Research Article

D-serine reduces memory impairment and neuronal damage induced by chronic lead exposure

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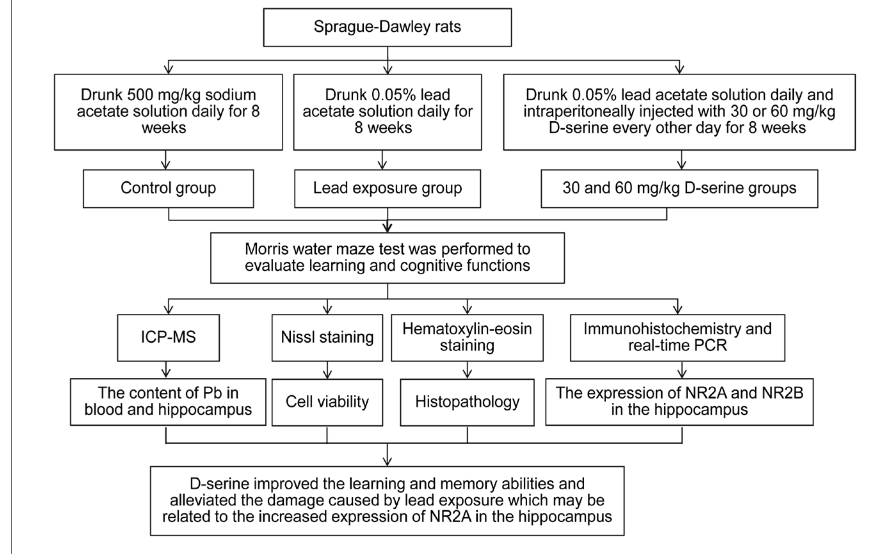
Jian-Zhu Bo[1, #], Ling Xue[1, #], Shuang Li[2], Jing-Wen Yin[1], Zheng-Yao Li[1], Xi Wang[1], Jun-Feng Wang[1], Yan-Shu Zhang[1, 2, *]

Graphical Abstract *D-serine reduces memory impairment and neuronal damage induced by chronic lead exposure*



Abstract

Although exogenous D-serine has been applied as a neural regulatory intervention in many studies, the role played by D-serine in hippocampal injuries caused by lead exposure remains poorly understood. Rat models of chronic lead exposure were established through the administration of 0.05% lead acetate for 8 weeks. Simultaneously, rats were administered 30 or 60 mg/kg D-serine, intraperitoneally, twice a day. Our results showed that D-serine treatment shortened the escape latency from the Morris water maze, increased the number of times that mice crossed the original platform location, and alleviated the pathological damage experienced by hippocampal neurons in response to lead exposure. Although D-serine administration did not increase the expression levels of the N-methyl-D-aspartate receptor subtype 2B (NR2B) in the hippocampi of lead-exposed rats, 60 mg/kg D-serine treatment restored the expression levels of NR2A, which are reduced by lead exposure. These findings suggested that D-serine can alleviate learning and memory impairments induced by lead exposure and that the underlying mechanism is associated with the increased expression of NR2A in the hippocampus. This study was approved by the Animal Ethics Committee of North China University of Science and Technology, China (approval No. LX2018155) on December 21, 2018.

Key Words: D-serine; hippocampus; lead; neurological function; N-methyl-D-aspartate; poisoning; protection; repair

Chinese Library Classification No. R453; R741; R135.1+1

Introduction

Lead, which is a ubiquitous environmental pollutant, causes a wide variety of long-lasting adverse effects in humans. Epidemiological and toxicological data have shown that lead exposure can impair the central nervous system (Sharma et al., 2015; Assi et al., 2016). A growing body of evidence has demonstrated that declining cognitive capacity and behavioral dysfunctions are the most common outcomes of lead exposure (Zeng et al., 2018; Santa Maria et al., 2019). Previous studies have shown that lead can induce learning and memory deficits associated with selective accumulation in the hippocampus, which is a basic anatomical structure associated with learning and memory (Zhou et al., 2018).

Evidence has suggested that damage to long-term potentiation (LTP) mechanisms, associated with N-methyl-D-aspartate receptor (NMDAR) injury, may represent the underlying mechanism that is responsible for neurologic damage observed in lead-exposed animals. Lead is a non-

[1]College of Public Health, North China University of Science and Technology, Tangshan, Hebei Province, China; [2]Laboratory Animal Center, North China University of Science and Technology, Tangshan, Hebei Province, China

***Correspondence to:** Yan-Shu Zhang, PhD, yangshu_zhang@163.com.

https://orcid.org/0000-0003-1207-7251 (Yan-Shu Zhang)

#Both authors contributed equally to this work.

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competitive NMDAR agonist capable of affecting the production and induction of LTP through the activation of the NMDAR, resulting in learning and memory impairments in rodents. Electrophysiological studies performed on isolated hippocampal neurons have shown that lead exposure can reduce the frequency of NMDAR single-channel opening. However, the mechanism through which lead causes NMDAR-mediated damage during the development of LTP in the hippocampus remains unclear. NMDAR is well-known to act as a crucial excitatory amino acid receptor in learning and memory processes. A previous study showed the removal of the NMDAR NR1 gene from the CA1 region in mice resulted in the development of learning and memory deficits associated with deficits in LTP (Wise and Lichtman, 2007). Lin et al. (2007) found that the disruption of the NMDAR downstream signaling pathway can prevent the induction of LTP and the formation of hippocampal-dependent memories. NMDAR is a hetero-oligomer, composed of seven subunits that belong to the NR1, NR2, and NR3 gene families (Rondi-Reig et al., 2006). The NR2 subunit represents a regulatory subunit for the NMDAR and is primarily distributed in the hippocampus (Scherzer et al., 1998). NR2 acts to modify NMDAR channel function, and at least one NR2 subunit is necessary to form a complete, functional NMDAR (Fodor and Nagy, 2007). Previous reports have shown that the various NR2 subunits are associated with different contributions to learning and memory functions (Sprengel et al., 1998; Fox et al., 2006; Gilmartin et al., 2013). Learning and memory deficits have been associated with reductions in NR2B gene expression during late-stage cerebral ischemia/reperfusion injuries. Repeated injections of ketamine in rats resulted in impaired learning and memory function and decreased NR2A and NR2B contents in the hippocampus (Wako et al., 1995). Evidence has suggested that NR2 may be an essential subunit during the process of learning and memory.

