Acute D-serine treatment produces antidepressant-like effects in rodents

Oz Malkesman1, Daniel R. Austin1, Tyson Tragon1, Gang Wang1, Gregory Rompala1, Anahita B. Hamidi1, Zhenzhong Cui2, W. Scott Young2, Kazu Nakazawa3, Carlos A. Zarate Jr.4, Husseini K. Manji1,5 and Guang Chen1,5

1 Laboratory of Molecular Pathophysiology, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA
2 Section on Neural Gene Expression, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA
3 Unit on Genetics of Cognition and Behavior, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA
4 Experimental Therapeutics and Pathophysiology Branch, Intramural Research Program, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA
5 Johnson and Johnson Pharmaceutical Research and Development, Titusville, NJ, USA

Abstract

Research suggests that dysfunctional glutamatergic signalling may contribute to depression, a debilitating mood disorder affecting millions of individuals worldwide. Ketamine, a N-methyl-D-aspartate (NMDA) receptor antagonist, exerts rapid antidepressant effects in approximately 70% of patients. Glutamate evokes the release of D-serine from astrocytes and neurons, which then acts as a co-agonist and binds at the glycine site on the NR1 subunit of NMDA receptors. Several studies have implicated glial deficits as one of the underlying facets of the neurobiology of depression. The present study tested the hypothesis that D-serine modulates behaviours related to depression. The behavioural effects of a single, acute D-serine administration were examined in several rodent tests of antidepressant-like effects, including the forced swim test (FST), the female urine sniffing test (FUST) following serotonin depletion, and the learned helplessness (LH) paradigm. D-serine significantly reduced immobility in the FST without affecting general motor function. Both D-serine and ketamine significantly rescued sexual reward-seeking deficits caused by serotonin depletion in the FUST. Finally, D-serine reversed LH behaviour, as measured by escape latency, number of escapes, and percentage of mice developing LH. Mice lacking NR1 expression in forebrain excitatory neurons exhibited a depression-like phenotype in the same behavioural tests, and did not respond to D-serine treatment. These findings suggest that D-serine produces antidepressant-like effects and support the notion of complex glutamatergic dysfunction in depression. It is unclear whether D-serine has a convergent influence on downstream synaptic plasticity cascades that may yield a similar therapeutic profile to NMDA antagonists like ketamine.

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Introduction

Depression is a chronic, severe mental illness, and a leading cause of disability worldwide (Belmaker & Agam, 2008; Duman, 2009; Hunsberger et al. 2009; Manji et al. 2001; Sanacora et al. 2008). Heritable genetic and epigenetic variants, emotional and social-psychological factors, and early life events have all been implicated in the pathogenesis of depression (Belmaker & Agam, 2008; Krishnan & Nestler, 2008). While the disorder is treatable, the effectiveness of current antidepressants, and the adverse effects associated with their use, remains far from satisfactory for most patients with depression (Belmaker & Agam, 2008; Manji et al. 2001; Sanacora et al. 2008). For example, data from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study,
the largest longitudinal randomized antidepressant treatment trial to date, showed that citalopram, a commonly used antidepressant, was associated with a remission rate of only 36.8% and an intolerance rate of 16% (Trivedi et al. 2006; Zisook et al. 2008). Data from the STAR*D study also showed that roughly 40% of patients with depression relapsed within 1 year (Trivedi et al. 2006; Zisook et al. 2008). It is also important to note that for those patients who do respond to currently available antidepressants, clinical benefits often take weeks to occur, suggesting that many of the therapeutic effects are produced by changes in downstream signalling cascades (Krishnan & Nestler, 2008; Manji et al. 2001). Indeed, a growing body of evidence supports the hypothesis that disruptions in neuronal synaptic plasticity cascades are critical to the onset of depression (Krishnan & Nestler, 2008). The limitations of current antidepressants have motivated researchers to identify new therapeutic strategies that act more quickly, and are more effective for a greater number of patients.

Extensive clinical and preclinical evidence suggests that monoaminergic disruptions contribute to depression. Monoamine depletion can lead to relapse in patients with a history of depression (Ruhe et al. 2007) and rapidly induces depression in patients who respond to monoamine oxidase inhibitors (MAOIs) (Shopsin et al. 1976). However, studies measuring monoamines and derived metabolites in the cerebrospinal fluid (CSF) of patients with depression have obtained mixed results (Hou et al. 2006; Lambert et al. 2000; Mann et al. 1996). In addition, preclinical animal studies found that pharmacological and genetic depletion of certain monoamines can abolish the response to antidepressants in depression-related behavioural tests such as the tail suspension test (TST) and forced swim test (FST) (Cryan et al. 2004; O’Leary et al. 2007; Page et al. 1999).

