024$. Further, there was no significant effect of drug, $F(1,39) = .40$, $p = .53$, $\eta^2_p = .010$, nor significant drug x block interaction, $F(2,78) = 2.37$, $p = .10$, $\eta^2_p = .057$. Restricting analyses to only patients performing above chance during the last block of trials, covarying for age, sex, IQ, and antipsychotic dose, or excluding patients who were on anti-depressants yielded a similar lack of effect of DCS on WPT performance. Thus, DCS did not enhance WPT performance (Fig. 12).

The IIT is a classification learning task in which patients used auditory feedback signaling correct versus incorrect responses to learn whether sine-wave grating stimuli that varied on bar width and bar orientation belonged to "Category A" or "Category B" (Ashby & Maddox, 2005). Patients were instructed to integrate information about the two dimensions and use the auditory feedback to learn whether stimuli belonged to Category A or B. There was a trend towards an effect of block across groups, $F(2,82) = 2.79$, $p = .07$, $\eta^2_p = .065$; however, this was not significant. There was also no significant effect of drug, $F(1,41) = .041$, $p = .841$, $\eta^2_p = .001$, nor drug x block interaction, $F(2,82) = .78$, $p = .46$, $\eta^2_p = .019$. Restricting analyses to patients who performed above chance during the last block of trials, covarying for age, sex, IQ, and antipsychotic dose, or excluding patients who were taking anti-depressants yielded similar results. Thus, DCS did not improve performance on the IIT (Fig. 13).

DCS Enhanced Working Memory Performance

In contrast to the lack of effect of DCS on the learning tasks in schizophrenia patients, DCS significantly enhanced working memory performance. The *n*-back is a spatial working memory task with 3 memory loads (0- to 2-back). In the 0-back control condition, patients indicated whether stimuli were on the left or right side of the screen. In the 1- and 2-back working memory conditions, patients indicated whether each new stimulus was in the same location on the screen as the stimulus shown 1 or 2 trials ago, respectively. Analyses including all patients indicated that both groups performed better at lower working memory loads, $F(2,82) = 40.97$, $p < 0.001$, $\eta^2_p = .50$. While the DCS group appeared to perform better than the Placebo group during the 2-back condition, the drug x block interaction, $F(2,82) = 1.00$, $p = .36$, $\eta^2_p = .024$, and overall drug effect were not significant, $F(1,41) = .10$, $p = .75$, $\eta^2_p = .003$. However, when analyses were restricted to DCS and Placebo patients who performed above chance, DCS significantly improved working memory performance (Fig. 14). Thus, there was a significant drug x block interaction, $F(2,62) = 3.24$, $p = .046$, $\eta^2_p = .095$, due to the DCS group performing significantly better than the Placebo group during the 2-back condition, $p = .045$. Although the DCS group showed higher mean performance than the Placebo group during the 1-back condition, this was not significant, $p = .36$; groups performed similarly during the 0-back control condition, $p = .49$. Controlling for sex, age, IQ, and antipsychotic dose yielded a similar effect of DCS on 2-back performance among patients who performed above chance, $p = .03$. Thus, while DCS showed no effect on the WPT or IIT in patients with schizophrenia, among Placebo and DCS patients who appeared able to successfully engage the *n*-back task, DCS significantly enhanced working memory performance.

DCS Did Not Significantly Affect Reaction Times on Cognitive Tasks

Investigation of reaction times for each cognitive task revealed no significant group differences. Thus, for the WPT, while there was a significant effect of block, $F(2,78) = 6.92$, $p = .004$, $\eta^2_p = .15$, there was no significant effect of drug, $F(1,39) = .07$, $p = .79$, $\eta^2_p = .002$, nor drug x block interaction, $F(2,78) = 1.43$, $p = .25$, $\eta^2_p = .035$ (Fig. 15A). On the IIT, there was a significant effect of block, $F(2,82) = 28.70$, $p < .001$, $\eta^2_p = .412$, and a significant drug x block interaction, $F(2,82) = 3.39$, $p = .040$, $\eta^2_p = .076$ (Fig. 15B). Follow-up tests for each block indicated that Placebo patients tended towards faster reaction time on the IIT than DCS patients during the second block of 80 trials, $F(1,41) = 3.27$, $p = .08$, $\eta^2_p = .074$; however, this was not significant, and DCS and Placebo patients showed similar reaction times on the IIT during the first, $F(1,41) = 1.77$, $p = .19$, $\eta^2_p = .041$, and third blocks of 80 trials, $F1,41) = .12$, $p = .74$, $\eta^2_p = .003$. The main effect of drug was not significant, $F(1,41) = 1.53$, $p = .22$, $\eta^2_p = .036$. Finally, on the *n*-back, while there was a significant effect of block, $F(2,82) = 73.88$, $p < .001$, $\eta^2_p = .643$, there was no significant effect of drug, $F(1,41) = .001$, $p = .94$, $\eta^2_p < .001$, nor drug x block interaction, $F(2,82) = .29$, $p = .75$, $\eta^2_p = .007$ (Fig. 15C).

Secondary Analyses Comparing Effects of DCS in Healthy Controls

Because the current study did not include healthy controls, the data presented thus far do not provide information about how the schizophrenia patients in each drug condition compared to healthy controls. However, given the importance of understanding how effects of DCS in patients with schizophrenia compare to effects of DCS in healthy controls, we re-analyzed data from our prior healthy control study to parallel the current study to facilitate comparisons of patterns of effects. Re-analysis of the healthy control data revealed that DCS had an opposite pattern of effects in healthy controls compared to patients with schizophrenia across the LTP and

learning measures of experience-dependent plasticity versus on baseline neural responsivity and working memory.

Thus, among healthy control participants, DCS did not significantly alter baseline amplitude of the C1, $t(63) = .762$, $p = .45$, $\eta^2_p = .009$, or P2, $t(63) = .00$, $p = .99$, $\eta^2_p = .00$, VEP components. However, DCS significantly enhanced plasticity of the VEP following HFvS in healthy controls. Thus, there was a significant effect of DCS on C1 potentiation across post-HFvS assessment blocks compared to Placebo, $F(1,63) = 5.38$, $p = .024$, $\eta^2_p = .079$. Similarly, DCS enhanced potentiation of P2 across post-HFvS blocks compared to Placebo, $F(1,63) = 4.46$, $p = .04$, $\eta^2_p = .066$. Finally, DCS also enhanced potentiation of C1-P2 peak-to-peak amplitude across post-HFvS blocks relative to Placebo, $F(1,63) = 7.59$, $p = .008$, $\eta^2_p = .107$. The effects of DCS on potentiation of C1, P2, and C1-P2 peak-to-peak amplitude following HFvS in healthy controls contrasts those in schizophrenia patients in which DCS enhanced baseline C1 amplitude without affecting potentiation of C1, P2, or C1-P2 peak-to-peak amplitude following HFvS (Supp. Fig. 1A-C).

Re-analyses of the first 240 trials of the WPT in healthy control participants confirmed enhanced WPT performance in healthy participants who received DCS across trial blocks, $F(1,62) = 6.04$, $p = .02$, $\eta^2_p = .089$, with no drug x block interaction, $F(2,124) = .19$, $p = .79$, $\eta^2_p = .003$ (Supp. Fig. 2). Similarly, on the IIT, there was a significant drug x block interaction for healthy control participants, $F(2,116) = 6.28$, $p = .004$, $\eta^2_p = .098$, due to the DCS group performing significantly better than Placebo participants during the first, $p = .038$, $\eta^2_p = .078$, and second blocks of 80 trials, $p = .041$, $\eta^2_p = .070$ (Supp. Fig. 3). Thus, DCS significantly enhanced acquisition of both learning tasks in healthy control participants; a pattern of findings

that contrasts those in schizophrenia patients in which DCS showed no effect on performance on the WPT or IIT.

In contrast to the beneficial effects of DCS on working memory in schizophrenia patients, in healthy control participants there was no effect of drug, $F(1,59) = .64$, $p = .43$, $\eta^2_p = .011$, nor interaction of drug x memory load during the *n*-back working memory task, $F(2,118) = .57$, $p = .54$, $\eta^2_p = .010$ (Supp. Fig. 4). All healthy participants performed above chance on the *n*-back, as defined above. The lack of effect of DCS on working memory in control participants therefore cannot be attributed to inclusion of healthy participants who did not understand the task or were unable to engage working memory during the task.

Thus, in healthy control participants, DCS significantly enhanced experience-dependent plasticity across the LTP and learning measures, without affecting baseline neural responsivity or working memory. This pattern of effects is in sharp contrast to those found in patients with schizophrenia, in which DCS enhanced baseline amplitude of the C1 early VEP component as well as working memory performance, without affecting plasticity on the EEG LTP task or learning on the WPT or IIT.

Given slight differences in study protocols and unmatched demographic characteristics between the healthy control and schizophrenia study samples, we do not present a direct statistical comparison of the effects of DCS in healthy control participants versus patients with schizophrenia. However, to facilitate comparison of the effects of DCS between the two populations, η^2_p effect sizes for baseline component amplitudes, working memory, and electrophysiological and learning measures of experience-dependent plasticity were converted to Cohen's d for each measure of interest and are summarized for each study population in Figure 16. Cohen's d was calculated to allow both effect size and direction of effect to be compared for

each measure. Among healthy controls, DCS showed a medium size effect across measures of experience-dependent plasticity, including C1 and P2 potentiation following HFvS, learning on the WPT across blocks, and learning during blocks 1 and 2 of the IIT. Conversely, among patients with schizophrenia, DCS showed a medium size effect on baseline C1 amplitude and 2-back working memory performance. Dissociation of benefits of DCS in healthy individuals versus in patients with schizophrenia is consistent with an emerging neurobiological framework that suggests that the mechanisms through which NMDAR signaling contributes to working memory and basic excitatory neurotransmission differs from those that contribute to experience-dependent plasticity. The dissociation of effects of DCS between the two populations also suggests that pro-cognitive effects of DCS may interact with baseline NMDAR neurotransmission and the integrity of intracellular signaling pathways to which NDMARs are linked.

Exploratory Analyses Comparing C1 Amplitude and 2-Back Performance in Schizophrenia Patients versus Placebo Healthy Controls

We undertook pairwise comparisons of C1 amplitude and 2-back working memory performance in placebo and DCS treated schizophrenia patients to placebo treated healthy controls to provide context for the effects of DCS in schizophrenia patients. While the LTP and *n*-back tasks were similar across the studies, they were not identical, and schizophrenia patients and healthy controls differed in demographic characteristics. Thus, these analyses are considered exploratory.