In the human brain, D-serine is converted from L-serine by serine racemase, which can act as a selective agonist of the glycine binding site of postsynaptic NMDARs (Klass et al., 2017). Previous studies have demonstrated that D-serine has significant prophylactic actions against learning and memory deficits in rat and mouse models (Mothet et al., 2006; Taylor et al., 2014; Han et al., 2015). By enhancing the activity of glutamate-activated NMDARs, D-serine can produce a series of effects, including depolarization, calcium influx, the release of neurotransmitters, and neuronal death (Montes., 2018). D-amino acid oxidase has been shown to selectively degrade D-serine in the rat brain, resulting in decreased NMDAR activity, which was completely reversed by the administration of exogenous D-serine (Rahman et al., 2012). D-serine can enhance fear extinction by increasing GluA2-containing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor endocytosis and may serve as a potential therapeutic agent against learning and memory disorders (Bai et al., 2014). However, few studies have examined the influence of lead exposure on D-serine in the central nervous system and the effects of D-serine interventions on lead exposure models. Therefore, this study aimed to investigate the potential neuroprotective effects of D-serine against lead exposure-induced injury in the rat hippocampus.

Materials and Methods

Animals and treatments

This study was approved by the Animal Ethics Committee of North China University of Science and Technology, China (approval No. LX2018155) on December 21, 2018. The animals were cared for in accordance with the guidelines issued by North China University of Science and Technology for the Care and Use of Laboratory Animals.

Sixty specific-pathogen-free, male, Sprague-Dawley rats, aged 21 days, were purchased from Beijing Hua Fukang Biological Polytron Technologies Inc. [License No. SCXK (Jing) 2014-0004]. The rats were fed a standard diet, allowed free access to double-distilled water, and were acclimatized for 1 week prior to experimentation, under a 12-hour light/dark cycle at 25 ± 2°C. The rats were randomly divided into four groups, with 15 rats in each group. The rats in the control group were treated with 500 mg/kg sodium acetate (Aladdin, Shanghai, China), administered in the drinking water. The rats in the lead, 30 mg/kg D-serine, and 60 mg/kg D-serine groups were treated with 0.05% lead acetate (Aladdin), administered in the drinking water, for 8 weeks. The rats in the 30 and 60 mg/kg D-serine groups were also intraperitoneally injected with D-serine (Sigma-Aldrich, St. Louis, MO, USA) at the indicated dose, twice a day for 8 weeks. The rats in the control and lead groups were intraperitoneally injected with the same amount of saline (500 mg/kg).

Morris water maze test

To test whether learning and memory abilities were impaired, rats were subjected to the Morris water maze test, 24 hours after the last D-serine injection. Briefly, both place navigation tests and space probe trials were performed (Zhao et al., 2018). In the place navigation test, rats underwent one trial per day for 4 consecutive days. Rats were placed in each of four quadrants and learned to find a hidden platform, which was located 2 cm below the water surface in a pool (1.8 m in diameter). Escape latency was defined as the time required to find and climb onto the platform. If the animal was unable to find the platform within 2 minutes, the time recorded for this test was 120 seconds. On day 5, the space probe trial test was performed. Briefly, after the hidden platform was removed, the rats were placed in the water, in a quadrant other than where the platform had been, and a 60-second trial was performed. The number of times of the rats crossed the previous platform location was recorded. The cumulative time, cumulative distance, and speed were recorded using a Water Maze Video Tracking System (Zheng Hua Biological Instrument Equipment Co., Huaibei, China). All cognitive tasks were performed between 10:00 and 17:00 during the light-off phase.

Inductively coupled plasma-mass spectrometry

After performing the learning and memory tasks, the rats were sacrificed by sodium pentobarbital injection (120 mg/kg, Sigma-Aldrich), and serum and brain tissue were collected. The lead contents in the hippocampus and blood were determined by inductively coupled plasma-mass spectrometry (Li et al., 2015). Tissue samples were digested with spectra-pure nitric acid, in a MarsX microwave mineralizer (CEM Corp., Charlotte, NC, USA). Then, samples were filtered through a 0.22-μm polyethersulfone syringe filter. Next, the lead contents of the tissue samples were determined using an inductively coupled plasma-mass spectrometry spectrometer (Agilent Technologies, Palo Alto, CA, USA).

Hematoxylin and eosin staining and Nissl staining

Hematoxylin and eosin staining was performed, according to the standard procedure, after an 8-week D-serine intervention (Zhang et al., 2015). The hippocampal samples were fixed in 4% paraformaldehyde, dehydrated with 75% alcohol, embedded in paraffin, and cut into 4-μm thick slices. Paraffin-embedded slices were stained with hematoxylin and eosin, dewaxed with xylene, and dehydrated with an alcohol gradient. Pathological reactions were observed using a high magnification lens on a light microscope (Leica, Wetzlar, Germany).

Nissl staining was performed according to the standard procedure. Hippocampal samples were post-fixed in 4% paraformaldehyde. Paraffin sections were coronally cut at a thickness of 5 μm. The sections were stained with Nissl stain. Nissl staining solution was purchased by Beyotime Biotechnology (Cat# C0117; Shanghai, China). Pathological reactions in hippocampal neurons were observed in Nissl-

stained tissue sections, using a high magnification lens on a light microscope.

