Effects of Organic Selenium on Lead-Induced Impairments of Spatial Learning and Memory as Well as Synaptic Structural Plasticity in Rats

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To study the effect of organic Se on spatial learning and memory deficits induced by Pb exposure at different developmental stages, and its relationship with alterations of synaptic structural plasticity, postnatal rat pups were randomly divided into five groups: Control; Pb (Weaned pups were exposed to Pb at postnatal day (PND) 21–42); Pb-Se (Weaned pups were exposed to Se at PND 43–63 after Pb exposure); maternal Pb (mPb) (Parents were exposed to Pb from 3 weeks before mating to the weaning of pups); mPb-Se (Parents were exposed to Pb and weaned pups were exposed to Se at PND 43–63). The spatial learning and memory of rat pups was measured by Morris water maze (MWM) on PND 63. We found that rat pups in Pb-Se group performed significantly better than those in Pb group (p<0.05). However, there was no significant difference in the ability of spatial learning and memory between the groups of mPb and mPb-Se (p>0.05). We also found that, before MWM, the numbers of neurons and synapses significantly decreased in mPb group, but not in Pb group. After MWM, the number of synapses, the thickness of postsynaptic density (PSD), the length of synaptic active zone and the synaptic curvature increased significantly in Pb-Se and mPb-Se group; while the width of synaptic cleft decreased significantly (p<0.05), compared to Pb group and mPb group, respectively. However, the number of synapses in mPb-Se group was still significantly lower than that in the control group (p<0.05). Our data demonstrated that organic Se had protective effects on the impairments of spatial learning and memory as well as synaptic structural plasticity induced by Pb exposure in rats after weaning, but not by the maternal Pb exposure which reduced the numbers of neurons and synapses in the early neural development.

Key words lead; organic selenium; learning; memory; synaptic structural plasticity

Lead (Pb), a well-established neurotoxicant, is found to impair the cognitive function in experimental animals and children. The learning and memory deficits induced by Pb exposure are probably due to impairments of synaptic functional plasticity. Synaptic structural plasticity is considered to be the physiological basis of synaptic functional plasticity and has been found to play very important roles in learning and memory. Synaptic structural plasticity is mainly manifested in the number and size of synapses, the width of synaptic cleft, the thickness of postsynaptic density, the length of synaptic active zone, and the synaptic curvature. The impairment of synaptic structural plasticity induced by Pb exposure at different developmental stages of the nervous system might be different. It is important to investigate whether the impairments of learning and memory induced by Pb exposure at different developmental stages can be repaired.

Metal chelators have been widely studied to observe whether they can reduce Pb-induced toxicity. Those metal chelators not only promote the excretion of lead but also lead to the loss of other essential trace elements in the human body, possibly due to the non-specificity of chelating Pb. Some metal chelators even have certain side effects. In addition, there is no evidence that metal chelators improve clinical outcomes, particularly cognitive function in children with Pb poisoning. In many countries, Pb-poisoning cases continue to occur. Thus, it is imperative to find alternative methods of preventing and/or attenuating the neurotoxicity induced by Pb because currently available therapies are very limited.

Selenium (Se), an essential trace element in the human body, has attracted many researchers’ attention due to its protective effects on Pb-induced neurotoxicity and cognitive dysfunction. Most of previous studies focus on the inorganic Se, however, the effect of organic Se on spatial learning and memory deficits induced by Pb exposure at different developmental stages, and its relationship with alterations of synaptic structural plasticity has not been reported yet. Organic Se may be a much safer and more effective supplement for the central nervous system (CNS) because it is easier to get to and much slower to disappear from CNS, compared to inorganic Se. Therefore, in the present study, we investigated the effects of organic Se on the spatial learning and memory deficits as well as the alterations of synaptic structural plasticity induced by Pb exposure in Wistar rats at different developmental stages. We found that organic Se had protective effects on impairments of spatial learning and memory as well as synaptic structural plasticity induced by Pb exposure in rats after weaning.

