

# Impaired Spatial Learning and Reduced MK-801 Associated Behavioral Deficits in Rodents Following Early Postnatal Exposure to Low-level Lead

Jing Guo  
Department of Life Sciences  
University of Toronto  
1265 Military Trail  
Toronto, Ontario M1C 1A4 CANADA

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## ABSTRACT

The current research aimed to investigate the effects of early postnatal exposure to low-level lead on the spatial learning of Long-Evans Hooded rats tested in the Morris water maze. To explore possible neurotoxic actions of lead on the N-methyl-D-aspartate (NMDA) receptors, the non-competitive NMDA receptor antagonist (+)-5-methyl-10,11-dihydroxy-5H-dibenzo(a,d)cyclohepten-5,10-imine (MK-801) was used. Two-day-old pups were randomly assigned to 0.1% lead carbonate diet or control diet and weaned onto regular food on postnatal day (PND) 23. Spatial acquisition was assessed from PND 24 to PND 27. Thirty minutes prior to behavioral testing, rats received an intraperitoneal (i.p.) injection of 0.1% MK-801 or saline. In the saline treatment group, Pb-exposed rats exhibited significantly longer overall mean escape latencies than those on the control diet, replicating the impaired spatial learning of Pb-exposed animals tested at a young age. Although MK-801 injection severely impaired animals' water maze performance regardless of diet, it affected the performance of Pb-exposed animals to a lesser extent than animals on the control diet. No main effects of diet and drug were found for probe trials on PND 28 and PND 36, but interestingly, lead diet/MK-801 animals performed significantly better than control diet/MK-801 animals during the first probe trial. Unfortunately, MK-801 not only caused animals to display higher activity levels in the activity box, but also significantly impaired animals' performance in the cued trial, suggesting non-specific sensorimotor deficits induced by MK-801 treatment might be responsible for animals' poor performance. However, a significant drug by diet interaction in the escape latencies and the significantly better probe trial performance of Pb-exposed animals within the MK-801 treatment group imply that lead exposure actually alleviated behavioral deficits induced by MK-801 injection, providing evidence for a possible interaction between lead and MK-801 at the molecular level.

## I. INTRODUCTION

Since the 1970s, the detrimental effect of chronic lead exposure on cognitive performance has raised concerns in society. Many studies show that developing brains with incomplete blood-brain-barriers are most susceptible to the devastating effect of lead [1-2], and there is a high correlation between children's blood lead concentration and their intellectual performance [3-6]. Numerous correlational studies also reveal that even low-level lead exposure during

early development can lead to reduced IQ, sensory deficits, associative deficits and impaired verbal performance [4-5,7-8]. Furthermore, these cognitive deficits may persist into late childhood [9-10]. Nevertheless, correlational studies, which involve no active manipulation of the variables, are inherently deficient in establishing a causal relationship between lead exposure and cognitive deficits. Unfortunately, formal controlled experiments that can better characterize the consequences of lead exposure have rarely

been carried out so far.

However limited in numbers, previous experiments that did examine the effect of lead have shown that lead exposure causes spatial memory deficits in rodents tested in the Morris water maze [1,11]. The Morris water maze is a standard protocol assessing rodents' ability to use external cues to remember the spatial location of a submerged platform [12-13]. Although the exact mechanism by which lead impairs spatial memory is not well understood, various studies have suggested that lead exerts its neurotoxic effect by altering N-methyl-D-aspartate (NMDA) receptors. NMDA receptors are critically involved in the production of long-term potentiation (LTP), a widely accepted cellular model for learning and memory [1, 14-17]. This makes NMDA receptors a particularly attractive site for studying lead effects.

NMDA receptors, which are highly expressed in the hippocampus, can be targeted by administering suitable antagonists. The drug (+)-5-methyl-10,11-dihydroxy-5h-dibenzo(a,d)cyclohepten-5,10-imine (MK-801), a non-competitive NMDA receptor antagonist that binds to the inside of the ion channel, is often used to assess the role of NMDA receptors in various learning paradigms. Previous studies have shown that systemic MK-801 administration produces impairments in acquired odor aversion, learned emotional responses, passive avoidance, spatial learning tasks, spatial working memory and reference memory in rodents [18-22]. If exposure to low-level lead results in alterations of NMDA receptors, one would expect an interaction between lead and MK-801 to manifest on the behavioral level.

