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Techniques and Methods

Low-level lead exposure effects on spatial reference memory and working memory in rats★

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Abstract

BACKGROUND: Studies have demonstrated that lead exposure can result in cognitive dysfunction and behavior disorders. However, lead exposure impairments vary under different experimental conditions.

OBJECTIVE: To detect changes in spatial learning and memory following low-level lead exposure in rats, in Morris water maze test under the same experimental condition used to analyze lead exposure effects on various memory types and learning processes.

DESIGN AND SETTING: The experiment was conducted at the Animal Laboratory, Institute of Psychology, Chinese Academy of Science between February 2005 and March 2006. One-way analysis of variance (ANOVA) and behavioral observations were performed.

MATERIALS: Sixteen male, healthy, adult, Sprague Dawley rats were randomized into normal control and lead exposure groups (n = 8).

METHODS: Rats in the normal control group were fed distilled water, and those in the lead exposure group were fed 250 mL of 0.05% lead acetate once per day. At day 28, all rats performed the Morris water maze test, consisting of four phases: space navigation, probe test, working memory test, and visual cue test.

MAIN OUTCOME MEASURES: Place navigation in the Morris water maze was used to evaluate spatial learning and memory, probe trials for spatial reference memory, working memory test for spatial working memory, and visual cue test for non-spatial cognitive function. Perkin-Elmer Model 300 Atomic Absorption Spectrometer was utilized to determine blood lead levels in rats.

RESULTS: (1) In the working memory test, the time to reach the platform remained unchanged between the control and lead exposure groups (F(1,1) = 0.007, P = 0.935). A visible decrease in escape latencies was observed in each group (P = 0.028). However, there was no significant difference between the two groups (F(1,1) = 1.869, P = 0.193). The working memory probe test demonstrated no change between the two groups in the time spent in the target quadrant during the working memory probe test (F(1,1) = 1.869, P = 0.193). However, by day 4, differences were observed in the working memory test (P < 0.01). (2) Multivariate repetitive measure and ANOVA in place navigation presented no significant difference between the two groups (F(1,1) = 0.579, P = 0.459). (3) Spatial probe test demonstrated that the time to reach the platform was significantly different between the two groups (F(1,1) = 4.587, P = 0.048), and one-way ANOVA showed no significant difference in swimming speed between the two groups (F(1,1) = 1.528, P = 0.237). (4) In the visual cue test, all rats reached the platform within 15 seconds, with no significant difference (F(1,1) = 0.579, P = 0.459). (5) During experimentation, all rats increased in body mass, but there was no difference between the two groups (F(1,1) = 0.05, P = 0.943). At day 28 of 0.05% lead exposure, the blood lead level was 29.72 μg/L in the lead exposure group and 5.86 μg/L in the control group (P < 0.01).

CONCLUSION: The present results revealed low-level lead exposure significantly impaired spatial reference memory and spatial working memory, but had no effect on spatial learning.

Key Words: lead; spatial learning; reference memory; working memory

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INTRODUCTION

Lead toxicity remains a significant public health problem because of the global pervasiveness and adverse effects to the nervous system. Epidemiological studies have revealed that low-level lead exposure is associated with a variety of cognitive and neurobehavioral dysfunctions in infants, children, and adults[1-2], such as attention deficit disorder with hyperactivity[3-5], deficits in learning

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and memory processes, discrimination dysfunction[6], deficits in inhibitory avoidance learning and reverse learning, and decreased probe behaviors[7-8].
Although there have been many attempts to explain the underlying, specific causes of these disturbances in terms of structures or target neurotransmitters, it has been difficult to establish a unifying explanation for the diverse effects of lead exposure. Moreover, although numerous publications have addressed the actions of lead on learning and memory, it is often difficult to compare these studies, because many observations lack confirmation from other laboratories. In addition, reports of lead exposure effects are highly variable, and even contrary, such as those on lead's effects on motor ability. Some reports have suggested that lead exposure leads to hyperactivity, others believe that lead exposure results in reduced activity[9], and another experiment reported no effects from lead exposure on motor ability[10].
Following a survey of publications, it is clear that lead-induced effects depend on a variety of aspects, such as exposure level, regimen (breast milk, food, and drinking water), duration of lead exposure, and age at exposure[11]. Therefore, consistent experimental conditions are very important when comparing these studies.
In the present study, the effects of low-level lead exposure (0.05% of lead acetate in the drinking water for 28 days) on the spatial learning and memory in rats were analyzed using the Morris water maze. This study aimed to develop an animal model of lead-induced cognitive deficits, and to provide behavior insight into the properties of lead exposure-induced learning and memory impairment.

