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## Dietary intake of magnesium-L-threonate alleviates memory deficits induced by developmental lead exposure in rats

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Elevation of brain magnesium enhances cognitive capacity. Chronic lead (Pb) exposure has been implicated in the impairment of cognition. Whether elevation of brain magnesium rescues the impairment of memory by chronic Pb exposure is still unclear. Here we investigated the effect and possible mechanisms of chronic dietary intake of magnesium-L-threonate (MgT) on impaired spatial memory by chronic developmental Pb exposure in rats. We found that MgT rescued impaired spatial memory ability in Pb-exposed rats. This positive effect is relative to increasing dendritic spine density and the expression of NR2A and GluR1.

### 1. Introduction

Lead (Pb) is one of the most widespread and potential neurotoxins in the environment. Early developmental Pb exposure results in cognitive deficits, especially learning and memory, which can even last into adulthood despite the removal of Pb exposure.<sup>1–4</sup> There are many reports showing that chronic Pb exposure impairs hippocampal long-term potentiation (LTP), which is the cellular model of learning and memory.<sup>5–7</sup> LTP is usually accompanied by spine formation and enlargement.<sup>8</sup> Our previous work show that Pb exposure exerts a decrease in spine density, as well as the mushroom shaped spines in hippocampal pyramidal neurons in rats.<sup>9</sup>

In the mammalian brain, excitatory synapses are mainly localized in dendritic spines that express postsynaptic density (PSD), which include a major component, the PSD-95 family of proteins. To anchor and stabilize *N*-methyl-D-aspartate receptor (NMDAR) complexes at synaptic sites, some of the NR1 splice variants and NR2 subunits of NMDARs directly interact with the PSD-95 family of proteins.<sup>10</sup> Pb exposure also inhibits the expression of the NR1 and NR2A subunit gene of NMDARs<sup>4</sup> which is necessary for the induction of LTP.<sup>8</sup>

According to these, Pb-induced cognitive deficits are related to impaired LTP, which are partly due to the reduction of dendritic spine density and the expression of some proteins anchored to PSD.

Mg<sup>2+</sup> is one of the most abundant cations found in the body, and there are a lot of functions of which found in cells, including

serving as a cofactor of enzymes, participating in the process of intracellular signal transduction as well as DNA and protein synthesis. In the central nervous system, Mg<sup>2+</sup> plays an important role in neurotransmission,<sup>11</sup> especially as a “gating” ion within the NMDAR complex<sup>12,13</sup> and the process of learning and memory.<sup>14,15</sup>

Many *in vitro* studies show that increasing Mg<sup>2+</sup> in neuronal cell cultures reduces oxidative cell death<sup>16</sup> and enhances the synaptic plasticity of cultured hippocampal neurons.<sup>17</sup> By using a newly developed Mg<sup>2+</sup> compound-magnesium-L-threonate (MgT) to elevate brain Mg<sup>2+</sup>, Slutsky *et al.* find it leading to significant enhancement of spatial and associative memory in rats.<sup>17</sup> At the cellular level, Mg<sup>2+</sup> increases the presynaptic boutons and enhances the protein level of NR2B-containing NMDARs in hippocampus thus resulting in enhancement of NMDAR signaling and synaptic plasticity. Furthermore, the effects of MgT on the protein expression of NR2B and brain derived neurotrophic factor (BDNF) are regional-special. All of these proteins are dependent on NMDAR signaling and leading to the enhancement of synaptic plasticity when elevated or activated.<sup>18</sup> Based on the studies above, we hypothesized that MgT may rescue the impaired learning and memory ability induced by Pb exposure through the NMDA receptor.

In this study, we performed the experiments to raise the brain Mg<sup>2+</sup> concentration to evaluate the effect of MgT on impaired learning and memory by chronic Pb exposure and to investigate whether the effect is related to spine density and the gene expression of NR2A, NR2B, GluR1 and PSD-95. MgT can be a potential complementary agent in the treatment of Pb intoxication.

### 2. Materials and methods

#### 2.1 Experimental animals and treatments

Sprague–Dawley (SD) rats were obtained from the Laboratory Animal Center, Anhui Medical University, China. The Pregnant

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SD rats were randomly divided into two groups: control and Pb-exposed group, drinking distilled water and Pb water (300 ppm Pb acetate in distilled water, 30 mL per day), respectively. The Pb-exposed pups then acquired Pb indirectly *via* their mother's milk during lactation period. After weaning until the postnatal day 30 (PND30), the Pb-exposed pups had access to the same solution as their mothers. At the PND31, male rats of control were randomly divided into two groups: control and MgT group, while male Pb-exposed group rats were randomly divided into two groups: Pb and Pb + MgT group. The MgT and Pb + MgT group received MgT for 30 days, while rats of control group and Pb group drank distilled water. The design of the experiment is shown in Fig. 1. All the animals were maintained on a 12 : 12 light/dark cycle in an air-conditioned constant temperature ( $24 \pm 1$  °C) room. At the age of 61–67 days, animals were carried out the Morris Water Maze (MWM) test or other experiments. All animal experiments were performed following the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Hefei University of Technology, China.