Exploratory independent samples t-tests comparing schizophrenia and healthy control participants on baseline C1 amplitude indicated that C1 amplitude was reduced in Placebo

schizophrenia patients relative to Placebo healthy controls, $t(51) = -2.31$, $p = .025$. Conversely, schizophrenia patients who received DCS showed similar C1 amplitude at baseline relative to Placebo healthy controls, $t(52) = .05$, $p = .96$, suggesting that DCS restored C1 amplitude in schizophrenia patients (Fig. 17A)

On the *n*-back working memory task, after excluding patients who performed at chance, Placebo schizophrenia patients showed significantly impaired performance during the 2-back condition relative to Placebo healthy controls, $t(43) = 3.56$, $p = .001$. Conversely, schizophrenia patients who received DCS performed similarly to Placebo healthy controls during the 2-back, $t(44) = 1.19$, $p = .24$, suggesting that DCS ameliorated working memory deficits in schizophrenia patients (Fig 17B).

The results of these exploratory analyses compliment the effect sizes reported above by suggesting that DCS normalized baseline C1 amplitude and performance in the 2-back condition of the working memory task in schizophrenia patients.

DISCUSSION

We carried out a randomized, double-blind DCS versus placebo study in patients with schizophrenia to test whether increasing signaling at the NMDAR using the partial NMDAR agonist DCS would ameliorate deficits in experience-dependent plasticity and working memory. We found that DCS increased baseline neural responsivity and working memory performance in patients with schizophrenia, without enhancing learning or electrophysiological measures of experience-dependent plasticity. Specifically, schizophrenia patients who received DCS showed augmented amplitude of the C1 VEP component across baseline and post-HFvS assessment blocks compared to patients who received placebo, as well as enhanced performance on the 2-

back condition of the *n*-back working memory task. Conversely, there were no differences between patients who received DCS and placebo in potentiation of C1, P2, or C1-P2 peak-to-peak amplitude following HFvS, nor in performance on the WPT and IIT incremental learning tasks. The pattern of DCS effects in schizophrenia patients is the opposite of those found in our prior study in healthy participants in which DCS enhanced potentiation of the VEP following HFvS and learning on the WPT and IIT, without significantly affecting baseline neural responsivity or working memory performance (Forsyth et al., 2015). There are thus two sets of intriguing dissociations in our results that provide insight into the role of NMDAR signaling on cognition: 1) in the current study, DCS enhanced working memory and baseline C1 but had no effect on learning on the WPT or IIT or on LTP in schizophrenia patients; and 2) in our prior study, DCS had no effect on working memory and baseline C1 but enhanced WPT and IIT learning performance and LTP in healthy controls.

DCS operates as a partial NMDAR agonist by increasing the open time and open probability of the NMDAR channel (Henderson et al., 1990; Dravid et al., 2010). Restored C1 amplitude and working memory performance in schizophrenia patients who received DCS is consistent with this mechanism and suggests that DCS ameliorated reductions in signaling across the NMDAR. Conversely, the lack of effect of DCS on experience-dependent plasticity in schizophrenia patients suggests a breakdown in the translation of increased electrical signaling across the NMDAR into the intracellular signal transduction cascades and structural changes at the synapse that support experience-dependent plasticity and normally follow activation of the NMDAR.

Effects of DCS on Baseline Neural Responsivity in Schizophrenia

Patients with schizophrenia who received DCS showed augmented C1 amplitude across baseline and post-HFvS assessments compared to patients who received placebo. The C1 component is an early pre-attentive evoked potential generated by neurons in primary visual cortex (Di Russo, Martinez, Sereno, Pitzalis, & Hillyard, 2002). Exploratory comparison of baseline C1 amplitude between schizophrenia and control participants who received placebo suggested that C1 was reduced in Placebo schizophrenia patients and that C1 amplitude was normalized in schizophrenia patients who received DCS. Reduced C1 in Placebo schizophrenia patients is consistent with prior findings of reduced amplitude of early VEP components in schizophrenia, including C1 (Schechter et al., 2005; Butler et al., 2007). To our knowledge, no studies have investigated potential mechanisms that could underlie early visual processing deficits in schizophrenia. However, it is plausible that such deficits could reflect reduced glutamate signaling or reduced responsivity of NMDARs or AMPARs in primary visual cortex neurons in schizophrenia. EEG signals are thought to primarily reflect excitatory postsynaptic potentials (EPSPs) at glutamatergic synapses in the cortex (Kirschstein & Kohling, 2009). Specifically, glutamate is released into the synaptic cleft following an action potential at excitatory synapses and binds to AMPA and NMDA receptors on the postsynaptic membrane, allowing Na^{+} and Ca^{2+} ions to cross the membrane. This influx of positive ions causes a positive potential at the postsynaptic membrane, which is thought to be detectable at the level of the scalp as EPSPs. EPSPs are therefore thought to be conveyed by both NMDARs and AMPARs. Our finding that DCS augmented C1 amplitude among schizophrenia patients in consistent with a role of NMDARs in the generation of visual evoked potentials. No other studies have assessed the effects of DCS on electrophysiological measures in patients with schizophrenia. However, one study using functional magnetic resonance imaging (fMRI) found that temporal lobe

activation was increased in schizophrenia patients who received DCS compared to placebo during a word production task (Yurgelun-Todd et al., 2005). This evidence of increased neural activation using fMRI following DCS compliments the current finding of increased C1 amplitude across VEP assessment blocks.

Interestingly, augmented C1 amplitude among DCS-treated schizophrenia patients contrasts with the results of our parallel study in healthy controls in which we found no difference in baseline C1 amplitude between participants who received DCS versus Placebo, and instead only found enhanced potentiation of C1 following HFvS in control participants who received DCS (Forsyth et al., 2015). The differential effect of augmenting NMDAR signaling using DCS on baseline C1 amplitude in schizophrenia patients compared to control participants could reflect lower glycine or D-serine availability or occupancy of the NMDAR co-agonist site among schizophrenia patients, allowing for greater effects of DCS on NMDAR transmission in schizophrenia patients relative to healthy controls. Alternatively, differential effect of DCS on C1 in schizophrenia patients could arise from increased expression of NMDAR glycine-binding sites in schizophrenia, similarly allowing for enhanced effects of DCS on NMDAR transmission. Indeed, evidence for both reduced availability of endogenous co-agonists for the NMDAR and increased number of NMDAR glycine-binding sites have been found in schizophrenia. Thus, patients with schizophrenia were found to have reduced D-serine levels and altered markers of D-serine metabolism (Hashimoto et al., 2005; Bendikov et al., 2007; Yamada et al., 2005; Madeira, Freitas, Vargas-Lopes, Wolosker & Panizzutti, 2008). Post-mortem studies of patients also found increased density of the NR1 subunit of the NMDAR to which glycine binds (Nudmamud-Thanoi & Reynolds, 2004; Kristiansen, Beneyto, Haroutunian, & Meador-Woodruff, 2006, Mueller, Haroutunian, David, & Meador-Woodruff, 2004), including an

increase in glycine binding sites in primary visual cortex (Ishimaru, Kurumaji & Toru, 1994; Dracheva et al., 2001). Although we cannot determine whether either of these mechanisms accounts for increased C1 amplitude in schizophrenia patients who received DCS, the differential effect of DCS on baseline C1 among patients compared to control participants is consistent with altered NMDAR-mediated transmission at visual cortex synapses in schizophrenia and suggests that increasing NMDAR neurotransmission using DCS partially ameliorated reductions in the early VEP response.

Effects of DCS on Working Memory in Schizophrenia

Schizophrenia patients who received DCS also showed enhanced performance during the 2-back condition of the *n*-back spatial working memory task. Spatial working memory depends on recurrent excitation in dlPFC microcircuits that involve glutamatergic pyramidal neurons with similar spatial tuning and GABAergic interneurons that inhibit neurons with dissimilar spatial tuning. A threshold of NMDAR activity is thought to be necessary to generate recurrent excitation to represent stimuli over delay periods. However, beyond this threshold, lateral inhibition from GABAergic interneurons and modulation from acetylcholine and dopamine is thought to sculpt network activity to refine the specifics of mental representation (Arnsten et al., 2012). Consistent with the role of NMDARs in generating the recurrent firing of dlPFC networks that is the "weakest link" of working memory function, NMDAR antagonists have been shown to suppress firing of dlPFC delay cells in monkeys performing spatial working memory tasks and thus to impair performance (Wang et al., 2013). Given convergent evidence that NMDAR signaling is impaired in schizophrenia, improved working memory in schizophrenia patients who

received DCS in the current study suggests that DCS ameliorated deficits in signaling at the NMDAR, possibly resulting in restored recurrent excitation in dlPFC working memory circuits.

While one prior study found beneficial effects of DCS on working memory in schizophrenia (Goff, Tsai, Manoach, & Coyle, 1995), several found no effects of DCS on working memory (Goff et al., 1999; Evins, Amico, Posever, Toker, & Goff, 2002; Goff et al., 2005; Goff et al., 2008; Buchanan et al., 2007; Cain et al., 2014; Duncan et al., 2004),. Differences in patient sample or DCS administration schedule may account for the differential effect of DCS in the current study. For example, the majority of prior studies used older and more chronic patient samples, used a lower dose of 50 mg DCS, and assessed working memory after 2-8 weeks of daily or weekly DCS dosing. Patients in the current study were relatively young and high-functioning, had relatively low symptom levels, and were administered a single dose of 100 mg DCS. It is unclear which of these differences might account for the benefits of DCS on working memory in the current study. However, it is notable that beneficial effects of DCS on the *n*-back only reached significance after patients who performed around or below chance were excluded from analyses. This suggests that positive effects of DCS were initially washed out by inclusion of patients who had difficulty understanding task instructions or engaging in the task. For prior studies that included more chronic and low-functioning patients with schizophrenia, this may have weakened power to detect beneficial effects of DCS. Additionally, multiple studies assessing the effects of DCS on cognition in rodents found that beneficial effects of DCS following a single dose did not persist with chronic dosing (Quartermain, Mower, Rafferty, Herting, & Lanthorn, 1994; Parnas, Weber, & Richardson, 2005; Mickley et al., 2012). This suggests that regulatory phenomena occur with prolonged DCS administration leading to desensitization of the NMDAR complex to effects of DCS; this could

also account for prior negative findings of DCS on working memory. Clarifying the effects of increasing NMDAR signaling following single dose DCS may be a more fruitful first step for probing the neurobiology of cognitive deficits in schizophrenia and identifying potential therapeutics than clinical trials involving chronic dosing of DCS. Taken together, our finding that increasing NMDAR signaling using acute DCS enhanced both baseline C1 amplitude and working memory performance in patients with schizophrenia is consistent with evidence that NMDAR signaling is impaired in schizophrenia, and suggests that enhancing NMDAR signaling may partially restore cognitive processes that are closely linked to changes in electrical signaling across the NMDAR.