MATERIALS AND METHODS

Design
One-way analysis of variance (ANOVA) and behavioral observation experiment in rats.

Time and setting
The experiment was performed at the Animal Laboratory, Institute of Psychology, Chinese Academy of Sciences.

Materials
Sixteen male, Sprague-Dawley rats, aged 2 months and weighing 405–500 g at the beginning of the experiment (Grade I, Permission no. 199036, Institute of Genetics, Chinese Academy of Sciences, Beijing, China), were housed in a controlled temperature (20–24 ℃) and humidity (40–70%) colony room with a light cycle of 7:00–19:00. The rats were housed in stainless steel, mesh cages (25 cm × 22.5 cm × 30 cm). Each cage contained eight rats, with free access to food and water. All experiments were conducted during the light phase (9:00–18:00). Rats were gently handled 3 days prior to formal experimentation. The experimental protocol and procedures were in compliance with the *Guide for Care and Use of Laboratory Animals*, Published by the Ministry of Science and Technology, China [12].

Reagent/device	Source
99% lead acetate	Xiangzhong Fine Chemicals Factory, Hunan
Model 300 Atomic Absorption Spectrometer	Perkin-Elmer, USA
Morris water maze	Animal Laboratory, Institute of Psychology, Chinese Academy of Sciences, Beijing

Experimental procedures
Animal grouping and intervention
Animals were randomly allocated into two groups, with eight rats in each group. The control group received distilled drinking water, 250 mL per day, for 28 days. The lead exposure group was administered 0.05% lead acetate in the drinking water, 250 mL per day, for 28 days. Body mass was recorded weekly during exposure. Following behavioral measurements, the animals were anesthetized by decapitation, and blood was collected for lead analysis using a Perkin-Elmer Model 300 Atomic Absorption Spectrometer (Health Science Center, Beijing University)

Apparatus
The Morris water maze consisted of a circular pool (180 cm in diameter, 50 cm in height) and a mobile platform. The pool was filled with water at (20 ± 2) ℃ to a depth of 30 cm, and the surface was covered with prepared Chinese ink. The escape platform was a Plexiglas square platform with scratches to provide traction (10 cm in diameter, 28 cm in height) supported by adjustable Plexiglas stands that enabled them to be hidden 2 cm below the water surface. The pool was placed in the center of the room, with several extra-maze visual cues, such as a picture on the left wall, door, window, lamp, computer, camera head, and wire. The auto-recording system included a camera head in the roof and a computer.

Morris water maze test
The experiment consisted of four phases: place navigation, probe test, working memory test, and visual cue test. One week prior to experimentation, all rats underwent handling for 5 minutes per day for acclimatization to handling.
Place navigation: During place navigation [13], the platform remained in a constant position in the center of the northeast quadrant, and was hidden 2 cm below the surface of the water. A trial consisted of placing the rat in the water facing the pool wall in a randomly chosen quadrant. The rat was allowed 60 seconds to locate the platform, after which the rats were placed on the platform by the observer if they were unsuccessful. After reaching or being placed on the platform, the rats were allowed to remain on the platform for 10 seconds, followed by a rest of 30 seconds prior to the start of the next trial. Each rat received one session of four trials per day, and the mean daily escape latency was calculated by averaging

latencies from each of the four daily trials to measure acquisition. Testing continued until the control group reached a pre-determined average escape latency criterion of 15 seconds.