Immunohistochemistry

Brain samples were obtained after an 8-week D-serine intervention, fixed in 4% paraformaldehyde (Aladdin), and placed in a 20% sucrose solution overnight at 4°C. Brain tissues were treated in xylene and embedded in paraffin, and sections (4-μm) were cut using a paraffin wax slicing machine (Leica, Heidelberg, Germany). Antigen retrieval was performed in citric acid buffer (pH 6.0), using a microwave oven. The sections were cooled to room temperature and transferred to 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase. The sections were washed three times with 0.01 M phosphate-buffered saline (5 minutes for each wash) and blocked with appropriate goat serum (blocking solution; 1:50; Beijing Boosen Biological Technology Co., Ltd., Beijing, China), for 1 hour at 37°C. The slices were incubated with primary antibodies, overnight at 4°C. The primary antibodies used included rabbit anti-NR2A antibody (1:50; Cat# bs-3304R; Beijing Boosen Biological Technology Co., Ltd.) and rabbit anti-NR2B antibody (1:200; Cat# bs-0222R; Beijing Boosen Biological Technology Co., Ltd.). Subsequently, the sections were equilibrated to room temperature, washed with phosphate-buffered saline, 3 times for 5 minutes each, and incubated with biotin-labeled goat anti-rabbit IgG (1:200; Cat# bs-0295G-Bio; Beijing Boosen Biological Technology Co., Ltd.), for 30 minutes at 37°C. The sections were washed with phosphate-buffered saline, three times for 5 minutes each. Next, horseradish peroxidase-labeled avidin (1:1000; Cat# bs-0437P-HRP; Beijing Boosen Biological Technology Co., Ltd.) was added, and the sections were incubated for 30 minutes at 37°C. Subsequently, the sections were developed with freshly prepared 3,3′-diaminobenzidine working fluid (1:1000; Cat# C-0003; Beijing Boosen Biological Technology Co., Ltd.). The samples were counterstained with hematoxylin. After being treated with alcohol and xylene, mounted on slides, and sealed with neutral balsam, the samples were observed under a light microscope (Nikon, Tokyo, Japan).

Real-time polymerase chain reaction

Total RNA was extracted from the hippocampus, after an 8-week D-serine intervention, using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed into complementary DNA. Real-time polymerase chain reaction was performed to determine the expression of genes using a Roche FastStart DNA Green Master kit (Roche LightCycler96, Basel, Switzerland). Polymerase chain reaction primers were obtained from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). The primer sequences were as follows: NR2A, 5′-GAA CAC AGA GCT CAT CCC CAA-3′ and 5′-AGA TCC CAA GAC CGT CTC TCA-3′; NR2B, 5′-TTG ATG AAA TCG AGC TGG CCT-3′ and 5′-AA GTC TCG GAG CCC TTC TTTG-3′; and glyceraldehyde-3-phosphate dehydrogenase, 5′-CCT GGA GAA ACC TGC CAA GTAT-3′ and 5′-AGC CCA GGA TGC CCT TTA GT-3′. The threshold cycle (Ct) value for each test gene was normalized to that for glyceraldehyde-3-phosphate dehydrogenase.

Statistical analysis

All data are expressed as the mean ± standard deviation (SD) for each group. Differences were analyzed using a one-way analysis of variance, followed by Tukey's *post hoc* test, using SPSS 19.0 software (IBM Corporation, Armonk, NY, USA). A value of $P < 0.05$ was considered significant.

Results

D-serine enhances the learning and memory performance of lead-exposed rats

To evaluate the effects of the D-serine intervention, we used the Morris water maze to analyze the spatial learning and memory abilities of the treated rats. On day 4, the mean escape latency for the lead group was significantly longer than that for the control group ($P < 0.05$). The mean escape latencies in the 30 and 60 mg/kg D-serine groups were shorter than those for the lead group ($P = 0.028$ and $P = 0.001$, **Figure 1A**). As shown in **Figure 1B**, the number of times that the animals in the 30 mg/kg D-serine and 60 mg/kg D-serine groups crossed the expected location of the hidden platform was significantly higher than that in the lead group ($P < 0.0001$). The results of the Morris water maze test showed that the D-serine intervention reversed the decline in learning and memory abilities induced by lead exposure. The effects of D-serine on the performance of lead-exposed rats in the place navigation test are shown in **Additional Tables 1** and **2**.

D-serine does not affect the lead contents in either the hippocampus or blood of lead-exposed rats

The lead contents in the hippocampus and blood samples were determined using inductively coupled plasma-mass spectrometry. The lead contents in hippocampus samples from rats in the lead group were significantly higher than those in the control group ($P = 0.001$). No significant difference was observed in the lead contents between the lead and D-serine treatment groups ($P > 0.05$). The evaluation of lead contents in the blood samples showed the same trend as was observed in the hippocampus ($P < 0.001$; **Figure 2**).

D- serine reduces the pathological damage observed in the hippocampus of lead-exposed rats

Alterations in hippocampal morphology were assessed by hematoxylin-eosin staining (**Figure 3A**). The cells in the hippocampus of control rats were arranged closely, with complete and plump structures, a clear nucleus, and homogeneous cytoplasm. In the lead-exposed rats, the number of cells in the hippocampus decreased, the cell arrangement was disordered, and a large number of cells had died, indicated by nuclear condensation and unclear cell boundaries. Compared with lead-exposed rats, the number of cells in the hippocampal samples from D-serine treated rats increased, and the disordered cell arrangement improved. The administration of 60 mg/kg D-serine resulted in a marked decrease in the number of dead cells when compared with the number of dead cells following the administration of 30 mg/kg D-serine. The Nissl staining method was used to observe the morphology of hippocampal neurons (**Figure 3B**). In the control group, the neurons of the hippocampus were arranged closely and neatly, with clear structures. For the lead-exposed rats, hippocampal neurons were arranged loosely and appeared disordered, whereas, in D-serine-treated rats, the neuronal arrangement was improved. The administration of 60 mg/kg D-serine resulted in a marked decrease in the disruption of the spatial arrangement of neuronal cells compared with that observed for the administration of 30 mg/kg D-serine.

Effects of D-serine on the expression of NMDAR in the hippocampus of lead-exposed rats

The expression patterns for NR2A and NR2B in the hippocampus were observed by immunohistochemical staining. The level of NR2A immunopositivity in hippocampal samples from rats in the lead group was lower than that in the control group ($P < 0.05$). Compared with the lead group, NR2A immunopositivity in the hippocampus of the 60 mg/kg D-serine group was significantly increased ($P = 0.003$), but no significant difference was observed for NR2A immunopositivity between the 30 mg/kg D-serine and lead groups ($P > 0.05$). No significant differences in NR2B immunopositivity were observed among the four groups ($P = 0.081$). Real-time polymerase chain reaction was used to detect the mRNA expression levels of NR2A and NR2B in the hippocampus. The resultant mRNA expression levels were consistent with the protein immunopositivity results (**Figure 4**).