The current study therefore investigates whether early postnatal exposure to low-level lead impairs spatial learning and memory of rats tested in the Morris water maze, and how this exposure interacts with the effect of MK-801. As some recent studies demonstrate that both lead exposure and MK-801 administration can induce hyperactivities in animals [23-24], this side effect on motor activity may confound behavioral data from the Morris water maze. Therefore, the hyperactivity associated with lead and MK-801 was controlled using an activity box in this study.

## II. METHOD

### a. Animals

Eight nursing mother rats with cross-fostered 2-day old pups (Long-Evans Hooded) were obtained from Charles River (Quebec, Canada). The 80 pups were culled by gender into 10 pups per litter, and each litter was housed in a standard plastic cage with ad lib access to food (ground chow) and water. The housing room was maintained on a 12 hour dark/light cycle. Pups were randomly assigned to two diet groups. The experimental group was fed food containing 0.1% lead carbonate, and the control group with 0.1% sodium acetate. Animals were weighed every 5 days starting from postnatal day (PND) 5. They were weaned on PND 23, housed in pairs, and fed on regular rodent chow thereafter. All animal treatments were in strict accordance with the Canadian Council on Animal Care's (CCAC) Guide to the Care and Use of Experimental Animals.

### a. Morris Water Maze Test

- *Standard water maze test.* Behavioral testing commenced on PND 24. The water maze consisted of a red plastic water tank (diameter 87 cm) filled with water to a depth of 17.7 – 18.2 cm. The water was maintained at room temperature (20-22 °C), and the tank was divided into four equally sized quadrants (East, South, West, North). A transparent plastic platform was submerged 1.5 - 2 cm below the surface of the water in the East quadrant and remained in the same position throughout all tests. The water maze was located in a cued environment. Salient external cues included a video camera on a tripod (next to the Northeast quadrant) and an experimenter standing at a fixed position (next to the Southwest quadrant).

Training was performed on four consecutive days with four trials taking place each day. Thirty minutes prior to behavioral testing on each day, each rat received an i.p. injection of either 0.1% MK-801 (Tocris, Ontario, Canada) or 0.1% saline solution. The solution to be injected was randomly assigned such that approximately half of each diet group received MK-801 and the other half received saline. On the first day,

Age	Diet Group	Body Weight (gm) <sup>a</sup>
PND 5	Control	11.6 ± 0.27
	Lead	10.6 ± 0.18*
PND 10	Control	21.0 ± 0.38
	Lead	16.8 ± 0.41*
PND 15	Control	33.0 ± 0.68
	Lead	24.2 ± 1.16*
PND 20	Control	44.0 ± 0.80
	Lead	35.2 ± 1.13*
PND 25	Control	60.5 ± 2.11
	Lead	59.2 ± 1.83

**Table 1.** Average body weight for lead-exposed and control rats at 5 weighing days.

<sup>a</sup> Body weight expressed as mean + S.E.M.

\* Significantly different from control rats,  $p < .05$

before the first trial, each rat received a priming trial whereby it was placed on the platform for 20 seconds. During each regular training trial, rats were dropped into a randomly assigned quadrant facing the wall of the pool. Animals were given a maximum of 60 seconds to find the platform and escape latencies were recorded by viewing a video monitor. If the platform was not found within 60 seconds, animals were placed on the platform for another 30 seconds, and the second trial only started after all rats had completed the first trial. Two replications of the experiment were performed with 24 rats in the first replication and 34 in the second (22 rats died before behavioral testing and were thus removed from the study).

- *Probe trials.* The first probe trial was performed on PND 28. Thirty minutes prior to testing, all rats received an i.p. injection of either 0.1% MK-801 or saline as previously assigned. In this trial, the platform was removed, and the amount of time animals spent in the East quadrant was recorded during a 60-second interval. A second probe trial was conducted on PND 36 and there was no injection prior to this test.

- *Cued trial.* Following the probe trial on PND 28, a cued trial was performed. In this trial the platform was submerged in the North quadrant and a flag was placed over the platform. Each rat was again given a maximum of 60 seconds to find the platform and escape latency was recorded.

#### c. Activity Box

After the cued trial was completed on PND28, all rats were individually evaluated on their motor activities in an activity box (base area 80cm x 80cm, height 32.45 cm), the bottom of which was marked by a grid of 25 equally sized squares (15cm x 15cm). Rats were placed onto the center square and their activity levels were measured in three 5-minute intervals. Activity was measured by counting the number of times the head and front two paws cross over one gridline or walk diagonally over an intercept. The testing box was cleaned following each 15-minute session.

