Availability of *N*-Methyl-D-Aspartate Receptor Coagonists Affects Cocaine-Induced Conditioned Place Preference and Locomotor Sensitization: Implications for Comorbid Schizophrenia and Substance Abuse

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ABSTRACT

Schizophrenia is associated with high prevalence of substance abuse. Recent research suggests that dysregulation of *N*-methyl-D-aspartate receptor (NMDAR) function may play a role in the pathophysiology of both schizophrenia and drug addiction, and thus, may account for this high comorbidity. Our laboratory has developed two transgenic mouse lines that exhibit contrasting NMDAR activity based on the availability of the glycine modulatory site (GMS) agonists D-serine and glycine. Glycine transporter 1 knockdowns (GlyT1^{+/-}) exhibit NMDAR hyperfunction, whereas serine racemase knockouts (SR^{-/-}) exhibit NMDAR hypofunction. We characterized the behavior of these lines in a cocaine-induced (20 mg/kg) conditioned place preference (CPP) and locomotor sensitization paradigm. Compared with wild-type mice, GlyT1^{+/-} mice displayed hastened extinction of CPP and robust cocaine-

induced reinstatement. SR^{-/-} mice appeared to immediately “forget” the learned preference, because they did not exhibit cocaine-induced reinstatement and also displayed attenuated locomotor sensitization. Treatment of GlyT1^{+/-} mice with gavestinel (10 mg/kg on day 1; 5 mg/kg on days 2–17), a GMS antagonist, attenuated cocaine-induced CPP and caused them to immediately “forget” the learned preference. Treatment of SR^{-/-} mice with D-serine (300 mg/kg on day 1; 150 mg/kg on days 2–17) to normalize brain levels caused them to avoid the cocaine-paired side of the chamber during extinction. These results highlight NMDAR dysfunction as a possible neural mechanism underlying comorbid schizophrenia and substance abuse. Also, these findings suggest drugs that directly or indirectly activate the NMDAR GMS could be an effective treatment of cocaine abuse.

Introduction

Schizophrenia is a debilitating psychiatric illness that affects 1% of the population worldwide (Perälä et al., 2007). The disease exhibits high heritability due to complex genetics, in which multiple risk genes of modest effect interact with environmental factors to cause the disorder. The results of postmortem and pharmacologic studies have led to the hypothesis that a core pathophysiologic feature of schizophrenia involves hypofunction of *N*-methyl-D-aspartate receptors (NMDARs; Javitt and Zukin, 1991; Coyle, 1996; Coyle et al., 2002; Javitt, 2007). A recent genome-wide association study involving 38,000 subjects and over 100,000 controls revealed 108 gene loci that achieved genome-wide significance (Ripke et al., 2014). Notably, over a dozen of these risk genes directly interact with the NMDAR or are downstream mediators of its activity, including serine racemase (SR), which synthesizes the

forebrain agonist for the NMDAR glycine modulatory site (GMS), D-serine. In addition, we have previously demonstrated that SR null mutant mice exhibit brain structural, neurochemical, and cognitive abnormalities closely resembling those described in schizophrenia (Balu et al., 2013; Puhl et al., 2014). Restoration of brain D-serine levels through exogenous treatment reverses these deficits (Balu et al., 2013).

Substance abuse disorders are three to five times more prevalent in patients with schizophrenia compared to healthy controls (Regier et al., 1990; Kavanagh et al., 2002; Coyle, 2006). That increased susceptibility comprises all classes of abused substances (Cantor-Graae et al., 2001; Green, 2007) and is associated with poor schizophrenia treatment outcomes due to reduced compliance, exacerbation of psychosis, homelessness, more frequent hospitalizations, violence, and increased cost of care (Erkiran et al., 2006; Green, 2007).