From an aetiological perspective, disrupted glutamatergic neurotransmission, resulting from altered N-methyl-D-aspartate receptor (NMDAR) function and deficient glial uptake of glutamate, has also been implicated in depression (Krishnan & Nestler, 2008; Sanacora et al. 2008). Consistent with glutamate dysfunction in the brains of some depressed patients, a sub-anaesthetic (0.5 mg/kg), intravenous infusion of the NMDA antagonist ketamine was found to result in rapid and long-lasting symptom relief in about 60–70% of patients with treatment-resistant depression or bipolar depression (Diazgranados et al. 2010a; Zarate et al. 2006). Preclinical studies further found that ketamine significantly up-regulates prominent synaptic plasticity cascades and promotes the formation of new synapses in vivo (Li et al. 2010). Despite its role as an NMDA antagonist, electrophysiological (Li et al. 2010) and neurochemical (Maeng et al. 2008b) evidence suggests that ketamine enhances glutamatergic neurotransmission.

Glia, particularly astrocytes, play an integral role at glutamatergic synapses where they are active, highly influential participants in neuronal signalling (Haydon, 2001). Reduced density of forebrain glia has been reported in clinical (Bremner et al. 2002; Cotter et al. 2001; Ongur et al. 1998), post-mortem (Cotter et al. 2001), and preclinical studies of depression (Banasr & Duman, 2008; Banasr et al. 2010). While originally considered exclusively a gliotransmitter, D-serine was recently found to be produced, stored, and released by neurons (Kartvelishvili et al. 2006; Rosenberg et al. 2010), suggesting that neurons may actually contribute more to its forebrain concentration than astrocytes (Yoshikawa et al. 2006, 2007). Regardless, D-serine’s role in glutamatergic throughput has key implications for regulating synaptic plasticity and may provide a nexus between neuronal and glial signalling (Billard, 2008).

Astrocytes are involved in the uptake of glutamate and the release of gliotransmitters like D-serine, which has a high affinity for the glycine binding site on the obligatory NR1 NMDAR subunit (Mothet et al. 2000). Notably, reductions in the NR1 subunit – which is a target of D-serine – have been implicated in mood disorders (Law & Deakin, 2001; McCullumsmith et al. 2007; Nudmamud-Thanoi & Reynolds, 2004). In addition, chronic corticosterone exposure and isolation stress significantly reduced NR1 expression in rodents (Cohen et al. 2011; Hermes et al. 2011). However, the therapeutic potential of compounds that target glia–neuron and inter-neuronal interactions to enhance glutamatergic signalling remains relatively unknown. Here, we used a series of behavioural experiments to evaluate the antidepressant-like effects of treatment with a single, acute dose of D-serine.

Method

Animals

All animal experimental procedures were approved by the Animal Care and Use Committee of the National Institute of Mental Health (NIMH) and followed NIMH guidelines. Three sets of animals were used: (1) male Wistar Kyoto (WKY) rats obtained from Taconic Farms (USA); (2) male 129S1/SvImJ wild-type (WT) mice obtained from Jackson Laboratories (USA); and (3) conditional NR1 knockout (KO) mutant mice,
which were produced by crossing an αCaM kinase II-driven Cre recombinase transgenic line, T29-1 (Tsien et al. 1996), with a loxP-flanked NR1 knock-in mouse line (Dang et al. 2006). Control animals for experiments involving NR1 KO mice were littermates where Cre recombinase was not expressed and sequences flanked by loxP (Flox) remained as homozygous (NR1 subunit expression persisted). Cre recombination was previously detected in the principal neurons of the entire forebrain, including cortex, hippocampus, amygdala, olfactory bulb, striatum, thalamus, and basal forebrain (Tsien et al. 1996), but was not observed in the Gad67-positive local interneurons (Jiang et al. 2010). Animals were housed in a facility with constant temperature (22 ± 1 °C) and a 12-h light/dark cycle (lights on 06:00 hours), with food and water available ad libitum.

Reagents and treatment doses

4-Chloro-D,L-phenylalanine (para-chlorophenylalanine, PCPA), ketamine, and D-serine were obtained from Sigma (USA). PCPA and ketamine were dissolved in phosphate-buffered saline (PBS), while D-serine was dissolved in 0.9% sterile saline. Pilot dose–response tests (data not shown) were used to determine optimally therapeutic doses of D-serine. All agents, including vehicle controls, were delivered intraperitoneally (i.p.; 800 mg/kg D-serine for rats and 2.7 g/kg for mice; 300 mg/kg PCPA; 2.5 mg/kg ketamine).