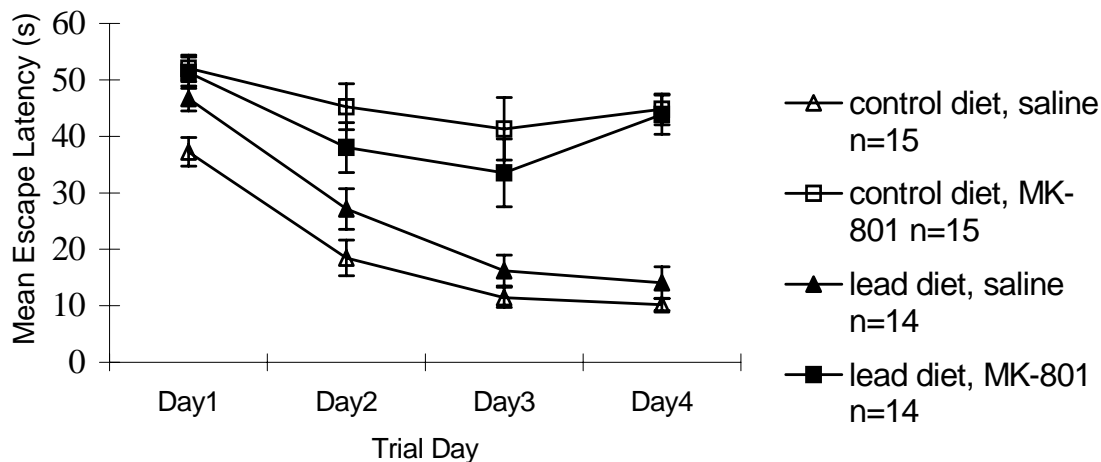
#### d. Statistical Analysis

All data were analyzed using SPSS 14.0. One-way analysis of variance (ANOVA) was performed on body weights and probe trial 2 performance. Overall mean escape latency, probe trial 1 performance, cued trial escape latency and activity levels were analyzed using 2 x 2 ANOVA, treating diet and drug as between-subject factors. Correlations between overall mean escape latency and other relevant data (cued trial escape latency and activity levels) were also calculated. All statistics were considered significant when  $p < .05$ .

### III. RESULTS

#### a. Weight

Animals were weighed every five days starting from PND 5 (Table 1). Pb-exposed rats weighed significantly less than control rats on the following weighing days: PND 5 [ $F(1, 60) = 8.505, p < .05$ ], PND 10 [ $F(1, 57) = 58.076, p < .05$ ], PND 15 [ $F(1, 57) = 44.013, p < .05$ ] and PND 20 [ $F(1, 56) = 40.562, p < .05$ ]. On PND 25, which was 2 days after weaning, there was no longer a significant difference in body weight between the two diet groups [ $F(1, 56) = 0.198, p = .658$ ].



**Figure 1.** Mean escape latency at each trial day for each treatment group (mean + S.E.M).

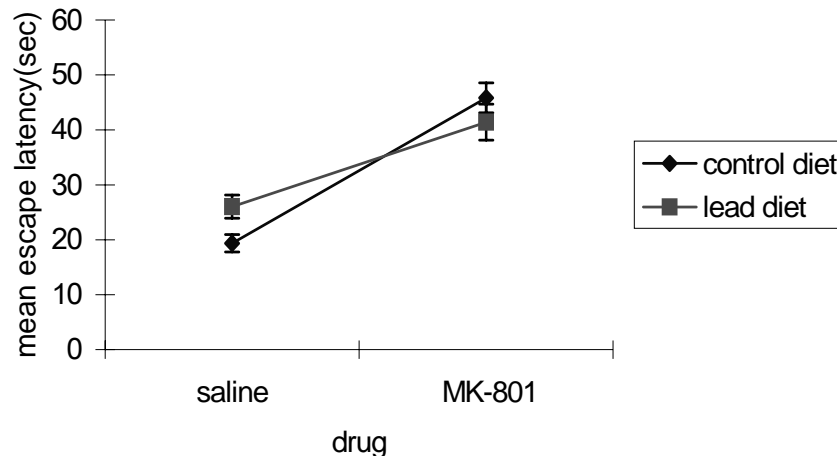
#### b. Morris Water Maze performance

- *Standard water maze test.* The trend of daily mean escape latencies for all treatment groups was shown in Figure 1. As can be seen in this figure, all animals regardless of diet or drug pretreatment showed decreased escape latency over days, indicating learning had taken place.