MATERIALS AND METHODS

Animals and Groups Adult specific pathogen free Wistar rats (40 females: 180–200 g, aged 2.5 months; 20 males: 200–240 g, aged 2 months) were purchased from the Experimental Animal Center of Sun Yat-Sen University, China (Institution license No. SYXK2007-0080). They were housed in controlled conditions of 12-h light: 12-h dark cycle, temperature (23°C), and humidity (60%). All the experimental procedures were performed in strict compliance with the guidelines for animal experiments of Sun Yat-Sen University, Guangzhou, China.

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The lead chloride (Pb, 2 mM via drinking water) (Guangzhou Chemical Reagent Factory, China) and the organic selenium enriched yeast, fermented by sodium selenite (Se, 6 µg/100 g body weight by gavage) (Hubei Angel Yeast Co., Ltd., China) were used in this study. The dosages of Pb were choosing according to literatures\(^\text{15)}\) and our pilot study.\(^\text{16)}\) The dosages of organic Se were choosing according to literatures.\(^\text{17)}\) The treatment of different groups of rats was summarized in Table 1. Since our pilot study did not find the difference in synapse number, synaptic structural parameters, spatial learning or memory between the group treated with organic Se alone and the control group, we decided to exclude the group treated with organic Se alone and to focus on the effects of organic Se on developmental Pb exposure. Female rats were randomly divided into Pb group (16 females) and Pb-free group (24 females) prior to mating. The female and male rats were mated by 2:1. The F1 generation pups were weaned at postnatal day 21 (PND 21). After weaning, the rat pups were divided into five groups and each group contained three subgroups (1 pup was selected per litter, total 8 pups per subgroup). The five groups (n=8) are as follows: Control (Both parents and weaned pups continuously had normal diet); Pb (Parents had normal diet and weaned pups were exposed to Pb for 3 weeks); Pb-Se (Parents had normal diet and weaned pups were exposed to Pb for 3 weeks and Se for another 3 weeks); mPb (Parents were exposed to Pb from 3 weeks before mating to the weaning of pups and weaned had normal diet); mPb-Se (Parents were exposed to Pb from 3 weeks before mating to the weaning of pups and weaned had normal diet for 3 weeks and Se for another 3 weeks). At the PND 63, pups of two subgroups of each group were killed to test the number of neurons (female pups) and synapses (male pups) in the rat hippocampus, respectively. The third subgroup pups (male pups) were trained with Morris water maze since PND 63 and then subjected to the measurement of the number of synapses and the synaptic structural plasticity in the CA1 region of rat hippocampus.

**Morris Water Maze (MWM) Test.** **Experimental Condition** The MWM consists of a cylindrical tank (diameter: 150 cm; height: 60 cm) and a set of video analysis system (Huabei Zhenghua Biological Instrument Equipment Co., Ltd., China). The platform is circular (12 × 35 cm) and has a rough surface. The tank is divided into four quadrants with different navigation marks for each quadrant. The midpoint of the wall in each quadrant acts as the starting location of releasing animals into the water. Keep all indoor lights on, put the curtain down and cover the tank with shade cloth.

**Spatial Acquisition: Learning Trials** Put the platform in a fixed location in the middle of one quadrant (defined as the target quadrant) of the tank and 1.5 cm below the water surface. The platform remained in the same place during learning trials. Place the animal at the starting position facing to the tank wall and released into the water horizontally. The computer tracking program was started as soon as the animal was released. The standard time limit is 90 s per trial. Stop the timer immediately when the animal reaches the platform. The animal would be placed on the platform if it could not find the platform within the time limit. Leave the animal on the platform for 15 s in order to allow the animal to orient itself to the spatial position and to remember the relative position of target platform and surrounding hints. Release the animal from a new starting position and repeat the learning trial. Animals were given 4 trials per day using different starting position in each quadrant and they were tested 5 d in total. The mean escape latency (time taken from the starting position to the platform) was measured to evaluate the spatial learning ability.

