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# Potential Association of Lead Exposure During Early Development of Mice With Alteration of Hippocampus Nitric Oxide Levels and Learning Memory

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**Objective** Chronic lead (Pb) exposure during development is known to produce learning deficits. Nitric oxide participates in the synaptic mechanisms involved in certain forms of learning and memory. This study was designed to clarify whether Pb-induced impairment in learning and memory was associated with the changes of nitric oxide levels in mice brains. **Methods** Sixty Balb/c mice aged 10 days were chosen. A model of lead exposure was established by drinking 0.025%, 0.05%, 0.075% lead acetate, respectively for 8 weeks. The controls were orally given distilled water. The ability to learn and memorize was examined by open field test, T-water maze test. In parallel with the behavioral data, NO level of hippocampus tissue was detected by biochemical assay. **Results** Compared with control groups, (1) the weight of 0.075% group was significantly reduced ($P<0.05$); (2) The number of times in mice attaining the required standards in T-water maze test was lower in 0.075% group ($P<0.01$). No significant difference was found between experimental and control groups in open field test ($P>0.05$); (3) NO level of mouse hippocampus tissue was decreased in 0.075% group ($P<0.01$). **Conclusions** The findings suggest that decreased hippocampus NO level may contribute to the Pb-induced deficits in learning and memory processes.

**Key words**: Lead exposure; Hippocampus; Learning and memory; Nitric oxide (NO)

## INTRODUCTION

Lead has been widely used for many purposes since ancient times and lead poisoning has been studied for centuries. Lead is an environmental toxicant that may deleteriously affect the nervous, hematopoietic, endocrine, renal, and reproductive systems. Lead poisoning has serious and even fatal consequences at any age, but young children are especially vulnerable. Lead exposure in young children is particularly serious because children absorb lead more readily than adults, for the developing nervous systems of children are more susceptible to the effects of lead. Children with greater lead levels may (also) have problems with learning and reading, and may also have growth retardation. Epidemiologic studies suggested that there was a negative correlation between long-term exposure to lead and learning memory[1]. The neurobehavioral toxicity of developmental exposure to lead (Pb) was investigated by conducting tests of spatial learning in the Morris Water Maze. In the Morris Water Maze, a statistically significant increase in the time required to find the hidden platform (escape latency) was observed when Pb-treated rats were tested in a reference memory paradigm[2]. However, the exact mechanisms of lead neurotoxicity remain to be determined. Nitric oxide (NO) is a free radical, which is produced in several tissues of the body and is thought to be the first of a new class of neural messenger molecules and a retrograde modulator of synaptic transmission in the brain. NO is now recognized as an important signaling molecule in the mammalian brain where it is thought to be involved in a number of important processes such as learning and memory. Inhibition of NO is known to possibly impair learning and memory. NO is a free radical synthesized by nitric oxide synthase (NOS) during the conversion of L-arginine to citrulline. It has a short half life and converts immediately into nitrite and nitrate. This study was designed to clarify whether Pb-induced impairment in learning and memory was associated with NO production by measuring nitrite and nitrate in mouse hippocampus.

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## MATERIALS AND METHODS

### *Animals and Experimental Design*

Sixty Babl/c mice (10-day-old) were purchased from Animal Center, Zhejiang University of Traditional Chinese Medicine. They were divided randomly into four groups. A model of lead exposure was established by drinking 0.025%, 0.05%, 0.075% lead acetate, respectively for 8 weeks. The controls were orally given distilled water. There were no significant differences in body weights at the beginning of the experiment. Body weights were recorded weekly.

### *Open Field Behavior*[3]

To test their locomotor function and exploratory behavior in a novel context, the animals were placed for 3 min in an open field circular tank (1 m diameter) with a wall 40 cm in height, and its floor was divided into equal-sized grids, recording the number of grid crossings and rearing (the number of times an animal was standing on its hind legs with forelegs in the air or against the wall) during prescribed time periods.