Interestingly, NMDARs also have been implicated in the aberrant regulation of synaptic plasticity that is critical for substance abuse and addiction. Specifically, modulation of the mesocorticolimbic dopamine system (the circuit in the brain responsible for reward processing) by glutamatergic inputs from cortical and subcortical regions appears to regulate

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ABBREVIATIONS: ANOVA, analysis of variance; CPP, conditioned place preference; GlyT1, glycine transporter 1; GMS, glycine modulatory site; NMDAR, *N*-methyl-D-aspartate receptor; SR, serine racemase; WT, wild-type.

aspects of maladaptive drug seeking (see Carlezon and Thomas, 2009, for a review). Furthermore, extinction of both cocaine-induced conditioned place preference (CPP) and cocaine self-administration has been shown to be NMDAR-dependent and can be enhanced by treatment with the partial GMS agonist D-cycloserine (Paolone et al., 2009; Thanos et al., 2011) or D-serine (Kelamangalath et al., 2009; Kelamangalath and Wagner, 2010; Hammond et al., 2013). Thus, we have hypothesized that the high comorbid substance abuse in schizophrenia results from shared NMDAR-mediated pathophysiology (Coyle, 2006; Benneyworth and Coyle, 2012).

To investigate the role of NMDAR GMS agonists in the pathophysiology of comorbid schizophrenia and substance abuse, we have used two transgenic mouse lines that exhibit contrasting levels of NMDAR activity based on the availability of the agonists D-serine and glycine. The first line is a constitutive knockdown of glycine transporter 1 (GlyT1), a glial transporter that regulates synaptic glycine (Tsai et al., 2004). Heterozygous mutants (GlyT1^{+/-}) exhibit NMDAR hyperfunction (Martina et al., 2005). The second is a constitutive knockout of SR (Basu et al., 2009). Homozygous mutants (SR^{-/-}) exhibit NMDAR hypofunction (Basu et al., 2009). We used a cocaine-induced CPP and locomotor sensitization paradigm to test the hypotheses that 1) GlyT1^{+/-} mice would exhibit hastened extinction of cocaine-induced CPP and enhanced locomotor sensitization to cocaine; 2) SR^{-/-} mice would exhibit prolonged extinction of cocaine-induced CPP and attenuated locomotor sensitization to cocaine; and 3) pharmacologic treatment with the GMS antagonist gavestinel and D-serine would restore GlyT1^{+/-} and SR^{-/-} mice, respectively, to wild-type (WT) phenotypes.

Materials and Methods

Animals. One hundred thirty-six adult (3 months of age) male mice, 64 WT, 37 GlyT1^{+/-}, and 36 SR^{-/-} were used for the current experiments. GlyT1^{+/-} mice (backcrossed onto a C57Bl/6J background for >10 generations) were bred in-house by crossing heterozygous (GlyT1^{+/-}) males or females with WT C57Bl/6J (GlyT1^{+/+}) males or females. Heterozygous (GlyT1^{+/-}) offspring expressed the GlyT1 gene with exons 2 and 3 (encoding regions common to all isoforms of the transporter protein) deleted (Tsai et al., 2004). SR^{-/-} mice (backcrossed onto a C57Bl/6J background for >10 generations) also were bred in-house by crossing heterozygous (SR^{+/-}) males and females. Homozygous (SR^{-/-}) offspring expressed the SR gene with exon 1 (encoding the catalytic domain of the enzyme) deleted (Basu et al., 2009). WT littermates also were used from each strain. Animals were housed in groups of four (two WT and two GlyT1^{+/-} or SR^{-/-}) in polycarbonate cages and maintained on a 12:12-hour light/dark cycle in a temperature (22°C) and humidity-controlled vivarium. Food and water were available ad libitum. All animal procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council of the National Academies, 2011) and were approved by the McLean Hospital Institutional Animal Care and Use Committee.