Open-field test (OFT)

The OFT was used with WKY rats and NR1 KO mice to monitor locomotor and exploratory activity as described previously (Shaltiel et al. 2008). Animals receiving experimental treatments were injected 30 min prior to the test. Animals were introduced into the center of a clear, Plexiglas 35 x 35 cm arena, and digital video was recorded. The videos were subsequently analysed using the Topscan video tracking system (Clever Systems Inc., USA) for total locomotor activity (distance travelled), time spent in the centre of the arena, and frequency of entries into the centre of the arena. Each arena was thoroughly cleaned with a 10% alcohol solution between testing sessions.

FST in rats

The FST was conducted with WKY rats as described previously (Einat et al. 2003). Briefly, the test involves two exposures to a cylindrical plastic tank, 60 cm high, 25 cm diameter, filled with water (22–23 °C) to a depth of 40 cm, during which the rodents cannot touch the bottom of the tank or otherwise escape. During the first exposure, rats were placed in the water and digitally recorded for 15 min. Approximately 24 h later, animals were injected with D-serine or saline and returned to their home cage. Thirty minutes later, rats were placed in their respective tanks and recorded for a 5-min test session. Immobility duration was scored using the ForcedSwimScan video tracking system (Clever Systems Inc., USA).

FST in mice

The FST was conducted with NR1 KO mice as described previously (Shaltiel et al. 2008). Briefly, transparent Plexiglas cylinders, 50 cm high, diameter 20 cm, were filled with tap water at 22–24 °C to approximately 25 cm in a way that mice were not able to touch the floor or escape. Animals receiving treatments were injected 30 min prior to the test with either D-serine or saline. Individual mice were placed in the water for a 6-minute session, and their behaviour was recorded for later analysis. Immobility duration was scored over the final 4 min of the test using the ForcedSwimScan video tracking system (Clever Systems Inc.).

Female urine sniffing test (FUST)

The FUST, a non-operant test for measuring reward-seeking behaviour in rodents based on interest in sniffing pheromone odours from the opposite sex, was conducted with WKY rats, 129S1/SvImJ WT mice, and NR1 KO mice as described previously (Malkesman et al. 2010, 2011). Animals receiving PCPA treatment or PBS (vehicle control) were injected between 09:00 and 10:00 hours on the day before the FUST experiment; animals receiving ketamine or PBS (vehicle control) were treated between 17:00 and 18:00 hours in the evening prior to FUST experiments. Animals receiving D-serine or saline (vehicle control) were injected 30–45 min before the FUST.

Prior to testing, animals were habituated to a sterile cotton-tipped applicator inserted into their home cage. At the onset of testing, animals were transferred into a dark room (3 lx lighting). The test had three phases: (1) a single exposure (3 min) to the cotton tip dipped in sterile water, during which the time spent sniffing the applicator was measured; (2) an interval of 45 min during which no applicator was presented to the animal; and (3) a single exposure (3 min) to a cotton tip infused with fresh urine collected from females of the same strain in oestrus, during which the time spent sniffing was measured.
Learned helplessness (LH) paradigm

The LH paradigm was conducted with 129S1/SvImJ WT mice using the Gemini Avoidance System (USA) as described previously (Maeng et al. 2008a), with slight modifications. The helplessness induction session was conducted on day 0 and mice were given 180 sessions of inescapable electric footshocks [0.45 mA, 24-s duration, inter-trial interval 20 s average (range 12–28 s)], accompanied by a cued light 3 s prior to footshock delivery in one of the closed compartments.

In the screening session, conducted 24 h after induction, the mice received 30 trials of a 3-s light (conditioned stimulus) followed by an additional 24 s of light plus shock [unconditioned stimulus, 0.45 mA, inter-trial interval 30-s average (range 22–38 s)]. Mice that developed helplessness – i.e. that had failed to escape more than 24 trials during the screening – were treated with D-serine or saline (vehicle control). D-Serine and saline injections were administered 30–45 min prior to the final phase of the LH paradigm, the active avoidance test (AAT). The parameters of the AAT were identical to those found in the screening section; latency to escape, as well as the number of escape failures, were recorded by the Gemini Avoidance System.

NR1 KO mice only underwent the AAT portion of the LH paradigm, without inducing or screening for LH first. For experiments involving D-serine and saline, mice received injections 30–45 min prior to the AAT.

Serotonin depletion and hippocampal level measurements

Serotonin levels were measured in WKY rats that received a single injection of PCPA or PBS (vehicle control) in the morning between 09:00 and 10:00 hours on the day prior to sacrifice. Serotonin amounts were measured in fresh, rapidly isolated hippocampal tissues. Samples were processed in buffer proportional to tissue mass using a glass dounce homogenizer and then centrifuged to remove nuclei and large debris (1000 g). Relative levels of serotonin in control and PCPA-treated rats were compared using a serotonin ELISA kit according to the manufacturer’s protocol (Rocky Mountain Diagnostics, USA).