**Reference Memory: Probe Trial (24 h after the Last Learning Trial)** The examiner removed the platform and placed the animal in the diagonal quadrant of the previous target quadrant and let it face the tank wall, ensuring that its spatial preference is a reflection of the memory of the target location rather than of a specific swimming path. The animal was returned to the cage after 60 s of swimming. The object of the probe trial is to determine whether or not the animal remembers where the platform was located. Only one probe trial was performed for each animal. The following indicators were measured as site crossings (number of animals crossing the original platform within the specified time), percent distance and time in target quadrant, and first bearing (animal's swimming angle at the start of the trial relative to a direct line from the start to the goal).

**Blood and Hippocampal Lead and Selenium Levels** The blood and hippocampi of treated rats were taken out and prepared as described previously.\(^\text{16)}\) The concentrations of lead and selenium in blood and hippocampal homogenates were measured by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500c, U.S.A.). The sample was dissolved directly by microwave digestion with HNO₃–H₂O₂ and the 2 elements were determined by ICP-MS. The equipment parameters were optimized as follows: RF power—13500W, RF matching—1.75V, Sample depth—8 mm, Carrier gas—0.7 L/min, Makeup gas—0.36 L/min, Nebulizer pump—0.1 Rps, S/C temp—2°C, Sample analysis time—5 min.\(^\text{19)}\)

**Measurement of the Number of Hippocampal Neurons** The rats at PND 63 were anesthetized with urethane (25%, 0.5 mL/100 g) and intracardially perfused with 0.9% saline, followed by 4% paraformaldehyde in a 0.01 M phosphate buffer (pH 7.4). The brain was carefully removed and post fixed

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**Table 1. The Grouping and Treatment of Wistar Rats and Their Offspring**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. (rat pups)</th>
<th>Parents (3 weeks before mating until PND 20)</th>
<th>Pups (PND 21–63)</th>
<th>1w</th>
<th>2w</th>
<th>3w</th>
<th>4w</th>
<th>5w</th>
<th>6w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
</tr>
<tr>
<td>Pb</td>
<td>24</td>
<td>Pb</td>
<td>Pb</td>
<td>Pb</td>
<td>Pb</td>
<td>Pb</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
</tr>
<tr>
<td>Pb-Se</td>
<td>24</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
</tr>
<tr>
<td>mPb</td>
<td>24</td>
<td>Pb</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
</tr>
<tr>
<td>mPb-Se</td>
<td>24</td>
<td>Pb</td>
<td>H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
<td>Se</td>
<td>Se</td>
<td>Se</td>
<td>Se</td>
</tr>
</tbody>
</table>

Note: PND=postnatal day.
in 4% paraformaldehyde at 4°C overnight. After standard dehydration and diaphanization procedures, the paraffin-embedded brain was cut into 5-μm thick coronal sections using a rotating microtome (Leica RM22445, Germany). The slides were subjected to Cresyl violet (Nissl) staining. Briefly, the sections were dewaxed, rehydrated, and then immersed in 0.1% Cresyl violet solution at 37°C for 30 min. After being rinsed with double distilled water, they were dehydrated and mounted with permount. Six equidistant sections per brain and 8 different images per region per section were used for cell counting. In each hemisphere, the number of neurons was counted throughout the CA1, CA3, and DG regions of the hippocampus at 400× magnification (Nikon TE2000-V, Japan) by a person who was blind to the groups of animals via the Imaging-Pro-Plus software 6.0.

Measurement of the Number of Synapses and Synaptic Structural Parameters The rats were anesthetized with urethane before and after MWM and intracardially perfused with 0.9% saline followed by fixative in the mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4) overnight at 4°C for the sample preparation of transmission electron microscopy (TEM).