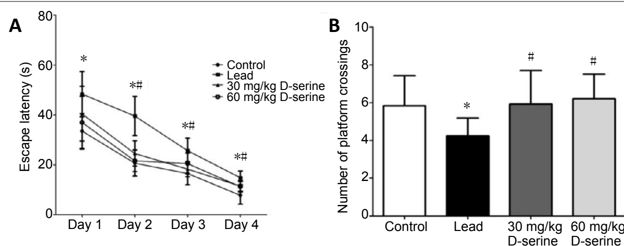

Figure 1 | Effects of D-serine on the cognitive performance of lead-exposed rats.
Cognitive performance was evaluated by the Morris water maze test. (A) Escape latency of rats during the place navigation test. (B) The number of times the expected hidden platform location was crossed during the space probe trial test. Data are expressed as the mean ± SD (*n* = 10). **P* < 0.05, *vs*. control group; #*P* < 0.05, *vs*. lead group (one-way analysis of variance, followed by Tukey's *post hoc* test).

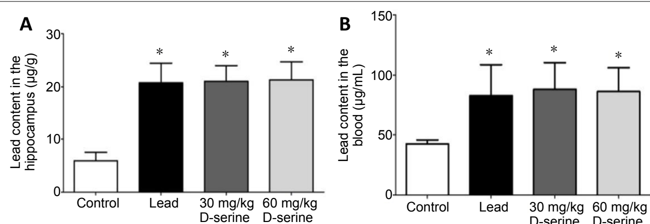

Figure 2 | Effects of D-serine on lead content in the hippocampus (A) and blood (B) samples from lead-exposed rats.
Data are expressed as the mean ± SD (*n* = 10). **P* < 0.05, *vs.* control group (one-way analysis of variance, followed by Tukey's *post hoc* test).

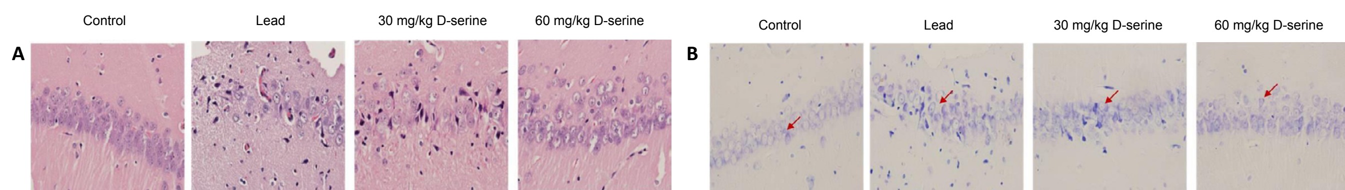

Figure 3 | Effects of D-serine on the hippocampal structure of lead-exposed rats.
(A) Hematoxylin-eosin staining. Compared with lead-exposed rats, the cells in the hippocampus of D-serine-treated rats increased in number, with some recovery of the disordered array. (B) Nissl staining. Treatment with D-serine increased neuronal repair (red arrows) in the hippocampus compared with the untreated lead-exposed group. Original magnification, 200×.

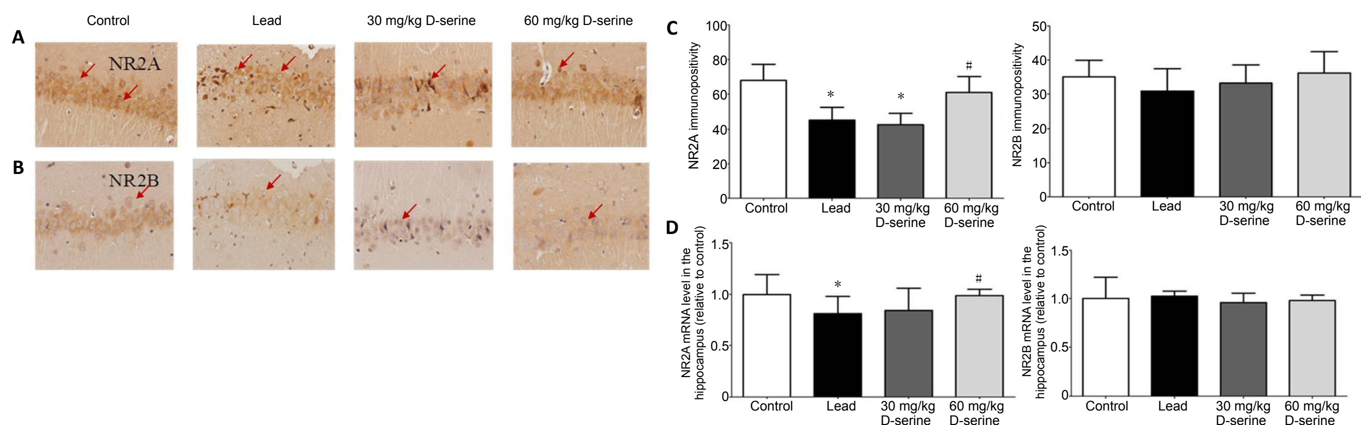

Figure 4 | Effects of D-serine on the expression of NR2A and NR2B in the hippocampus of lead-exposed rats.
(A, B) NR2A (A) and NR2B (B) immunopositivity (red arrows) in the hippocampus (original magnification, 200×). Compared with the lead group, NR2A immunopositivity in the hippocampus of the group treated with 60 mg/kg D-serine significantly increased, although no significant difference was observed between the 30 mg/kg D-serine and lead groups. No significant differences in NR2B immunopositivity were observed among the four groups. (C) Quantitative results for NR2A and NR2B immunopositivity levels in the hippocampus. (D) Quantitative results for NR2A and NR2B mRNA expression levels in the hippocampus. Data are expressed as the mean ± SD (*n* = 5). **P* < 0.05, *vs*. control group; #*P* < 0.05, *vs*. lead group (one-way analysis of variance, followed by Tukey's *post hoc* test).