In-situ hybridization histochemistry

To detect mouse NR1 mRNAs, a complementary RNA (cRNA) probe derived from the AtrII-SphI 0.4 kb antisense DNA fragment of rat NR1 cDNA from exon 13 to exon 16 was labeled with [35S]UTP. In-situ hybridization histochemistry was conducted as described previously (Young & Mezey, 2004).

Statistics

Statistical analyses were performed by either two-way ANOVA (multiple variables) or Student’s t tests (single variable, two comparable groups). When values were quantified multiple times in distinct conditions for the same animal (e.g. time spent sniffing water vs. urine in the FUST), two-way repeated-measures ANOVA was used with Bonferroni post-hoc comparison tests where appropriate. χ² statistics were computed to assess the percentage of mice that remained helpless in the LH paradigm after D-serine or saline treatment. Data are expressed as mean ± S.E.M.; significance was evaluated at p < 0.05 for all statistical measures.

Results

D-Serine reduced immobility in the FST without affecting motor activity

The FST is frequently used to examine the antidepressant-like properties of experimental compounds (Malkesman et al. 2009; Porsolt et al. 1977). To eliminate the potential confound of altered locomotor output, we first measured open-field activity in a stress-sensitive strain of rats (WKY) (De La Garza & Mahoney, 2004) treated with D-serine (Fig. 1a). Time significantly affected distance travelled \((F_{5,80}=18.53, p<0.0001)\), but D-serine treatment did not significantly alter the locomotion of rats in the OFT \((F_{1,80}=0.3840, p=0.5493; n=6 \text{ per group})\). No significant interaction was noted between the two variables \((F_{5,80}=1.363, p=0.2258)\). D-Serine treatment neither activated nor suppressed motor activity, and rats habituated to the novel environment in a similar way to animals treated with vehicle control. The same dose of D-serine used in the OFT did, however, significantly reduce immobility duration in the FST \((t=3.473, p=0.0025; D\text{-serine}, n=10; \text{vehicle, } n=9; \text{Fig. 1b})\).

Serotonin depletion reduced time spent sniffing urine in the FUST

Here, we used a pharmacological approach to deplete serotonin and then conducted a behavioural test to evaluate sexual reward-seeking behaviour. Working with the same rat strain (WKY), we blocked serotonin synthesis with the tryptophan hydroxylase inhibitor PCPA and conducted the FUST the following day.
Previous studies from our laboratory found that sniffing urine elicited behavioural and neurochemical responses that suggest this is a rewarding activity to rodents, and the model was sensitive to environmental, genetic, and pharmacological manipulations relevant to depression (Malkesman et al. 2010).

We first assessed whether a single injection of PCPA was sufficient to deplete serotonin levels in WKY rats and found that PCPA administration significantly reduced serotonin levels in hippocampal tissue compared to vehicle-treated control rats (Fig. 2a; t test: \( t_{16} = 2.220, p = 0.0421; n = 8 \) per group).

Next, we evaluated whether the FUST was sensitive to the effects of serotonin depletion in WKY rats (Fig. 2b). Consistent with our previous work, time spent sniffing was found to be significantly affected by odour (\( F_{1,16} = 277.9, p < 0.0001; \) PCPA, \( n = 20 \); vehicle, \( n = 16 \)). Treatment also significantly affected sniffing duration (\( F_{1,36} = 14.80, p = 0.0005 \)), and a significant interaction was noted between the two variables (\( F_{1,36} = 17.81, p = 0.0002 \)). Post-hoc comparisons indicated that PCPA significantly reduced time spent sniffing urine (\( t = 5.502, p < 0.001 \)), but not water (\( t = 0.06133, p > 0.05 \)).

**Ketamine and \( \alpha \)-serine rescued PCPA-induced sniffing deficits in the FUST**

Given the antidepressant-like effects of \( \alpha \)-serine in the FST, we next examined how \( \alpha \)-serine affected reward-seeking behaviour in serotonin-depleted rats. Evidence from other behavioural measures suggested that PCPA disrupts response to certain classes of antidepressants (Page et al. 1999); therefore, we first evaluated the effects of the novel antidepressant ketamine in the FUST. Ketamine targets NMDAR-mediated signalling, increases synaptogenesis, and its therapeutic effects are probably mediated by signals downstream of glutamatergic neurotransmission (DiazGranados et al. 2010b; Li et al. 2010; Sanacora et al. 2008). Animals received an acute sub-anesthetic dose of ketamine (2.5 mg/kg), mirroring previous preclinical rodent studies from our laboratory (Maeng et al. 2008b).