The fixed brains were sectioned coronally at 400 μm using a vibrating microtome (Leica VT 1000S, Germany). The CA1 region of hippocampus in the sectioned slice was dissected using a stereoscopic microscope and followed by post-fixation with 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) for 1 h. The tissue was infiltrated with PELCO Eponate 12 kit (Ted Pella, Inc.) following dehydration with a graded ethanol series. The 80nm sections (8 sections per animal) at similar planes were cut and mounted on copper grids. Samples were stained with 4% uranyl acetate and 0.4% lead citrate, and then viewed by a TEM (Zeiss 10C, Germany) at an acceleration voltage of 80 kV. Three photos of each sub-region (top, center and bottom) per ultrathin series per animal were taken at 13500× and 37000× magnification, respectively. All the pictures at 13500× magnification were used to observe the number of synapses and all the pictures at 37000× magnification were used to measure the synaptic structural parameters by selecting typical Gray I synapses. The number of synapses was expressed as the average of all the synapses in each photo taken at 13500× magnification. The measurement of synaptic cleft, the thickness of postsynaptic density, the length of synaptic active zone and the synaptic curvature was performed as described.

Statistical Analysis Data were expressed as mean±S.D. Data obtained from MWM tests were analyzed by repeated measures ANOVA, one-way ANOVA and Bonferroni test. One-way ANOVA followed by Bonferroni test, was employed for testing the significance of the indexes of synaptic structural plasticity among different groups. Values of \( p \leq 0.05 \) were considered statistically significant.

### RESULTS

**Developmental Pb Exposure Impairs the Spatial Learning and Memory in Rat Pups** The time required to locate the hidden platform (escape latency) decreased with practices in all groups (Fig. 1). There was a significant time-effect on escape latency within each group, suggesting that the rat pups improved their spatial learning over the 5-d training period \( (F=14.78, p<0.001) \). The mean escape latency of the Control group during Days 2–5 was significantly shorter than that of Pb and mPb group \((p<0.05)\), while there was no significant difference between Pb and mPb group \((p>0.05)\). The results suggest that both postnatal and maternal Pb exposures may impair the spatial learning in rat pups. This is probably not due to the difference in sensory or motor functions between Pb exposure group and the Control group because there is no difference in swimming speed or cued trials between them (data not shown).

In the space probe trial, site crossings, the percentage of time and distance spent in the target quadrant, and first bearing of Pb and mPb group were significantly different from those of the Control group \((p<0.05)\). But there were no significant difference in the above four measures between Pb and mPb group \((p>0.05)\) (Table 2). The results showed that the Control group rats developed a stronger bias for the platform and had clear memory of the platform position, whereas Pb exposure at different developmental stages impaired the spatial memory of rat pups.

**Organic Se Cannot Rescue the Spatial Learning and Memory Deficits Due to Maternal Pb Exposure in Rat Pups** The escape latency of each group rats gradually decreased by repeated training (Fig. 2). The time-effect of escape latency within each group was significant \( (F=12.78, p<0.001) \). The escape latency of the Control group was significantly shorter than that of Pb and mPb group during Days 2–5, suggesting that the rat pups improved their spatial learning over the 5-d training period \( (F=14.78, p<0.001) \). The mean escape latency of the Control group during Days 2–5 was significantly shorter than that of Pb and mPb group \((p<0.05)\), while there was no significant difference between Pb and mPb group \((p>0.05)\). The results suggest that both postnatal and maternal Pb exposures may impair the spatial learning in rat pups. This is probably not due to the difference in sensory or motor functions between Pb exposure group and the Control group because there is no difference in swimming speed or cued trials between them (data not shown).

### Table 2. Effects of Different Developmental Pb Exposure on the Performance of Rat Pups in the Probe Trial of MWM

<table>
<thead>
<tr>
<th>Group ((n=8))</th>
<th>Site crossings</th>
<th>Percent distance in target quadrant (%)</th>
<th>Percent time in target quadrant (%)</th>
<th>First bearing (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.75±0.55</td>
<td>32.10±1.30</td>
<td>32.20±5.46</td>
<td>8.46±1.47</td>
</tr>
<tr>
<td>mPb</td>
<td>1.50±0.57**</td>
<td>17.74±1.02**</td>
<td>18.09±0.99**</td>
<td>29.38±4.83***</td>
</tr>
<tr>
<td>Pb</td>
<td>1.35±0.35**</td>
<td>16.75±2.93**</td>
<td>17.13±4.26**</td>
<td>28.09±5.70***</td>
</tr>
</tbody>
</table>

Note: Values are expressed as mean±S.D. \(*p<0.01\) and \(**p<0.001\), compared with the Control group (one-way ANOVA and Bonferroni test).
than that of mPb and mPb-Se group (p<0.05), while there was no significant difference between mPb and mPb-Se group (p>0.05), although there was a trend of decreasing the escape latency in mPb-Se group compared to mPb group.