Drugs. Cocaine (cocaine HCl; Medisca, Plattsburgh, NY) and D-serine (Sigma-Aldrich, St. Louis, MO) were prepared in 0.9% bacteriostatic saline. Gavestinel [4,6-dichloro-3-([1E]-3-oxo-3-[phenylamino]-1-propenyl)-1H-indole-2-carboxylic acid sodium salt] (GV 150526A; Tocris Bioscience, Ellisville, MO) was prepared in 5% β -cyclodextrin [(2-hydroxypropyl)- β -cyclodextrin] (Sigma-Aldrich, St. Louis, MO). Cocaine and gavestinel were administered intraperitoneally at injection volumes of 10 and 5 ml/kg, respectively. D-Serine was administered subcutaneously at an injection volume of 5 ml/kg.

Apparatus. Place conditioning was conducted in four identical acrylic chambers (30 cm $\times$ 15 cm $\times$ 15 cm), described previously by Smith et al. (2014). Chamber floors consisted of interchangeable

halves with distinct textures (hole or grid). Hole floors were made from perforated 16-gauge stainless steel mounted on acrylic rails with 6.4-mm round holes placed at 9.5-mm staggered centers. Grid floors were made from 2.3-mm stainless steel rods mounted on acrylic rails 6.4 mm apart.

Procedure. Place-conditioning procedures were conducted, as previously described (Cunningham, 1995; Cunningham et al., 1999; Bechtholt et al., 2004; Smith et al., 2014). Specifically, animals were randomly assigned to GRID⁺ or GRID⁻ conditioning groups, with genotype (WT, GlyT1^{+/-}, SR^{-/-}) and treatment (gavestinel, D-serine, vehicle) counterbalanced. Conditioning consisted of five phases, as follows: habituation, conditioning, preference testing, extinction, and cocaine-induced reinstatement (see Fig. 1). Prior to each session, animals were transported from the colony room to the place-conditioning room where they were allowed to habituate for 1 hour. Lighting was dimmed (~10 lux). On day 1 (habituation), all animals received injections (i.p., injection volume 10 ml/kg) of 0.9% bacteriostatic saline and were immediately placed in the conditioning chambers on a smooth Plexiglas floor with white matting beneath it for 30 minutes. On days 2 and 3 (conditioning), animals received injections (i.p., injection volume 10 ml/kg) of saline in the morning and cocaine (20 mg/kg) in the afternoon (4 hours later) and were immediately placed in the conditioning chambers for 30 minutes. GRID⁺ animals were placed on the hole side for saline injections and on the grid side for cocaine injections, whereas GRID⁻ animals were placed on the grid side for saline injections and on the hole side for cocaine injections. On day 4 (preference testing), all animals received injections (i.p., injection volume 10 ml/kg) of 0.9% bacteriostatic saline and were immediately placed in the conditioning chambers with access to both the grid and hole sides for 30 minutes. Days 5–16 (extinction) were identical to day 4 (preference testing). On day 17 (cocaine-induced reinstatement), all animals received injections (i.p., injection volume 10 ml/kg) of cocaine (20 mg/kg) and were immediately placed in the conditioning chambers with access to both the grid and hole sides for 30 minutes. Characterization of cocaine-induced CPP in WT, GlyT1^{+/-}, and SR^{-/-} mice and the effects of pharmacologic treatment on GlyT1^{+/-} and SR^{-/-} mice were assessed in separate experiments. For treatment studies, GlyT1^{+/-} mice received injections (i.p., injection volume 5 ml/kg) of gavestinel (10 mg/kg on day 1; 5 mg/kg on days 2–17) or vehicle (5% β -cyclodextrin), whereas SR^{-/-} mice were treated with injections (s.c., injection volume 5 ml/kg) of D-serine (300 mg/kg on day 1; 150 mg/kg on days 2–17) or vehicle (0.9% bacteriostatic saline). Treatment occurred for 17 days, concurrent with place conditioning.

Data Collection and Analysis. All place-conditioning sessions were video recorded and analyzed in real-time using EthoVision XT software (version 7.1; Noldus Information Technology, Leesburg, VA). Time spent on the cocaine-paired side of the chamber and the total distance traveled were calculated. Mixed factorial and one-way analysis of variance (ANOVA) tests were conducted using Statistica software (Version 12; StatSoft, Tulsa, OK). Fisher's least significant difference post hoc tests were conducted when indicated.