## 2.2 Materials

Magnesium-L-threonate powder (brand name Magtein™; MgT) was obtained from Center for Learning and Memory, School of Medicine, Tsinghua University, and its chemical structure is shown in Fig. 2. The protocol was adopted as that described previously.<sup>18,19</sup> Simply, MgT was mixed in the rats' drinking water, and the concentration of MgT liquids was adjusted according to body weight measured every 3 days and the water intake on daily basis. Doing like these is to get as close as possible to the target dosage of 604 mg per kg per day (50 mg per kg per day elemental magnesium), for this doses and time courses of MgT exposure have been shown to be effective in elevating brain magnesium and enhancing hippocampal-dependent learning and memory in rats.<sup>19</sup>

## 2.3 Tissue collection

At the age of 70 days old, rat pups were deeply anesthetized with CO<sub>2</sub> and decapitated. Brains were then removed from the skull quickly within 1 min. Some brains were longitudinally cut into two halves; the left hemibrain was used for Golgi-Cox assay, while the right part was frozen and stored at –80 °C for examining special protein expression.

## 2.4 Pb determination in hippocampus

Pb concentration in the hippocampus was estimated on the animals which had been used for behavioral experiment. After

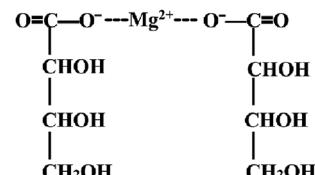


Fig. 2 The chemical structure of MgT.

decapitation of the animals, the hippocampus were isolated and digested with nitric acid. The Pb in the hippocampus was measured by Inductively Coupled Plasma Atomic Emission Spectrometer (CS600, LECO, USA) in Instruments' Center for Physical Science, University of Science & Technology of China.

## 2.5 Morris water maze test

For assessing the effect of MgT and Pb on spatial memory ability, the MWM experiment was carried out. Each animal performed four consecutive trials per day for 5 consecutive days in its 61–67 days of life. Before being gently released into the water at one of four starting location which was randomly selected, each individual rat was placed on the platform for 30 s. Between trials the animals were allowed to rest for 30 s. Recording was automatically terminated as escape latency when the animal found the platform and reached the platform. If rats could not find the platform within 60 s, they were guided to the platform by hand and allowed to remain there for the equal time, and their escape latency was accepted as 60 s. The mean latency of four trials each day was measured for individual animals every day.

The day after the acquisition phase, a probe test was conducted by removing the platform. Rats were allowed to swim freely in the pool for 60 s. The time spent in the target quadrant which had previously contained the hidden platform was recorded to indicate the degree of memory consolidation.

## 2.6 Morphometric analysis of dendritic spine density

The Golgi-Cox staining method, which is a well-known method to stain whole neuron dendrites and spines *in vivo*<sup>20</sup> and the method has been used in our study,<sup>21</sup> was used to process the brains of rats. In brief, the brains were first stored in the dark for two days (37 °C) in Golgi-Cox solution, and then were sectioned at a thickness of 200 µm in 6% sucrose with a vibratome (VT1000S, Leica, Germany). The coronal sections containing hippocampal dentate gyrus (DG) neurons were used in the present study. We collected one out of every 3 sections and got 4–5 sections per animal. Those sections were mounted to 2% gelatin-coated slides and stained with ammonia for 60 min,

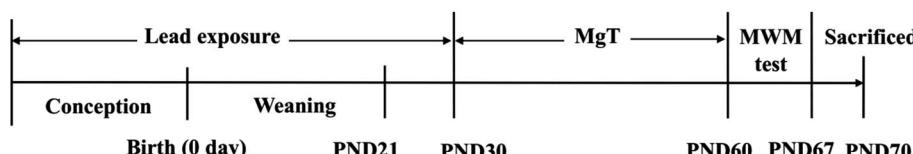


Fig. 1 Scheme of experiment design.



washed with water three times, followed by Kodak Film Fix for 20 min, and then washed with water, dehydrated, cleared, and mounted using a resinous medium. At last, DG neurons in hippocampus were imaged with a Nikon widefield microscope (Eclipse 80i, Nikon, Japan) by using a  $40\times$  objective. From all sections in each rat, about 6 neurons per section were chosen. Then, spine density (spine number per  $10\text{ }\mu\text{m}$ ) and dendrite number for each neuron were analyzed by sholl analysis using MATLAB software. The spines counted in the present study were on 2–3 stretches of the secondary dendrite about  $20\text{ }\mu\text{m}$  in length.

## 2.7 Real-time quantitative PCR

The total RNAs were extracted using the RNA kit (Axy-gen, Silicon Valley, USA) from the hippocampus DG homogenate of control and Pb rats with or without MgT treatment respectively ( $n = 4$  for each group). Subsequently, First-Strand cDNA Synthesis SuperMix were used to complete the reverse transcription reaction according to the manufacturer's instructions (TransGene, Biotech), generating the first strand of total cDNA.