In the space probe trial, site crossings, the percentage of time and distance spent in the target quadrant, and first bearing of mPb and mPb-Se group were significantly different from those of the Control group (p<0.05). But there were no significant difference in the above four measures between mPb and mPb-Se group (p>0.05) (Table 3). The results suggest that organic Se cannot rescue the spatial learning and memory deficits due to maternal Pb exposure in rat pups.

**Organic Se Rescues the Spatial Learning and Memory Deficits in Rat Pups Exposed to Pb after Weaning**

The escape latency of rats in all the three groups shortened by repeated training (Fig. 3). The time-effect of escape latency of rat pups in all the three groups shortened by repeated training (Fig. 3). The time-effect of escape latency was statistically significant (F<12.46, p<0.001). The mean escape latency of the Control group and Pb-Se group were significantly shorter than that of the Pb group (p<0.05), while there was no significant difference between the Control and Pb-Se group (p>0.05).

Unlike the escape latency in place navigation test, site crossings, the time and distance spent in the target quadrant, and first bearing in the space probe trial were all significantly different between any two groups (p<0.05). The first three measures in the Control group were significantly higher than Pb group, while first bearing was significantly lower. Meanwhile all measures in the Pb-Se group fell in between the Control and Pb group (Table 4). The results showed spatial learning and memory deficits due to Pb exposure after weaning in rat pups could be improved, but not fully recovered by organic Se.

**Blood and Hippocampal Pb and Se Concentrations in Rat Pups after Different Treatments**

The concentrations of Pb and Se in blood and hippocampus of rat pups with different treatments were measured on PND 21 and PND 63 (Table 5). It showed that the concentration of blood and hippocampal Pb in all treatment groups was significantly higher than that of the Control group (p<0.05). The blood and hippocampal Pb concentration in mPb-Se and Pb-Se group was significantly lower than that of mPb and Pb group, respectively, but still higher than that of the Control group (p<0.05). As for the concentration of blood and hippocampal Se, rat pups in mPb-Se and Pb-Se groups had significantly higher blood and hippocampal Se concentrations compared to the Control group (p<0.05). This result demonstrates that organic Se can significantly reduce the concentration of blood and hippocampal Pb in rat pups.

**Effects of Organic Se on the Number of Neurons in Hippocampus of Rat Pups with Different Developmental Pb Exposures**

The Nissl staining showed that the number of neurons in hippocampus was significantly lower in Pb-exposed rat pups compared to the Control group (p<0.05). This result suggests that organic Se can rescue the hippocampal damage caused by Pb exposure.

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**Table 3. Organic Se Does Not Improve the Performance of Maternal Pb-Exposed Rat Pups in the Probe Trial of MWM**

<table>
<thead>
<tr>
<th>Group (n=8)</th>
<th>Site crossings</th>
<th>Percent distance in target quadrant (%)</th>
<th>Percent time in target quadrant (%)</th>
<th>First bearing (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>mPb</td>
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<td>18.09±0.99**</td>
<td>29.38±4.83***</td>
</tr>
<tr>
<td>mPb-Se</td>
<td>1.45±0.50**</td>
<td>18.25±2.21**</td>
<td>18.10±1.47**</td>
<td>28.52±6.20***</td>
</tr>
</tbody>
</table>

Note: Values are mean±S.D. (n=8). *p<0.05 and **p<0.01, compared with the Control group (one-way ANOVA and Bonferroni test).