Effects of DCS on Experience-Dependent Plasticity in Schizophrenia

In contrast to the effects of DCS on working memory in patients with schizophrenia, DCS showed no effects on electrophysiological or learning measures of experience-dependent plasticity. Potentiation of VEPs following HFvS is thought to reflect NMDAR-dependent LTP at visual cortex synapses (Clapp et al., 2006). Relatedly, the WPT and IIT are incremental learning tasks in which encoding of stimulus-outcome contingencies is thought to be mediated by NMDAR-dependent LTP at cortico-striatal synapses (Yin & Knowlton, 2006; Kreitzer & Malenka, 2008). Consistent with NMDAR-dependent mechanisms of plasticity on these measures, in our prior study among healthy control participants, increasing NMDAR signaling using DCS enhanced potentiation of the C1 and P2 VEP components following HFvS and enhanced performance on the WPT and IIT learning tasks. In the current study of schizophrenia patients, controlling for antipsychotic dose, sex, age, and IQ did not alter the effects of DCS, nor did excluding patients who continued to perform near or below chance by the last block of 80

trials for each learning task. Given that the current sample of schizophrenia patients were relatively high-functioning, had low levels of positive and negative symptoms, and that DCS improved working memory performance in the same patients, it is unlikely that confounding effects such as lack of motivation, inattention, or difficulty understanding the tasks accounts for this lack of effect of DCS on experience-dependent plasticity. To our knowledge, no other studies have directly tested the hypothesis that DCS would enhance experience-dependent plasticity in patients with schizophrenia. Several studies found minimal effects of DCS on brief neuropsychological tests of short-term verbal or spatial learning such as on the California Verbal Learning Test (Goff et al., 2005), the Hopkins Verbal Learning Test (Goff et al., 2008; Cain et al., 2014), the Auditory Verbal Learning Test (Buchanan et al., 2007) and the Brief Visuospatial Memory Test (Buchanan et al., 2007). However, a minority of studies found benefits of DCS in patients with schizophrenia on select learning measures. Thus, one study found reduced delusional severity in patients when DCS was combined with a first cognitive-behavioral therapy (CBT) training session to generate alternative explanations for neutral social scenarios (Gottlieb et al., 2011). An additional study found that DCS improved performance on an auditory discrimination task on which patients were trained over 8 weeks (Cain et al., 2014). We cannot rule out that patients would have showed benefits of DCS over a longer period of practice with stimuli and feedback on the WPT or IIT. However, it is notable that DCS enhanced C1 amplitude and working memory in the same patients and enhanced plasticity in healthy adults on the same tasks over the same period of practice with stimuli. The lack of effect of DCS in patients with schizophrenia on any measure of plasticity in the current study suggests that increased electrical signaling at the NMDAR may not have been translated into the intracellular signaling cascades and structural synaptic changes that underlie experience-dependent plasticity.

Abnormalities in the Broader NMDAR Complex in Schizophrenia May Explain the Lack of Effect of DCS on Experience-Dependent Plasticity

Recent developments in the neurobiology of schizophrenia implicating abnormalities in the broader glutamatergic postsynaptic density in which NMDARs are embedded may explain the weak effects of DCS on experience-dependent plasticity in the current study. Thus, glutamatergic synapses are localized to dendritic spines and are characterized by an electron-thick structure beneath the plasma membrane, called the postsynaptic density (PSD). PSDs are intracellularly organized multiprotein complexes comprised of several hundred proteins that physically link the membrane-bound NMDAR to kinases, phosphatases and downstream signaling proteins (Rao & Finkbeiner, 2007). Abundant scaffold proteins assemble and hold the complex together by binding to membrane-bound receptors, cell adhesion molecules, signaling enzymes, and actin cytoskeletal elements. Insertion or removal of AMPARs from synapses is thought to underlie the expression of changes in synaptic strength associated with LTP and LTD, respectively. Rises in intracellular Ca^{2+} mediated by NMDAR channel activation regulates these changes by triggering the intracellular signaling cascades that lead to protein synthesis and AMPAR trafficking to the membrane (see Fig.18 for summary depiction; Derkach, Oh, Guire, & Soderling, 2007). The expression of experience-dependent plasticity is therefore critically dependent on not only the integrity of the NMDAR but also the integrity of these intracellular signaling pathways and the broader machinery of the synapse.

Recent large scale genomic studies indicate that schizophrenia is associated with alleles both intrinsic to the NMDAR and alleles affecting the broader PSD in which NMDARS are embedded (Hall, Trent, Thomas, O'Donovan, & Owen, 2015). For example, a genome-wide

association study of over 36,000 schizophrenia patients and 100,000 controls found that schizophrenia was associated with common genetic variants involved in both NMDAR neurotransmission and the machinery of the broader PSD, including variants for GRIN2A which encodes the NR2A NMDAR subunit, SRR which encodes the enzyme that catalyzes L-serine to create the NMDAR co-agonist D-serine, NLGN4X which encodes neuroligins that are involved the formation of glutamatergic synapses, and CNKSR2 which encodes a scaffold protein involved in coupling signal transduction to cytoskeletal remodelling in the PSD (PGC-SCZ, 2014). Additional large scale genomic studies (i.e. 600-2500 patients) found that rare and de novo genetic mutations associated with schizophrenia disproportionately affected genes involved in the broader NMDAR signaling complex. Mutations were found in genes encoding the membrane-associated guanylate kinases (MAGUK) family of scaffold proteins involved in trafficking and clustering glutamate receptors at the PSD, the activity-regulated cytoskeleton-associated protein (ARC) which localizes to NMDAR-activated synaptic sites and is central to synaptic remodeling and long-term maintenance of synaptic changes, and those involved in actin filament bundle assembly, which is a dynamic process involved in regulating structural changes that support experience-dependent plasticity in dendrites (Fromer et al., 2014; Purcell et al., 2014; Kirov et al., 2012).

Consistent with genetic risk for schizophrenia involving mutations affecting the broader PSD, post-mortem brain studies indicate that both the NMDAR itself and the broader NMDAR-associated signaling complex are abnormal in schizophrenia. Post-mortem studies repeatedly found altered mRNA expression of various NMDAR subunits in schizophrenia, including the NR1, NR2A, NR2C and NR3A subunits (Sokolov, 1998; Beneyto & Meador-Woodruff, 2008; Mueller & Meador-Woodruff, 2004; Meador-Woodruff, Clinton, Beneyto, McCullumsmith,

2003; Dracheva et al., 2001). In addition, multiple NMDAR-associated proteins were found to have altered expression or phosphorylation in schizophrenia including PSD-95, a member of the MAGUK family of PSD scaffold proteins, NF-L, a PSD molecule that binds to the NMDAR and maintains the stability of NMDARs in the PSD by binding cytoskeletal actin filaments in dendritic spines, and SynGAP, a PSD protein that interacts with the NMDAR and regulates downstream signaling (Funk, Rumbaugh, Harotunian, McCullumsmith, & Meador-Woodruff, 2009; Ohnuma et al., 2000; Dracheva et al., 2001; Funk, McCullumsmith, Haroutunian, & Meador-Woodruff, 2012). Indeed, a recent unbiased proteomic study of the PSD revealed that 143 out of over 700 PSD proteins showed differential expression in schizophrenia, with NMDAR-interacting proteins showing the most notable alterations in schizophrenia compared to healthy brains (Focking et al., 2015).

Thus, accumulating genomic and post-mortem brain findings implicate broad alterations to the PSD in schizophrenia. If a breakdown in NMDAR signaling occurs in the broader cellular machinery that is coupled to NMDARs, increasing the probability of NMDAR channel opening and open time using the partial agonist DCS would be insufficient to ameliorate deficits in experience-dependent plasticity in schizophrenia. This may explain why DCS had limited effects on measures of experience-dependent plasticity in the current study of schizophrenia patients, despite the capacity of DCS to enhance experience-dependent plasticity using the same measures in healthy control participants, and the capacity of DCS to enhance baseline neural transmission and working memory performance in the same sample of schizophrenia patients.

Limitations

There are several limitations to the current study that should be noted. First, the 100 mg DCS dose could have been sub-optimal for augmenting experience-dependent plasticity in schizophrenia patients. The current dose of DCS improved working memory in the same patient sample and was selected to match that used in our parallel study of healthy participants in which DCS augmented experience-dependent plasticity. DCS at 100 mg has been shown to have beneficial effects on learning and cognition in other studies of healthy individuals (Nitsche et al., 2004; Kuriyama, Honma, Koyama, Kim, 2011), and in individuals with anxiety disorders (Wilhelm et al., 2008) and Alzheimer's disease (Tsai, Falk, Gunther, & Coyle, 1999), as well as on negative symptoms in patients with schizophrenia (van Berckel et al., 1996). Nevertheless, we cannot rule out that a different dose of DCS would have yielded different effects on experience-dependent plasticity. Second, the majority of patients in the current study were on psychotropic medication, which could potentially interact with effects of DCS on NMDAR signaling. Most prior studies of DCS in schizophrenia involved patients who were taking anti-psychotics, including those showing some beneficial effects of DCS on learning or negative symptoms (Cain et al., 2014; Goff et al., 2008; Gottlieb et al., 2011). Further, controlling for antipsychotic dose did not alter the effects of DCS in the current study. Given that anti-depressant medication has been suggested to alter the efficacy of DCS (Andersson et al., 2015), we also re-analyzed the data excluding patients on anti-depressants; no change in effects of DCS were detected. However, it is possible that different effects of DCS on experience-dependent plasticity would have been found on a sample of un-medicated patients. Third, due to programming error, there were slight variations in the number of trials and probability structure of cue combinations that patients were presented with on the WPT. However, the number of trials per cue combination was similar between schizophrenia patients who received DCS versus placebo. While this

additional variance could have obscured our ability to detect effects of DCS on the WPT, it is notable that when healthy control data was restricted to the first 240 trials to parallel that in the current study, beneficial effects of DCS were still evident. Given that DCS did not enhance performance on the IIT and or potentiation following HFvS on the EEG LTP task, it is unlikely that the variation in cue combination trials on the WPT accounts for the lack of effect of DCS on this measure. Finally, it is possible that beneficial effects of DCS might have emerged from a larger study of patients or if cognitive training had occurred over a more extended period of time. We assessed effects of DCS on the VEP response across approximately two hours in the current study, and learning tasks each involved 240 trials. The number of trials per cognitive task was selected to balance potential fatigue in patients while ensuring that testing covered the period of time in which control participants showed the largest effects of DCS on incremental learning. We cannot rule out the possibility that effects of DCS would have emerged later in training on the incremental learning tasks. However, overall, these results suggest that effects of DCS on experience-dependent plasticity are much weaker in patients with schizophrenia than among healthy control participants.