For real-time quantitative PCR (Q-PCR), the total reaction pool is  $20\text{ }\mu\text{L}$  which is composed of:  $10\text{ }\mu\text{L}$  of SYBR Green;  $0.4\text{ }\mu\text{L}$  of forward and reverse primer each;  $2\text{ }\mu\text{L}$  of cDNA template ( $100\text{ ng }\mu\text{L}^{-1}$ ) and  $7.2\text{ }\mu\text{L}$  of deionized water. The primers used in this protocol were listed as follows:

forward, 5'-GACGCAGCCGTCTTGAACTA-3' and  
reverse, 5'-CAGCGCAATTCCATAGCCT-3' for NR2A,  
forward, 5'-AATCAGAACGCCCTAACGC-3' and  
reverse, 5'-GCTGACAATCTCAAGTCGGTAG-3' for GluR1,  
forward, 5'-CTGTGCTATGTTGCCCTAGACTTC-3' and  
reverse, 5'-CATTGCCGATAGTGTGACCTG-3' for  $\beta$ -actin,  
respectively. The real-time fluorescence PCR was performed with Real-Time PCR System machine (LightCycler® 96, Roche). Data analysis was done by using the  $C_t$  method and results were normalized to  $\beta$ -actin in software by Real-Time PCR System machine.

## 2.8 Western blot

Samples of hippocampal DG homogenate from four groups, control, MgT, Pb and Pb + MgT, were obtained, then the protein concentration was determined by using the bicinchoninic acid (BCA) method and equal amount of samples were resolved on polyacrylamide gels. Protein was then transferred to PVDF membrane and incubated in 5% non-fat dry milk, after that protein was probed with such primary antibody as anti-NR2A (#4205S Cell Signaling), anti-NR2B (#5580 Cell Signaling), anti-GluR1 (#8850 Cell Signaling), anti-PSD-95 (Abcam 2723), and anti- $\beta$ -actin (Abcam 16039) overnight followed by the appropriate HRP-coupled secondary antibody. Visualization of immunoreactive bands was detected by enhanced chemiluminescence and captured on chemiluminescence imaging instrument (GE ImageQuant LAS4000mini). For the western blot analyses, digital images were quantified using ImageJ software. The integrated optical density (IOD) of each immunoreactive band was measured. IOD was normalized to the IOD of  $\beta$ -actin band also in the same lane.

## 2.9 Statistical analysis

All data were expressed as mean  $\pm$  SEM. Statistical analysis was performed in GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA). Statistical comparisons were made using One-way repeated ANOVA or Two-way repeated ANOVA when appropriate. A value of  $p < 0.05$  was considered to be statistically significant.

## 3 Results

### 3.1 MgT rescues spatial memory deficits by chronic Pb exposure

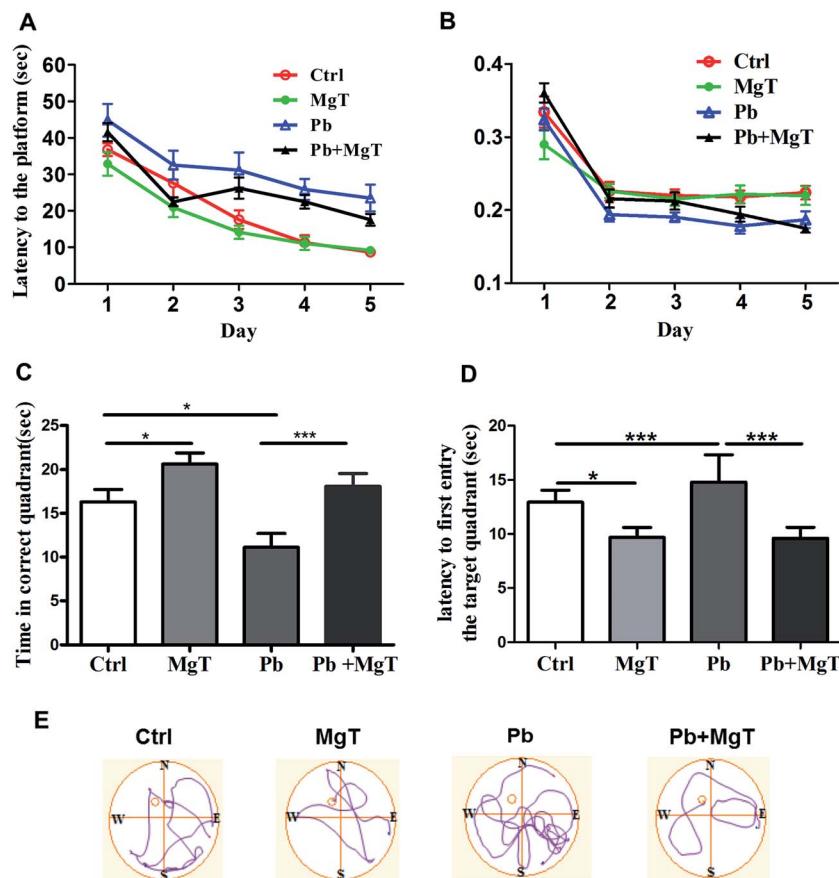
Chronic Pb exposure is implicated in the impairment of learning and memory<sup>1,22</sup> and elevation of brain magnesium enhances the cognitive capacity and synaptic plasticity.<sup>19</sup> To determine whether MgT rescues the cognitive capacity impaired by chronic Pb exposure, we tested rats for hippocampus-dependent spatial memory using the MWM experiments. All rats showed a progressive reduction of the average latency to find the hidden platform during the 5 training days (Fig. 3A). In addition, four groups of rats had no significant differences at the swimming speed between any two groups (Fig. 3B). So MgT has no learning effects on Pb rats.