**Table 4. Organic Se Improves the Performance of Pb-Exposed Rat Pups after Weaning in the Probe Trial of MWM**

<table>
<thead>
<tr>
<th>Group (n=8)</th>
<th>Site crossings</th>
<th>Percent distance in target quadrant (%)</th>
<th>Percent time in target quadrant (%)</th>
<th>First bearing (°)</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Pb</td>
<td>1.35±0.35**</td>
<td>16.75±2.93**</td>
<td>17.13±4.26**</td>
<td>28.09±5.70***</td>
</tr>
<tr>
<td>Pb-Se</td>
<td>3.25±0.55</td>
<td>27.98±2.77*</td>
<td>29.05±3.59</td>
<td>12.05±2.41*</td>
</tr>
</tbody>
</table>

Note: Values are mean±S.D. *p<0.05, **p<0.01 and ***p<0.001, compared with the Control group (one-way ANOVA and Bonferroni test).
of neurons in the regions of CA1, CA3 and DG of rat hippocampus in mPb group was much less than that of the Control group ($p<0.05$). But there was no significant difference in the number of neurons between mPb and mPb-Se groups ($p>0.05$) (Fig. 4, Table 6). The results suggested that organic Se could not rescue the decrease of neuron numbers in the hippocampus due to maternal Pb exposure in rat pups. Meanwhile, there was no significant difference of neuron numbers between the Pb group and Control group ($p>0.05$) (Fig. 4, Table 6), indicating the postnatal Pb exposure did not affect the number of neurons in the hippocampus of rat pups.

### Effects of Organic Se on the Number of Synapses in the CA1 Region of Rat Hippocampus with Different Developmental Pb Exposures

Before MWM, the number of synapses in the CA1 region of hippocampus in mPb group was significantly less than that of the Control group ($p<0.05$), but no significant difference was found between the Pb group and the Control group ($p>0.05$). After MWM, the numbers of synapses in hippocampal CA1 region of mPb and Pb group rats were significantly less than that in the Control group ($p<0.05$). The number of synapses in Pb-Se group was significantly higher than that in Pb group ($p<0.05$), however,
there was no significant difference in the number of synapses between mPb and mPb-Se groups ($p>0.05$) (Fig. 5, Table 7). The results suggested that after MWM organic Se has the protective effect on the decrease of synapse number in the hippocampus due to Pb exposure after weaning, but not maternal Pb exposure in rat pups.

**Effects of Organic Se on the Synaptic Structure in the CA1 Region of Rat Hippocampus with Different Developmental Pb Exposures**

The representative electron micrographs of CA1 regions of rat hippocampus at 37000× magnification were showed as Fig. 6. During the process of learning and memory, the synaptic cleft decreases, while the thickness of postsynaptic density, the length of synaptic active zone and the synaptic curvature increase. The measurement of synaptic structural parameters in the representative series of electron micrographs showed that the synaptic cleft in mPb and Pb group was significantly wider than that of the control group; and the thickness of postsynaptic density, the length of synaptic active zone, and the synaptic curvature were significantly smaller than those of the control group ($p<0.05$) (Table 8). These results suggest that both early and later developmental Pb exposure can impair the synaptic structure in the CA1 regions of rat pups. The synaptic structural parameters in both Pb-Se group and mPb-Se group were improved significantly compared to Pb group and mPb group ($p<0.05$). It suggests

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**Table 7. The Number of Synapses in the CA1 Region of Rat Hippocampus**

<table>
<thead>
<tr>
<th>Group (n=8)</th>
<th>Before MWM</th>
<th>After MWM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.43±2.54</td>
<td>41.26±1.79</td>
</tr>
<tr>
<td>mPb</td>
<td>19.85±1.76**</td>
<td>25.78±1.28**</td>
</tr>
<tr>
<td>mPb-Se</td>
<td>19.66±2.09**</td>
<td>25.23±1.33**</td>
</tr>
<tr>
<td>Pb</td>
<td>29.78±1.83</td>
<td>34.53±0.84*</td>
</tr>
<tr>
<td>Pb-Se</td>
<td>30.59±2.42</td>
<td>39.64±1.14</td>
</tr>
</tbody>
</table>

Note: Values are mean±S.D.; *$p<0.05$ and **$p<0.01$, compared with the Control group (one-way ANOVA and Bonferroni test).
that organic Se has the protective effect on the impairments of synaptic structure in the hippocampus caused by both early and later developmental Pb exposure.