Probe test: A probe test was performed to introduce the rats to the tank at the farthest southern point from the initial release point, with the platform removed. The rats were allowed to swim for 2 minutes. The swimming path, time to reach the target, and swimming speed were obtained by dividing total distance traveled by time to determine spatial reference memory.

Visual cue test: Following the probe test, the platform was placed 2 cm above the surface in a constant position at the center of the northeast quadrant, with a red marker. Latencies were recorded at four release points to calculate the average latency to determine non-spatial cognitive function.

Working memory test: In the working memory test, rats were required to find a hidden platform that was placed at a new position in each session. The rats performed four sessions (20, 30, 40, and 50 cm from the edge of the pool) per day. Each session consisted of two 60-second trials (trial 1 and trial 2) to locate the escape platform. In the first trial, the rats were randomly placed in the water facing the pool wall at one of the four starting positions, and were given 60 seconds to locate the platform, or were placed on the platform after an unsuccessful 60-second swimming time. After a 15-second rest on the platform, trial 2 began by placing the rat in the same starting position as trial 1 and recording escape latency to locate the platform. The latencies in trial 1 or trial 2 in each of the four sessions were averaged. The test continued until the control group reached a pre-determined average escape latency criterion of 15 seconds.

Determination of blood lead level [14]: 50.0 μL blood sample was added into a sample cup with 50.0 μL tritonox-100 and 400.0 μL double-distilled water. After mixing well, blood lead levels were measured using an atomic absorption spectrometry with a graphite furnace. During measurements, 25.0 g/L ammonium phosphate was utilized as a matrix modifier. Absorbance value was measured at 283.3 nm, with 0.5 nm slit width and 5.0 mA lamp current.

Main outcome measure

Changes in rats following working memory test, place navigation, probe test, and visual cue test; changes in body mass and blood lead levels during the trials.

Design, enforcement, and evaluation

The experiment was designed by the second author, operated by the first author, and evaluated by the third author. All authors received training, and blood lead levels measurements were blind.

Statistical analysis

Statistical comparisons were made using student *t*-tests and one-way repetitive measure ANOVA to determine differences between control and experimental groups (SPSS 11.5). Differences between groups were considered significant if $P < 0.05$.

RESULTS

Working memory test

Escape latency

As expected from random variation of platform placement and animal start positions between sessions, the latencies to find the hidden platform on the first trial varied randomly within, as well as between the groups, over the 4 days of testing ($F(1,1) = 0.007$, $P = 0.935$). The latencies to find the platform on the second trial were considerably shortened in each session, but there remained no difference between the two groups ($F(1,5) = 0.089$, $P = 0.361$). The results are illustrated in Figure 1.

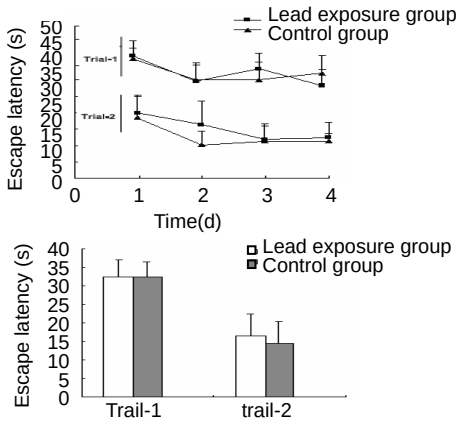
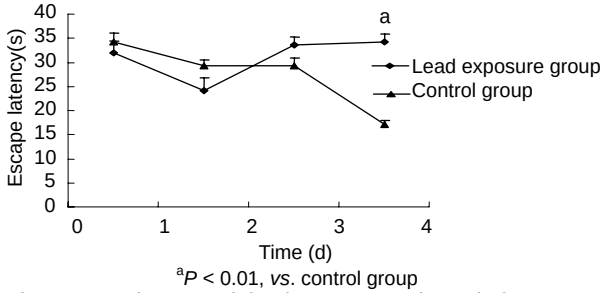


Figure 1 Average latency in control and lead exposure groups

Probe test in the working memory test

There was no significant difference in the time to stay on the target quadrant during the working memory probe test between the two groups ($F(1,1) = 1.869$, $P = 0.193$) until the fourth day working memory testing ($P < 0.01$, Figure 2).