Results

Nineteen animals, three WT, eight GlyT1^{+/-}, and eight SR^{-/-} mice, did not display a preference for the cocaine-paired side of the chamber during preference testing and were

Fig. 1. Timeline of behavioral testing and pharmacologic treatment.

excluded from statistical analyses. WT littermates of GlyT1^{+/-} and SR^{-/-} mice from characterization and treatment studies did not differ statistically in time spent on the cocaine-paired side of the chamber and were combined into one group ($n = 60$). Likewise, GlyT1^{+/-} mice from characterization studies did not differ statistically from vehicle-treated GlyT1^{+/-} mice from treatment studies and were combined into one group ($n = 20$), and SR^{-/-} mice from characterization studies did not differ statistically from vehicle-treated SR^{-/-} mice from treatment studies and were combined into one group ($n = 18$).

Cocaine-Induced CPP. Mixed factorial ANOVAs were conducted for WT mice varying chamber side (saline or cocaine) by trial (1–14), as well as for untreated and treated GlyT1^{+/-} and SR^{-/-} mice varying group (untreated or treated) and chamber side (saline or cocaine) by trial (1–14). WT mice showed a significant main effect of chamber side, $F(1,112) = 72.09$, $P < 0.01$, indicating that, overall, animals spent more time on the cocaine-paired side of the chamber than the saline-paired side of the chamber. Also, there was a significant chamber side $\times$ trial interaction, $F(13,1456) = 7.90$, $P < 0.01$. Post hoc analysis indicated that WT mice spent more time on the cocaine-paired side of the chamber throughout all trials (P values < 0.01 ; see Fig. 2, left). GlyT1^{+/-} mice also showed a significant main effect of chamber side, $F(1,54) = 18.82$, $P < 0.01$, as well as a significant group $\times$ chamber side $\times$ trial interaction, $F(13,702) = 3.33$, $P < 0.01$. Post hoc analysis indicated that GlyT1^{+/-} mice spent more time on the cocaine-paired side of the chamber during preference testing, extinction trials 1–8, and cocaine-induced reinstatement (P values < 0.05 ; see Fig. 2, middle). Finally, SR^{-/-} mice also showed a significant group $\times$ chamber side $\times$ trial interaction, $F(13,598) = 1.90$, $P < 0.05$. Post hoc analysis indicated that SR^{-/-} mice spent more time on the cocaine-paired side of the chamber during preference testing and extinction trials 1, 3, 8, and 11 (P values < 0.05 ; see Fig. 2, right). Together these results indicate that all three genotypes expressed a strong place preference for a 20 mg/kg dose of cocaine. Extinction of that place

preference was hastened in GlyT1^{+/-} mice compared to WT mice (that never extinguished) and occurred even more quickly in SR^{-/-} mice. Finally, GlyT1^{+/-} mice showed robust cocaine-induced reinstatement of CPP, whereas SR^{-/-} mice did not.

Post hoc analysis of GlyT1^{+/-} mice treated with gavestinel indicated that animals spent more time on the cocaine-paired side of the chamber during preference testing, extinction trial 1, and cocaine-induced reinstatement (P values < 0.05 ; see Fig. 3, left). In addition, gavestinel-treated mice tended to spend less time on the drug-paired side than nontreated mice during preference testing ($P = 0.07$). Post hoc analysis of SR^{-/-} mice treated with D-serine indicated that animals spent more time on the cocaine-paired side of the chamber during preference testing, but spent more time on the saline-paired side during extinction trials 5–10 and 12 (P values < 0.05 ; see Fig. 3, right). These results indicate that gavestinel treatment caused GlyT1^{+/-} mice to extinguish place preference for a 20 mg/kg dose of cocaine more quickly than WT mice, but did not prevent cocaine-induced reinstatement of CPP. Also, gavestinel treatment tended to attenuate cocaine-induced CPP and had no effect on cocaine-induced reinstatement, whereas D-serine treatment appeared to cause an aversion to the cocaine-paired side of the chamber during extinction training in SR^{-/-} mice, but also had no effect on cocaine-induced reinstatement.