Summary and Future Directions

In summary, we found that acute DCS administration enhanced neural responsivity and working memory in patients with schizophrenia without enhancing learning or electrophysiological measures of experience-dependent plasticity. Thus, patients receiving DCS showed enhanced amplitude of the C1 VEP component across assessments and improved performance on the 2-back condition of the working memory task relative to patients who received placebo. These results need to be replicated in a larger sample of patients and effects of

acute and chronic DCS on broader aspects of symptomatology and cognition need to be investigated before any practical applications of DCS can be realized. Nevertheless, the current findings offer encouraging support for using NMDAR agonists to target deficits in working memory and baseline neural transmission in patients with schizophrenia. Conversely, there were no differences between schizophrenia patients who received DCS versus Placebo on VEP potentiation following HFvS or on performance on the WPT or IIT learning tasks. The lack of effect of DCS on experience-dependent plasticity in schizophrenia contrasts with our prior findings in healthy participants in which DCS enhanced potentiation of the VEP following HFvS and enhanced acquisition the WPT and IIT incremental learning tasks. As a partial agonist at the glycine co-agonist site of the NMDAR, DCS augments NMDAR signaling by increasing channel open time and open probability. Sufficient NMDAR channel opening is necessary to generate the recurrent excitation in dlPFC circuits that represents stimuli over delay periods during working memory. However, in other regions of healthy brains, influx of Ca^{2+} ions following NMDAR channel opening also triggers intracellular signaling cascades that lead to protein synthesis, AMPAR insertion into the membrane, and ultimately increased synaptic strength between neurons encoding important inputs and outputs. The current dissociation of effects of DCS on working memory versus experience-dependent plasticity in patients with schizophrenia suggests a breakdown in the cellular machinery that allows increased NMDAR channel opening to be translated into increases in synaptic strength. This is consistent with growing evidence from genomic and post-mortem brain studies indicating that the integrity of the broader PSD complex in which NDMARs are embedded is compromised in schizophrenia. Interventions that capitalize on compensatory mechanisms that are intact in schizophrenia may offer a more effective route for countering deficits in experience-dependent plasticity in schizophrenia. Alternatively, given

patient-to-patient heterogeneity in the NMDAR-associated proteins affected by risk alleles in schizophrenia, identifying protein pathways that compromise the integrity of the NMDAR signaling complex on an individual patient basis and using patient-specific interventions to restore these pathways may offer a powerful route for intervention. The current results highlight the importance of considering how different biophysical properties of the NMDAR contribute to cognitive deficits in schizophrenia in distinct ways and suggest that effective therapeutics for cognitive deficits may require greater specificity of targets based on a clearer understanding of the neurobiology of individual cognitive deficits in schizophrenia.

Table 1. Probability Structure of the Weather Prediction Task. Probability of sun and mean number of trials out of 240 total trials on the Weather Prediction Task for each cue combination for the Placebo and DCS group. For each cue combination, each card could be present (1) or absent (0). Grey rows indicate cue combinations that predicted sun and rain equally and were therefore excluded from analyses.

	Cue				Probability of Sun || Combination		Mean Trials || Combination	
Combination	1	2	3	4	Placebo (min-max)	DCS (min-max)	Placebo (min-max)	DCS (min-max)
1	1	0	0	0	0.862 (0.806-0.947)	0.867 (0.794-0.919)	35.789 (29-44)	33.696 (28-40)
2	0	0	1	0	0.312 (0.200-0.429)	0.321 (0.100-0.417)	14.421 (10-19)	14.174 (10-19)
3	1	0	1	0	0.745 (0.588-0.941)	0.726 (0.652-0.824)	18.895 (15-22)	19.217 (14-24)
4	0	1	0	0	0.681 (0.563-0.769)	0.669 (0.563-0.889)	14.737 (11-20)	15.000 (9-19)
5	1	1	0	0	0.748 (0.632-0.850)	0.733 (0.625-0.875)	19.211 (13-23)	18.696 (14-22)
6	0	1	1	0	0.498 (0.222-0.667)	0.468 (0.143-0.778)	8.632 (3-11)	9.957 (6-20)
7	1	1	1	0	0.499 (0.286-0.750)	0.480 (0.250-0.667)	9.526 (7-14)	10.435 (8-14)
8	0	0	0	1	0.126 (0.061-0.195)	0.120 (0.056-0.176)	38.789 (33-46)	37.391 (27-45)
9	1	0	0	1	0.520 (0.250-0.667)	0.504 (0.222-0.875)	9.737 (8-13)	9.087 (5-11)
10	0	0	1	1	0.156 (0.063-0.241)	0.148 (0.086-0.206)	31.684 (26-39)	32.870 (24-39)
11	1	0	1	1	1.00 (1.00-1.00)	1.00 (1.00-1.00)	4.947 (1-7)	4.913 (1-7)
12	0	1	0	1	0.460 (0.200- 0.667)	0.477 (0.273-0.833)	11.000 (6-19)	10.261 (6-20)
13	1	1	0	1	0.484 (0.267-0.600)	0.528 (0.167-0.778)	10.684 (7-17)	9.609 (6-14)
14	0	1	1	1	0.310 (0.182-0.417)	0.339 (0.167-0.500)	13.632 (10-18)	14.696 (11-20)

Table 2. Demographic and Clinical Characteristics of Placebo and DCS Groups.

	Placebo (*n* = 21)	DCS (*n* = 24)
Age (*SD*)	28.14 (6.62)	26.88 (6.58)
Sex	5 F/16 M	3 F/21 M
WASI (*SD*)	103.95 (13.64)	101.79 (16.74)
BPRS Thinking Disturbance (*SD*)	5.81 (3.40)	5.21 (2.98)
BPRS Withdrawal-Retardation (*SD*)	6.14 (3.86)	6.76 (3.50)
BPRS Total (*SD*)	40.52 (10.87)	40.75 (12.80)
Chlorpromazine Equivalence (mg)	229.89 (133.11)	224.05 (170.74)
Antidepressant (*n*)	5	8
Antipsychotic-free (*n*)	3	3
Schizophrenia (*n*)	16	17
Schizoaffective (*n*)	3	3
Schizophreniform (*n*)	2	4

Figure 1. LTP Task. (A) Standard circle black and white checkerboard stimulus presented at .83 Hz during visual evoked potential (VEP) assessment blocks and at ~8.87 Hz during high-frequency visual stimulation (HFvS). (B) Time course of the long-term potentiation (LTP) paradigm.

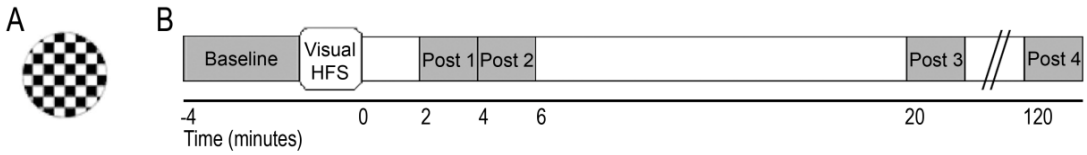


Figure 2. Weather Prediction Task. Two example Weather Prediction Task (WPT) trials.

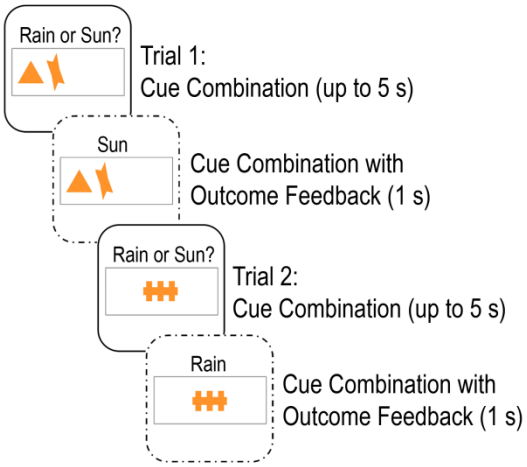


Figure 3. Example Information Integration Categories (reproduced from Spiering & Ashby, 2008). Example categories of circular sine-wave gratings for an information–integration category-learning task. The diagonal line is the category boundary.

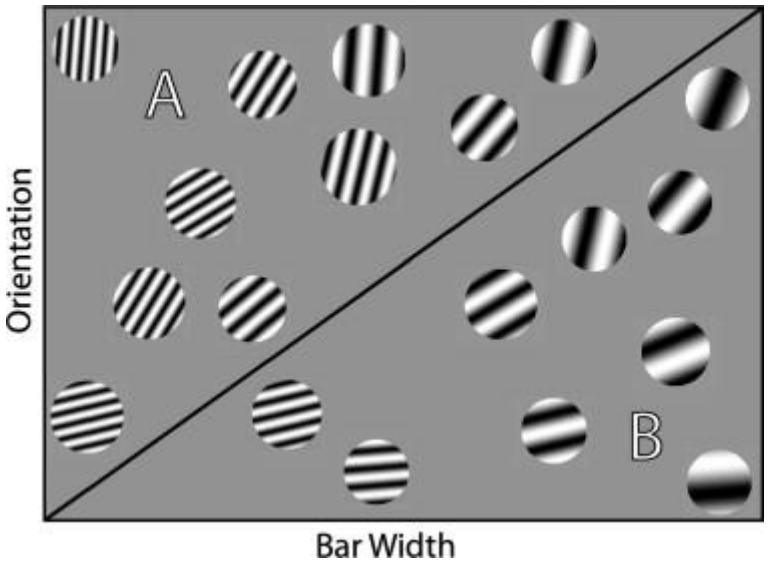


Figure 4. Information Integration Task and *N*-back Task. (A) Two example Information Integration task (IIT) trials and (B) two example trials for the 1-back condition of the *n*-back task. The IIT and *n*-back task were identical in stimuli, trial structure, and feedback such that the only difference participants experienced was whether they were asked to learn about the stimuli (i.e. for the IIT) or recall whether stimuli were in the same location on the screen as recently shown stimuli (i.e. for the *n*-back).

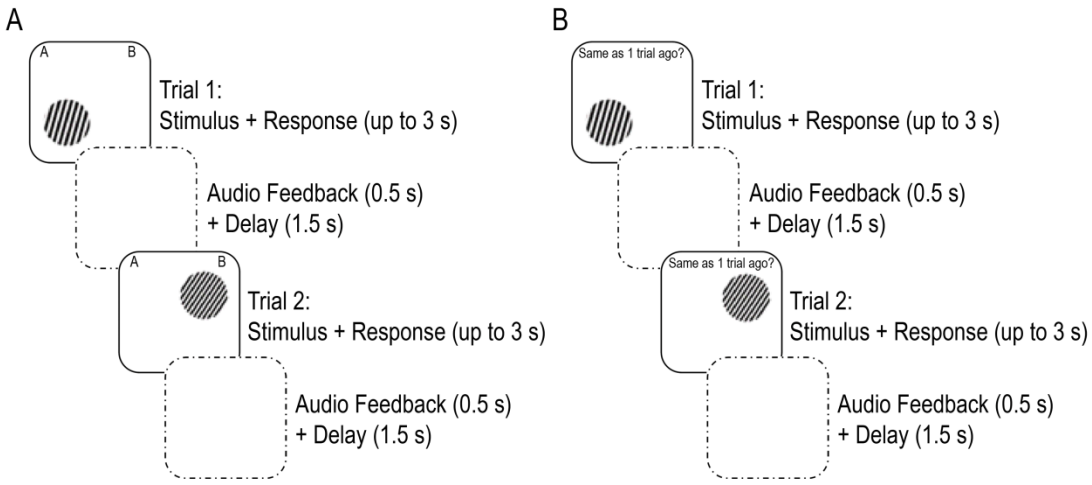


Figure 5. LTP Task Visual Evoked Potentials. (A) Grand average visual evoked potentials (VEPs) elicited by the standard checkerboard stimulus in Oz for Placebo (left panel) and DCS (right panel) schizophrenia patients across VEP assessment blocks. The VEP included a prominent negative component, C1, as well as a positive component, P2. (B) Scalp topography of C1 and P2 for Placebo (top panel) and DCS (bottom panel) patients across VEP assessment blocks.