[a]$P < 0.01$, *vs*. control group

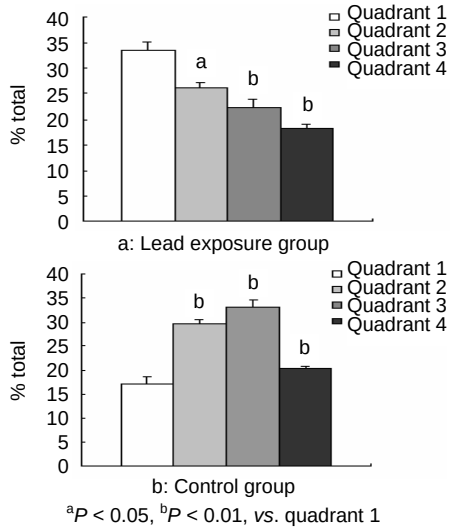
Figure 2 Time remaining in target quadrant during working memory probe test

On day 4, further analysis to determine the time spent in the four quadrants demonstrated that lead exposure rats spent significantly more time in the target quadrant, compared with the other quadrants during the previous session ($P = 0.031$, $P = 0.001$, $P = 0.002 < 0.01$, Figure 3), Furthermore, as shown in Figure 3, the percentage in the target quadrant was significantly greater in the other three quadrants ($P = 0.031$, $P = 0.001$, $P = 0.002$; Figure 3a). Moreover, the time spent in the target quadrant was

significantly shorter in the control subjects, compared with the other three quadrants ($P < 0.01$; Figure 3b). These results indicated that lead-exposure rats searched for the platform in the target quadrant, while the controls looked for the platform in the other three quadrants.

Place navigation

During water maze acquisition, all groups behaved similarly. Multivariate repetitive measure ANOVA revealed no differences between the groups ($F(1,1) = 0.579$, $P = 0.459$). However, there were differences in trials from different trial days in each group ($P < 0.05$). The average latencies were approximately 15 seconds in the two groups after 4 days of training, indicating that lead exposure did not impair acquisition of spatial reference memory (Figure 4).



[a]$P < 0.05$, [b]$P < 0.01$, *vs*. quadrant 1

Figure 3 Time remaining in the four quadrants on day 4

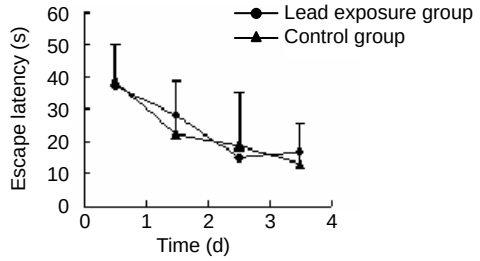


Figure 4 Average latency in place navigation

Spatial probe test

Significant differences in the time to reach the target were determined between the two groups ($F(1,1) = 4.587$, $P = 0.048$), indicating that lead exposure impaired spatial reference memory in rats. One-way ANOVA demonstrated no significant difference in swimming speed ($F(1,1) = 1.528$, $P = 0.237$), suggesting that lead exposure did not affect motor ability or coordination in the rats.

Visual cue test

One-way ANOVA revealed no significant differences in the time to reach the platform between the two groups ($F(1,1) = 0.129$, $P = 0.725$), and all rats reached the platform within 15 seconds. These results indicated that the non-spatial components of the water maze task were normal in the lead-exposure rats, and the effect of lead intoxication in the water maze test was not attributed to non-cognitive aspects of the task.