The baseline effects of ketamine on sniffing duration were measured in a group of rats that received the vehicle-control PBS (Fig. 3a). Sniffing time was significantly affected by odour (\( F_{1,17} = 225.1, p < 0.0001 \); ketamine, \( n = 8 \); vehicle, \( n = 9 \)) but not by treatment (\( F_{1,17} = 0.1272, p = 0.7258 \)) or by the interaction between the two variables (\( F_{1,17} = 0.1591, p = 0.6949 \)). However, in serotonin-depleted rats (Fig. 3b), both odour and treatment significantly affected time spent sniffing (odour: \( F_{1,18} = 120.9, p < 0.0001 \); treatment: \( F_{1,18} = 6.522, p = 0.0199 \); ketamine, \( n = 10 \); vehicle, \( n = 9 \)), as did the interaction between the two variables (\( F_{1,18} = 5.913, p = 0.0251 \)). Post-hoc comparisons revealed that ketamine significantly increased time spent sniffing urine (\( t = 3.471, p < 0.01 \)) but not water (\( t = 0.1401, p > 0.05 \)) in serotonin-depleted rats.

Rats treated with \( \alpha \)-serine had a similar sniffing duration profile to those treated with ketamine. In the group receiving PBS vehicle (Fig. 3c), time spent sniffing was significantly affected by odour (\( F_{1,10} = 199.4, p < 0.0001 \)), but not by treatment (\( F_{1,10} = 0.5065, p = 0.4947 \); \( n = 6 \) per group) or by the interaction between the two variables (\( F_{1,10} = 0.7860, p = 0.3984 \)). In rats administered PCPA (Fig. 3d), sniffing duration was significantly affected by odour (\( F_{1,22} = 115.9, p < 0.0001 \)), but not by treatment (\( F_{1,22} = 2.161, p > 0.05 \)).
Serotonin depletion led to reward-seeking deficits in rats. 

\[ p = 0.1556; \ n = 11 \text{ per group}; \] there was also a trend towards significance in the interaction between the two variables \( F_{1,22} = 4.040, p = 0.0568 \). Post-hoc analysis found that in serotonin-depleted rats, d-serine significantly increased time spent sniffing urine \( t = 2.502, p < 0.05 \) but not water \( t = 2.420, p > 0.05 \).
After evaluating the efficacy of D-serine in the FST and serotonin depletion model, we turned to another well-known and widely used model of antidepressant efficacy, the LH paradigm (Krishnan & Nestler, 2008). WT mice underwent a series of random, inescapable footshocks and were screened for LH behaviour the following day. Those mice that developed helplessness were randomly divided into two groups and treated with either D-serine or saline (vehicle) prior to the AAT. Escape latency (Fig. 4a) was significantly influenced by treatment ($F_{1,406} = 5.812, p = 0.0302; n = 7$ per group) but not by trial number ($F_{29,406} = 0.57, p = 0.9647$) or by the interaction of the two variables ($F_{29,406} = 1.35, p = 0.1102$). LH mice treated with D-serine also had a significantly greater number of escapes (Fig. 4b) than those treated with saline ($t_{14} = 2.177, p = 0.0235$). Upon screening for spontaneous recovery (Fig. 4c), it was found that D-serine significantly increased the percentage of mice that recovered from helplessness ($\chi^2 = 4.267, p = 0.0389$).

**Fig. 4.** D-Serine improved measures of helplessness and sexual reward-seeking deficits in wild-type (WT) mice that developed helplessness. (a) D-Serine treatment significantly reduced escape latencies in male WT mice that developed helplessness, (b) increased the number of escapes, and (c) increased the number of mice that recovered from helplessness. (d) D-Serine treatment significantly increased sniffing duration in learned helplessness (LH) mice vs. saline-treated LH mice. Data are mean $\pm$ S.E.M. (* $p < 0.05$, *** $p < 0.001$).

**D-serine improved measures in the LH paradigm**

After evaluating the efficacy of D-serine in the FST and serotonin depletion model, we turned to another well-known and widely used model of antidepressant efficacy, the LH paradigm (Krishnan & Nestler, 2008). WT mice underwent a series of random, inescapable footshocks and were screened for LH behaviour the following day. Those mice that developed helplessness were randomly divided into two groups and treated with either D-serine or saline (vehicle) prior to the AAT. Escape latency (Fig. 4a) was significantly influenced by treatment ($F_{1,406} = 5.812, p = 0.0302; n = 7$ per group) but not by trial number ($F_{29,406} = 0.57, p = 0.9647$) or by the interaction of the two variables ($F_{29,406} = 1.35, p = 0.1102$). LH mice treated with D-serine also had a significantly greater number of escapes (Fig. 4b) than those treated with saline ($t_{14} = 2.177, p = 0.0235$). Upon screening for spontaneous recovery (Fig. 4c), it was found that D-serine significantly increased the percentage of mice that recovered from helplessness ($\chi^2 = 4.267, p = 0.0389$).