On day 6, we tested the spatial memory by the probe trial. Compared with Pb-exposed rats, MgT treatment significantly increased the time spent in the target quadrant (Fig. 3C, Pb,  $11.11 \pm 1.58$ , Pb + MgT,  $18.1 \pm 1.46$ ,  $p < 0.001$ ) and reduced the latency to first entry to correct quadrant (Fig. 3D, Pb,  $14.8 \pm 2.5$ , Pb + MgT,  $9.60 \pm 1.02$ ,  $p < 0.001$ ). So, MgT can rescue the spatial memory deficits resulting from chronic Pb exposure during development. In addition, consistent with previous study (Slutsky, *et al.*, 2010), MgT also enhanced the spatial memory in control rats (Fig. 3C, Control,  $16.30 \pm 1.42$ ; MgT,  $20.63 \pm 1.26$ ,  $p < 0.05$ ) and reduced the latency to first entry to correct quadrant (Fig. 3D, Control,  $12.96 \pm 1.09$ ; MgT  $9.69 \pm 0.92$ ,  $p < 0.05$ ).

### 3.2 MgT increases spine density and dendrite number of tertiary branch order in hippocampal DG area in Pb-exposed rats

Exposure to Pb in rats during the critical window for rodent nervous system development leads to decreased spine density<sup>21</sup> and loss of synapses, disrupts synaptic signaling and thus leads to progressive loss of cognition.<sup>23</sup> Slutsky *et al.*<sup>19</sup> demonstrates that increase in brain  $Mg^{2+}$  leads to increase in functional connectivity, synaptic plasticity, and enhancement of learning and memory. To determine whether MgT increases spine density in Pb-exposed rat, Golgi-Cox staining method was performed. Golgi-Cox impregnated dendritic arborization and dendritic spines were showed in Fig. 4A and representative sections of Golgi-Cox stained dendrites of granule cell in hippocampus dentate gyrus were showed in Fig. 4B. Pb-exposed with MgT-treated rats increased the number of dendritic spine in hippocampal DG area by 53.6% compared with Pb-exposed rats ( $p < 0.001$ , Fig. 4C). Consistent with our recent study,<sup>21</sup> the number of dendritic spine in Pb-exposed rats exhibited 10.33% decrease ( $p < 0.001$ ) compared with control group. In





**Fig. 3** MgT rescues spatial memory deficits in Pb-exposed rats. (A) Mean escape latencies to find the hidden platform for each of the four groups during the water maze training trials. (B) Swimming speed of the four groups during the water maze training trials. (C) Time spent in the correct quadrant and (D) latency to first entry to the target quadrant of the four groups in the probe trial on the sixth day. (E) Representative swimming paths of the four groups in probe test of the MWM experiment. The directions "North", "South", "East", and "West" are indicated as "N", "S", "E", and "W", respectively. The "North-West" quadrant was the target quadrant. There were 11, 11, 9, 9 male rats in Control, MgT, Pb and MgT + Pb, respectively. (\* $p < 0.05$ , \*\*\* $p < 0.001$ ).

addition, the number of dendritic spine increased about 14.4% in MgT-treated rats compared with control group ( $p < 0.001$ ).

Moreover, as shown in Fig. 4D, Pb-exposed with MgT-treated rats significantly increased the dendrite number of tertiary branch order compared with Pb-exposed rats (Pb,  $2.26 \pm 0.34$ ; Pb + MgT,  $5.08 \pm 0.37$ ,  $p < 0.001$ ) and MgT induced a significant increase in dendrite number of tertiary branch order compared with control rats (control,  $2.90 \pm 0.39$ ; MgT  $4.43 \pm 0.48$ ,  $p < 0.05$ ). No obvious changes were observed in the dendrite number at primary branch and secondary branch order (Fig. 4D).

Taken together, MgT can increase spine density and dendrite number of tertiary branch order in hippocampal DG area in Pb-exposed rats which may in turn influence the neuronal connection and the whole network in hippocampus, thus resulting in rescued spatial memory.