One-way repeated measure analysis showed that all four groups displayed significant or nearly significant savings on learning across the four training days (control diet/saline [F(3, 42) = 43.369,  $p < .01$ ]; control diet/MK-801 [F(3, 42) = 2.823,  $p = .0502$ ]; lead diet/saline [F(3, 39) = 40.107,  $p < .01$ ]; lead diet/MK-801 [F(3, 39) = 5.702,  $p < .01$ ]). The overall mean escape latency (average of all 16 trials) was computed for each rat. The ANOVA revealed a significant main effect of drug [F(1, 54) = 70.483,  $p < .01$ ], with MK-801-treated rats displaying significantly longer overall escape latency ( $43.70 \pm 2.119$  s;  $n = 29$ ) than saline treated rats ( $22.58 \pm 1.439$  s;  $n = 29$ ). This significant difference was consistent throughout four trial days. Although there was no significant main effect of diet on overall escape latency, a significant simple effect of diet was revealed when data analysis was performed on results from saline treated rats only [F(1, 28) = 6.411,  $p < .05$ ]. Among rats that were injected with

saline prior to behavioral testing, Pb-exposed rats took a significantly longer time ( $26.03 \pm 2.134$  s;  $n = 14$ ) than controls ( $19.35 \pm 1.588$  s;  $n = 15$ ) to find the hidden platform (Figure 2). Further analysis revealed a significant difference in the escape latency between the two groups on the first day of training [F(1, 27) = 7.75,  $p = .010$ ] even though the difference fell to a non-significant level starting from training day 2.

Interestingly, the data analysis indicated a significant drug by diet interaction [F(1, 54) = 4.954,  $p < .05$ ], implying differential effects of lead exposure on the two injection groups. While Pb-exposed animals showed significantly worse performance than animals on the control diet within saline-treated group, among MK-801-treated rats, Pb-exposed rats even displayed slightly shorter escape latencies ( $41.41 \pm 3.273$  s;  $n = 14$ ) than those on control diet ( $45.84 \pm 2.720$  s;  $n = 15$ ) even though the difference was not statistically significant [F(2, 37) = 1.09,  $p = .305$ ] (Figure 2). It was surprising that lead exposure did not exacerbate behavioral deficits induced by MK-801 injection, and even exhibited a tendency to alleviate the degree of impairment caused by MK-801 injection from baseline condition (saline injection).



**Figure 2.** Overall mean escape latency for all treatment groups (mean + S.E.M). \*\* Significantly different from saline treated rats,  $p < .01$ ; \* Significantly different from saline treated rats on control diet,  $p < .05$

- *Probe trials.* Probe trials measured the amount of time rats spent in the East quadrant, the quadrant in which the hidden platform was previously located. There was no significant main effect of either diet or drug on both probe trials. Nevertheless, a significant simple effect of diet was revealed when considering probe trial 1 performance among MK-801-treated rats only [ $F(1, 27) = 5.20, p < .05$ ]. Control diet/MK-801 rats spent significantly less time ( $11.27 \pm 1.123$  s;  $n = 15$ ) in the target quadrant than lead diet/MK-801 rats ( $15.86 \pm 1.703$  s;  $n = 14$ ) (Figure 3).

- *Cued trial.* A strong correlation was found between cued trial escape latency and mean escape latency ( $r = 0.721, p < .01$ ) (Figure 4). Also, there was a significant main effect of drug on animals' performance in this visual platform trial [ $F(1, 54) = 44.311, p < .01$ ]. MK-801-treated rats took a significantly longer time ( $35.62 \pm 4.043$  s;  $n = 29$ ) than saline treated rats ( $8.41 \pm 0.685$  s;  $n = 29$ ) to find the platform. Moreover, 31% ( $n = 9$ ) of MK-801-treated rats were unable to find the platform within 60-second time limit, suggesting that severe visual deficits had been caused by the drug administration. There was no main effect of diet found in this trial. Neither did any drug by diet interaction exist.