Discussion

To confirm whether D-serine intervention was sufficient to improve learning and memory abilities in lead-exposed rats, we conducted an animal study using a D-serine intervention. Our data showed that lead exposure could induce damage to hippocampal cells, associated with learning and memory deficits. After D-serine injection, the learning and memory abilities of treated rats were significantly enhanced, and the injury to hippocampal neurons was partially alleviated, which is likely related to the observed increase in NR2A expression in the hippocampus of D-serine-treated rats. These findings indicated that exogenous D-serine treatment was able to repair damage to the hippocampus induced by lead exposure in rats, which improved learning and memory abilities. Our results showed that the lead contents in the hippocampal and blood samples from treated rats increased significantly after 8 weeks of lead exposure. The data also showed that D-serine did not affect either the lead contents in either hippocampal or blood samples of lead-exposed rats. These results suggested that D-serine antagonizes lead neurotoxicity, possibly by affecting the lead contents in other parts of the body. A previous study showed that exogenous D-serine administration relieved chronic lead exposure-induced deficits in synaptic plasticity via NMDAR activation (Jiang et al., 2017). NMDAR activation is necessary for LTP induction in the central synapse (de Lima et al., 2005). LTP is believed to represent the cellular and molecular mechanism that underlies hippocampus-dependent learning and memory functions (Baudry et al., 2015). Therefore, NMDAR regulation may represent an important target for the treatment of nerve injuries associated with lead exposure.

NMDARs are primarily composed of NR1 and NR2 subunits, and NMDAR activation is necessary for cognitive functions and memory formation in animals (Boomhower and Newland,

2017; Wang et al., 2019; Liu et al., 2020). NR2 serves as the regulatory subunit of the NMDAR (Bickler et al., 2003). The present study indicated that D-serine can reverse the lead exposure-induced decrease in NR2A expression in a dose-dependent manner. A previous study showed that NMDAR structure and function did not mature until the third week after birth, and during the process of NMDAR maturation, the NMDAR structure transformed from an NR1/NR2B receptor into and NR1/NR2A receptor (Yashiro and Philpot, 2008).

Evidence has suggested that NMDAR-dependent currents and LTP were decreased in the hippocampus of NR2A knockout mice, which also displayed cognitive deficits. Mice with the targeted deletion of NR2A subunit C presented visual-spatial working memory impairments (Boyce-Rustay and Holmes, 2006; Brigman et al., 2008).

In addition, NR2B plays an important role in the structure and function of NMDARs (Morrone et al., 2007). In a transgenic mouse model that overexpressed NR2B in the forebrain, specific voltage-dependent stimulation of NMDAR activity and synaptic transfer were enhanced (Neal et al., 2011). These results suggested that NR2B may play an important role in the modulation of synaptic transmission and electrical activity. Lead is a potent, specific, non-competitive antagonist of the NMDAR, which interacts at the Zn regulatory site of NMDAR complexes that contain the NR2A subunit but not those that contain the NR2B subunit (Usuda et al., 2018). However, a previous study found that the protein and mRNA levels of NR2A and NR2B were decreased in the hippocampal neurons of lead-exposed rats (Han et al., 2015). In the present study, the expression of NR2B in the hippocampus did not change following either lead exposure or exogenous D-serine administration.

D-serine is a D-amino acid that can be found at high levels in the mammalian brain, where it is required for excitatory neurotransmission. A previous study showed that D-serine is the dominant endogenous co-agonist for NMDAR neurotoxicity (Bai et al., 2014). D-serine can enhance the glutamate-induced activation of NMDAR by binding to the glycine site, which is essential for NMDAR-dependent potentiation and the depression of synaptic transmission. Exogenous D-serine has also been shown to rescue the impairment of hippocampal LTP associated with sodium fluoroacetate exposure (Duffy et al., 2008).

A previous study has shown that increasing D-serine levels, through pharmacological or genetic means, can reverse learning deficits (Matsuda et al., 2010). In the present study, we found that exogenous D-serine administration dramatically improved spatial learning and memory, as assessed by the Morris water maze test, and had a positive effect on hippocampal impairments associated with lead exposure. In addition, the increased expression of NR2A was observed in rats subjected to 60 mg/kg D-serine injection. Although the rats injected with 30 mg/kg D-serine showed the reduction of learning and memory impairment, they did not show any significant changes in NR2A or NR2B expression levels in the hippocampus. Therefore, determining the mechanism through which 30 mg/kg D-serine alleviates the learning and memory impairments of rats after lead exposure requires further research. Low levels of D-serine may rescue some signaling pathways. These results implied that D-serine may enhance spatial learning and memory abilities after surpassing a threshold concentration, through the upregulation of NR2A expression. Our results were not consistent with those reported by Andersen and Pouzet (2004), which may be due to the use of different toxicants or neurobehavioral tests that measure different aspects of spatial learning and memory (Andersen and Pouzet, 2004).

This study has certain limitations. Although this study suggests that D-serine may represent a candidate drug with certain recovery effects for memory impairment and neuronal damage caused by lead exposure, whether it can be used to cure center nervous system diseases still requires further research. For example, D-Serine should be used to treat rats after lead exposure to verify whether it can rescue lead-induced damage as well as prevent it. In addition, NR2A knockdown mice can be used to identify the mechanisms through which D-serine functions to alleviate hippocampal damage. Additional *in vivo* studies and possibly even clinical trials are necessary to clarify whether D-serine can be applied to improve memory impairments (Liu et al., 2009; Fuchs et al., 2012).

Taken together, our results showed that interventions using certain doses of D-serine were able to prevent learning and memory deficits induced by lead exposure. NR2A mRNA and protein levels were up-regulated, which may alleviate damage to hippocampal cells, suggesting that D-serine may improve the learning and memory abilities and alleviate the damage caused by lead exposure through the increased expression of NR2A in the hippocampus. Moreover, our findings also indicated that D-serine administration may represent a useful clinical application for alleviating central nerve damage caused by environmental pollutants in the future.