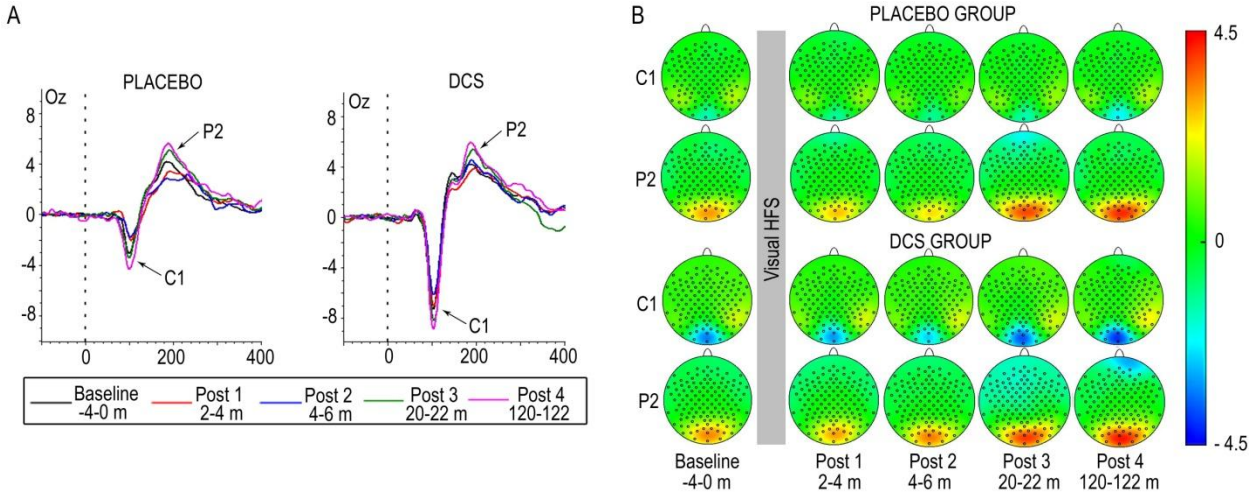


Figure 6. C1 Amplitude. Mean ± SE C1 amplitude across VEP assessment blocks for schizophrenia patients who received Placebo or DCS. *C1 amplitude was enhanced across baseline and post-HFvS assessment blocks in patients who received DCS compared to patients who received Placebo, $p = .03$.

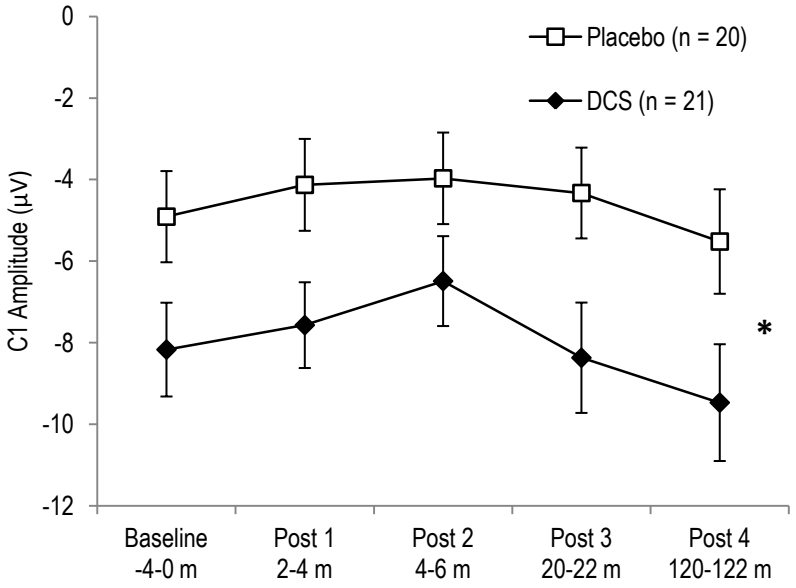


Figure 7. P2 Amplitude. Mean ± SE P2 amplitude across VEP assessment blocks for schizophrenia patients who received Placebo or DCS.

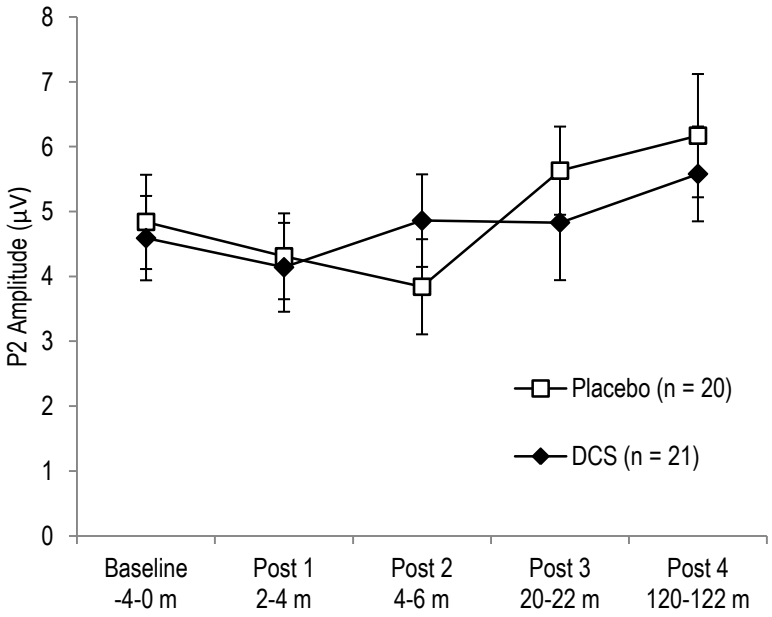


Figure 8. C1-P2 Peak-to-Peak Amplitude. Mean ± SE C1-P2 peak-to-peak amplitude across VEP assessment blocks for schizophrenia patients who received Placebo or DCS.

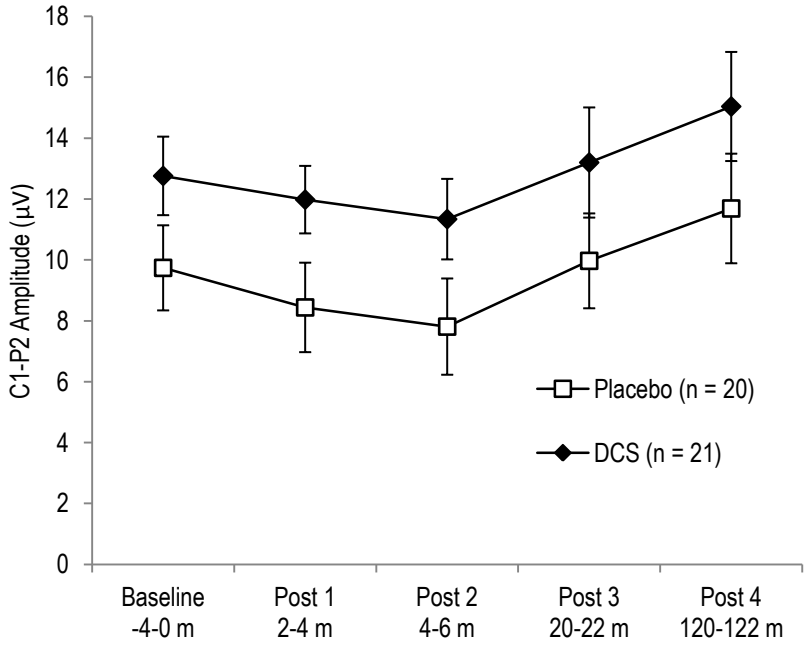


Figure 9. C1 Plasticity. Mean ± SE C1 amplitude change from baseline following HFvS for schizophrenia patients who received Placebo or DCS.

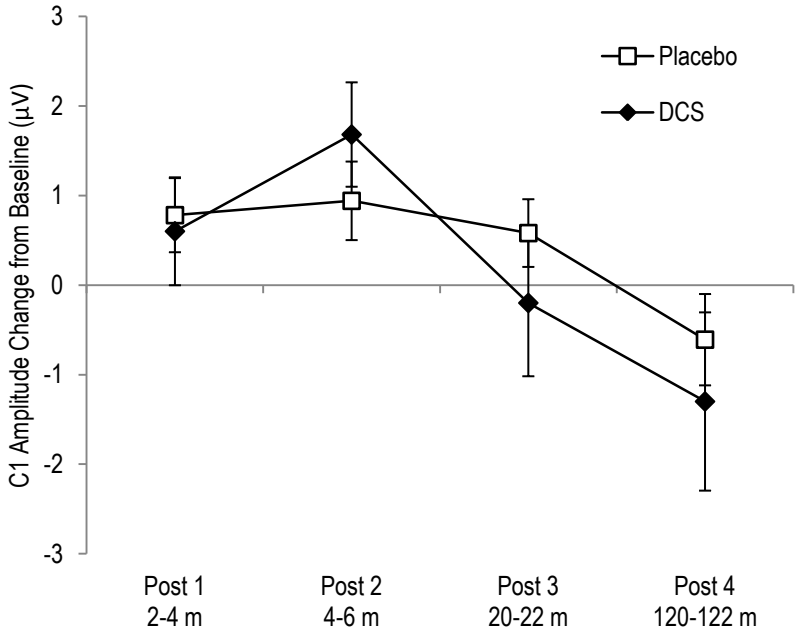


Figure 10. P2 Plasticity. Mean ± SE P2 amplitude change from baseline following HFvS for schizophrenia patients who received Placebo or DCS.

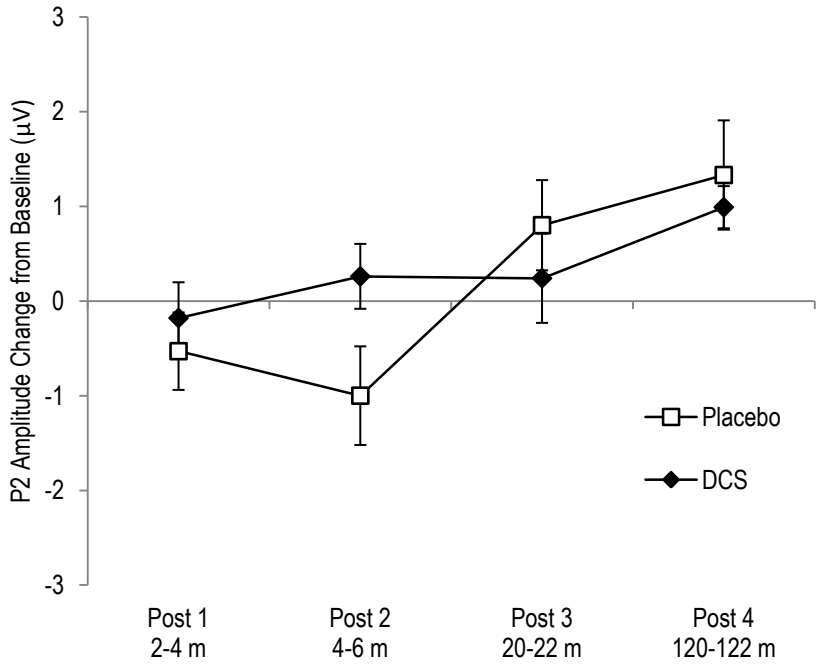


Figure 11. C1-P2 Plasticity. Mean ± SE C1-P2 peak-to-peak amplitude change from baseline following HFvS for schizophrenia patients who received Placebo or DCS.