Our previous work found that LH mice exhibited sniffing deficits in the FUST, and that such deficits could be rescued by treatment with the conventional antidepressant citalopram (Malkesman et al. 2010). We thus investigated whether D-serine altered time spent sniffing urine or water in WT mice that had developed LH (Fig. 4d). Sniffing duration was significantly affected by odour ($F_{1,8} = 121.5, p < 0.0001$), treatment ($F_{1,8} = 18.38, p = 0.0027$), and the interaction between the two variables ($F_{1,8} = 18.39, p = 0.0027; n = 5$ per group). Post-hoc analysis indicated that D-serine significantly increased time spent sniffing urine ($t = 5.798, p < 0.001$) but not water ($t = 0.2649, p > 0.05$) in LH mice.

**NR1 ablation altered behaviour in the FUST and AAT**

D-Serine is an endogenous agonist of the obligatory NR1 subunit of the NMDAR and has a high affinity to the glycine-binding site (Mothet et al. 2000). Because of D-serine’s antidepressant-like profile in the FST, FUST,
latencies and (male NR1 KO mice had significantly elevated escape avoidance test portion of the learned helplessness paradigm, compared to control (Flox) littermates. (male NR1 KO mice exhibited a sexual reward-seeking deficit throughout the forebrain. (b) littermates had persistent, normal NR1 expression entire forebrain region. In comparison, control (Flox) mice (Supplementary Fig. S1a), often used as a measure of anxiety, was lower in NR1 KO mice, but the difference was not statistically significant (t test: t_{15}=1.248, p=0.2342). In the FST (Supplementary Fig. S1c), the immobility duration of NR1 KO mice did not differ from that of Flox control mice (t test: t_{15}=1.558, p=0.1402).

We next evaluated NR1 KO and Flox control mice in depression-related behavioural tests. In the FUST (Fig. 5b), sniffing duration was significantly influenced by odour (F_{1,13}=719.2, p=0.0010), genotype (F_{1,34}=382.0, p=0.0097; KO, n=7; Flox, n=8), and the interaction between the two variables (F_{1,34}=222.8, p=0.0349). Post-hoc analysis revealed that NR1 KO mice spent significantly less time than Flox control mice sniffing urine (t=3.778, p<0.01) but not water (t=0.506, p>0.05).

NR1 KO mice were also compared with Flox controls in the AAT portion of the LH paradigm. Escape latency (Fig. 5c) and number of escapes (Fig. 5d) were analysed in accordance with our previous analysis of WT mice. Trial number did not significantly influence escape latency (F_{28,435}=1.265, p=0.1647; KO, n=6; Flox, n=11), but genotype (F_{1,435}=7.255, p=0.0167) and the interaction between the two variables (F_{28,435}=2.068, p=0.0011) did significantly affect latency. NR1 KO mice had significantly fewer escapes than Flox controls (t test: t_{15}=2.543, p=0.0225).

**d-Serine had no antidepressant-like effects in conditional NR1 KO mice**

After characterizing the depression-like phenotype of the conditional NR1 KO mice, we lastly investigated whether these mice would be sensitive to d-serine treatment. We examined immobility duration in the FST (Fig. 6a), sniffing duration in the FUST (Fig. 6b), and escape latency (Fig. 6c) and number of escapes (Fig. 6d) in the AAT portion of the LH paradigm. In contrast to the results seen in WT mice, d-serine had no significant influence on immobility in the FST (t test: t_{10}=0.8822, p=0.3984; n=5 per group). In the FUST, sniffing duration was not significantly altered by treatment (two-way repeated-measures
female urine sniffing test, D-serine had no impact on sniffing the learned helplessness paradigm. Data are mean ± S.E.M. (vs. animals receiving saline (vehicle) treatment. (NR1 KO mice (0.2973, p = 0.5423). In the forced swim test, NR1 KO mice treated with D-serine had no significant difference in immobility duration or on the number of escapes in male NR1 KO mice. (c) Animals treated with D-serine had no significant difference in escape latency, or (d) number of escapes in the active avoidance test portion of the learned helplessness paradigm. Data are mean ± S.E.M.

ANOVA: $F_{1,3} = 1.141, p = 0.3133$). In the AAT, D-serine had no significant effect on escape latency ($F_{1,29} = 0.2973, p = 0.5975$) or on the number of escapes in NR1 KO mice ($t_{10} = 0.6308, p = 0.5423$).