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Additional files:
Additional Table 1: *Effect of D-serine on the performance of lead-exposed rats in place navigation test of Morris water maze.*
Additional Table 2: *Original data of Additional Table 1.*
Additional file 1: *Open peer review reports 1–3.*

References

Andersen JD, Pouzet B (2004) Spatial memory deficits induced by perinatal treatment of rats with PCP and reversal effect of D-serine. Neuropsychopharmacology 29:1080-1090.

Assi MA, Hezmee MN, Haron AW, Sabri MY, Rajion MA (2016) The detrimental effects of lead on human and animal health. Vet World 9:660-671.

Bai Y, Zhou L, Wu X, Dong Z (2014) D-serine enhances fear extinction by increasing GluA2-containing AMPA receptor endocytosis. Behav Brain Res 270:223-227.

Baudry M, Zhu G, Liu Y, Wang Y, Briz V, Bi X (2015) Multiple cellular cascades participate in long-term potentiation and in hippocampus-dependent learning. Brain Res 1621:73-81.

Bickler PE, Fahlman CS, Taylor DM (2003) Oxygen sensitivity of NMDA receptors: relationship to NR2 subunit composition and hypoxia tolerance of neonatal neurons. Neuroscience 118:25-35.

Boomhower SR, Newland MC (2017) Effects of adolescent exposure to methylmercury and D-amphetamine on reversal learning and an extradimensional shift in male mice. Exp Clin Psychopharmacol 25:64-73.

Boyce-Rustay JM, Holmes A (2006) Ethanol-related behaviors in mice lacking the NMDA receptor NR2A subunit. Psychopharmacology (Berl) 187:455-466.

Brigman JL, Feyder M, Saksida LM, Bussey TJ, Mishina M, Holmes A (2008) Impaired discrimination learning in mice lacking the NMDA receptor NR2A subunit. Learn Mem 15:50-54.

de Lima MN, Laranja DC, Bromberg E, Roesler R, Schröder N (2005) Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. Behav Brain Res 156:139-143.

Duffy S, Labrie V, Roder JC (2008) D-serine augments NMDA-NR2B receptor-dependent hippocampal long-term depression and spatial reversal learning. Neuropsychopharmacology 33:1004-1018.

Fodor L, Nagy J (2007) Pharmacological analysis of recombinant NR1a/2A and NR1a/2B NMDA receptors using the whole-cell patch-clamp method. Methods Mol Biol 403:3-13.

Fox CJ, Russell KI, Wang YT, Christie BR (2006) Contribution of NR2A and NR2B NMDA subunits to bidirectional synaptic plasticity in the hippocampus in vivo. Hippocampus 16:907-915.

Fuchs SA, Peeters-Scholte CM, de Barse MM, Roeleveld MW, Klomp LW, Berger R, de Koning TJ (2012) Increased concentrations of both NMDA receptor co-agonists D-serine and glycine in global ischemia: a potential novel treatment target for perinatal asphyxia. Amino Acids 43:355-363.

Gilmartin MR, Kwapis JL, Helmstetter FJ (2013) NR2A- and NR2B-containing NMDA receptors in the prelimbic medial prefrontal cortex differentially mediate trace, delay, and contextual fear conditioning. Learn Mem 20:290-294.

Han H, Peng Y, Dong Z (2015) D-Serine rescues the deficits of hippocampal long-term potentiation and learning and memory induced by sodium fluoroacetate. Pharmacol Biochem Behav 133:51-56.

Jiang M, Polepalli J, Chen LY, Zhang B, Südhof TC, Malenka RC (2017) Conditional ablation of neuroligin-1 in CA1 pyramidal neurons blocks LTP by a cell-autonomous NMDA receptor-independent mechanism. Mol Psychiatry 22:375-383.

Klass A, Glaubitz B, Tegenthoff M, Lissek S (2017) d-Cycloserine facilitates extinction learning and enhances extinction-related brain activation. Neurobiol Learn Mem 144:235-247.

Li Q, Liu H, Alattar M, Jiang S, Han J, Ma Y, Jiang C (2015) The preferential accumulation of heavy metals in different tissues following frequent respiratory exposure to PM2.5 in rats. Sci Rep 5:16936.

Lin YB, Zhao TJ, Zhan Y (2007) Molecular structure and physiological function of N-methyl-D-aspartic acid receptor. Shengming Kexue 19:57-62.

Liu H, Gong XD, Zhao X, Qian Y, Gu XP, Xia TJ (2020) GLYX-13 pretreatment ameliorates long-term isoflurane exposure-induced cognitive impairment in mice. Neural Regen Res 15:128-135.

Liu YH, Wang L, Wei LC, Huang YG, Chen LW (2009) Up-regulation of D-serine might induce GABAergic neuronal degeneration in the cerebral cortex and hippocampus in the mouse pilocarpine model of epilepsy. Neurochem Res 34:1209-1218.

Matsuda S, Matsuzawa D, Nakazawa K, Sutoh C, Ohtsuka H, Ishii D, Tomizawa H, Iyo M, Shimizu E (2010) D-serine enhances extinction of auditory cued fear conditioning via ERK1/2 phosphorylation in mice. Prog Neuropsychopharmacol Biol Psychiatry 34:895-902.

Montes de Oca Balderas P (2018) Flux-independent NMDAR signaling: molecular mediators, cellular functions, and complexities. Int J Mol Sci 19:3800.

Morrone LA, Rombolà L, Pelle C, Corasaniti MT, Zappettini S, Paudice P, Bonanno G, Bagetta G (2007) The essential oil of bergamot enhances the levels of amino acid neurotransmitters in the hippocampus of rat: implication of monoterpene hydrocarbons. Pharmacol Res 55:255-262.

Mothet JP, Rouaud E, Sinet PM, Potier B, Jouvenceau A, Dutar P, Videau C, Epelbaum J, Billard JM (2006) A critical role for the glial-derived neuromodulator D-serine in the age-related deficits of cellular mechanisms of learning and memory. Aging Cell 5:267-274.