Blood lead levels and body mass

Body mass increased during experimentation. However, one-way repetitive measure ANOVA revealed no significant difference in body mass between the two groups ($F(1,1) = 0.05$, $P = 0.943$), suggesting that lead acetate did not decrease food intake or weight gain. Exposure to 0.05% lead acetate in the drinking water for 28 days resulted in significantly increased blood lead levels, (29.72 ± 3.14) μg/L in the lead exposure group and (5.86 ± 0.56) μg/L in the control group. A *t*-test of mean blood lead levels demonstrated a significant effect of lead exposure between the two groups ($P < 0.01$).

DISCUSSION

In the present study, lead exposure led to increased blood lead levels, and behavioral results revealed that lead intoxication impaired spatial reference memory and working memory in rats, with no effects on spatial learning.

In the place navigation test, control and lead-exposure subjects gradually found a shorter path to the target with decreased latencies to reach the hidden platform averaging between 14–15 seconds. This suggested that the ability to retain information about the target location remained intact in lead-exposure rats. However, in the probe test, lead-exposure rats exhibited a longer latency to locate the hidden platform at day 7, although these subjects also swam to the target area similar to the controls. This clearly suggested that lead exposure can impair spatial reference memory, because spatial accuracy is determined during the probe test, where a well-trained animal will show high preference to the target quadrant.

More interestingly, analysis of working memory performance determined that the results from the second trial and probe test were not consistent. Previous experimental animal studies showed that sufficient learning curves could be obtained by repeatedly performed probe tests after each training session by means of variable interval probe test as a tool for repeated measurements of spatial memory [15]. There was no difference in trial 2 between the two groups, which might be due to familiarity with the experimental context or a cognitive map correlating the contextual information. Although tasks changes under the experimental context remained intact (extra-maze cues), the rats still completed the task. It is possible that the spatial information during space learning might have assisted in locating the submerged platform during the working memory task. Therefore, performance in the second trial of lead-exposure rats was similar to that of

the control rats.

Differences were measured between groups in the probe test without affecting the second trial. In the fourth probe test, lead-exposure rats spent significantly more time in the quadrant where the platform had been located in the previous session, compared with the remaining three quadrants. In comparison, control rats spent significantly less time in the platform quadrant, compared with the other three quadrants. These results suggested that lead exposure impairs working memory in rats. Working memory functions occurred for a particular trial, but were then forgotten or ignored during subsequent trials.

The deficits observed during the Morris water maze training phase could not be attributed to impairment of non-cognitive processes. There are several reasons why the authors believe the effects of lead were primarily due to the effect on cognitive processes. First, the visual cue test results indicated that motor, motivational, and visual abilities did not significantly differ between the treatment groups. If functional disabilities involving these parameters were present in the lead-exposed rats, the rats should have needed significantly longer to locate the visual platform. Second, we believe that swim speed is a good measure of motor and coordination abilities, and the lack of difference in swim speed during the probe tests indicated that differences in motor function could not have accounted for differences in performance. Last, experimental evidence exists that body weight did not correlate with poor performance in the Morris water maze. Many studies have reported that lead exhibits an inhibitory effect on long-term potentiation [16]. However, partial long-term potentiation remains, because of the existence of other neural circuits. Therefore, it is plausible that lead exposure affects spatial reference and working memory, but not spatial learning. Nevertheless, further studies are needed to determine these mechanisms. A further understanding of the mechanisms affected by lead will advance the design of strategies to prevent and treat lead intoxication.

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Background: The study was supported by the Hunan Provincial Bureau of Science and Technology, No.99SSY-2002-8 for compound preparation effects on learning and memory of low-level lead exposure rats.

Professional evaluation: The present study detected low-level lead exposure effects on spatial learning and memory in rats using the Morris water maze test, as well as lead effects on cognitive strategies, memory types, and memory processes. This study was rationally designed with novel operating methods, attained reliable data, and possessed evident innovation.

Bias or limitations: There is a close association between learning and memory in the psychological field. However, findings from the present study suggest that lead exposure results in memory impairment, with no effects on learning. Accordingly, further studies are needed to determine whether lead exposure only impairs memory, and not learning, as well as the internal relationship between learning and memory.

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