### *T-water Maze*

Learning abilities of mice were assessed in a T-water maze. The water maze consisted of a metal T-shaped pool (vertical arm: 30 cm×14 cm×25 cm, horizontal arm: 50 cm×14 cm×25 cm), and filled with water (25℃) in which a circular escape platform was located on the surface of the 10-cm-deep water. In a T-water maze experiment the correct reflex of mice was directly turning their right paws to reach the platform. After the mice reached the platform, they were allowed to stay on it for 30 sec. The mice were trained in two daily sessions consisting of four trials and given 60 s to find the escape platform in the pool. If the mice did not find the platform within this limit, they were guided onto it. An experiment was made on day 3 and the mice were given 25 trials to find it. If 8 trails were successful in 25 trials, the experiment was considered as "success" (attaining the required standards) and the number of right reflexes (learning score, LS) in 25 trials was recorded. Otherwise it was recorded as "failure" (failing to reach the required standards).

### *NO Measurements*

After the T-water maze test and open field test, these mice were decapitated, and their brain hippocampi were quickly removed and homogenized in 154 mmol/L NaCl using a polytron. NO concentration in brain homogenates was analyzed with a NO assay kit (Nanjing Jianchen Company, China) and absorbance was read spectrophotometrically at 540 nm (UV-160A, Japan).

$$\text{NO (μmol/g protein)} = \frac{(OD_{sample} - OD_{blank})}{(OD_{standard} - OD_{blank})} \times 20\ (\mu mol/L) \div \text{protein of sample g/L}$$

### *Statistical Analysis*

Data were analyzed by SPSS 10.0. Results were expressed as $\bar{x} \pm s$. Differences in each parameter among the four groups were analyzed by analysis of variance (F test). Differences between two groups were examined for significance with the Student-Newman-Keuls (SNK) test. Furthermore, T-water maze data were analyzed with $\chi^2$ test.

## RESULTS

### *Assessment of Lead Levels*

Blood lead levels (BLLs) were measured by atomic absorption spectrometry (PE-700AA), which were significantly higher in the 0.075%, 0.05%, 0.025% groups than in the control ($P$<0.05, Table 1).

TABLE 1

Blood Lead Levels after Exposure to Lead

| Group | n | BLLs（$\bar{x} \pm s$, μg /dL） |
|---|---|---|
| 0.075% | 17 | 68.9±12.9 |
| 0.05% | 16 | 30.4±16.5 |
| 0.025% | 14 | 15.7±7.9 |
| Control | 15 | 7.8±2.1 |

### *Growth*

Body weight was affected by lead exposure. Within 21 days there was a decreasing tendency of the body weight of mice in 0.075% and 0.05% groups (Fig. 1). Body weight remained significantly lower until d 35 and d 56 in 0.075% group, compared with the mice in 0.025% and control groups ($a$<0.05, Table 2).

TABLE 2

Effect of Lead on the Weights in Mice

| Group | n | Weight（$\bar{x} \pm s$, g） | | |
|---|---|---|---|---|
| | | d 7 | d 35 | d 56 |
| 0.075% | 17 | 13.94±2.57 | 19.20±2.28[a,b] | 21.95±2.31[a, b] |
| 0.05% | 16 | 14.32±2.61 | 20.30±1.57 | 23.39±1.18 |
| 0.025% | 14 | 14.48±2.01 | 21.51±2.07 | 23.93±2.27 |
| Control | 15 | 13.49±2.26 | 21.20±2.28 | 24.15±2.60 |

*Note.* [a]$P$<0.05, compared with control. [b]$P$<0.05, compared with 0.025% group.

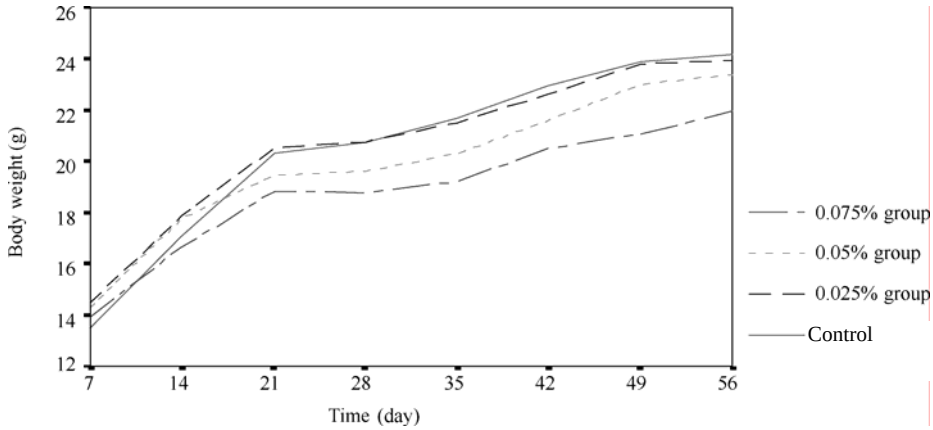


FIG. 1. Effect of lead exposure on body weight.