Neal AP, Worley PF, Guilarte TR (2011) Lead exposure during synaptogenesis alters NMDA receptor targeting via NMDA receptor inhibition. Neurotoxicology 32:281-289.

Rahman A, Khan KM, Al-Khaledi G, Khan I, Al-Shemary T (2012) Over activation of hippocampal serine/threonine protein phosphatases PP1 and PP2A is involved in lead-induced deficits in learning and memory in young rats. Neurotoxicology 33:370-383.

Rondi-Reig L, Petit GH, Tobin C, Tonegawa S, Mariani J, Berthoz A (2006) Impaired sequential egocentric and allocentric memories in forebrain-specific-NMDA receptor knockout mice during a new task dissociating strategies of navigation. J Neurosci 26:4071-4081.

Santa Maria MP, Hill BD, Kline J (2019) Lead (Pb) neurotoxicology and cognition. Appl Neuropsychol Child 8:272-293.

Scherzer CR, Landwehrmeyer GB, Kerner JA, Counihan TJ, Kosinski CM, Standaert DG, Daggett LP, Veliçelebi G, Penney JB, Young AB (1998) Expression of N-methyl-D-aspartate receptor subunit mRNAs in the human brain: hippocampus and cortex. J Comp Neurol 390:75-90.

Sharma P, Chambial S, Shukla KK (2015) Lead and neurotoxicity. Indian J Clin Biochem 30:1-2.

Sprengel R, Suchanek B, Amico C, Brusa R, Burnashev N, Rozov A, Hvalby O, Jensen V, Paulsen O, Andersen P, Kim JJ, Thompson RF, Sun W, Webster LC, Grant SG, Eilers J, Konnerth A, Li J, McNamara JO, Seeburg PH (1998) Importance of the intracellular domain of NR2 subunits for NMDA receptor function in vivo. Cell 92:279-289.

Taylor AM, Bus T, Sprengel R, Seeburg PH, Rawlins JN, Bannerman DM (2014) Hippocampal NMDA receptors are important for behavioural inhibition but not for encoding associative spatial memories. Philos Trans R Soc Lond B Biol Sci 369:20130149.

Usuda K, Kawase T, Shigeno Y, Fukuzawa S, Fujii K, Zhang H, Tsukahara T, Tomonaga S, Watanabe G, Jin W, Nagaoka K (2018) Hippocampal metabolism of amino acids by L-amino acid oxidase is involved in fear learning and memory. Sci Rep 8:11073.

Wako K, Ma N, Shiroyama T, Semba R (1995) Glial uptake of intracerebroventricularly injected D-serine in the rat brain: an immunocytochemical study. Neurosci Lett 185:171-174.

Wang KW, Ye XL, Huang T, Yang XF, Zou LY (2019) Optogenetics-induced activation of glutamate receptors improves memory function in mice with Alzheimer’s disease. Neural Regen Res 14:2147-2155.

Wise LE, Lichtman AH (2007) The uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist memantine prolongs spatial memory in a rat delayed radial-arm maze memory task. Eur J Pharmacol 575:98-102.

Yashiro K, Philpot BD (2008) Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. Neuropharmacology 55:1081-1094.

Zeng Z, Huo X, Zhang Y, Xiao Z, Zhang Y, Xu X (2018) Lead exposure is associated with risk of impaired coagulation in preschool children from an e-waste recycling area. Environ Sci Pollut Res Int 25:20670-20679.

Zhang DS, Liu YL, Zhu DQ, Huang XJ, Luo CH (2015) Point application with Angong Niuhuang sticker protects hippocampal and cortical neurons in rats with cerebral ischemia. Neural Regen Res 10:286-291.

Zhao ZH, Zheng G, Wang T, Du KJ, Han X, Luo WJ, Shen XF, Chen JY (2018) Low-level gestational lead exposure alters dendritic spine plasticity in the hippocampus and reduces learning and memory in rats. Sci Rep 8:3533.

Zhou JJ, Li DP, Chen SR, Luo Y, Pan HL (2018) The α2δ-1-NMDA receptor coupling is essential for corticostriatal long-term potentiation and is involved in learning and memory. J Biol Chem 293:19354-19364.

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1 **Additional Table 1 Effect of D-serine on the performance of lead-exposed rats in place navigation test of Morris**
2 **water maze**

	Control	**Lead**	**30 mg/kg D-serine**	**60 mg/kg D-serine**
Day 1				
Cumulative time (s)	163.89±29.92	126.54±89.82	148.97±52.04	113.82±58.65
Cumulative distance (m)	42.63±9.30	30.63±13.97	37.02±12.82	26.89±13.52
Speed (m/s)	0.95±0.15	0.94±0.16	0.98±0.11	0.91±0.13
Day 2				
Cumulative time(s)	95.05±34.62	76.65±38.45	109.37±42.90	126.37±61.11
Cumulative distance (m)	25.48±10.63	17.52±8.50	27.33±11.24	35.15±17.10
Speed (m/s)	0.97±0.14	0.88±0.16	0.92±0.11	1.00±0.12
Day 3				
Cumulative time (s)	91.43±57.18	61.02±24.61	64.64±20.49	64.53±40.24
Cumulative distance (m)	19.53±10.76	14.64±0.15	15.35±5.39	16.16±11.43
Speed (m/s)	0.90±0.06	0.90±0.15	0.89±0.12	0.86±0.17
Day 4				
Cumulative time (s)	120.00±18.63	119.75±0.61	120.00±0.08	120.02±14.76
Cumulative distance (m)	33.64±3.79	31.13±5.52	31.93±3.56	35.22±2.87
Speed (m/s)	0.28±0.03	0.26±0.05	0.27±0.03	0.29±0.03