Locomotor Sensitization to Cocaine. Locomotor sensitization was calculated by obtaining a difference score of the total distance traveled during the first cocaine exposure (i.e., day 2 conditioning) and the final cocaine exposure (i.e., day 17 cocaine-induced reinstatement). A one-way ANOVA indicated that untreated SR^{-/-} mice exhibited significantly less locomotor activity than untreated WT and GlyT1^{+/-} mice (P values < 0.05 ; see Fig. 4). Gavestinel treatment tended to decrease locomotor activity of GlyT1^{+/-} mice compared with untreated animals, and D-serine treatment tended to increase locomotor activity of SR^{-/-} mice compared with untreated animals, although those differences did not reach statistical significance. These results indicate that SR^{-/-} mice exhibited attenuated locomotor

Fig. 2. Characterization of cocaine-induced CPP in WT (left), GlyT1^{+/-} (middle), and SR^{-/-} (right) mice. Mean time (seconds) spent on the cocaine-paired side (●) and saline-paired side (○) of the CPP chamber during preference testing (T; day 4), extinction (Ext; days 5–16), and cocaine-induced reinstatement (R; day 17). * $P < 0.01$ and * $P < 0.05$, respectively, between time spent on the cocaine-paired side and the saline-paired side.

Fig. 3. Effects of gavestinel and D-serine treatment on cocaine-induced CPP in GlyT1^{+/-} (left) and SR^{-/-} (right) mice, respectively. Mean time (seconds) spent on the cocaine-paired side (●) and saline-paired side (○) of the CPP chamber during preference testing (T; day 4), extinction (Ext; days 5–16), and cocaine-induced reinstatement (R; day 17). * $P < 0.01$ and # $P < 0.05$, respectively, between time spent on the cocaine-paired side and the saline-paired side.

sensitization to a 20 mg/kg dose of cocaine compared with WT and GlyT1^{+/-} mice and that neither gavestinel treatment nor D-serine treatment significantly altered locomotor sensitization in GlyT1^{+/-} and SR^{-/-} mice, respectively.

Discussion

The current studies demonstrated that while WT, GlyT1^{+/-}, and SR^{-/-} mice all acquired CPP using a 20 mg/kg dose of cocaine, the alteration of NMDAR coagonist availability via genetic manipulation or pharmacologic treatment profoundly affected extinction learning and cocaine-induced reinstatement.

Fig. 4. Locomotor sensitization to cocaine in WT, GlyT1^{+/-}, and SR^{-/-} mice. Mean difference in distance traveled (centimeters) following cocaine administration on the first conditioning trial (day 2) and cocaine-induced reinstatement (day 17). * $P < 0.05$, compared to WT and untreated/vehicle-treated GlyT1^{+/-} mice.

Place preference did not extinguish in WT mice, even after 12 days of extinction training. Extinction was hastened in untreated GlyT1^{+/-} mice, followed by robust cocaine-induced reinstatement, but there was no difference in locomotor sensitization compared to WT mice. Untreated SR^{-/-} mice appeared to extinguish even more quickly than GlyT1^{+/-} mice, but place preference was not reinstated after a cocaine prime. Also, locomotor sensitization to cocaine was attenuated in untreated SR^{-/-} mice. In GlyT1^{+/-} mice, treatment with the GMS antagonist gavestinel caused a nonsignificant trend toward decreased time spent on the cocaine-paired side of the chamber during preference testing, enhanced extinction, and caused a nonsignificant trend toward decreased locomotor sensitization. In SR^{-/-} mice, treatment with the GMS agonist D-serine increased time spent on the saline-paired side of the chamber during extinction trials, had no effect on cocaine-induced reinstatement, and caused a nonsignificant trend toward increased locomotor sensitization.