### 3.3 Effect of MgT on the expression of some synaptic proteins in Pb-exposed rats

Dendritic spines are the locus of the vast majority of excitatory glutamatergic synapses where NMDARs,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and

PSD-95 are critical constituents.<sup>24</sup> Given that MgT increases spine density in Pb-exposed rats is real, we wondered whether MgT could increase the expression of some synaptic proteins in Pb-exposed rats. Here we checked the protein levels of NR2A, NR2B, PSD-95 and GluR1 in hippocampal DG area.

MgT significantly increased NR2A proteins in Pb-exposed rats ( $p < 0.05$ , Fig. 5A and B), but had no effect on NR2B proteins in Pb-exposed rats (Fig. 5A and C). Consistent with previous study,<sup>4,25</sup> chronic Pb exposure significantly decreased the protein level of NR2A ( $p < 0.05$ , Fig. 5A and B) without altering NR2B subunits (Fig. 5A and C). In addition, MgT significantly increased NR2A and NR2B proteins relative to control respectively ( $p < 0.01$ , Fig. 5B;  $p < 0.05$ , Fig. 5C).

As a molecular organizer, PSD-95 not only anchors NMDARs to the postsynaptic membrane but also clusters GluR1-containing AMPARs at glutamatergic synapses. Thus, based on the effect of MgT on the expression of NR2A, NR2B, we inferred that there should be an associated alteration in the level of PSD-95 and GluR1. The data showed that MgT elevated GluR1 protein expression in Pb-exposed rats (Fig. 5A and D,  $p < 0.05$ ) without altering PSD-95 proteins in Pb-exposed rats



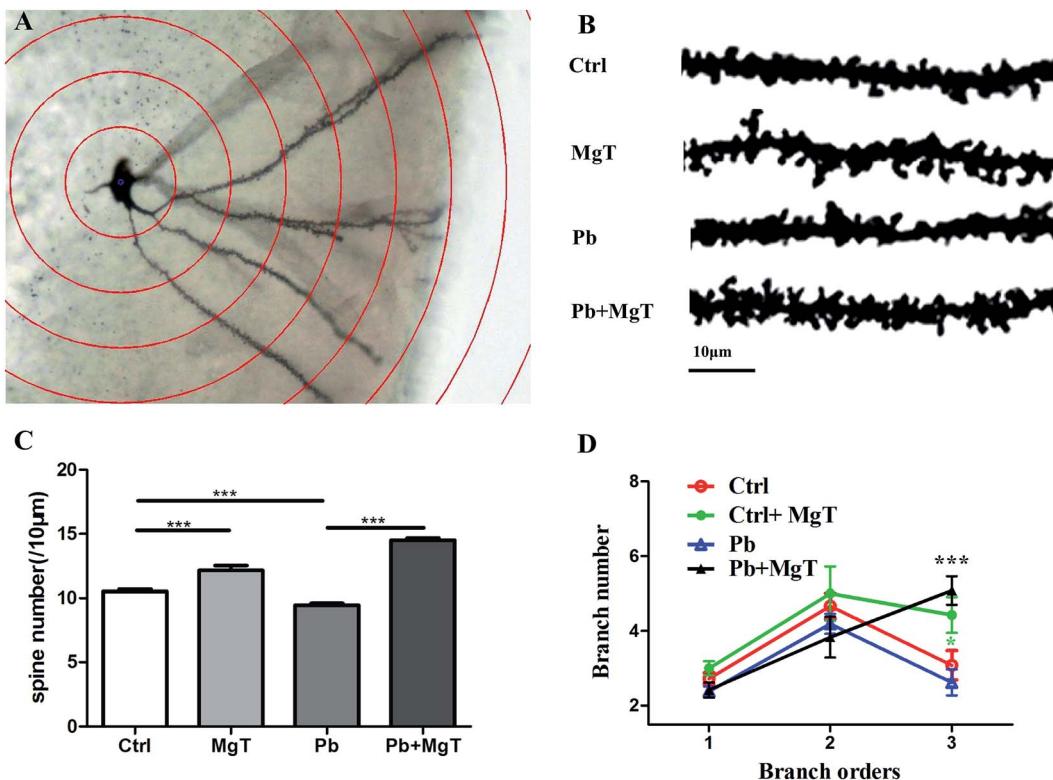


Fig. 4 MgT increases spine density and dendrite number of tertiary branch order on hippocampal DG area in Pb-exposed rats. (A) Representative images of Golgi-Cox impregnated granulosa cell in hippocampus dentate gyrus measured by Sholl analysis. (B) Representative sections of Golgi-Cox stained dendrites of granulosa cell in hippocampus dentate gyrus in four groups: control, MgT, Pb (lead) and Pb + MgT (scale bar = 10  $\mu$ m) (C) histograms plot showing the alteration of dendritic spine density (spines/10  $\mu$ m) after lead exposure or/and MgT treatment. (D) Quantification of dendrite number at different branch order in DG neurons of SD rats (\*\*p < 0.001).