3 Data are expressed as the mean ± SD (n =10), and analyzed by one-way analysis of variance followed by Tukey's post
4 hoc test).
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	Total time (ms)	Total distance (m)	Mean speed (m/s)		Total time (ms)	Total distance (m)	Mean speed (m/s)		Total time (ms)	Total distance (m)	Mean speed (m/s)		Total time (ms)	Total distance (m)	Mean speed (m/s)
	day1				day2				day3				day4		
	Control				Control				Control				Control		
	175267	39.71	0.75		62355	15.37	0.85		215640	56.04	0.97		120027	33.39	0.28
	168277	46.58	1.07		98719	26.78	0.96		119576	28.74	0.85		120074	31.25	0.26
	162381	43.46	1		39967	7.11	0.66		76363	19.85	0.91		120012	34.4	0.29
	171882	54.44	1.24		117173	31.5	1.08		52088	12.86	0.97		120012	32.64	0.27
	212286	54.64	1.02		53618	13.98	0.99		52089	13.45	0.95		120028	34.03	0.28
	167731	46.84	0.95		99466	29.48	1.1		80153	19	0.86		120027	28.94	0.24
	93256	25.28	0.86		91948	24.05	0.96		140105	37.73	0.98		120027	33.28	0.28
	178652	44.3	0.83		151353	41.95	0.89		25226	5.19	0.79		120011	33.73	0.28
	161836	40.39	0.96		128421	35.8	1.12		112367	30.03	0.88		120027	43.36	0.36
	147312	30.66	0.81		107454	28.8	1.05		40732	8.91	0.85		120012	31.41	0.26
mean	163888	42.63	0.949	mean	95047.4	25.482	0.966	mean	91433.9	23.18	0.901	mean	120025.7	33.643	0.28
SD	29921.907	9.2997491	0.1445645	SD	34623.745	10.628473	0.1395389	SD	57184.773	15.370707	0.0648845	SD	18.631216	3.7886264	0.0316228
	Lead				Lead				Lead				Lead		
	89216	24.3	0.92		61589	14.97	0.84		50124	14.71	1.06		120011	43.5	0.36
	93804	25.84	1.1		59079	14.28	0.95		25335	6.84	1.03		120011	32.59	0.27
	126563	28.05	0.75		117172	22.91	0.78		98062	20.66	0.7		120012	28.74	0.24
	136501	40.77	1.01		69451	11.15	0.64		47394	10.16	0.81		120012	28.76	0.24
	55146	15.03	0.9		165001	38.17	0.9		88671	21.57	0.96		120011	28.03	0.23
	146111	42.4	1.17		81354	21.43	1.02		92914	23.03	0.89		120012	32.56	0.27
	83648	26.67	1.13		49468	14.72	1.2		66068	19.31	1.15		120012	31.73	0.26
	78406	17.89	0.81		40732	9.46	0.91		50778	11.13	0.86		118156	30.27	0.26
	369098	61.27	0.68		41932	10.54	0.86		52416	11.93	0.88		119247	33.5	0.28
	86925	21.41	0.96		80699	17.56	0.69		38328	7.03	0.67		120011	21.66	0.18
mean	126541.8	30.363	0.943	mean	76647.7	17.519	0.879	mean	61009	14.637	0.901	mean	119749.5	31.134	0.259
SD	89817.354	13.966134	0.1641172	SD	38453.661	8.4974852	0.1612073	SD	24610.432	6.1006175	0.1533659	SD	609.27119	5.5229787	0.0455705
	30mg/kg				30mg/kg				30mg/kg				30mg/kg		
	182240	45.18	0.87		92056	20.8	0.85		40295	7.6	0.7		120027	28.07	0.23
	128310	34.46	1.09		58095	14.75	0.8		42916	9.39	0.76		120011	32.91	0.27
	186624	49.39	0.96		123616	32.94	1.02		53179	13.38	0.97		120012	38.18	0.32
	144690	38.47	1.19		146984	35.26	0.8		71635	16.97	0.94		120012	36.09	0.3
	172863	43.88	1.01		187170	51.31	1.09		63226	17.44	1.03		120011	30.38	0.25
	132787	32.98	0.99		87251	22.68	0.97		65519	15.68	0.91		119794	31.58	0.26
	108765	27.28	0.94		88999	19.52	0.89		53835	11.79	0.78		120012	27.51	0.23
	94242	19.21	0.77		79064	21.36	0.93		71417	16.62	0.8		120027	30.88	0.26
	82667	20.15	0.96		68250	18.73	1.06		71527	17.63	0.94		120012	28.83	0.24
	256514	59.17	0.99		158887	35.99	0.8		112804	26.97	1.06		120105	34.88	0.29
mean	148970.2	37.017	0.977	mean	109037.2	27.334	0.921	mean	64635.3	15.347	0.889	mean	120002.3	31.931	0.265
SD	52042.751	12.818922	0.1134362	SD	42900.224	11.242329	0.1105994	SD	20487.245	5.3859448	0.1217876	SD	78.658262	3.5544102	0.0302765
	60mg/kg				60mg/kg				60mg/kg				60mg/kg		
	184003	38.06	0.89		285093	77.18	1.08		52743	13.41	0.93		120027	34.9	0.29

	59733	14.09	0.82		124599	33.33	0.93		161291	41.02	0.85		120028	33.18	0.28
	93476	20.75	0.91		113897	32.23	0.97		34507	5.81	0.63		120059	35.98	0.3
	160635	43.55	1.12		148951	44.38	0.98		95767	28.51	1.1		120027	36.29	0.3
	72400	18.41	0.97		114911	37.5	1.22		29156	7.3	1.03		120028	37.21	0.31
	167731	45.86	1.1		114693	29.81	0.86		28503	4.68	0.62		120011	39.16	0.33
	180617	36.75	0.76		104318	28.98	1.07		64210	17.13	1.01		120011	35.78	0.3
	49031	13.51	0.91		50344	11.07	0.83		63883	15.61	0.93		120011	38.16	0.32
	126017	29.14	0.87		94239	24.77	0.95		74163	19.97	1.03		120011	31.19	0.26
	44555	8.75	0.72		112696	32.26	1.08		41060	8.12	0.72		120027	30.38	0.25
mean	113819.8	26.887	0.907	mean	126374.1	35.151	0.997	mean	64528.3	16.156	0.885	mean	120024	35.223	0.294
SD	56454.95	13.523648	0.1301324	SD	61114.545	17.099716	0.1170043	SD	40238.423	11.430554	0.1737335	SD	14.757296	2.8692587	0.0250333