*Open Field*

Both groups (0.075%, 0.05%) showed signs of habituation to the new environment, as indicated by a tendency for a decrease in the total distance moved, and a reduction in rearing, but no significant difference was detected among the four groups ($P$>0.05, Table 3).

TABLE 3

Effect of Lead Exposure on Open Field in Mice ( $\bar{x} \pm s$ )

| Group | n | Crossing (3 min) | Rearing |
|---|---|---|---|
| 0.075% | 17 | 52.76±21.65 | 0.58±0.24 |
| 0.05% | 16 | 53.94±18.34 | 0.88±1.54 |
| 0.025% | 14 | 62.21±39.13 | 2.90±0.61 |
| Control | 15 | 62.60±18.07 | 3.01±0.56 |

*T-water Maze*

There was an effect of lead treatment on mice in T-water maze and the mice with lead exposure had a trend to lower the correct reflex compared with control group. There was a significant difference between 0.075% group and control group in T-water maze test ($P$<0.01, Table 4).

TABLE 4

Effect of Lead Exposure on Learning and Memory in Mice

| Group | *n* | Success | | Failure | |
|---|---|---|---|---|---|
| | | *n* | % | *n* | % |
| 0.075%[a] | 17 | 5 | 29.49 | 12 | 70.51 |
| 0.05% | 16 | 6 | 37.50 | 10 | 62.50 |
| 0.025% | 14 | 6 | 44.44 | 8 | 55.56 |
| Control | 15 | 10 | 66.67 | 5 | 33.33 |

*Note.* [a]$P$<0.01, compared with control.

*Hippocampus NO Levels*

Hippocampus NO levels in the lead exposure groups showed a decreasing tendency and were significantly lower in the 0.075% group than those in the control group ($P$<0.01, Table 5).

TABLE 5

Effect of Lead Exposure on NO Levels in Mice Hippocampi ( $\bar{x} \pm s$, μmol/g prot.)

| Group | n | NO |
|---|---|---|
| 0.075%[a] | 17 | 3.28±1.64 |
| 0.05% | 16 | 4.03±1.51 |
| 0.025% | 14 | 4.52±1.48 |
| Control | 15 | 5.13±0.86 |

Note. [a]$P$<0.01, compared with control.

## DISCUSSION

It has been reported that NO is involved in neurosynaptic transmission[4-5] and plays an important role in the control of cerebral blood flow. Pharmacological inhibition of NO synthesis could reduce spontaneous and stimulant-induced activities in animals. The nitric oxide/c GMP system has been shown to play a crucial role in the mechanism of learning and memory. Calcium is a common intracellular mediator for these regulatory processes. Thus significant activities in neurosynaptic transmission of animals could be completely blocked in the absence of calcium or in the presence of a calmodulin inhibitor[3]. This study showed that mice drinking 0.075% lead acetate decreased their learning memory and hippocampus NO levels. The reasons are as follows. First, lead may substitute for calcium in many intracellular regulatory events[6]. Since lead

interacts with calcium-calmodulin, nitric oxide synthase (NOS) in brains is regulated by calcium-calmodulin. Thus, possible replacement of calcium with lead and decreasing intracellular calcium may be the reasons for the observed alterations[7]. Second, previously it was reported that lead acetate could cause a significant reduction in stimulated flow rate, total protein and some electrolyte disturbances[8]. Interaction of heavy metal cations may exert effects on the constitutive nitric oxide synthase catalytic site(s) by direct binding to it or by interfering with the electron transfer during catalysis[9]. Considering the properties of lead to interact with calcium- and calmodulin-dependent processes, its interaction with constitutive nitric oxide synthase could be anticipated[10]. The decrease of NO may explain the Pb-mediated cognitive deficits because NO regulates many neurophysiological events in the developing nervous system. We suggest that lead exposure in developing mice contributes to the decrease of hippocampus NO which is associated with lead-induced impairment in learning memory. The controversy on the exact role of lead in the impairment of learning and memory remains to be elucidated by further studies.

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