(Fig. 5A and E). Moreover, Pb exposure had no significant effect on expression of PSD-95 proteins (Fig. 5A and E) and GluR1 proteins (Fig. 5A and D), and MgT increased them obviously in control rats (Fig. 5D and E,  $p$  < 0.05).

These results indicated that NR2A and GluR1 play a critical role in MgT induced alterations in dendritic spine density impaired by chronic Pb exposure.

Further we examined the levels of NR2A and GluR1 mRNA in hippocampal DG area using Q-PCR. There showed significantly increased NR2A mRNA level in Pb-exposed with MgT-treated rats compared with Pb-exposed rats ( $p$  < 0.001, Fig. 6A). In addition, Pb decreased NR2A mRNA expression significantly ( $p$  < 0.001) and MgT increased the expression of NR2A mRNA in control rats significantly ( $p$  < 0.01).

MgT did not change GluR1 mRNA level in Pb-exposed rats (Fig. 6B). Meanwhile, MgT did not increase the expression of GluR1 mRNA significantly in control rats and Pb did not show obvious effect on this expression.

Taken together, these results indicated that MgT increased the expression of NR2A at the transcriptional and translational level while it increased GluR1 expression only at translational level.

### 3.4 Pb concentration in hippocampus

Finally, we examined Pb concentration within the hippocampus. In Table 1, Pb concentration in hippocampus of

control and Pb-exposed rats on PND30 and four groups on PND70 are listed. There are significant difference in Pb concentration between control and Pb-exposed group ( $p$  < 0.05) on PND30. On the PND70, there is no significant difference between control group and MgT group, and the same result between the Pb group and Pb + MgT group. Compared with control rats, the Pb group and Pb + MgT group have significant difference in hippocampal Pb concentration, respectively ( $p$  < 0.001). These results indicate that Pb-exposed rats had much higher hippocampal Pb concentration than control rats and MgT can not inhibit the fixation of Pb in the brain, that is, MgT did not exhibit chelating effect on Pb.

## 4. Discussion

In this study, our results show that MgT can rescue the spatial memory deficits resulting from Pb exposure during development.

The cognitive function of rats exposed to Pb in “developmental window” are impaired and this effects can persist into adulthood.<sup>1</sup> Based on this study, we exposed the rats with Pb during gestation and lactation until PND30. When rats grew up to young adult (60–70 days of age), their spatial memory were checked and hippocampal Pb concentration were measured. Consist with previous study,<sup>1</sup> though Pb intake were abolished

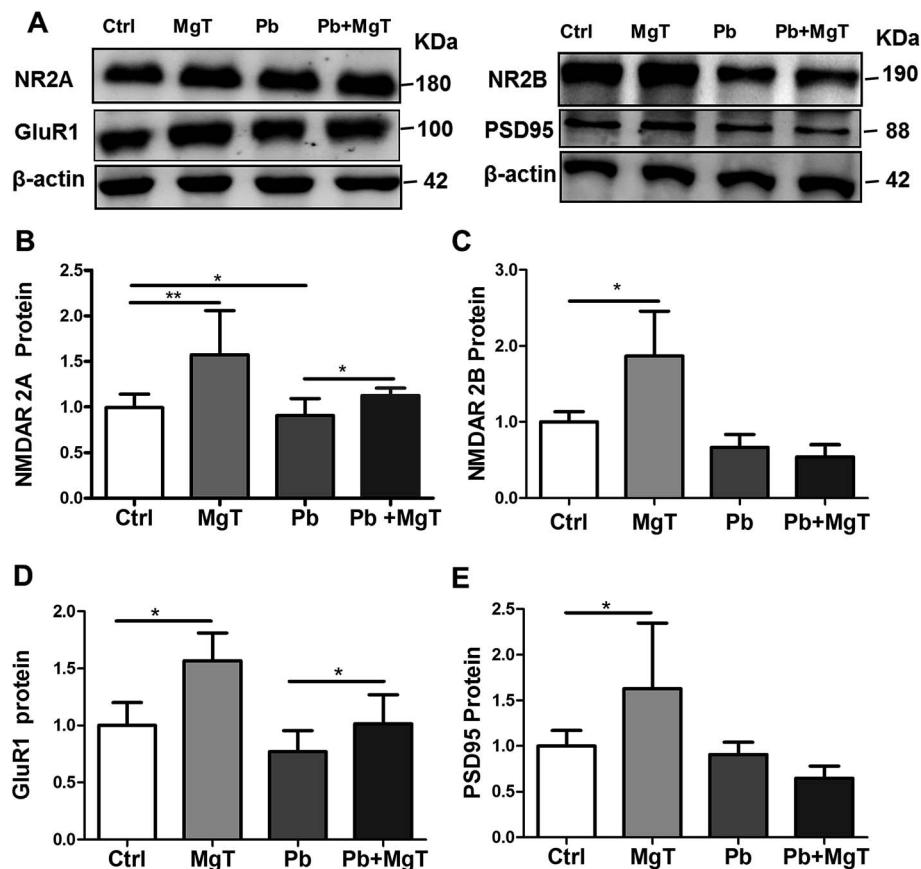


Fig. 5 Effects of MgT on protein expression of NR2A, NR2B, GluR1 and PSD-95 in hippocampal DG region in control and Pb-exposed rats. Representative immunoblot (A) and corresponding densitometric analysis showed relative expression of NR2A (B), NR2B (C), GluR1 (D) and PSD-95 (E) in four groups: control, MgT, Pb and Pb + MgT respectively. Blot images were cropped for comparison.  $\beta$ -Actin was used as a loading control (\* $p < 0.05$ , \*\* $p < 0.01$ ).

from PND31 to young adult, the impairment of the cognitive function also existed.