The lack of extinction observed in WT mice was most likely due to the dose of cocaine administered. This dose was chosen to maximize the acquisition of CPP, and, as expected, over 85% of all animals tested expressed a preference for the cocaine-paired side of the chamber during preference testing. Extinction rates of place preference in mice following conditioning with high doses of cocaine vary. CD-1 mice treated with 20 mg/kg doses largely extinguished after 8 days, whereas some never extinguished after 12 days (Brown et al., 2010). C57Bl/6J mice treated with 16 mg/kg extinguished after 6 days (Itzhak and Anderson, 2012), but did not extinguish after 8 days when treated with 20 mg/kg (Bernardi and Lattal, 2010). Such persistence in conditioned response is most likely due to the formation of stronger drug–cue associations at higher doses of cocaine, leading to enhanced memory consolidation during conditioning trials and/or enhanced memory retrieval during preference testing and extinction trials (Hilderbrand and Lasek, 2014).

GlyT1^{+/-} mice largely behaved as hypothesized. Extinction of place preference occurred more quickly compared to WT mice, and reinstatement of the extinguished preference was precipitated by cocaine. The finding that GlyT1^{+/-} mice did not exhibit enhanced locomotor sensitization compared with WT mice was most likely due to a ceiling effect on locomotor activity resulting from the dose of cocaine that was used. It is possible that a lower dose of cocaine would cause an enhancement of locomotor sensitization in GlyT1^{+/-} mice, given that locomotor sensitization is observed in C57Bl/6 mice at doses as low as 5 mg/kg (Turner et al., 2013).

We hypothesized that SR^{-/-} mice would exhibit prolonged extinction of cocaine-induced CPP and attenuated locomotor sensitization. The extinction hypothesis was based on previous studies by others showing that NMDAR blockade prevents extinction of amphetamine-induced CPP (Hsu and Packard, 2008), as well as studies from our laboratory showing that SR^{-/-} mice exhibit impaired extinction of behavioral sensitization to amphetamine (Benneyworth and Coyle, 2012). The locomotor sensitization hypothesis was based on studies showing that NMDAR antagonism resulting from pharmacologic blockade or genetic manipulation attenuates locomotor sensitization to psychostimulants (Haracz et al., 1995; Kim et al., 1996; Li and Wolf, 1999; Horio et al., 2012). Locomotor sensitization data from untreated SR^{-/-} mice in the current studies replicate previous results.

Although one interpretation of the extinction data in the current studies is that NMDAR hypofunction results in enhanced extinction of cocaine-induced CPP, it seems more likely that these animals were able to make the association between the effects of cocaine and the side of the chamber where drug was administered (see Fig. 2, right), but then rapidly forgot that relationship. This alternative interpretation seems particularly plausible given that untreated $SR^{-/-}$ mice did not exhibit cocaine-induced reinstatement of CPP, a finding that is not surprising given that NMDARs have been shown to be involved in drug-induced reinstatement (see Ma et al., 2009, for a review).

The data from gavestinel-treated $GlyT1^{+/-}$ mice further support this notion, because NMDAR hypofunction resulting from genetic manipulation and pharmacologic treatment produced similar effects. However, it is interesting that gavestinel-treated $GlyT1^{+/-}$ mice showed cocaine-induced reinstatement, albeit to a somewhat lesser extent than untreated and vehicle-treated animals. This discrepancy may highlight the inherent differences between negative pharmacologic modulation of receptor activity and constitutive genetic knockout of a coagonist-producing enzyme. Furthermore, the effects of gavestinel treatment seem to indicate competitive binding between glycine, which is increased in $GlyT1^{+/-}$ mice, and gavestinel, given that treatment attenuated the acquisition of CPP, resulting in quicker extinction due to the weakened drug-cue association, and blunted cocaine-induced reinstatement. Although we have previously demonstrated the ability of gavestinel to reverse phencyclidine-induced attenuation of prepulse inhibition (Benneyworth et al., 2011), these studies are the first to investigate its effects on psychostimulant-induced place preference and locomotor sensitization. As such, additional studies must be conducted to examine whether gavestinel affects baseline behavioral motivation or interacts with other substances of abuse.