Discussion

The present study used three distinct behavioural paradigms relevant to depression to evaluate the preclinical antidepressant-like profile of the glutamatergic NR1 subunit agonist D-serine. Acute treatment with D-serine was found to decrease immobility in the FST, increase the time that serotonin-depleted rodents spent sniffing female urine in the FUST, and improve escape measures in the LH paradigm. We then examined the behaviour of mice with forebrain-specific ablation of the NR1 subunit in excitatory neurons and found that these KO mice exhibited a depression-like phenotype in the FUST and AAT behavioural measures. Finally, we found that D-serine treatment did not rescue the deficits of NR1 KO mice in these depression-related behavioural tests. Taken together, the results of this series of experiments offer consistent evidence that D-serine – and the NR1 subunit of NMDARs – deserve further investigation as a putative pharmacological strategy for treating depression.

One of the major limitations of conventional antidepressants is that chronic treatment is needed before therapeutic benefits are seen. However, recent clinical studies have demonstrated that a single intravenous administration of ketamine (Ibrahim et al. 2011; Zarate et al. 2006), as well as some NR2B antagonists (Preskorn et al. 2008), exert rapid antidepressant effects. Preclinical studies have noted similar antidepressant effects associated with a single injection of ketamine or a NR2B antagonist (Li et al. 2010, 2011; Maeng et al. 2008b). Accordingly, and in keeping with standard practice for the initial assessment of a compound’s antidepressant effects, the current study used a single, acute injection of D-serine to assess its antidepressant-like effects in a series of preclinical tests. Previous studies demonstrated that a single i.p. injection of D-serine is sufficient to elicit a rapid (within 30 min), transient increase in D-serine levels in the prefrontal cortex (Fukushima et al. 2004), lead to a significant haemodynamic response in the hippocampus (Panizzutti et al. 2005), and trigger significant changes in gene expression in brain tissues (Davidson et al. 2009). Subcutaneous injection of D-serine also increases cortical levels of the compound, although the effect is more delayed (Smith et al. 2009).

We first used two well-established preclinical behavioural models – the FST and the LH paradigm – to examine the putative antidepressant-like effects of D-serine. To further refine our investigation, we also used a more recently developed paradigm, the FUST, to measure sniffing duration in monoamine-depleted animals; this newly developed paradigm has predictive and face validity for measuring depressive-like symptoms in rodents (Hunsberger et al. 2011; Malkesman et al. 2009, 2010). Unlike the FST and LH paradigms, which focus on despair-related measures (DiazGranados et al. 2010b; Krishnan & Nestler, 2008; Maier, 1984), the FUST measures sexual reward-seeking behaviour, which is distinct and relevant to anhedonia, a hallmark symptom of depression. The first portion of our experiment assessed the injection of PCPA and whether the FUST was sensitive to serotonin depletion, which is known to affect rodent
response to certain antidepressants (O’Leary et al. 2007; Page et al. 1999) in the FST and TST. We confirmed, biochemically and behaviourally, that PCPA delivery altered serotonin levels in the hippocampus, one of many brain regions involved in reward-seeking behaviours (Malkesman et al. 2010), and one that is known to contain serotonin-sensitive downstream signalling cascades (Krishnan & Nestler, 2008). Administration of PCPA significantly reduced serotonin levels in the hippocampus and markedly reduced the time that rodents spent sniffing urine, but did not affect time spent sniffing water. Time spent sniffing urine remained significantly greater than time spent sniffing water in rodents that received PCPA, which suggests that olfactory function is unimpaired in serotonin-depleted animals. These findings suggest that serotonin depletion causes acute sexual reward-seeking deficits.

We next used the FUST to examine the antidepressant-like effects of D-serine on serotonin-depleted animals, which has not been previously investigated. Because monoamine depletion has a mixed response to conventional antidepressants in behavioural tests like the FST and TST (Cryan et al. 2004; O’Leary et al. 2007; Page et al. 1999), we used ketamine, a novel, rapid-acting compound with antidepressant properties that does not rely on traditional monoaminergic neurotransmission. Ketamine, which was found to have antidepressant-like effects in other depression-related behavioural measures such as the FST and LH paradigm (Maeng et al. 2008b), alters glutamate release. In the present study, both ketamine and D-serine significantly increased the time that serotonin-depleted rats spent sniffing urine, but not water, in the FUST. Baseline measures were not affected, and neither ketamine nor D-serine significantly affected time spent sniffing water or urine in rats with normal serotonin levels. Our findings in the FUST, in combination with the results from the FST and LH paradigm, support D-serine’s potential as an endogenously expressed antidepressant compound.