There are many drugs used for treating chronic Pb-induced deficits early, especially the learning and memory. These drugs are common chelating agents, such as triethylene tetramine and penicillamine which exhibited partly treating effect

on the Pb neurotoxicity, nevertheless, they showed many adverse effects at the same time.<sup>26,27</sup> Latest animal studies<sup>28</sup> show that some chemical substances, such as clioquinol and vitamin B<sub>12</sub>, can reverse the learning impairments induced by chronic Pb exposure in behavioral and electrophysiological experiment, but the action mechanisms of which are not been

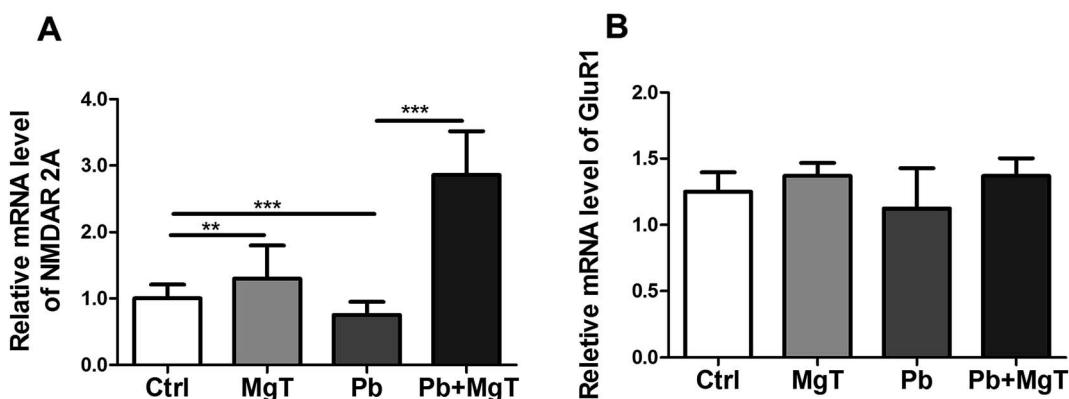


Fig. 6 Effect of MgT on mRNA levels of NR2A and GluR1 in hippocampal DG region in four groups. (A) mRNA levels of NR2A in four groups, (\*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). (B) mRNA levels of GluR1 in four groups, there were no statistical significance between any two groups of the four groups. The results were calculated as averages of triplicate experiments.

Table 1 Pb concentrations in hippocampus ( $\mu\text{g g}^{-1}$ )

	Control ( <i>n</i> = 4)	Control + MgT ( <i>n</i> = 4)	Pb-exposed ( <i>n</i> = 4)	Pb + MgT ( <i>n</i> = 4)
PND30	0.0459 ± 0.147		0.812 ± 0.147 <sup>a</sup>	
PND70	0.0852 ± 0.024	0.0731 ± 0.079	0.713 ± 0.11 <sup>b</sup>	0.557 ± 0.133 <sup>b</sup>

<sup>a</sup> Significantly different from controls of PND30 ( $p < 0.05$ ). <sup>b</sup> Significantly different from controls of PND70 ( $p < 0.001$ ).

studied further. Besides, environmental enrichment have been used by researchers to reverse learning impairments in the cognitive function induced by Pb. Though the reversal effect on the cognitive function was obvious, the mechanism of that has yet to be defined.<sup>10,29</sup>

In current study, MgT treatment for 30 days improved rats spatial memory in control group, which is consistent with study by Slucky *et al.*<sup>19</sup> While the important finding is the improvement effect of MgT on spatial memory on Pb-exposed rats.

Dendritic spines are post-synaptic locations of excitatory synapses, receiving and processing synaptic information, which protrude from the shaft or branch of the dendrite.<sup>24</sup> Loss of synapses disrupts synaptic signaling and thus leads to progressive loss of cognition.<sup>23</sup> We have previously shown that dendritic spine density decreased in hippocampal CA1 area in Pb-exposed rats on postnatal day 14 and postnatal day 21.<sup>21</sup> This decreased effect still exists on rat postnatal day 70 though Pb had been removed from the drinking water on postnatal day 30. We firstly used MgT on Pb-exposed rats and found that MgT reversed the decrease of dendritic spine density and dendrite number mediated by chronic Pb exposure. And these changes are consistent with the changes in memory ability exhibited in MWM experiment. Thus we conclude that the rescue effect of MgT on memory ability impaired by Pb is partly for their reversed effect on spine density decreased by Pb at least.