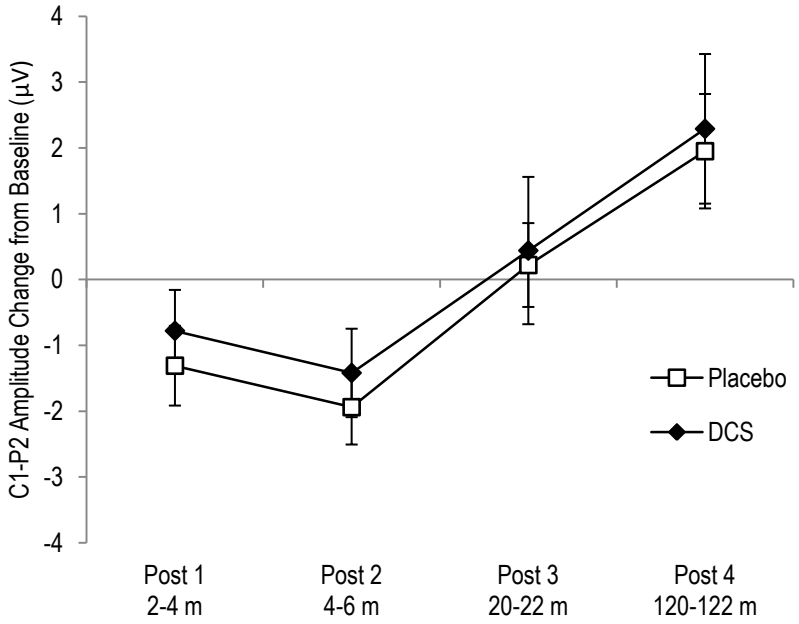


Figure 12. Weather Prediction Task Accuracy. Mean ± SE percent correct responses per 80-trial blocks of the Weather Prediction Task (WPT) for schizophrenia patients who received Placebo or DCS.

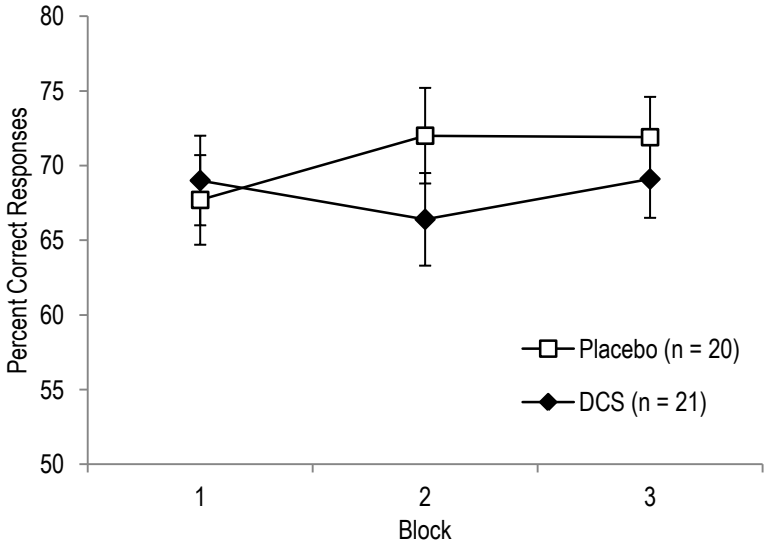


Figure 13. Information Integration Task Accuracy. Mean ± SE percent correct responses per 80-trial blocks of the Information Integration Task (IIT) for schizophrenia patients show received Placebo or DCS.

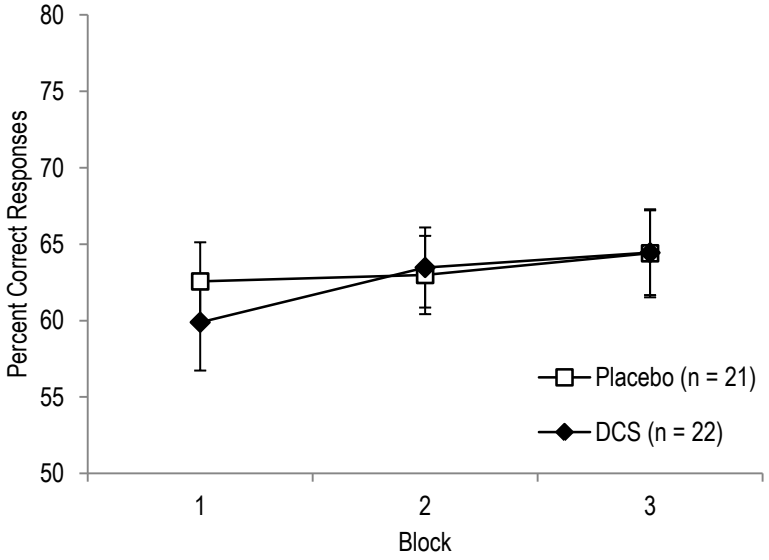


Figure 14. *N*-Back Task Accuracy. Mean ± SE percent correct responses per 80-trial blocks for the 0-back (0B), 1-back (1B), and 2-back (2B) conditions for schizophrenia patients who performed above chance and received Placebo or DCS. *Patients who received DCS performed significantly better than patients who received Placebo during the 2-back condition. Inset figure presents mean ± SE percent correct responses when all schizophrenia patients were included.

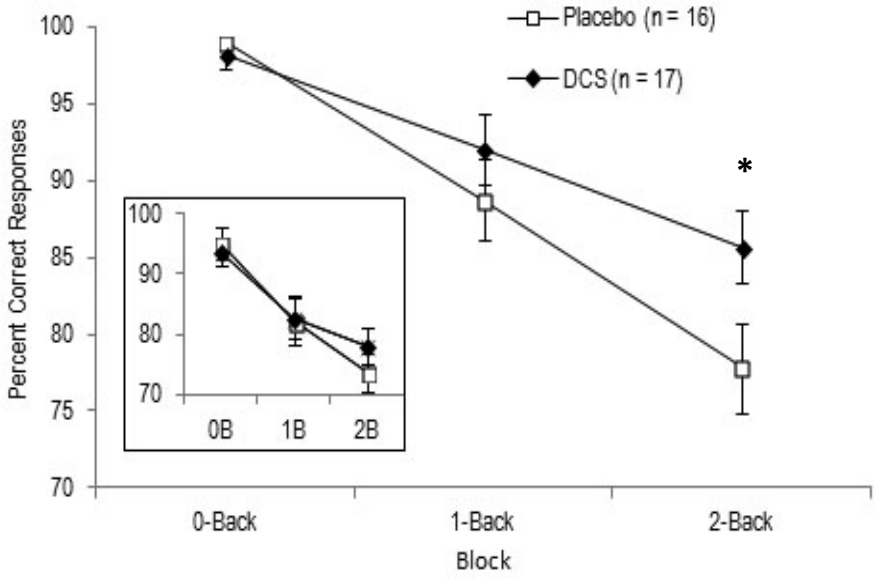
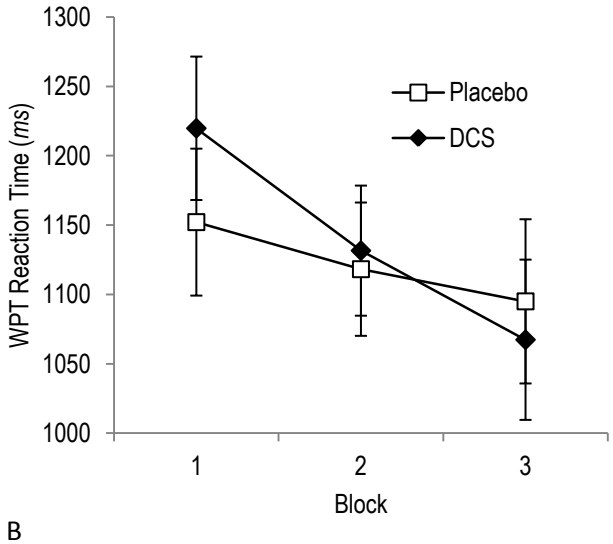
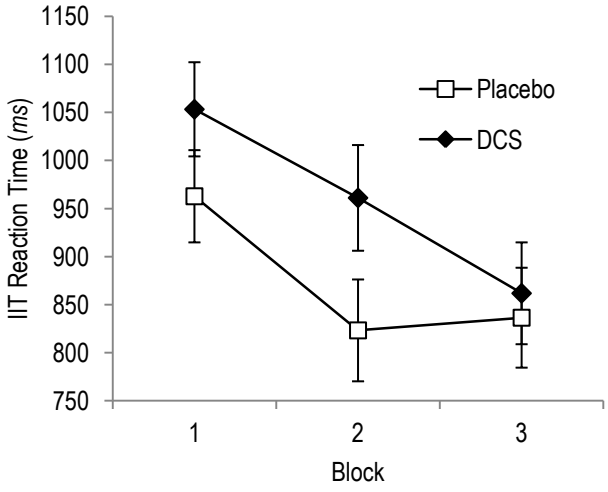


Figure 15. Cognitive Task Reaction Times. Mean ± SE reaction time per 80-trial blocks for schizophrenia patients who received Placebo and DCS for the (A) Weather Prediction Task (WPT), (B) Information Integration Task (IIT), and (C) *n*-back task.

A



B



C

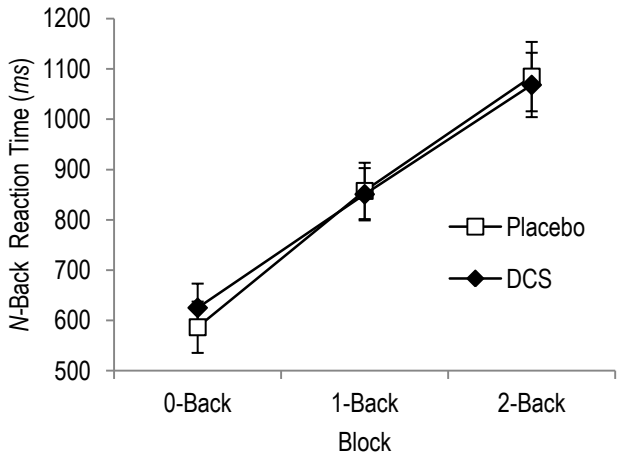

1200
1100
1000
900
800
700
600
500
N-Back Reaction Time (ms)
Placebo
DCS
0-Back
1-Back
2-Back
Block

Figure 16. Effect Sizes by Study Population. Cohen's d effect size with 95% confidence interval for effect of DCS in schizophrenia patients (SZ) and healthy control (HC) participants on each outcome measure. Effect sizes for 1-back and 2-back performance are shown for participants who performance above chance. *Significant effect of DCS in SZ. †Significant effect of DCS in HC.