DISCUSSION

The cognitive impairment is one of major clinical symptoms of lead-poisoned children. Both human and animal studies have strongly indicated that Pb-exposure may cause learning and memory deficits. In the present study, we used MWM to evaluate the effect of Pb and/or organic Se exposure at different developmental stages on the ability of spatial learning and memory in rat pups. Our results showed that organic Se significantly improved the rats’ ability of spatial learning and memory, i.e., shorter time of finding out the hidden platform in the place navigation test (Note: It is not due to the sensory or motor function difference of rat pups, data not shown), and more memorization of the location of the hidden platform in the probe trial than rats with Pb exposure after weaning but not with maternal Pb exposure. Thus, organic Se had protective effects on the spatial learning and memory deficits induced by Pb exposure after weaning, but not by the maternal Pb exposure in rat pups.

The mechanisms underlying the neuroprotection of Se against Pb-induced neurotoxicity are supposed to be related to the inhibition of the gastrointestinal absorption of Pb, reduction in the accumulation of Pb in the body, and the acceleration of Pb excretion. Thus, we first want to investigate whether organic Se could affect the Pb concentration in Pb-exposed rat pups. Blood Pb concentration is a valid evaluation index of body Pb levels. Our results demonstrated that the treatment of organic Se significantly reduced the concentrations of blood Pb in Pb-exposed rat pups, however, the blood Pb concentrations in rat pups treated with Pb and Se were still much higher than that in the Control group. These results suggest that the neuroprotection of Se against Pb-induced neurotoxicity may not mainly due to its effect on the absorption, accumulation or excretion of Pb.

The early developmental stage (gestation through lactation) of CNS in rats is found to be a sensitive period of low-level Pb exposure. During this stage, neurogenesis is the self-proliferation of neural progenitor cells and their differentiation into neuronal cells. Thus, the exposure of Pb at the early developmental stage (maternal Pb exposure) can inhibit the proliferation and differentiation of neural progenitor cells, resulting in the reduction of neuron numbers as shown in the results, which is consistent with the work from two other independent groups. Synapse is a highly specialized connection between two neurons, which contributes to their functional connection and signal transmission. Hence, neurons are the basis of synapse formation. In our study, the decrease of neurons induced by maternal Pb exposure led to the irreversible inhibition of synapse formation and synaptic plasticity, and therefore the irreversible learning and memory deficits. That’s why we did not find the significantly protective effect of organic Se on maternal Pb exposure-induced the spatial learning or memory deficits, although we did observe a trend of increasing synapse numbers after MWM and enhancing the ability of spatial learning in mPb-Se group compared to those in the mPb group.

At the late developmental stage of CNS, the number of neurons relatively keeps stable and neural progenitor cells usually stay at a resting state without proliferation unless they are stimulated under certain conditions. Therefore, we found that the numbers of neurons and synapses before MWM were not affected by Pb exposure after weaning in rat pups. After MWM, however, the number of synapses in Pb group was significantly less than that of the Control group. Given that the number of neurons were not affected by Pb exposure after weaning, the reduction of synapse numbers might be due to the irreversible inhibition of synapse formation and synaptic plasticity, resulting in the irreversible learning and memory damaged by Pb exposure after weaning in rat pups.

In addition to the change of synapse numbers, synaptic structure plasticity is mainly manifested in alterations of the synaptic structural parameters. Synaptic cleft is the structure responsible for the information communication between

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### Table 8: Synaptic Structural Parameters in the CA1 Region of Rat Hippocampus