As post-synaptic terminals, dendritic spines are the locus of the vast majority of excitatory glutamatergic synapses containing NMDARs and AMPARs.<sup>24</sup> Functional NMDARs are necessary for spatial learning and memory, as assessed by the MWM.<sup>30</sup> Though exposure to Pb impaired cognitive function and hampered the expression of glutamate receptors, the underlying mechanisms remain elusive.<sup>4,22</sup> As one of main glutamate receptors, the NMDARs are composed of an obligatory NR1 subunit and one or more accessory subunits from the NR2 or NR3 families. NR2A and NR2B are the most abundant NR2 family members in the hippocampus.<sup>31-33</sup> Several lines of studies show that chronic developmental Pb exposure results in decreased NR2A content in the hippocampus while NR2B mRNA levels either remain unchanged or exhibit a slight increase in Pb-exposed rats.<sup>4,25,34</sup> Consistent with these studies, our study further confirmed these changes of NMDAR subunits expression affected by Pb. In this study, we showed that chronic dietary intake of MgT reversed NR2A protein and mRNA expression levels by chronic Pb exposure. And the reverse effect is consistent with the rescue effect of MgT on memory ability and on spine density. Meanwhile MgT increased NR2A protein and mRNA levels in hippocampal DG subregions in control rats, but there are two studies<sup>18,19</sup> which exhibited that NR2A protein

did not change by MgT, the reason should be the different region studied.

As for NR2B we have the same result with Abumaria, N., *et al.* that MgT increased NR2B expression in control rats.<sup>18,19</sup> But considering the two contradictory results that MgT increase NR2B expression and spatial learning ability and that Pb exposure result in NR2B mRNA levels either remained unchanged or exhibited a slight increase<sup>4,25,34,35</sup> and decrease spatial learning ability, we can not explain their relationship to our knowledge. In our study MgT have not exhibited significant effect on NR2B expression in Pb-exposed rats, but there is a decreasing trend which maybe suggest a changeover effect on Pb.

AMPARs are tetramers comprised of four glutamate receptor subunits, GluR1–GluR4. Accumulated evidence has demonstrated that synaptic insertion of GluR1-containing AMPARs contributes to the synaptic potentiation during LTP, experience-dependent neuronal plasticity as well as learning and memory.<sup>36-38</sup> By examining the levels of GluR1 protein expression in the hippocampus of aged animals, many studies have shown the role of GluR1-containing AMPARs in aging-associated cognitive deficits. To our surprise, the results of these studies are contradictory, for some studies report that GluR1 expression in the hippocampus is age-related decrease or increase, while others show no significant difference between young adult and aged rodents.<sup>39-41</sup> Using *in situ* hybridization, Guilarte *et al.* have found that there is no change of GluR1 mRNA in Pb-exposed animals at 50 days of age,<sup>10</sup> likewise we cannot find the mRNA change in hippocampus DG region in Pb-exposed rats though there is a decreased trend. But on protein level, MgT treatment increased GluR1 in control and Pb-exposed animals. Thus if only taking GluR1 protein expression level in account, MgT exhibited the rescue effect. Of course, what we tested is the total GluR1 protein level including the protein intracellular and inserting in cell membrane, and only inserting into postsynaptic membrane can it play a part in synaptic transduction. That is, to declare the relationship between GluR1 and synaptic plasticity, the expression on postsynaptic membrane of GluR1 protein level should be tested.

As a major component of PSD, PSD-95 is a central postsynaptic scaffold and signaling hub that promotes formation and maturation of dendritic spines.<sup>42</sup> PSD-95 is dispensable for synaptic anchoring NMDARs to the postsynaptic membrane and facilitates clustering of GluR1-containing AMPARs at glutamatergic synapses on both excitatory and inhibitory neurons. Besides these, PSD-95 controls the different physiological roles played by the NMDAR complex.<sup>43</sup> Pb exposure did not affect PSD-95 protein expression which is consistent with a previous



study.<sup>10</sup> MgT significantly increased PSD-95 protein level in control but not in Pb-exposed rats.

In summary, we show that MgT is important in rescuing memory ability impairment by Pb. By reversing the expression of such synaptic proteins as NR2A and GluR1 decreased by Pb, MgT increases the number of dendritic spines and thus the number of synapse which is benefit to improve memory ability deficits. Because MgT does not exhibit chelating effect on Pb and Mg<sup>2+</sup> can serve as a cofactor of enzymes, participating in the process of intracellular signal transduction, the possible mechanism how MgT rescues impaired memory ability by Pb may be that Mg<sup>2+</sup> promotes some enzymes activity decreased by Pb by elevating brain Mg<sup>2+</sup>. Of course the mechanism should be explored further. Our study suggest that MgT can be a potential complementary agent in the treatment of Pb intoxication.

## Conflict of interests

The authors declare no competing financial interests.

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