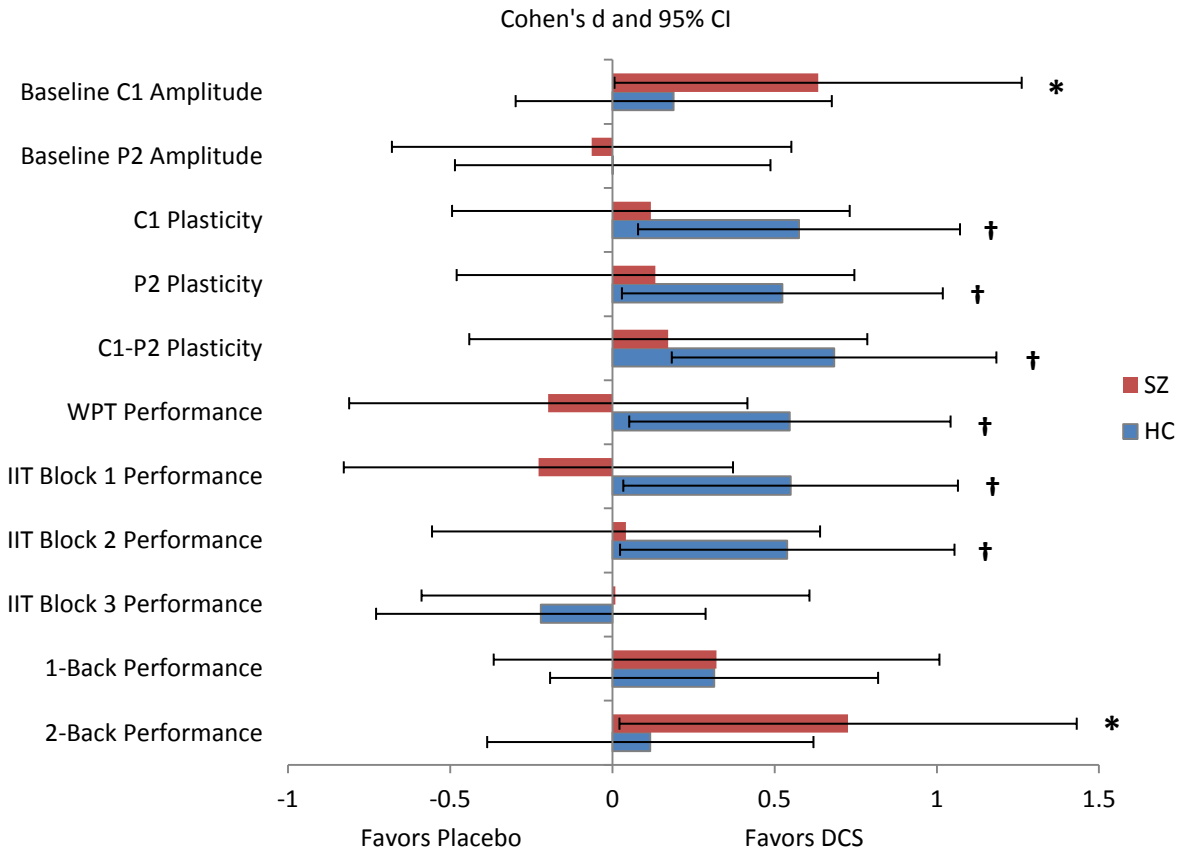


Figure 17. Schizophrenia Comparisons to Healthy Controls on C1 and *N*-Back. (A) Baseline C1 Amplitude for healthy control (HC) participants who received Placebo and schizophrenia patients (SZ) who received Placebo and DCS. *SZ-Placebo showed significantly reduced C1 amplitude relative to HC-Placebo, $p = .025$ and SZ-DCS, $p = .049$. (B) Percent correct responses on the 2-back condition of the *n*-back task among healthy control (HC) participants who received Placebo and schizophrenia patients (SZ) who received Placebo and DCS. *SZ-Placebo showed impaired 2-back performance relative to HC-Placebo, $p = .001$, and SZ-DCS, $p = .045$.

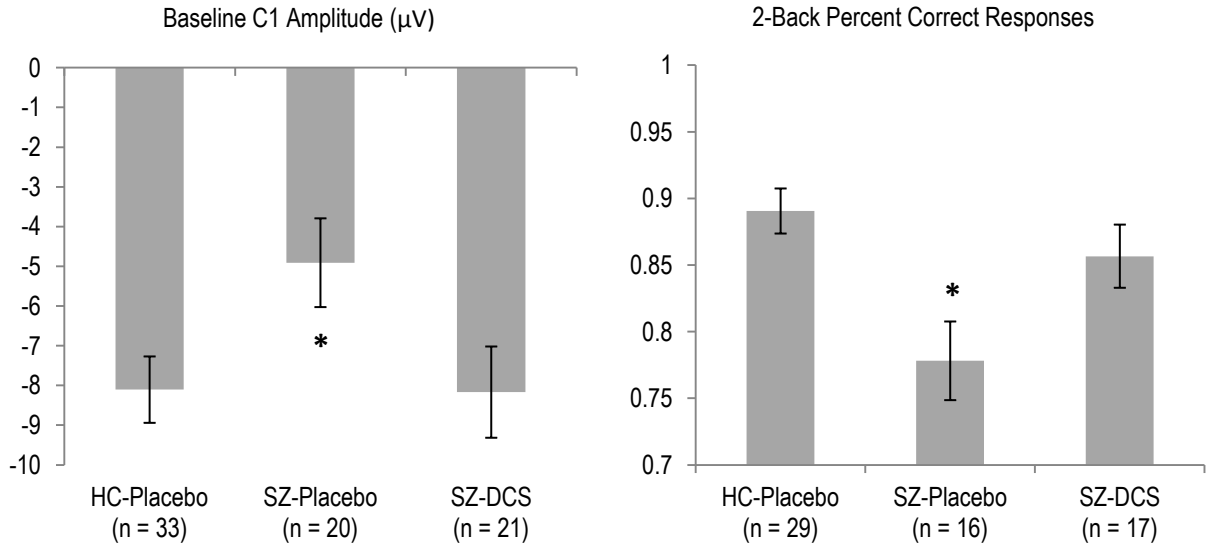
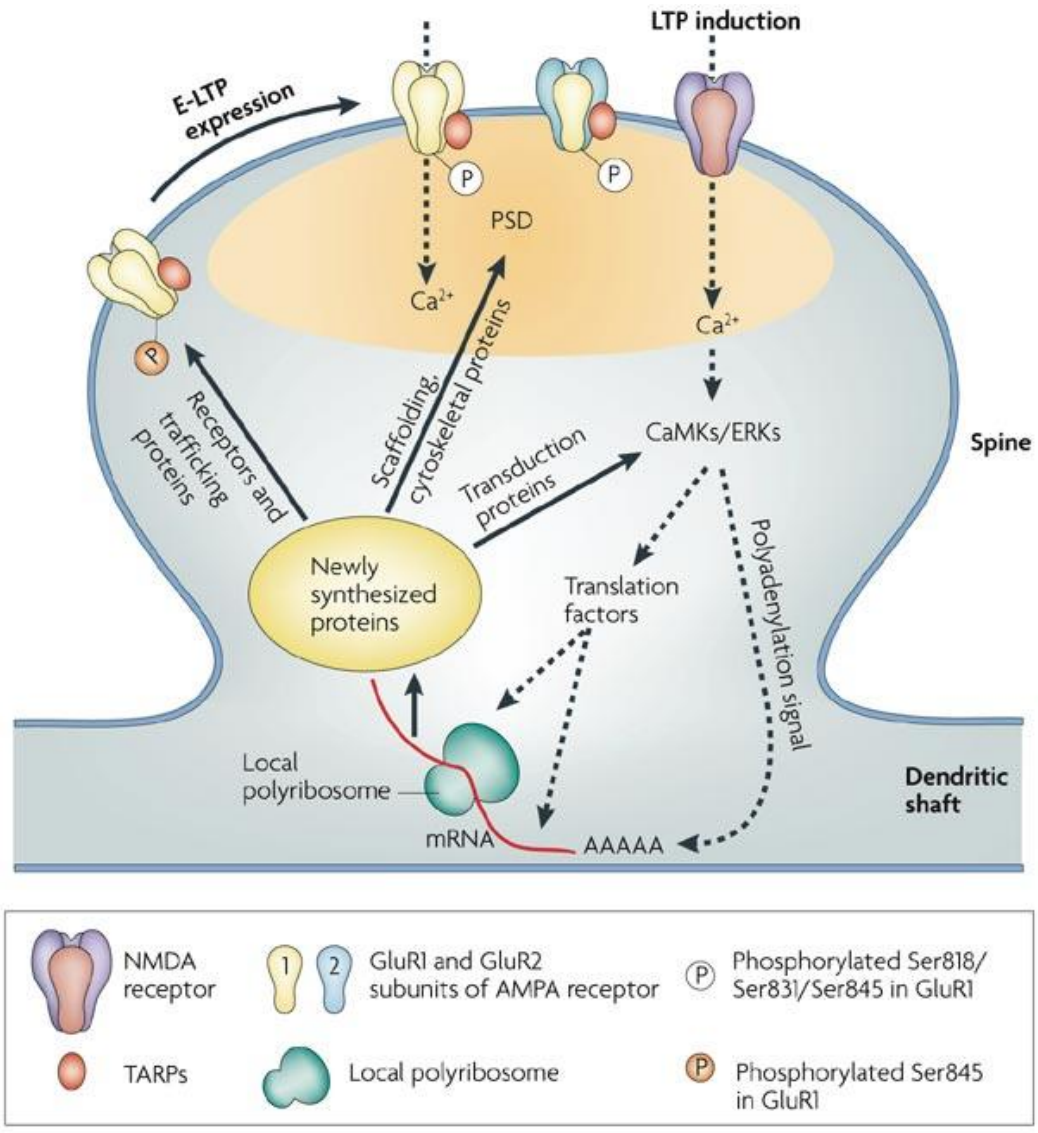


Figure 18. Mechanisms of Experience-Dependent Plasticity (reproduced from Derkach et al., 2007). Stimulation of synaptic NMDA (N-methyl-D-aspartate) receptors (NMDARs) (for example, long-term potentiation (LTP) induction) promotes Ca2+ influx that activates calcium/calmodulin-dependent protein kinases (CaMKs) and extracellular signal-related kinases (ERKs) in dendritic spines. These kinases phosphorylate and activate translation factors (for example, eIF4E, 4E-BP1 and cytoplasmic polyadenylation element binding protein, CPEB) that are required for the stabilization of local mRNAs through their polyadenylation (for example, CaMKII mRNA), and to initiate translation of mRNAs that have been selectively transported into the dendrites and/or spines. This local protein synthesis provides a feedforward mechanism to increase receptor numbers, receptor trafficking, levels of scaffolding and cytoskeleton proteins that promote surface expression, and lateral diffusion and stabilization of AMPA (-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-type glutamate receptors (AMPARs) at potentiated synapses. E-LTP, early phase LTP; PSD, postsynaptic density; TARPs, transmembrane AMPAR regulatory proteins.