Finally, we explored the mechanisms underlying D-serine’s therapeutic profile, and found that D-serine is mediated through the NR1 subunit of NMDARs on excitatory neurons. D-serine’s role as a gliotransmitter, neurotransmitter, and allosteric modulator of NMDARs suggests that it is an important regulator of excitatory-inhibitory balance (Billard, 2008). D-Serine is a co-agonist of glutamate that binds with high affinity to the glycine site of the obligatory NR1 subunit of NMDARs. NR1 is required for the channel to function properly and regulate ion movement through the receptor complex (Nakanishi, 1992). Forebrain-specific ablation of NR1 expression in excitatory neurons led to a depression-like phenotype in mice. Compared with Flox control animals, NR1 KO mice exhibited reduced sniffing of female urine in the FUST, increased escape latencies, and reduced number of escapes in the LH paradigm. In the present series of experiments, genotype had no significant effect on the FST, but this finding is confounded by the hyperactivity observed in NR1 KO mice in the OFT; significant changes in motor activity can influence immobility performance in the FST. We find it unlikely that NR1 KO hyperactivity would significantly affect less motor-sensitive paradigms like the LH and FUST, although we cannot eliminate the possibility entirely. In the FUST, for example, no significant difference was noted between NR1 KO and control mice in time spent sniffing water; only time spent sniffing female urine was affected. It is also important to note that treatment of NR1 KO mice with D-serine did not significantly affect behavioural measures in the FST, FUST, or AAT portion of the LH paradigm. These findings strongly support the hypothesis that D-serine’s antidepressant-like effects are mediated via the NR1 subunit of NMDARs on excitatory neurons, and that disruptions in NMDAR-mediated signalling probably contribute to depressive-like behaviours.

Our overall results from this series of experiments, together with recent clinical findings in schizophrenia and post-traumatic stress disorder (PTSD) (Heresco-Levy et al. 2009; Tsai & Lin, 2010), suggest that D-serine has antidepressant-like properties in rodents as well as in humans. Nevertheless, these clinical studies also noted that while doses of D-serine ranging from 30 to 120 mg/kg.d were well tolerated in patients, adverse kidney side-effects were also reported (Heresco-Levy et al. 2005; Kantrowitz et al. 2010). Our current data from NR1 KO mice also support the hypothesis that these antidepressant-like effects occur through the NR1 subunit of NMDARs. Additional studies with D-serine-like agents capable of penetrating the blood–brain barrier (BBB) are needed in order to further explore the therapeutic potential of NR1 glycine-site agonists for the treatment of depressive symptoms.

We found that an NMDAR co-agonist as well as an NMDA antagonist produced similar antidepressant-like effects in mice, despite their opposing effects on NMDARs. Clinical findings support modulation of NMDA-dependent signalling as a possible new approach to treating depression. A recent preclinical Phase-I trial of GLYX-13, a glycine-site NMDAR partial agonist, found that this agent had antidepressant-like
effects in rodent paradigms of depression (http://www.naurex.com/media/Naurex_ACNP_release_120310_FINAL-2.pdf). Clinical studies have noted that ketamine treatment significantly improved suicidal ideation (DiazGranados et al. 2010b; Larkin & Beautrais, 2011; Price et al. 2009). Reconciling the similar effects of compounds with opposing roles on NMDARs will require further investigation of the convergent downstream effects of these putative treatments.

Recent studies have also significantly expanded our understanding of the downstream targets of NMDAR antagonists. Interestingly, these compounds promote synaptogenesis and enhance prominent synaptic plasticity cascades, which can also be activated by NMDAR stimulation. Ketamine’s transient blockade of NMDARs activates brain-derived neurotrophic factor (BDNF) by rapidly deactivating eukaryotic elongation factor 2 (eEF2) kinase (Autry et al. 2011) and induces synaptogenesis by indirectly activating the extracellular signal-regulated kinase (ERK), phosphoinositide-3 kinase-AKT (PI3K/AKT), and mammalian target of rapamycin (mTOR) pathways (Li et al. 2010). Physiological NMDAR activation requires glutamate and the co-agonists glycine or D-serine, and can activate the ERK pathway (Lenz & Avruch, 2005; Sweatt, 2004). ERK pathway deficits have also been discovered in the brains of individuals who committed suicide (Dwivedi et al. 2001, 2006, 2009) and those suffering from certain mood disorders (Yuan et al. 2010). These findings raise the possibility that ketamine and D-serine activate common, convergent downstream targets, thereby producing similar effects on protein expression and promoting comparable changes in synaptic plasticity and dendritic remodelling. Such a result may explain how both NMDA agonists and antagonists elicit a therapeutic response in rodent and clinical studies. Overall, our work supports the hypothesis that modulation of glutamatergic signalling is a promising avenue for the development of novel, rapidly acting antidepressants.

Note

Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

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Statement of Interest

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References


Davidson ME, Kerepesi LA, Soto A, Chan VT (2009). D-Serine exposure resulted in gene expression changes...
implicated in neurodegenerative disorders and neuronal dysfunction in male fischer 344 rats. Archives of Toxicology 83, 747–762.


Maeng S, Hunsberger JG, Pearson B, Yuan P, et al. (2008a). BAG1 plays a critical role in regulating recovery from both manic-like and depression-like...