<table>
<thead>
<tr>
<th>Group</th>
<th>Width of synaptic cleft (nm)</th>
<th>Length of synaptic active zone (nm)</th>
<th>PSD thickness (nm)</th>
<th>Curvature of synaptic interface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.33±0.47</td>
<td>259.32±4.92</td>
<td>74.64±3.21</td>
<td>1.407±0.0081</td>
</tr>
<tr>
<td>mPb</td>
<td>23.54±0.60*</td>
<td>219.86±6.15*</td>
<td>51.64±3.08*</td>
<td>1.080±0.0031*</td>
</tr>
<tr>
<td>mPb-Se</td>
<td>19.47±0.34*</td>
<td>241.38±9.74*</td>
<td>61.79±3.23*</td>
<td>1.275±0.0026*</td>
</tr>
<tr>
<td>Pb</td>
<td>23.76±0.42*</td>
<td>221.12±5.89*</td>
<td>51.48±5.25*</td>
<td>1.1020±0.0029*</td>
</tr>
<tr>
<td>Pb-Se</td>
<td>19.34±0.61*</td>
<td>245.94±3.28*</td>
<td>63.47±2.97*</td>
<td>1.2761±0.0058*</td>
</tr>
</tbody>
</table>

Note: Values are mean±S.D.; *p<0.05, compared with the Control group; †p<0.05, compared with the mPb group; ‡p<0.05, compared with the Pb group (one-way ANOVA and Bonferroni test).
neurons, and its size and shape are very important for the synaptic activity. PSD provides the structure basis for the stability of ion channels and aggregation of signal molecules, and its thickness and length (the length of synaptic active zone) are supposed to control the transmitting efficiency of neurotransmitters. Synaptic curvature, defined as the curvature of the interface connecting presynaptic components and postsynaptic components of synapses, is associated with synaptic activity state. In the present study, we observed that organic Se had protective effects on the changes of synaptic structural parameters induced by both early and later developmental Pb exposure, such as the decrease of the width of synaptic cleft and the increases of the thickness of PSD, the length of synaptic active zone and synaptic curvature. These alterations of synaptic structural parameters may decrease the distance and increase the contact area between presynaptic components and postsynaptic components of synapses so as to enhance the transmitting efficacy of neurotransmitters and synaptic functional plasticity, resulting in further improving the ability of learning and memory. The changes of the above synaptic structural parameters may be due to the regulation of neural cell adhesion molecules (NCAMs) because our another study found that organic Se had protective effects on Pb-induced decrease of NCAM expression and increase of the activity of sialyltransferase in the hippocampus, which might affect the synaptic structure during development. At the resting state, NCAM undergoes posttranslational modifications that involve adding several α-2,8-polysialic acid (PSA) residues in its extracellular domains, which is beneficial for keeping the structural stability of synapses. During the process of learning and memory, the nerve impulse will remove those PSA residues from NCAM so that astrocytes surrounding the synapses will shrink because of the change of their adhesion strength, resulting in the decrease of synaptic cleft and the increase of PSD, the length of synaptic active zone and the synaptic curvature.

But it should be pointed out that although organic Se significantly reversed the changes of synaptic structural parameters caused by maternal Pb exposure, it only showed a trend of increasing synapse numbers after MWM and enhancing the spatial learning ability without significance. This might be due to the fact that a significant loss of neurons and synapses already happened in maternal Pb exposure rat pups. Previous study reported that inorganic Se (sodium selenite) significantly alleviated the cognitive dysfunction caused by Pb exposure from lactation until weaning in Sprague-Dawley (SD) rats. Our data together with data from other groups suggest that the protection of organic Se against Pb-mediated spatial learning and memory deficits is dependent on the time of Pb exposure, i.e., prenatal Pb exposure-mediated learning and memory damage is irreversible, while learning and memory damage caused by postnatal Pb exposure is still reversible.

In conclusion, we have shown that organic Se affected the synaptic structure plasticity and improved spatial learning and memory deficits induced by Pb exposure in rat pups after weaning. The food rich in organic Se may be used for the prevention and control of Pb intoxication in children. Since the loss of neurons and the cognitive impairment are basically irreversible induced by early developmental Pb exposure, we must pay more attention to women before and during gestation. The exact mechanisms underlying the protective effects of organic Se on Pb-induced learning and memory deficits and whether the protection is permanent need more in-depth studies in the future.

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