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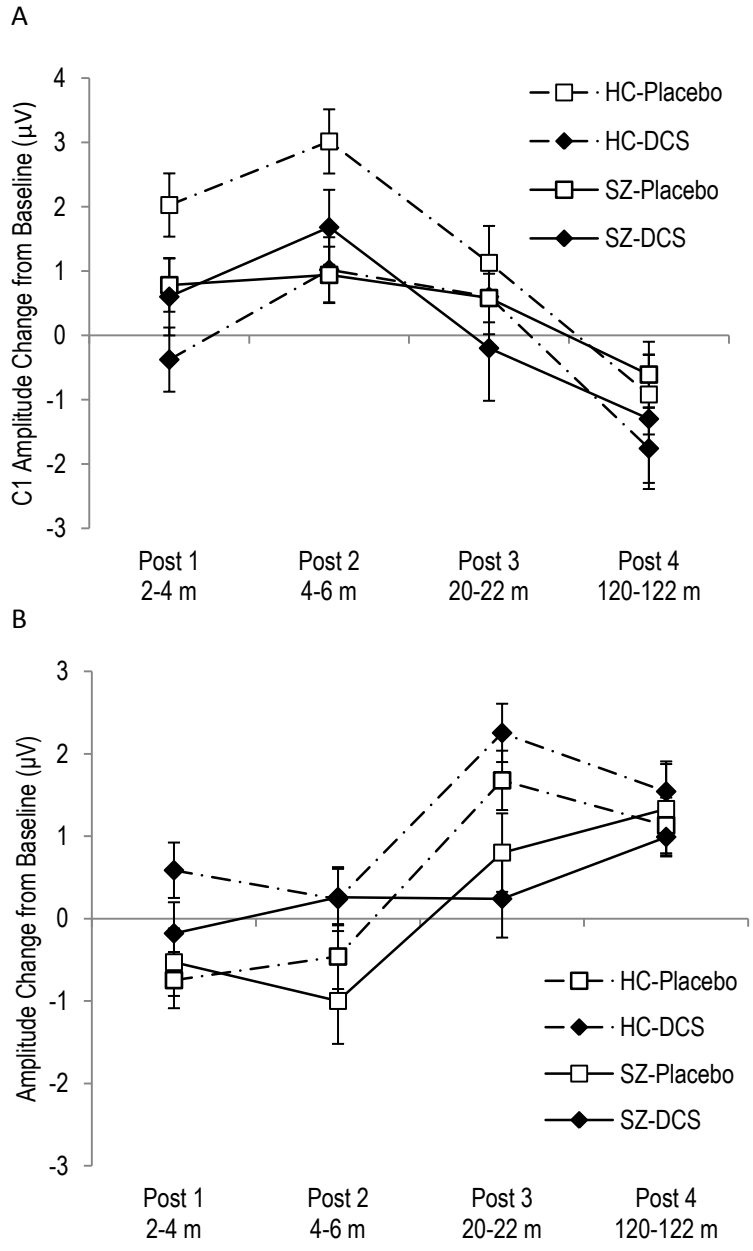
Appendix A

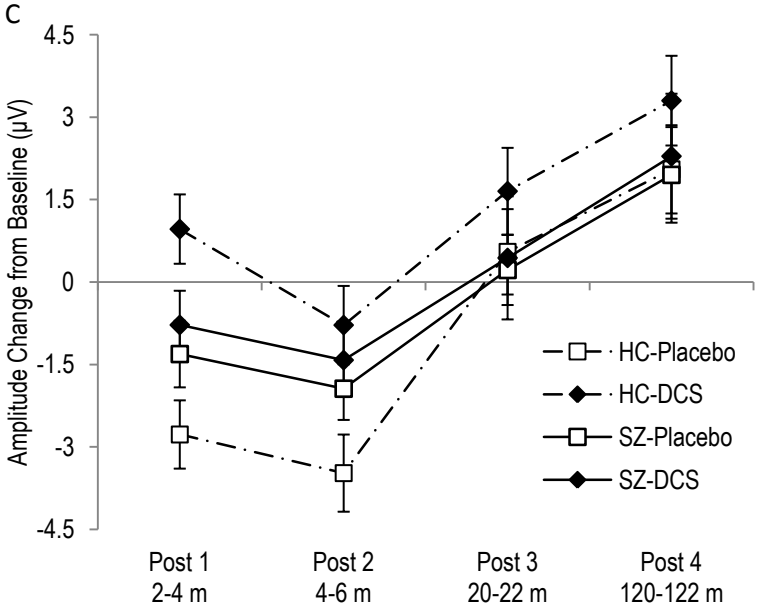
Supplementary Table 1. Demographic characteristics of Healthy Control participants who received Placebo and DCS.

	n	Age (*SD*)	Sex	WASI (*SD*)
Placebo	33	20.55 (2.41)	19 F/14 M	120.42 (9.33)
DCS	32	20.59 (2.69)	18 F/14 M	120.78 (8.23)

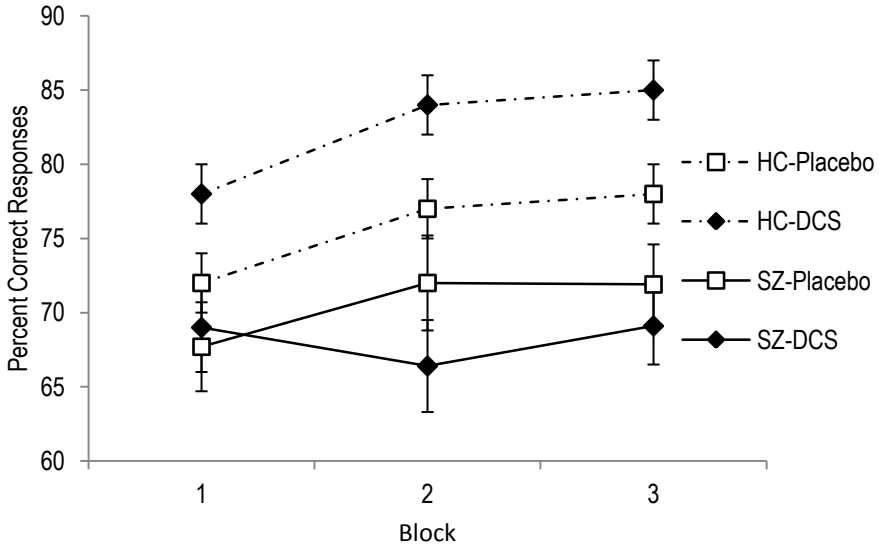
Supplementary Figure 1. Mean ± SE (A) C1, (B) P2, and (C) C1-P2 peak-to-peak amplitude change from baseline for Healthy Control (HC) and Schizophrenia patients (SZ) who received Placebo or DCS.



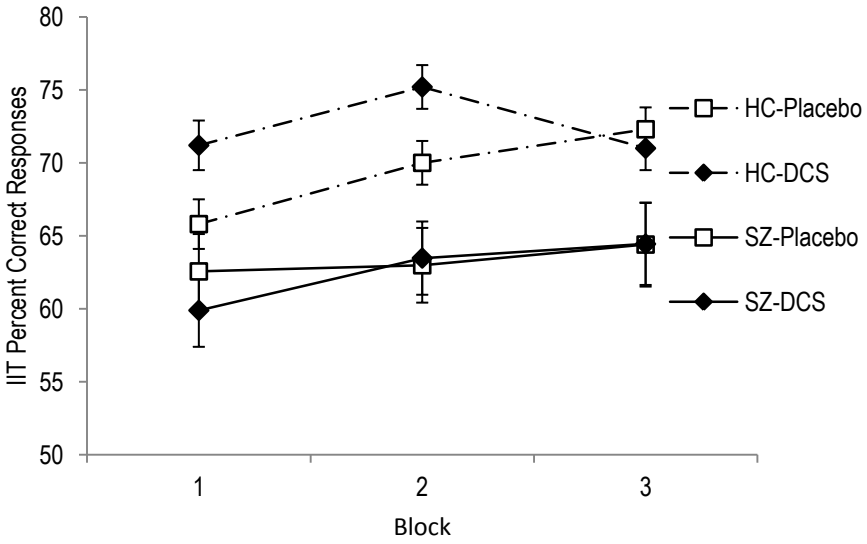
C


4.5
3
1.5
0
-1.5
-3
-4.5
Amplitude Change from Baseline (μV)
HC-Placebo
HC-DCS
SZ-Placebo
SZ-DCS
Post 1
2-4 m
Post 2
4-6 m
Post 3
20-22 m
Post 4
120-122 m

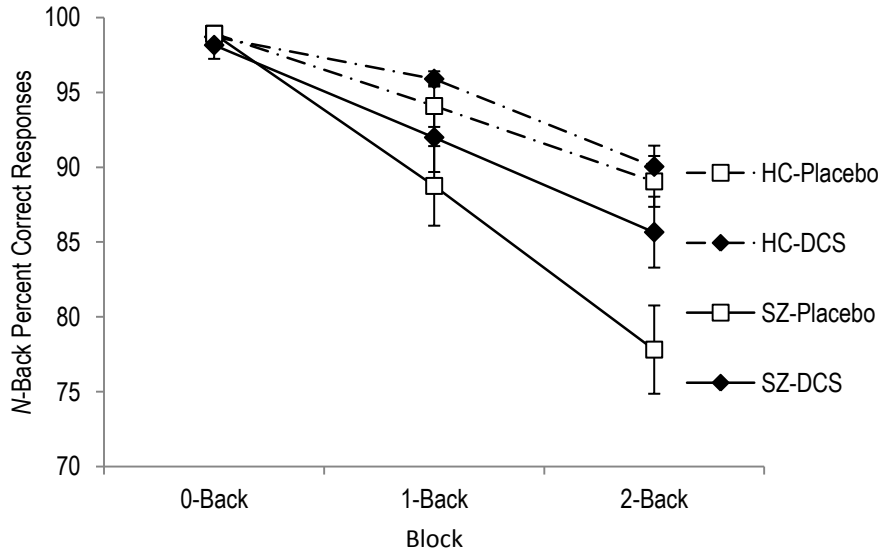
Supplementary Figure 2. Mean ± SE percent correct responses per 80-trial blocks of the Weather Prediction Task (WPT) for Healthy Control (HC) and Schizophrenia patients (SZ) who received Placebo or DCS.



Supplementary Figure 3. Mean ± SE percent correct responses per 80-trial blocks of the Information Integration Task (IIT) for Healthy Control (HC) and Schizophrenia patients (SZ) who received Placebo or DCS.



Supplementary Figure 4. Mean ± SE percent correct responses per 80-trial blocks for the 0-back, 1-back, and 2-back conditions of the *n*-back task for Healthy Control (HC) and Schizophrenia patients (SZ) who received Placebo or DCS.



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