The most interesting findings from the current studies concern the D-serine-treated $SR^{-/-}$ mice. Previously, we demonstrated that chronic D-serine treatment at the dose used in the present studies corrected several cellular, molecular, and behavioral consequences of NMDAR hypofunction in $SR^{-/-}$ mice (Balu et al., 2013, 2014). In the current studies, D-serine treatment caused a nonsignificant trend toward increased locomotor sensitization compared to untreated and vehicle-treated animals, as hypothesized. However, it is the effect of D-serine on place preference during extinction training that was most intriguing. D-Serine significantly decreased the time spent on the cocaine-paired side of the chamber, shifting preference to the saline-paired side of the chamber.

These findings suggest that D-serine treatment caused $SR^{-/-}$ mice to learn an operant association more robustly than WT mice, manifesting in increased drug seeking. During preference testing (when conditions were identical to extinction trials), D-serine-treated $SR^{-/-}$ mice quickly learned that drug was no longer present on the cocaine-paired side of the chamber, as it had been on the conditioning days. This lack of cocaine reinforcement seems to have led to an increase in drug-seeking behavior, resulting in a disproportionate amount of time spent on the saline-paired side of the chamber during preference testing (day 4) and extinction training (days 5–16). Administration of the priming dose of cocaine during reinstatement testing thus led to competing motivations, as treated mice were reminded of the drug-environment

association on the cocaine-paired side of the chamber, but also recalled that there was no cocaine available on that side of the chamber during the previous 13 days of testing, thereby significantly attenuating, but not eliminating, the reinstatement of the preference for the cocaine-paired side of the chamber. Alternatively, D-serine treatment may have resulted in an aversive association between cocaine and the drug-paired side of the chamber, but that interpretation is inconsistent with the clear place preference exhibited during preference testing.

These results are consistent with other studies showing that administration of the GMS partial agonist D-cycloserine facilitates extinction learning in fear-conditioning paradigms (Myers and Davis, 2007), as well as in drug self-administration (Thanos et al., 2011) and CPP paradigms (Paolone et al., 2009; Myers and Carlezon, 2010). Furthermore, D-serine administration during extinction training has been shown to attenuate drug-induced reinstatement of cocaine self-administration (Kelamangalath et al., 2009; Kelamangalath and Wagner, 2010; Hammond et al., 2013). It also is possible that D-serine treatment had no effect on cocaine-induced reinstatement due to upregulation of its degradation enzyme, as cocaine has been shown to increase D-amino-acid oxidase levels (Curcio et al., 2013). These findings suggest that D-serine, GMS agonists, or NMDAR-positive modulators may be effective treatments for individuals with schizophrenia who also suffer from comorbid substance dependence, as they may decrease the salience of drug-associated cues in the absence of drug.

The current studies identify the NMDAR as a possible neural substrate for comorbid schizophrenia and substance abuse, at least for individuals with schizophrenia who express a NMDAR hypofunction endophenotype. These findings have important implications for the treatment of cocaine abuse, especially given that there are currently no Food and Drug Administration–approved treatments. It seems plausible that the reason individuals with schizophrenia are more vulnerable to addiction disorders is that they experience blunted reward when using substances of abuse, which drives heavier use. Similar effects are seen in individuals with a family history of alcohol abuse (Schuckit, 1984, 1985). It will be important for future studies to identify the molecular pathways downstream of the NMDAR that are involved in mediating these effects.

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Authorship Contributions

Participated in research design: Puhl, Bechtholt.

Conducted experiments: Puhl, Berg.

Performed data analysis: Puhl.

Wrote or contributed to the writing of the manuscript: Puhl, Berg, Bechtholt, Coyle.

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