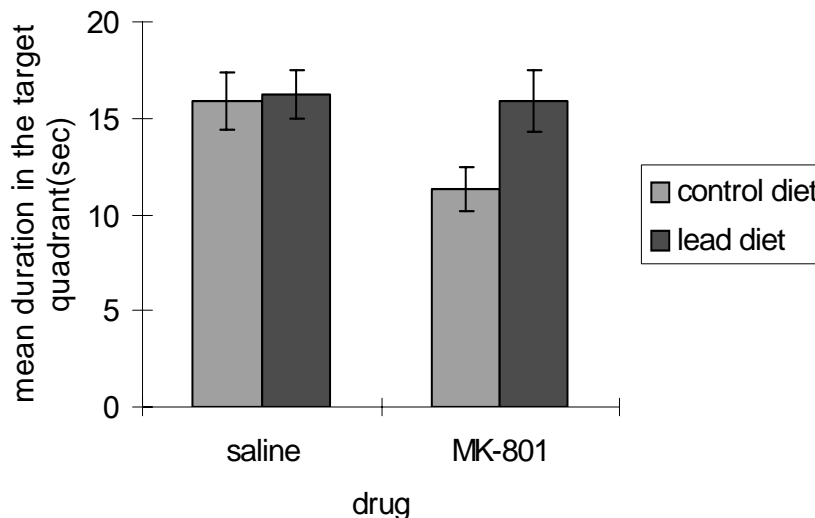
#### c. Activity Box

Since the activity levels at each 5-minute interval were significantly correlated with the total activity levels (first interval:  $r = 0.857, p < .01$ ; second interval:  $r = 0.774, p < .01$ ; third interval:  $r = 0.728, p < .01$ ), only the total activity levels were analyzed. The ANOVA indicated a significant main effect of drug on animals' activity levels [ $F(1, 54) = 23.667, p < .01$ ] (Figure 5).

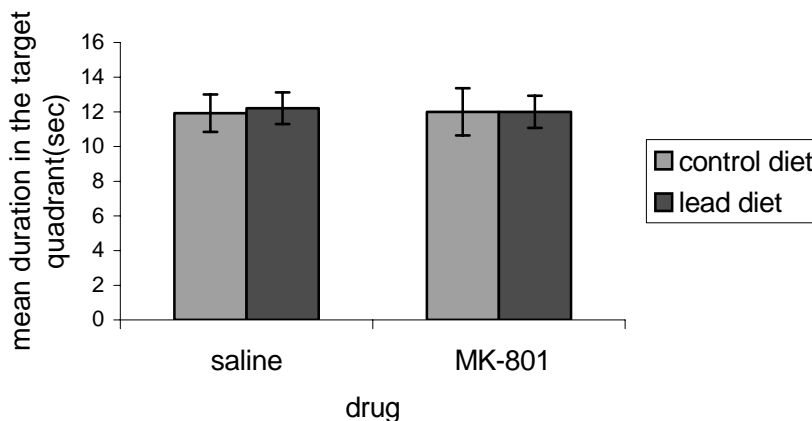
Rats injected with MK-801 displayed significantly greater activity levels than those injected with saline. There was neither a main effect of diet nor any drug by diet interaction found. Animals' activity levels were also uncorrelated with the overall mean escape latency.

#### IV. DISCUSSION

The present study shows that early postnatal exposure to low-level lead impaired the performance of drug-free animals in the Morris water maze, but not MK-801-treated animals. Among saline treated rats, the ones exposed to the lead diet exhibited significantly longer overall mean escape latency than those on the control diet. Although MK-801-treated rats were all severely impaired during training, Pb-exposed ones showed comparable, if not better, performance as compared to those



**Figure 3a.** Mean duration in the target quadrant for all treatment groups (control diet/saline, lead diet/saline, control diet/MK-801, lead diet/MK-801) during probe trial 1, shown as mean + S.E.M. \* Significantly different from lead diet/MK-801 rats,  $p < .05$



**Figure 3b.** Mean duration in the target quadrant for all treatment groups (control diet/saline, lead diet/saline, control diet/MK-801, lead diet/MK-801) during probe trial 2, shown as mean  $\pm$  S.E.M.

on the control diet. More importantly, lead diet/MK-801 animals performed significantly better than control diet/MK-801 animals in probe trial 1, further suggesting a possible alleviation of MK-801 induced deficits as a result of low-level lead exposure.

Together with previous studies [2], the findings suggest that both prenatal and early postnatal exposure to low-level lead affect animals' spatial learning ability tested at a young age (between PND 20 and PND 30). In Jett et al.'s experiment, lead-exposed

animals that started behavioral testing on PND 21 were also impaired in the probe trial. In this current study, however, Pb-exposed animals performed equally well with control animals in both probe trials, suggesting that low-level lead only slows down the acquisition of spatial memory without affecting its consolidation and retrieval. These inconsistent results might be obtained due to differences in the starting time and route of lead administration. In this experiment, instead of implementing